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La Portada

El ratón de la Sierra Madre Occidental (*Peromyscus schmidlyi*) se encuentra en los estados de Sinaloa, Sonora y Durango en la Sierra Madre Occidental de México en altitudes mayores a los 2,000 msnm. Los individuos están asociados a vegetación donde predominan pinos y encinos. de matorral costero y chaparral. Se describió en 2004, en honor a David J. Schmidly, y puede distinguirse de otras especies del género por medio de análisis genéticos. Fotografía tomada del Banco de Imágenes de la Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO), con número de referencia CLG0065. (Autor de la fotografia Celia López González).

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."

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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, artículos de revisión y notas científicas son bienvenidas.

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Editorial

Issue dedicated to David J. Schmidly, Ph.D., in recognition of his contributions to mammalogy in México

In May 2020, we were invited by Dr. Sergio Ticul Álvarez–Castañeda, editor of Therya, to serve as guest editors of the May 2021 issue to be published in recognition of Dr. David J. Schmidly's many contributions to mammalogical research in México and his involvement with and support of the Asociación Mexicana de Mastozoología A. C. (aka the "Mexican Society of Mammalogists"). Accepting this role was an honor and privilege, and we enthusiastically supported the idea for this honorary issue, for a number of reasons. First, Dr. Schmidly (Figure 1a, b) spent a significant portion of his research career, beginning with his first trip to México in 1968 as a Master's student at Texas Tech University, and continuing to this day, studying the systematics and natural history of Mexican mammals and he has contributed significantly to the scientific literature in that context. He has published extensively on the mammalian fauna of México; several of these studies are mentioned herein. Second, Dr. Schmidly has been instrumental in the lives and professional careers of many students of Mexican mammalogy, whether they were citizens of México or the United States. Through personal interest and friendship, Dr. Schmidly encouraged a cohort of undergraduates to seriously contemplate a professional career in mammalogy. Many of those who heeded Dr. Schmidly's encouragement would become the "movers and shakers" that generated an explosion in Mexican mammalogy and followed in the footsteps of preeminent Mexican mammalogists such as Drs. Bernardo Villa, Ticul Álvarez, and José Ramírez-Pulido. Third, Dr. Schmidly was instrumental in helping to encourage a group of young, enthusiastic, and forward-thinking mammalogists to establish the Asociación Mexicana de Mastozoología AC. We discuss this topic in more detail later in this paper.

Finally, from a personal standpoint, both of us have had a long-term association with Dr. Schmidly that would not have been developed if not for Dr. Schmidly's interests in mammalogy of México and the adjacent area to the north, aka Texas. Robert was recruited as a Master's student from Dr. Schmidly's mammalogy class at Texas A&M University and conducted his thesis work in 1983–1986, working on a taxonomic revision of Mexican populations of the *Peromyscus boylii* species complex. That experience provided Robert with the opportunity to conduct extensive fieldwork in México. This began a long-term friendship and scientific collaboration with Dr. Schmidly, resulting in several research endeavors on Mexican *Peromyscus* that continue to this day. Lisa began working for Dr. Schmidly in 1992, as an editorial assistant on Texas Natural History: A Century of Change as well as the fifth, sixth, and seventh editions of The Mammals of Texas. Further, beyond being colleagues and collaborators, we both count Dr. Schmidly and his wife Janet as two of our dearest friends. So for us,

Figure 1. David Schmidly in the field in Mexico, 1984, sporting his typical field attire (left). David Schmidly examining *Peromyscus* in the Mammal Collection of the Natural Science Research Laboratory, Texas Tech University, 2016 (right).

agreeing to help with this honorary volume was an easy path toward saying "thank you" to Dr. Schmidly for his many contributions to mammalogy in México and the influence he has had on our lives!

Contributions to Education and the Science of Mammalogy in México

Students Influenced. In our opinion, perhaps the greatest contribution that Dr. Schmidly has made to Mexican mammalogy has been his influence and impact on young biologists. As a young professor at Texas A&M University, Dr. Schmidly began taking field biology classes to México in the mid-1970s, many with the famous herpetologist Dr. James Dixon. During the early 1980s, Dr. Schmidly, along with Drs. Ira F. Greenbaum and C. William Kilpatrick, received a National Science Foundation grant to study the systematics of the *Peromyscus boylii* species complex (Figure 2). This project entailed several extended field trips throughout México to collect research material for morphometric, karyotypic, and allozymic studies. During many of these trips, undergraduate students —primarily several students that were affiliated with Dr. José Ramirez-Pulido and the mammal collections at Universidad Nacional Autónoma de México (UNAM) (Figure 3)— participated in collecting specimens, karyotyping, obtaining tissues, and preparing vouchers. Dr. Schmidly befriended many of these budding young mammalogists and encouraged them to pursue graduate degrees, whether it be in the United States or México. Several of the students that participated in these trips, including Gerardo Ceballos, Rodrigo Medellín, Livia León-Paniagua, Víctor Sánchez-Cordero, Aurora Alondra Castro-Campillo, and the late Daniel Navarro-López, became outstanding researchers of Mexican mammals in their own right. Further, several also were involved in the early stages of developing the Asociación Mexicana de Mastozoología A. C. (see below). Later, during Dr. Schmidly's terms as President at Texas Tech University and then at Oklahoma State University, he developed partnerships with several universities in México and encouraged a new generation of Mexican students to pursue mammalogical research, such as Irene Tiemann-Boege, who received her Master's degree with RDB at Texas Tech University and is now an Associate Professor at Johannes Kepler University in Austria. Further, Dr. Schmidly has a wonderful ability to connect with students and he never fails to show interest in their research. For example, it was not uncommon at the annual meetings of the American Society of Mammalogists and the Association of Southwestern Naturalists to see Dr. Schmidly enthusiastically discussing systematics or natural history of Mexican mammals with a young student from México. Dr. Schmidly had the uncanny ability to make students feel special and that their research was significant and of personal interest to him. These interactions gave students confidence in their abilities as young mammalogists.

Although the focus of this paper pertains to Dr. Schmidly's contributions to mammalogy in México, it must be stated that this devotion to and love of the mammalian fauna of México had an impact on students north of the border, as well. No fewer than 20 graduate students at Texas A&M University "cut their teeth" on field mammalogy as a result of Dr. Schmidly's field trips to México (Figure 4). Whether it was conducting mammalogical surveys along the Rio Grande drainage and the mountains of San Carlos, Tamaulipas, pursuing *Peromyscus hooperi* in Coahuila and Zacatecas, or collecting *Peromyscus* in the pine-oak forests throughout México's many montane regions, Dr. Schmidly's students received the hands-on training in tax-

Figure 2. David Schmidly with students and faculty at Universidad Nacional Autónoma de México, ca. 1982, during NSF-funded work on the *Peromyscus boylii* species group in Mexico. Left to right: Timothy Houseal, Juan Carlos Morales, Kathy Davis, Ira Greenbaum (front), Federico Romero (behind Greenbaum), Luis Miguel Mota (behind Federico), Steve Smith, Gerardo Ceballos (front), Rodrigo Medellín (behind Gerardo), David Schmidly, Livia León-Paniagua, Jan Ensink, Esther Romo-Vázquez, and Laurel Treviño Murphy.

Figure 3. David Schmidly with a group of students, many affiliated with Universidad Nacional Autónoma de México, ca. 1982. First line: María Canela, Víctor Sánchez-Cordero, Gerardo Ceballos, Daniel Navarro, David Schmidly, Livia León, Héctor Arita. Second lines: Rosario Manzanos, Lena Paula Urrutia, Alondra Castro, Juan Carlos Morales, Álvaro Miranda, Francisco Sour, Sara Quiroz, Miguel Mártinez Ramos, Jesús Maldonado.

onomy and natural history that would form the basis of their professional careers. Further, this extensive field project provided Schmidly's graduate students with an opportunity to develop long-lasting friendships with that cadre of fellow students from México (Figure 5).

Papers Devoted to Mammals of México. In perusing Dr. Schmidly's curriculum vitae, it appears that, to date, he has published at least 49 scientific papers pertaining directly to mammals occurring in México. In addition, he has published papers on mammals of the southwestern United States whose distributions include México, and there are other papers pertaining to the Rio Grande Corridor or the Gulf of México (marine mammals) that would be pertinent to studies of mammalogy in México, but these were not included in the 49 total papers mentioned above. The majority of these papers pertain to systematics, natural history, and distributional information, and they have contributed significantly to the knowledge of the mammalian fauna of México. Specifically, Dr. Schmidly has published numerous papers on the systematics of *Peromyscus*, and he is recognized as an authority on several of these species groups, such as the *P. boylii* and *P. truei* complexes.

Impact on Mexican Mammal Taxonomy. As mentioned above, Dr. Schmidly's expertise in rodent systematics is widely known and demonstrated by his many published scientific articles. During his career, he has authored or coauthored manuscripts resulting in numerous taxonomic

Figure 4. David Schmidly and students from the United States and México in Patzcuaro, Michoacán, in July 1983. Left to right: Jan Ensink, Timothy Houseal, Steve Smith, Robert Bradley, Gerardo Ceballos, David Schmidly, Scott Kilpatrick, Marc Allard, Alvaro Dávila, Juan Carlos Morales, Kathy Davis, Livia León-Paniagua, Esther Romo-Vázquez. Photo by C. William Kilpatrick, whose son Scott is in the photo.

revisions for Mexican mammals. New taxa that occur in México that Dr. Schmidly has described with coauthors include: *Peromyscus hooperi* (Lee and Schmidly 1977); *Antrozous pallidus packardi* (Martin and Schmidly 1982); *Peromyscus carletoni* (Bradley *et al.* 2014); *Peromyscus pectoralis zimmermani* (Bradley *et al.* 2015); and *Peromyscus kilpatricki* (Bradley *et al.* 2017). Species elevated from subspecies status include *Peromyscus beatae* from *P. boylii beatae* (Schmidly *et al.* 1988), *Peromyscus levipes* from *P. boylii levipes* (Schmidly *et al.* 1988), and *Peromyscus laceianus* from *P. pectoralis laceianus* (Bradley *et al.* 2015). In addition, *Peromyscus sagax* was elevated from synonomy with *P. boylii levipes*, in part (Bradley *et al*. 1996).

Other revisions by Schmidly and colleagues to the taxonomic status of Mexican mammals include: *Dipodomys ordii compactus* to *Dipodomys compactus*, *D. compactus largus* to *D. compactus compactus*, *D. compactus parvabullatus* to *D. compactus compactus*, and *D. ordii durranti* to *D. ordii obscurus* (Schmidly and Hendricks 1976; Baumgardner and Schmidly 1981); *Scalopus inflatus* to *S. aquaticus inflatus* and *Scalopus montanus* to *S. aquaticus montanus* (Yates and Schmidly 1977); *Antrozous pallidus cantwelli* to *A. p. pallidus*, and *Antrozous pallidus obscurus* to *A. p. pallidus* (Martin and Schmidly 1982); *Heteromys temporalis* to *H. desmarestianus temporalis*, *Heteromys longicaudus* to *H. desmarestianus longicaudus*, and *Heteromys goldmani lepturus* to *H. desmarestianus lepturus* (Rogers and Schmidly 1982); *P. boylii ambiguus* to *P. levipes ambiguus* (Castro-Campillo *et al.* 1999); and *P. boylii sacarensis* to *P. beatae sacarensis* (Bradley *et al.* 2000). In recognition of his contributions to systematics and taxonomy, Dr. Schmidly has been honored by his colleagues with two patronyms of Mexican mammals: *Peromyscus schmidlyi* (Bradley *et al.* 2004) and *Habromys schmidlyi* (León-Paniagua *et al.* 1993).

Establishment of and Service to the Asociación Mexicana de Mastozoología. Dr. Schmidly was instrumental in encouraging young Mexican mammalogists to create a society for the study of Mexican mammals, using the format of the American Society of Mammalogists as a guide. Below is an excerpt (translated to English) of the article by Juan Pablo Gallo-Reynoso (2014) that summarizes the organization of the Asociación Mexicana de Mastozoología. We took the liberty to highlight (in bold) mammalogists who at the time were graduate or postdoctoral students that had attended field trips with Dr. Schmidly in the early 1980s.

"In 1983, a group of students met by chance of fate in the Mammozoology Laboratory of the Institute of Biology, some with interests in bats, others in rodents, others in aquatic mammals, some more in ecology, others in taxonomy, or paleontology; we formed a network of acquaintances that eventually formed a critical mass. We were graduates of the Faculty of Sciences of the UNAM, of the UAM (Metropolitan Autonomous University) Iztapalapa and Xochimilco, of the INAH (National Institute of Anthropology and History) and of other universities, so we got together: **Alondra Castro**, **Esther Romo**, **Livia León**, María Canela, Rosario Manzanos, Silvia Manzanilla; Álvaro Miranda, **Federico Romero**, Héctor Arita, Hiram Barrios, **Juan Carlos Morales**, Juan Pablo Gallo, **Rodrigo Medellín**; some still students, others already graduated, some were in postgraduate studies abroad such as **Daniel Navarro**, Fernando Cervantes, **Gerardo Ceballos** and **Víctor Sánchez Cordero**; others pursuing postgraduate studies at the Faculty of Sciences. From all of us came the firm proposal to go forward, to found the Mexican Association of Mammozoology, why not?"

Figure 5. American and Mexican students at Universidad Nacional Autónoma de México, July 1983. Front, left to right: Kathy Davis, Livia León-Paniagua, Federico Romero, Esther Romo-Vázquez, Jan Ensink. Back, left to right: Steve Smith, Marc Allard, Robert Bradley.

Further, Dr. Schmidly helped host and organize the Joint International Meeting between the American Society of Mammalogists and Asociación Mexicana de Mastozoología, in Cancun, Quintana Roo, México, in 1987. Dr. Schmidly and Michael Mares edited a proceedings of many of the important papers presented at that joint conference, entitled "Latin American Mammalogy: History, Biodiversity, and Conservation", that was published in 1991 (Mares and Schmidly 1991). Dr. Schmidly also served as Associate Editor for Revista Mexicana de Mastozoología, the journal of the Asociación Mexicana de Mastozoología, from 1990 to 1991.

Honors and Recognitions from the Asociación Mexicana de Mastozoología and Other Institutions in México. Dr. Schmidly's contributions to mammalogy and education in México have been identified and acknowledged by several scientific and civic organizations. First, from a professional mammalogical standpoint, Dr. Schmidly was recognized by the Asociación Mexicana de Mastozoología in 2010 with the presentation of the prestigious Ticul Álvarez Solorzano Award. Dr. Schmidly was the second winner of this award, which is the highest distinction awarded by the society, in recognition of his professional career, his impact on Mexi-

can mammalogy, and the training of professionals with the highest standards in México. Second, over the course of his professional and academic career, Dr. Schmidly has received special recognition for his efforts to form collaborations between multiple universities and cities in México and the United States. For example, he was: appointed as Maestro Emerito (Professor Emeritus), Universidad Popular Autónoma del Estado de Puebla (UPAEP), México, for establishing a series of joint degrees between Oklahoma State University and Mexican institutions in the Puebla region; appointed as Visiting Professor at the Universidad de las Americas, Chollulo, Puebla, México, and invited to teach a natural history course on mammals in the biology department; designated as Vistante Distinguido by the city of Puebla, México; and granted Diplomas of Recognition from universities in Guadalajara and Tamaulipas, México.

Organization of Contributions to this Honorary Issue

For this honorary issue, we received 17 contributions from 67 authors. Based on the scientific content of each contribution, we organized this issue into four categories: Editorial, Conservation, Natural History, and Systematics and Taxonomy. Interestingly, Dr. Schmidly's major scientific contributions are in the disciplines of conservation, natural history, and systematics. Below, we list the categories, contribution titles, and authors of these articles.

Editorial

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Conservation

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Vaquita: Beleaguered Porpoise of the Gulf of California, México, by *Bernd Würsig, Thomas A. Jefferson, Gregory K. Silber, and Randall S. Wells*.

Natural History

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Concluding Remarks

Dr. Schmidly has had a long-term connection to both the mammals and the people of México. He began collecting and studying the mammals of México beginning in 1968 with Dr. Robert Packard at Texas Tech University, and his interest in the mammalian fauna of México intensified during his research for his PhD at the University of Illinois with Drs. Donald Hoffmeister and Raymond Lee. The impact of those early trips to México were a profound influence on the career of that young mammalogist, and they formed the foundation of his professional career at Texas A&M University, through three stints as university president (Texas Tech University, Oklahoma State University, and University of New Mexico), and continues to this day. Although his systematic and natural history studies of mammals in México represent significant contributions to Mexican mammalogy, perhaps his influence on the students of Mexican mammalogy represents his great contribution. From all of us who benefited from Dr. Schmidly's vast mammalogical knowledge, mentorship, research opportunities, and personal friendship, we dedicate this issue to his legacy. Finally, we would like to take this opportunity to sincerely thank the many individuals who contributed to this honorary issue of Therya. This project would not have been possible without: 1) the high-quality manuscripts enthusiastically contributed by friends and colleagues of Dr. Schmidly, 2) the incredible responses by numerous individuals who agreed to review the submitted manuscripts in a timely fashion, and in some cases with extraordinary turn-around, and 3) Sergio Ticul Álvarez–Castañeda, not only for inviting us to serve as Guest Editors but especially for his vision for this issue of Therya and for coordinating and spear-heading this endeavor.

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Neither stable nor pristine: American bison populations were long influenced by humans

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Populations of North American bison (*Bison bison*) are widely presumed to have remained stable, numbering in the tens of millions, right up until the hide hunts of the 1870s nearly brought about extinction. Recent scholarship from various disciplines consistently undermines this presumption. Indigenous people likely affected bison populations from their arrival toward the end of the Pleistocene. By the time of Columbus, indigenous populations were high and their impacts were felt keenly. As documented in the 16th century journals of Cabeza de Vaca, big game populations, including bison, were suppressed by hunting. That changed, however, with arrival of Old World diseases that are estimated to have reduced indigenous populations in the Americas by 90 % within a century of contact with Europeans. Such drastic reductions in indigenous human populations allowed bison populations to expand. Gradually, increased pressure from human hunters, along with competition from feral horses, introduced infectious diseases, habitat changes, and droughts, all suppressed bison populations well before the notorious hide hunts began in the 1870s. The hide hunts were the final blow to free-ranging bison, but reduced populations in the decades prior paved the way and helps explain why bison were reduced to near extinction within a few years.

Se considera ampliamente que las poblaciones del bisonte Americano (*Bison bison*) se han mantenido estables, llegando a decenas de millones, hasta que la caza por sus cueros en la década de 1870 casi provocó la extinción. Estudios recientes de diversas disciplinas socavan consistentemente esta presunción. Los pueblos indígenas probablemente afectaron a las poblaciones de bisontes desde su llegada hacia el final del Pleistoceno. En la época de Colón, la población indígena era alta y sus impactos se sintieron profundamente. Como se documenta en el diario de Cabeza de Vaca del siglo XVI, las poblaciones de grandes mamíferos, incluido el bisonte, fueron disminuidas por su caza. Sin embargo, eso cambió con la llegada de enfermedades del Viejo Mundo, las que reducieron a las poblaciones indígenas en las Américas estimadamente un 90 %, en un siglo de contacto con los europeos. Tales reducciones drásticas en las poblaciones de los pueblos americanos permitieron que las poblaciones de bisontes se expandieran. Gradualmente, el aumento de la presión de los cazadores humanos, junto con la competencia de los caballos salvajes, introdujo enfermedades infecciosas, cambios de hábitat y sequías. Todo ello suprimió a las poblaciones de bisontes mucho antes de que comenzaran las notorias cacerías de pieles en la década de 1870. La caza de cueros fue el golpe final para los bisontes en libertad, pero la reducción de las poblaciones en las décadas anteriores allanó el camino y ayuda a explicar por qué los bisontes se redujeron a casi a la extinción en unos pocos años.

Keywords: Bison; hunting; indigenous; populations.

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Introduction

"It ain't what you don't know that gets you in trouble. It's what you know for sure that just ain't so." - Anonymous

The story of the American bison is well known, particularly as a case study on the need for conservation. In its most popular version, it goes something like this: American bison thrived for millennia in North America, in numbers of 60 million or more. American Indians hunted bison for their meat and skins in the late Pleistocene and throughout the Holocene. But Indians were always few in number and, being ecologically sensitive, took only what they needed, thus having a negligible impact on bison populations. It was only with the arrival of the hide hunters in the 1870s that the human impact was finally felt, resulting in a sudden crash in bison populations in the span of a few years, nearly causing extinction.

Historians and biologists have evaluated evidence of human impacts on North America's bison population. In doing so, they have challenged many popular elements of the bison story. Humans have affected bison populations for millennia primarily through hunting, although other forces of human origin were being introduced by the early 19th century. Hunting impacted bison demographically, through reductions in numbers or skewing of sex ratios, and indirectly by concentrating bison in areas in which they were relatively safe. Similarly human hunting pressure excluded bison from areas in which they were particularly vulnerable to hunters.

Bison entered North America during the Pleistocene and underwent rapid and extensive speciation into at least four species: (*Bison alaskensis, B. priscus, B. latifrons,* and *B. antiquus;* McDonald 1981). Near the end of the Pleistocene, human hunters invaded North America, and some 35 genera of large mammals became extinct. Using improved dating techniques for fossils, Martin (1966) concluded that human hunters had caused these extinctions. Similar patterns of megafaunal extinctions following invasions of human hunters have been documented for Australia (Van Der Kaars *et al.* 2017), Madagascar (Martin 1966), and New Zealand (Perry *et al.* 2014).

Martin's "overkill" hypothesis has been challenged over the years by researchers who favor climate change at the

end of the Pleistocene as the primary cause for the megafauna extinctions. Yet climate change was not concurrent with extinctions in Madagascar, Australia, and New Zealand. A thorough and detailed review of the controversy for the Americas (Haynes 2007) firmly concluded that overkill was the major force for megafauna extinctions.

Although bison survived the late Pleistocene, they became notably smaller in size during the last few millennia of the Holocene. This "Holocene dwarfing" made the extant bison of North America a newly evolved species (McDonald 1981). Human hunting pressure was likely a significant force behind the dwarfing, as smaller bison would reach reproductive age earlier than larger ones, and thus provide a demographic advantage in the face of higher mortality.

The journey of Cabeza De Vaca. In 1528, Spain sent the Panfilo de Narvaez expedition to Florida with the objective of establishing a Spanish colony. The expedition, consisting of five ships and 600 people, failed miserably. The second in command, Alvaro Nuñez Cabeza de Vaca and three other men were the lone survivors who, over the course of eight years, finally made their way to Mexico City (De Vaca 1542). After landing near present-day Tampa, the expedition marched into central Florida, then north and west to Florida panhandle. From there they traveled in barges along the Gulf coast from Florida to coastal Louisiana. After spending six years as captives among Indians on Last Island, De Vaca and his companions escaped to the mainland and traveled through southwestern Louisiana then through central Texas, New Mexico, and southeastern Arizona before turning south toward México City.

Although the expedition failed, the long journey of De Vaca provided perhaps the best insight into conditions in the continental United States prior to settlement by Europeans. The journey occurred before the onslaught of European diseases that over the next three centuries would kill millions of American Indians. Moreover, De Vaca was a Spanish nobleman, and like others of that time, was well acquainted with two pastimes: big game hunting and falconry, activities that fostered interest in and knowledge of wildlife (Geist 2018). Most importantly, upon returning to Spain, De Vaca published a long and detailed account of his experiences.

Geist (2018) reviewed the journals written by De Vaca (De Vaca 1542) using his accounts to test the notion that North America before European settlement was, as popularly believed, pristine, with few Indians and exceptionally abundant wildlife, particularly big game. What he found directly contradicted the popular view. Instead, throughout his journeys from Florida to Arizona and south into México, De Vaca reported abundant Indians and wildlife that was either absent or quite rare. Moreover, the Indians themselves lived on the edge of starvation. Some grew crops of maize, beans, or squash and hunted opportunistically. Others subsisted for months at a time on insects, snakes, lizards, rabbits, and even fruit of prickly pear (*Opuntia* spp.).

Throughout their travels, De Vaca and his men likewise suffered from insufficient food that, combined with inadequate clothing and shelter, resulting in much suffering and death. But what Geist (2018) found particularly striking was hardly any mention whatsoever of what later were common large species of wildlife, including wild turkeys (*Meleagris gallopavo*), American alligator (*Alligator mississippiensis*), American elk (*Cervus elaphus*), and pronghorn (*Antilocapra americana*). The only species of big game that was regularly mentioned was white-tailed deer (*Odocoileus virginianus*) that were scarce most places and abundant in only a few. Finally, passenger pigeons (*Ectopistes migratorius*), a species that would darken the skies of 19th century America, were never mentioned at all.

De Vaca did observe live bison, but only three times, all apparently in southern or southwestern Louisiana (De Vaca 1542). Describing them as "cows", he noted that they were about the size of Spanish cattle and reported that he had eaten their meat. Farther west, he and his companions, based on their communications with various Indian groups, thought that bison were more common to the north of their route and encountered bison skins and robes that had arrived apparently along trade routes. Descriptions of bison by two of De Vaca's companions gave the distinct impression that they had not observed bison either frequently or at close quarters. One of them described bison as a "tapir" while the other thought that bison had a single horn emerging from their foreheads, unicorn-style (Guengerich 2013).

Although De Vaca's route was south of what in the 19th century would be the heart of bison country, it would nonetheless have passed through the southern edge of the primary bison range as defined by McDonald (1981:104, fig 23). Thus, it seems safe to conclude that bison, like most other larger species of wildlife, were scarce. Given the large numbers of Indians encountered, and their constant quest for food, it also seems reasonable to conclude that bison were likely considerably less abundant in 16th century America than in 19th century America.

The "great dying". Geist's (2018) conclusions from analyzing the journals of Cabeza De Vaca were bolstered by a thorough and detailed analysis (Koch *et al.* 2019) that linked marked declines in populations of American Indians with ecological succession following abandonment of intensively used lands, a process that removed enough carbon dioxide from the atmosphere to trigger the "Little Ice Age". The authors first estimated the indigenous population of the Americas at the time Columbus first landed. They then evaluated the impacts of that population's land use. Next, they calculated the reductions in Indian populations from 1,500 CE through 1,600 CE, roughly the first century following Columbus' voyage. Finally, they estimated the recovery of indigenous agricultural sites following the population decline in terms of carbon sequestration to infer effects that those changes would have had on climate.

Koch *et al.* (2019) compiled estimates from extensive interdisciplinary literature reviews, cross-combined and sampled them using two different statistical methods, breaking down estimates for each of seven regions. Their model concluded that the mid-point for the pre-Columbia's population of the Americas was about 60.5 million and that the total land use area required to sustain them was roughly 61.9 million ha. A century later, the population of indigenous people had dropped by 90 % to an estimated 6.1 million and the land use area proportionally declined to 6.1 million ha. As the estimated 55.8 million ha of abandoned cropland reverted primarily to forests, carbon dioxide was removed from the atmosphere.

These changes were deemed sufficient to trigger the Little Ice Age, beginning about 1610 when atmospheric carbon dioxide dropped to 272 ppm (Koch *et al.* 2019). In terms of bison and other American wildlife, the 90 % reduction in indigenous populations set the stage for the large populations recorded in the nearly three centuries following.

Bison population reductions prior to the hide hunts. Most of the actual observations of free-living North American bison are from the 19th century, from the time of Lewis and Clark in the century's first decade to the commercial extinction of the northern herd in the early 1880s. Bison specialists have tried to estimate population sizes from historical accounts by three methods: estimates of herd sizes, estimates of numbers killed, and estimates of carrying capacity, and none of them generate reliable numbers (Shaw 1995).

One the most commonly cited estimate for 19th century bison numbers was 60 million from Seton (1929). That number began with the observation of a single herd in 1871, which, taken at face value, would have contained 12 to 18 million bison. It was arbitrarily altered by Hornaday (1889) to four million perhaps to bring it in line with Hornaday's own estimates based on hide shipping records by one of three railroads (Roe 1970). An arbitrary assumption, likely a guess, was then made by Seton (1929) of the area required for a herd of that adjusted size, and the results projected on to the range map for North America (Roe 1970; Shaw 1995).

In more recent years, historians have favored estimates based on carrying capacity (Flores 2001; West 1995). Although these estimates may seem more "scientific", they are nonetheless seriously flawed. For one thing, carrying capacity is based upon the amount of forage required to sustain a population. Given the heavy human impact of hunting for subsistence and for market as well as the potential impacts of infectious diseases, droughts, and habitat alterations, it seems reasonable to infer that at least some bison populations of the 19th century were held at levels below carrying capacity.

Even the estimates for carrying capacity themselves contain serious flaws. They were based upon a United States Department of Agriculture report published in 1910 that tallied 24 million horses and cattle plus 6 million sheep over an area of roughly half the bison range on the Great Plains (Seton 1929). Indian-set fires were common on the Great Plains when bison were free-ranging. Moreover, free-ranging bison practiced short-term intensive grazing, rather than the more common continuous grazing of livestock whose movements by 1910 were restricted by fencing. Estimates of carrying capacity based on continuous grazing with fire suppression simply cannot be converted into reliable estimates of carrying capacity for free-ranging bison in frequently burned grasslands. Because of the limitations of historical estimates of bison population sizes, assessment of population reductions in the 19th century in this paper are based upon range contractions, relative abundance, declines in bison robes traded, and accounts of serious food shortages by plains Indian tribes.

Predating the hide hunts of the 1870s by decades, the robe trade was a force with the potential to suppress bison herds. For one thing, harvest was restricted to females, given their lighter and more pliable skins. For another, the demand was high and rose steadily. Declines in the robe trade in the face of high demand provided evidence of declines in bison at least in some regions.

Isenberg (2000) compiled records of bison robes traded. Between 1825 and 1830, fur traders shipped a total of 785,000 robes through New Orleans. By the 1840s, western plains nomadic tribes brought more than 100,000 robes annually to steamboats for shipment east. The American Fur Company shipped 45,000 robes to Saint Louis in 1839. By 1847, the number had risen to 110,000. Shipping records showed a marked decline in the 1850s, likely reflecting reduced bison populations. The Upper Missouri Outfit of the American Fur Company shipped 89,000 robes in 1853, 75,000 in 1857, and 50,000 in 1859 (Isenberg 2000). Fort Pierre tallied 75,000 robes in 1849, but by 1857 shipped only 19,000 (Isenberg 2000).

The robe trade's dependence upon female bison skewed the sex ratios of herds toward more males, thereby depressing calf production. In his classic work, "The Oregon Trail", Francis Parkman wrote of his observations in 1846, "Thousands of (bull bison) might be slaughtered without causing any detriment to the species, for their numbers greatly exceed those of the cows; it is the hides of the latter alone which are used for the purposes of commerce and for making the lodges of the Indians; and the destruction among them is therefore greatly disproportionate" (Parkman 1945:294). Those same steamboats that carried bison robes eastward along rivers imposed significant changes to riparian habitats as they were deforested to provide wood for fuel (Isenberg 2000). In addition, livestock pulling wagon trains of settlers along those same rivers consumed grasses in areas vital to bison in winter (West 1995).

Anthrax (*Bacillus anthracis*) struck bison populations particularly hard beginning in about 1800, though its effects were restricted to Canada and there is no evidence that it occurred south of the Canadian border (Isenberg 2000). Bovine tuberculosis (*Mycobacterium bovis*) probably was brought to bison by infected cattle from Texas. Brucellosis

(*Brucella abortus*), although a controversy for the management of the Yellowstone bison in the 20th century, probably arrived too late to impact 19th century bison populations (Flores 2001). Drought struck the Great Plains starting in 1846 and lasted for a decade (Isenberg 2000). Its end coincided with the conclusion of the Little Ice Age (West 1995). The Little Ice Age ended after atmospheric carbon dioxide increased through deforestation and the burning of fossil fuels during the industrial revolution. Bryson (1974) estimated that in the decades following the end of the Little Ice Age, the changes would have reduced bison populations on the Great Plains by 50 to 75 %. Both the short-term drought and the end of the Little Ice Age would have reduced forage production on the Great Plains and lowered the carrying capacity for bison.

Spatial distribution of bison in the 19th century was influenced by conflicts between warring tribes that left buffer zones. Lewis and Clark found that a sparsely inhabited region of about 120,000 square km along the upper Missouri River contained an abundance of big game, including bison, elk, deer, and pronghorn. Clark realized that the game abundance was due to the buffer zone (Martin and Szuter 1999). Decades later, other buffer zones in Kansas and Colorado provided safe havens for bison. These buffer zones collapsed after peace was made in 1840 between the Comanches and Kiowas with their former enemies, the Cheyenne and Arapahos (Flores 2001). When the buffer zones disappeared, so did the bison (West 1995).

Thus, several significant forces—the robe trade, habitat alterations along rivers, introduction of infectious diseases, and loss of buffer zones—all began in the first half of the 19th century, suppressing bison populations from earlier levels and destabilizing conditions on the Great Plains. In the absence of reliable estimates of actual bison numbers, reductions in bison abundance can be inferred through range contractions and through reports of starvation and hardship among plains Indians who relied upon bison for subsistence and trade. Range contraction began first in the western (shortgrass prairie) portions of the southern plains between 1821 and 1833 (Shaw and Lee 1997). By 1857, bison were displaced first from the west to about 240 km east of the Rocky Mountains (West 1995). This happened in a region still dominated by Indian hunters, not by white hunters who had long been presumed to be the cause (West 1995).

To the east in the tallgrass prairie regions, bison were displaced during the period 1833 to 1849 (Shaw and Lee 1997), although in that case the relative role of Indian vs white hunters is less clear. The forced transfer of Indian tribes from the southeast into Oklahoma Territory no doubt added to the pressure that displaced bison from tallgrass prairie regions (Flores 2001). By about 1850, Comanche were reported to be eating their horses and increasing their raids into Mexico, both signs of depleted bison. For four consecutive years, 1849, 1850, 1851, and 1852, the Kiowa and Comanche hunting grounds contained few if any bison (Flores 2001).

The hide hunts. The hide hunts began suddenly following the 1871 development of a field technique to preserve bison hides using arsenic (Martin 1973). The industrial revolution created enormous demand for bison hides for use as belts in large machinery, and the hide hunts were on. By then breach-loading rifles had replaced muzzleloaders, enabling more firepower and greater ranges than ever before. The southern herd was essentially gone by late 1875 and the northern herd by about 1882 (McHugh 1972). Although there is no doubt that the hide hunts finished off free-living bison in North America, the impacts by humans earlier in the 19th century reduced the populations to a significant extent, and made it easier for hide hunts to finish the job.

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Modern extirpation of the Texas kangaroo rat, *Dipodomys elator*, in Oklahoma: changing land use and climate over a century of time as the road to eventual extinction

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Surveys conducted during three years (2014-2017) provide the most extensive documentation to date for the possible presence of the Texas kangaroo rat (*Dipodomys elator*), a Tier II species considered to be of greatest conservation need, in seven counties in southwestern Oklahoma. The project encompassed 15 surveys on 93 nights; 266 localities were surveyed for a total of 9,094 trap nights and more than 32,428 km of paved and unpaved roads were surveyed for potential habitat and activity. No Texas kangaroo rats were captured or observed. However, 2,178 individuals of 17 mammal species were captured and individuals of 12 additional mammal species were collected and/or observed. New locality and natural history information for mammal species was obtained and six county records were recorded based on specimens and/or observations. Project results and historical information suggest that the Texas kangaroo rat (*D. elator*) is likely extirpated from the state of Oklahoma.

Estudios conducidos en Oklahoma durante tres años (2014-2017) proveen los datos extensivos de la posible presencia de la Texas kangaroo rat (*Dipodomys elator*), una especie considerado como Tier II que requiere la máxima atención para conservación en siete condados del suroeste de Oklahoma. El estudio incluyó 15 muestreos por 93 noches; 266 localidades distintas fueron muestreados con 9,094 trampa-noches y más de 32,428 km de caminos pavimentados y de tierra fueron examinados para el hábitat y actividad potencialmente. Ningún ejemplar de *D. elator* fue visto o capturado. Sin embargo, 2,178 individuos de 17 especies de mamíferos fueron colectados o observados. Nuevas localidad e información de la historia natural de las especies fue obtenida, se registran datos ejemplares o observaciones para seis condados. Los resultados de este estudio y datos históricos sugieren que el Texas kangaroo rat (*D. elator*) fue extirpado del estado de Oklahoma.

Keywords: *Dipodomys elator*; extinction; land use; Oklahoma; Texas kangaroo rat.

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Introduction

Human activities during the last century have affected the distribution of mammal species throughout the world (Ceballos *et al.* 2017; Ceballos *et al.* 2020). Occasionally these impacts are shown through major geographic range restrictions of megafauna, such as elephants or tigers and other predators (Ceballos *et al.* 2017; Ceballos *et al.* 2020). Certainly, these are charismatic species for conservation groups or for governments seeking to preserve high profile fauna, though being a high-profile animal may prove problematic for conservation (see Courchamp *et al.* 2018). Research on small mammals in danger of extinction due to habitat and climate change is less common. When a new genus and species of small salt-dwelling rodent (*Pipanacoctomys aureus*) was discovered in an isolated valley in northwestern Argentina, both climate change and habitat effects were hypothesized to greatly limit the species and eventually to affect its viability in the salt desert (Mares *et al.* 2000). Other small mammal studies also have implicated climate and land use in rarefaction of species (Cameron and Scheel 2001).

The Texas kangaroo rat, *Dipodomys elator*, is a geographically limited small mammal that has been documented in southern Oklahoma and adjacent Texas. This species was described in 1894 from Henrietta, Clay County, Texas (Merriam 1894). It was reported for Oklahoma in the early 1900s when two specimens were collected in November 1904 and July 1905 in southwestern Oklahoma near Chattanooga, Comanche County (Bailey 1905). Bailey (1905:149) reported that "while not numerous, they seem to be well distributed in the vicinity" and were found or known to be living under houses and outbuildings and feeding on Kafir corn (a predecessor of milo and grain sorghums). Despite its putative ubiquity, this species was only known for Oklahoma from these two specimens, until a specimen was collected in 1969 immediately north of the Red River in Cotton County in association with Ord's kangaroo rat, *Dipodomys ordii* (Baumgardner 1987).

Previous researchers (*e. g*., Baumgardner 1987; Moss and Mehlhop-Cifelli 1990; Stangl *et al.* 1992) have suggested that the Texas kangaroo rat has been extirpated from Oklahoma. However, only modest efforts had been made to determine its presence in the state. For example, road surveys were conducted two nights in 1970 (Martin and Matocha 1972), road surveys totaling 637 km were made in Comanche (99.6 km), Tillman (119.7 km), and Cotton (417.9 km) counties between 1985 and 1987 (Jones *et al.* 1988), and an undetermined amount of sampling was conducted by personnel of Midwestern State University in the area where the specimen was reported from Cotton County (Baumgardner 1987). During summer 1988, a

survey conducted by Moss and Mehlhop-Cifelli (1990) consisted of 354 trap nights, 66 km of night road surveys, and the examination of aerial photographs and soil maps to determine potential habitat. Martin (2002) reported conducting road surveys during June to August from 1996 to 2000 in 12 Texas and two Oklahoma counties; however, no data are presented in the report for the Oklahoma counties.

In contrast, records of *D. elator* have been reported from localities in 11 counties in northern Texas (Archer, Baylor, Childress, Clay, Cottle, Foard, Hardeman, Montague, Motley, Wichita, and Wilbarger); an additional Texas county record (Coryell) is unverified (Dalquest and Collier 1964; Packard and Judd 1968; Martin and Matocha 1972, 1991; Cokendolpher *et al.* 1979; Baumgardner 1987; Jones *et al.* 1988; Martin 2002). Extensive research has been conducted in some of these counties to better understand the distribution, ecology, diet, behavior, reproduction, natural history, and genetic diversity of the Texas kangaroo rat (*e. g.,* Dalquest and Collier 1964; Chapman 1972; Martin and Matocha 1972, 1991; Packard and Roberts 1973; Roberts and Packard 1973; Webster and Jones 1985; Jones *et al.* 1988; Stangl and Schafer 1990; Stangl *et al*. 1992, 2005; Goetze *et al.* 2007, 2008; Nelson *et al.* 2009, 2013; Stasey *et al.* 2010; Nelson and Goetze 2013; Goetze *et al.* 2015; Pfau *et al.* 2019). These data constitute the majority of the knowledge of the biology of the Texas kangaroo rat.

In 1996, the International Union for Conservation of Nature (IUCN) listed *D. elator* as vulnerable based on its decline throughout its historic range (Wahle *et al.* 2018). Habitat degradation, fragmentation, and loss of habitat from conversion to agricultural uses and development were cited by the IUCN as major threats. Although the species was listed as a category two candidate species by the United States Fish and Wildlife Service (USFWS) in 1982 (47 FR 58454), the practice of maintaining a category two candidate list was discontinued in 1996 (61 FR 64481). In Texas, *D. elator* was listed as a threatened protected non-game species in 1977 (Texas 1977), a threatened non-game species in 1985 (Texas 1985), and as a threatened species in 1987 (Texas 1987). In Oklahoma, *D. elator* was identified as a Tier II species of greatest conservation need by the Oklahoma Department of Wildlife Conservation (Appendix E, Oklahoma Comprehensive Wildlife Conservation Strategy 2016). In 2010, WildEarth Guardians petitioned the USFWS to federally list the Texas kangaroo rat (WildEarth Guardians 2010). In 2011, the USFWS determined that "the petition presents substantial scientific or commercial information indicating that listing the Texas kangaroo rat throughout its entire range may be warranted" and a status review was initiated (FWS-R2-ES-2011-0011; USFWS 2011).

To evaluate the status of this species of greatest conservation need in Oklahoma and to develop and implement scientifically sound management and conservation initiatives if its presence was documented, information was needed by the Oklahoma Department of Wildlife Conservation. Thus, the purpose of this study was to address a critical and immediate need by assessing the presence, distribution, and habitat of the Texas kangaroo rat to determine its status in the State of Oklahoma where it was little known and presumed extirpated.

Materials and Methods

Observation and trapping surveys were conducted in seven counties in southwestern Oklahoma, including Harmon, Jackson, Tillman, Cotton, Greer, Kiowa, and Comanche from 1 October 2014 to 30 September 2017 (Figure 1). These counties were selected based on their proximity to areas in Texas where *D. elator* is or was known to occur and because two of them are the reference sites for the only known specimens from Oklahoma. Localities that were accessible (*e. g*., roadsides, private land where permission was secured, parks, state and city property), one historic site (Bailey 1905), and one recent site (Baumgardner 1987) were surveyed for the presence of burrows and activity of the Texas kangaroo rat. Surveys also were conducted along paved and unpaved roads and by walking potential habitat.

Dipodomys elator is not reported to hibernate and is active year-round (Dalquest and Collier 1964); thus, the survey and inventory approach included surveys during all seasons. Localities surveyed were selected based primarily on soil and vegetation preferences described for *D. elator* in Texas (Martin 2002; Nelson *et al.* 2013). Texas kangaroo rats have been reported to inhabit arid areas not prone to flooding (Martin 2002), characterized by short, sparse grasses (Goetze *et al.* 2007; Nelson *et al.* 2009), and containing little woody canopy cover (Goetze *et al.* 2007). Although they have been reported to occur only in localities where the soil contains a significant clay component (Bailey 1905; Dalquest and Collier 1964; Roberts and Packard 1973; Martin and Matocha 1991), they are not restricted to such soils (Martin and Matocha 1991).

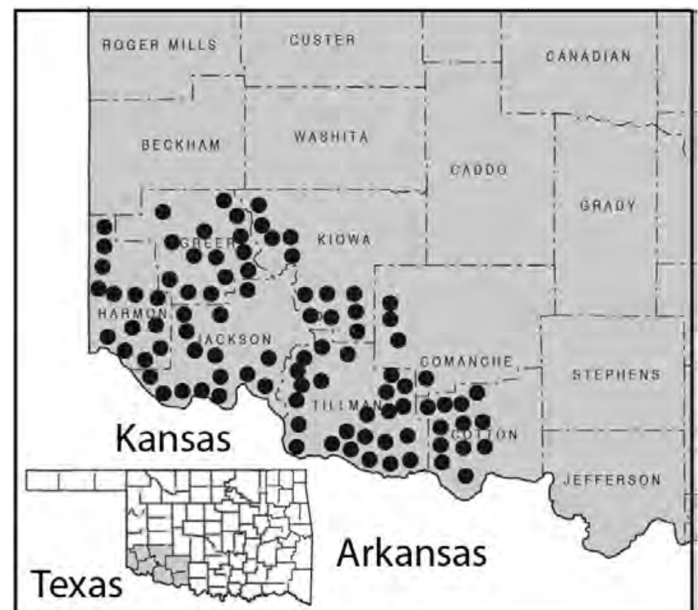

Figure 1. Map of 266 localities surveyed for *D. elator* in southwestern Oklahoma. Dots may represent more than one locality.

Localities were examined for the presence of burrows, distinct trails, and dust-bathing areas. Trapping to test for burrow occupancy was conducted by placing 7.5 X 8.8 X 30 cm folding Sherman Live Traps (extended length to minimize damage to tails) within 0.10 to 0.50 m of each burrow entrance, with the open end of each trap facing the burrow entrance. If no burrows were present, traps were placed in survey lines. Traps were baited with oatmeal each evening and checked each morning.

Small mammal species that were captured were released or euthanized, prepared as scientific voucher specimens including tissue samples, and deposited in the Collection of Mammals and Oklahoma Collection of Genomic Resources at the Sam Noble Oklahoma Museum of Natural History, respectively. All protocols followed guidelines described by Sikes *et al.* (2011, 2016) for the use of wild mammals in research and were approved by the University of Oklahoma Institutional Care and Use Committee (R13-010, R16-011).

Because Ord's kangaroo rat (*D. ordii*) also is known to occur in the seven counties that were surveyed, all individuals of *Dipodomys* that were captured were carefully identified. *Dipodomys elator* and *D. ordii* are easily distinguished from each other using external characteristics. The Texas kangaroo rat has a white-tipped tail and four toes on the hind feet, whereas the tail seldom is white-tipped in Ord's kangaroo rat and the hind feet have five toes (Carter *et al.* 1985; Caire *et al.* 1989).

Specific methods and procedures were developed should *D. elator* be captured or observed. For burrows, the diameter and orientation of entrance/exit hole would be recorded (see Figure 3 in Stangl *et al.* 1992) and the specific location of each burrow recorded in decimal degrees using a GPS unit, set to WGS84 datum. For captures, individuals of *D. elator* would be photographed, sexed, checked for reproductive condition, relative age and condition, and marked with hair dye in order to determine recapture rates. A small ear biopsy would be taken to provide a small tissue sample for genetic studies. Contents of check pouches would be extracted and analyzed to determine diet. The site of each capture would be recorded using a GPS and the animals released at the point of capture. Soil and vegetation would be sampled at each site and analyzed. The habitat would be photographed and described in general terms; the capture or sighting site also would be described according to its association in the landscape. A 1m^2 quadrat would be placed directly over burrows or the capture location. Within each quadrat, vegetative richness would be recorded as the total number of species present. Percentage cover of grass, forbs, bare ground, woody vegetation, and rocks would be recorded. Average herbaceous vegetation height would be obtained by averaging the height of the herbaceous vegetation 15 cm interior to each corner of the quadrat. The height of woody vegetation also would be recorded as the height of the lowest branch. Specimens of dominant plants would be collected, placed in a plant press, and deposited as voucher specimens. Vegetation and soil data between quadrats would be analyzed and compared.

Results

Fifteen surveys were conducted from 2014 to 2017 to document the presence (or absence) of the Texas kangaroo rat (*D. elator*) in seven counties in southwestern Oklahoma. Surveys were conducted during a total of 93 nights in October 2014 (10 nights), February 2015 (7 nights), May 2015 (4 nights), July 2015 (6 nights), August 2015 (5 nights), April 2016 (7 nights), May 2016 (7 nights), June 2016 (6 nights), July 2016 (7 nights), August 2016 (5 nights), September 2016 (3 nights), May 2017 (7 nights), June 2017 (7 nights), July 2017 (7 nights), and August 2017 (5 nights). A total of 266 localities was surveyed (Table 1; Figure 1), with the total number of localities for each county varying from 7 (Comanche) to 83 (Tillman). Although fewer localities were surveyed in Year 1 (84), 91 localities were surveyed in both Year 2 and Year 3.

A total of 9,094 trap nights (a trap night is equal to one trap set for one night) of effort was achieved during the three-year project (Year 1: 2,302; Year 2: 3,022; Year 3: 3,770). This effort is about 25.7 times the effort of the previous survey by Moss and Mehlhop-Cifelli (1990; 354 trap nights). Trap success for all small mammals varied from 0 to 100 %, but averaged 24.6 % across all sites (25.04 Year 1; 33.86 % Year 2; 9.76 % Year 3).

Visual surveys also were conducted along roads for large portions of each of the seven counties. Habitats along more than 32,428 km of paved and unpaved roads were surveyed for the presence of potential *D. elator* habitat, burrows, and activity: 11,265 km for Year 1; 11,265 km for Year 2; 9,898 km for Year 3. This effort is estimated at more than 43.6 times the efforts from previous surveys (Martin and Matocha 1972; Jones *et al.* 1988; Moss and Mehlhop-Cifelli 1990).

No *D. elator* was captured or observed in Years 1, 2, or 3. However, 2,178 individuals of 17 mammal species were captured (Table 2). Of these, 563 were prepared as scientific voucher specimens including tissue samples and deposited in the Collection of Mammals and Oklahoma Collection of Genomic Resources at the Sam Noble Oklahoma Museum of Natural History, respectively. The remaining 1,617 individuals of the 2,178 individuals captured were identified and released. Although no *D. elator* was captured or observed, this project provides new locality and natural history information on 30 other mammal species in seven counties in southwestern Oklahoma, including six new county records (Braun *et al.* 2020; Braun *et al*. pers. observ.). This information continues to expand the knowledge of mammal species throughout the state.

Discussion

The known historical distribution of the Texas kangaroo rat is limited to two localities in two counties in southwestern Oklahoma and localities in 11 counties in northern Texas; as noted previously, an additional Texas county record (Coryell) is unverified (Dalquest and Collier 1964; Packard and Judd 1968; Martin and Matocha 1972; Cokendolpher

Table 1. The number of localities surveyed by county during all years of this study. Y=Year, T=Trip. Year 1: 1 October 2014-30 September 2015; Year 2: 1 October 2015-30 September 2016; Year 3: 1 October 2016-30 September 2017.

YearTrip	County							Total
	Comanche	Cotton	Greer	Harmon	Jackson	Kiowa	Tillman	
Y1T1				5	18			23
Y1T2		8					12	20
Y1T3							9	9
Y1T4			10			8		18
Y1T5	3					6	5	14
Total Y1	3	8	10	5	18	14	26	84
Y2T1			7	4	7		2	20
Y2T2			1	7	7			15
Y2T3		4					8	12
Y2T4			12		7		3	22
Y2T5	3	4				2	4	13
Y2T6		9						9
Total Y2	3	17	20	11	21	2	17	91
Y3T1			8	8	2		3	21
Y3T2				7	7		11	25
Y3T3		18					6	24
Y3T4	1						20	21
Total Y3	1	18	8	15	9		40	91
Grand Total	7	43	38	31	48	16	83	266

et al. 1979; Martin and Matocha 1991; Martin 2002). This geographic region encompassed arid areas of short or overgrazed grass with open areas of bare ground and clay or sandy loam soils, such as mesquite-buffalo grass pastures, which research indicates is the preferred habitat of *D. elator* (Dalquest and Collier 1964; Stangl *et al.* 1992; Goetze *et al.* 2007; Nelson *et al.* 2009). However, research also suggests that the species may be somewhat opportunistic in its habitat requirements and have broader habitat tolerance than generally supposed (Stangl *et al.* 1992; Martin 2002).

Over nearly 125 years, much of the suitable habitat in the historic range of *D. elator* has been destroyed or modified (Stangl *et al.* 1992; Nelson *et al.* 2009). Key factors that have contributed to changes in the suitable habitat for *D. elator* include habitat degradation, fragmentation, habitat loss from conversion to agriculture, fire suppression, the disappearance of bison, decreased grazing, and loss of historical ecological processes (Figure 2; Stangl *et al.* 1992; Nelson *et al.* 2009; Holt 2018). In 1988 in Oklahoma, Moss and Mehlhop-Cifelli (1990) found only 2.6 % of the total area surveyed consisted of potential Texas kangaroo rat habitat and at many sites no suitable habitat was found after an initial identification using aerial photographs and soil survey maps. Between 1985 and 2000 in Texas, significant changes in the habitat in the historic range of *D. elator* were found to correspond to an increase in Conservation Reserve Program fields that resulted in increasing the density and coverage of grasses, an increase in cultivated areas, and a transition to monocultures (Martin 2002). Martin (2002) noted that, in Texas, the "habitat in much of the historic range of the species is not suitable to maintain viable populations." By 2002 in Texas, Martin (2002) suggested that Texas kangaroo rats were present in only five of the 11 counties where it previously had been reported. During the 2014 to 2017 study reported herein, surveys of 266 sites and observations along more than 32,428 km of paved and unpaved roads in Oklahoma found no individuals and few areas of what might be considered suitable habitat for *D. elator*.

Although there is little information on the historical distribution of *D. elator* in Oklahoma, it may have overlapped or coincided with the area known as the Big Pasture (Figure 3), located in what is now parts of Comanche, Cotton, and Tillman counties (Cooper 1957). The surplus lands of the Apache, Comanche, and Kiowa nations were opened to white settlement by lottery from 9 July to 6 August 1901, but the 488,000-acre Big Pasture was set aside for grazing reserves of the Apache, Comanche, and Kiowa nations. In December 1906, however, the Big Pasture, the last large tract of land unavailable for white settlement in Oklahoma Territory, was opened by sealed bids (Cooper 1957). The Big Pasture had been grazed by bison herds and supported wolves recently enough for Theodore Roosevelt to hunt them there (Wynn 2011).

The impact of opening this area to settlement cannot be overstated. Within a year of opening of Big Pasture in 1906, 2,337 families had settled the area (Cooper 1957). Even before the opening of the Apache, Comanche, and Kiowa lands in 1901, the Big Pasture had been leased to Texas ranchers for grazing and quarter sections were leased for agriculture. Stipulations in agricultural leases included

Figure 2. Examples of habitats surveyed for *D. elator* in southwestern Oklahoma. A) Cotton field. Oklahoma: Tillman Co.: 1 mi N, 6 mi W Chattanooga, 350 m (Photo taken 10 August 2017 by J. K. Braun). B) Harvested grain field. Oklahoma: Harmon Co: 3.25 mi S, 0.5 mi W Gould, 471 m (Photo taken 15 June 2017 by J. K. Braun). C) Mesquite grassland in late summer. Oklahoma: Tillman Co.: 4 mi N Loveland, 341 m (Photo taken 9 August 2017 by J. K. Braun). D) Mesquite grassland in early late spring. Oklahoma: Tillman Co.: 2.25 mi S Loveland, 260 m (Photo taken 4 May 2015 by J. K. Braun).

the provisions that at least 120 acres had to be "broken out" and quarter sections fenced with a four-wire fence. Agriculture developed very rapidly in the Big Pasture (*e. g*., cotton, wheat, sorghum, and milo) as well as statewide. Between 1890 and 1900, the number of farms in Oklahoma increased from 8,826 to 108,000 and to 190,192 by 1910, making Oklahoma one of the most rapidly settled agricultural frontiers in the history of the United States (Fite 2009). Habitat conversion has been shown to have major effects on many wildlife species (Sykes *et al.* 2019).

Habitat suitable for *D. elator* in Oklahoma was likely fragmented due to the rapid conversion of native habitat to agricultural use and agricultural practices that became more intensive over time. In 1910, 80 to 95 % of the acres in these seven counties were in farms and the percent acres in cultivation ranged from 34 % to 41 %. The trend toward habitat conversion continues today as the percent land in farms (cropland and pastureland), excluding Comanche County, increased an average of 7.5 % (1-17 %) from 2007 to 2012. Presently, the percent of acres in farms in 2012 for six of the seven counties that were surveyed ranged from 91 to 99 % (2012 USDA Census of Agriculture); Comanche County had 68 % in farms, a number lower likely due to the presence of a large military installation and a national wildlife refuge.

Although some small mammals may utilize the interiors of agricultural fields, *D. elator* generally avoids them because deep plowing disturbs or destroys animals and their burrow systems (Martin and Matocha 1972; Martin 2002). In Texas, *D. elator* may, however, inhabit the undisturbed edges and road banks bordering pastures or cultivated fields (Martin 2002; Goetze *et al.* 2016). However, unlike Texas, most areas in southwestern Oklahoma are cultivated from section line to section line or have roadsides covered with dense areas of native and non-native grasses (Figure 2). Thus, even edges and road banks, which are used by *D. elator* in Texas—and may have been used historically in Oklahoma—disappeared or became narrower over time with expanded plowing as well as from the activities of blading and road construction.

Grazing and associated disturbances also have been suggested as important factors in maintaining suitable habitat for *D. elator* (Stangl *et al.* 1992; Stasey 2005; Goetze *et al.* 2007). But, changes in grazing practices and control of wildfires also have resulted in modifications in suitable habitat for *D. elator* (Diamond and Shaw 1990). Many uncultivated fragments that were fenced and grazed by cattle are no longer intensively grazed, resulting in an increased abundance of mesquite, shrubs, grasses, and forbs, the invasion of introduced plant species, and a decrease in the presence of bare ground (Diamond and Shaw 1990; Stangl *et al.* 1992; Martin 2002; Stasey 2005; Goetze *et al.* 2007; Nelson *et al.* 2009; Stasey *et al.* 2010). The control of wildfires has allowed the increase of woody vegetation (specifically mesquite) that, as it matures, increases the amount of shade and changes the composition of the vegetation, often in favor of dense introduced grasses (Nelson *et al.* 2009). Uncultivated lands allowed to attain a mature mesquite stage do not provide preferred or historical habitat for *D. elator* (Goetze *et al.* 2007; Stasey 2005). In addition, extensive modification of mesquite pastures through mesquite eradication or reduction has been shown to reduce available suitable habitat (Lewis 1970; Martin and Matocha 1972).

Several small mammal species known to be associated with *D. elator* were captured during this project. These include: *Ictidomys tridecemlineatus*, *Chaetodipus hispidus, Perognathus merriami, Peromyscus leucopus, Peromyscus maniculatus cf P. sonoriensis* (Bradley *et al.* 2019), *Reithrodontomys* sp., and *Neotoma micropus* (Roberts and Packard 1973; Stangl *et al.* 1992; Martin 2002; Goetze *et al.* 2007; Stasey *et al.* 2010). But, the largest numbers of captures were that of *S. hispidus* (1,200 of 2,178 captures), a species with which *D. elator* rarely co-occurs and, indeed, actively avoids (Chapman 1972; Goetze *et al.* 2007; Packard and Roberts 1973; Rob-

Table 2. Mammal species and total individuals captured for seven counties surveyed during all years, 2014-2017.

Species	County							Total
	Comanche	Cotton	Greer	Harmon	Jackson	Kiowa	Tillman	
Didelphis virginiana						1	1	2
Sylvilagus floridanus		1	1					2
Ictidomys tridecemlineatus		1		1			1	3
Xerospermophilus spilosoma							2	2
Perognathus merriami			3	1	20			24
Chaetodipus hispidus	9	28	23	28	81	10	32	211
Dipodomys ordii		7	36	41	30	1	38	153
Reithrodontomys fulvescens			3					3
Reithrodontomys montanus			3				1	4
Peromyscus leucopus		23	27	15	20	7	42	134
Peromyscus maniculatus cf P. sonoriensis	3	36	18	6	16	5	101	185
Baiomys taylori					1			1
Onychomys leucogaster			19	9	5	4	14	51
Sigmodon hispidus	45	608	83	40	163	89	172	1,200
Neotoma floridana		12					6	18
Neotoma micropus	2		36	30	85	15	9	177
Mus musculus				1	3	3	1	8

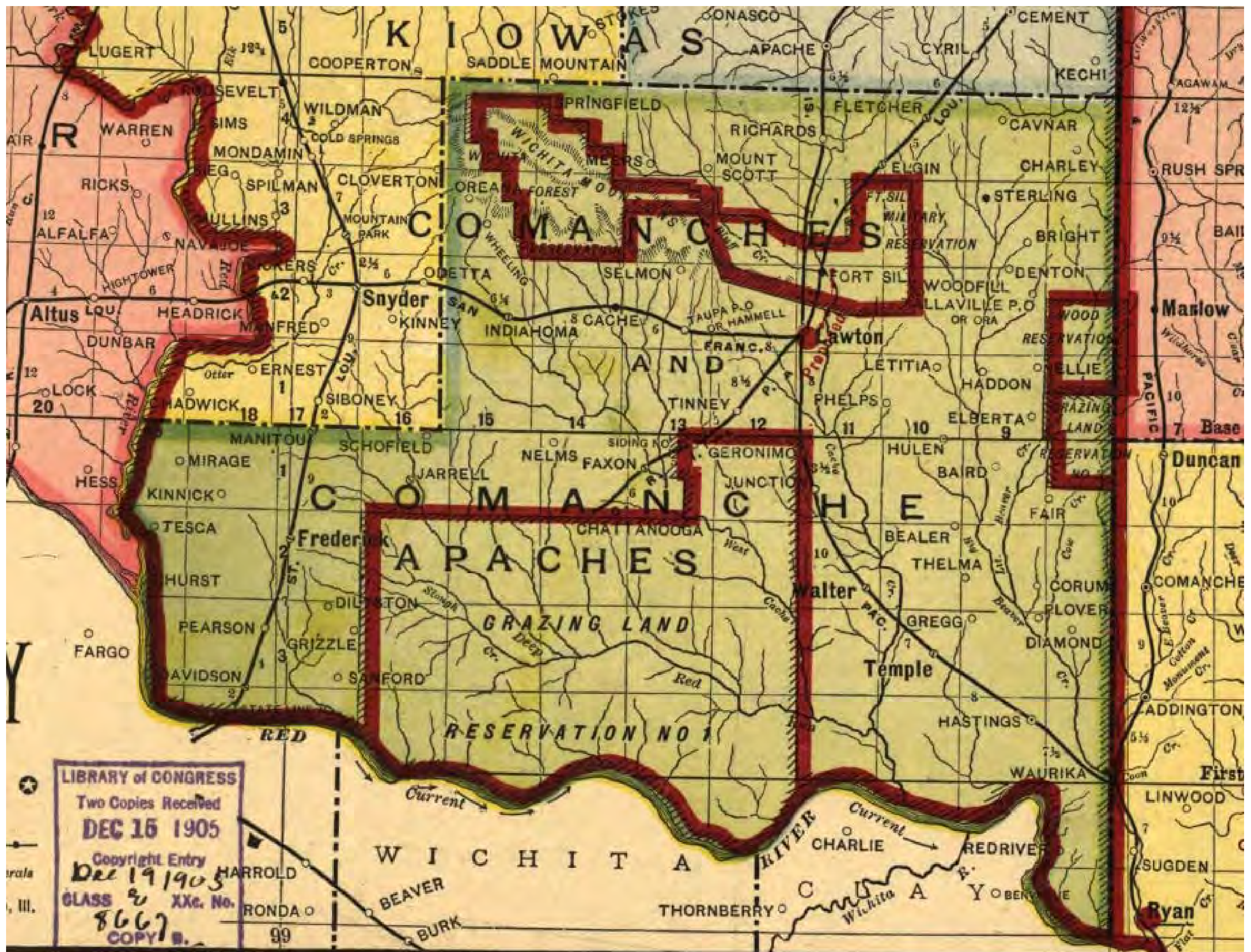

Figure 3. Map of the Big Pasture (labeled as Grazing Land Reservation No. 1) from the Library of Congress and published in The Daily Oklahoman in 1905.

erts and Packard 1973; Stasey *et al.* 2010). That *S. hispidus* was such a commonly captured species may be a reflection of changes in grazing practices and control of wildfires. The cotton rat, *Sigmodon hispidus*, has increased both its population density and geographic range with changing grazing practices over many decades (Slabach and Krupa 2018). Indeed, climate change has been implicated in cotton rat populations in recent years, with species range expansions expected over the coming decades (https://www.fs.fed.us/rmrs/sites/default/files/documents/Sigmodon%20hispidus%20Species%20Report.pdf). The projections for cotton rat expansion suggest that *D. elator* will not be expected to recolonize Oklahoma in the near future.

Other factors that may have altered the distribution of *D. elator* include the elimination of two mammal species (*Bison bison* and *Cynomys ludovicianus*) that significantly impact the environment and are known to create and maintain a disturbed, altered habitat preferred by *D. elator* (Stangl *et al.* 1992). The elimination of prairie dog mounds and other naturally occurring habitat heterogeneity features during the transition to agriculture also may have reduced the distribution of *D. elator* (Goetze *et al.* 2007).

The discovery of *D. elator* in Oklahoma in 1904 and 1905, and then a complete lack of records thereafter (with a single exception) corresponds directly to these major events in Oklahoma history. The capture of the two specimens from Chattanooga, Oklahoma (Savage, www.okhistory.org), which were not captured in native habitat, but in an area converted to agriculture (Kafir corn) and human habitation, represent a pivotal moment in the conservation history of this species. The rapid rate of human agricultural activities, habitat degradation, fragmentation, conversion of habitat, suppression of fire, and decreased grazing likely had an immediate impact on any populations of this habitat specialist in the state.

The results of this 3-year project provide the most extensive documentation of the absence of populations of *D. elator* in Oklahoma, particularly relative to its known historical locations, since 1988 (Moss and Mehlhop-Cifelli 1990). Although, more recently, Martin (2002) reported conducting road surveys during June to August from 1996 to 2000, no data for Oklahoma were presented in the report. In

Oklahoma, this Tier II species is of greatest conservation need, with a low population status and an unknown population trend. It has been petitioned for potential listing as an endangered or threatened species under the United States Endangered Species Act. These results and historical information suggest that the Texas kangaroo rat (*D. elator*) has been extirpated from Oklahoma, but these results will be useful to the State of Oklahoma and USFWS in making decisions about the status of this species and will provide scientific data for the basis for the development and implementation of scientifically sound conservation measures and management strategies in areas where populations are present, such as Texas.

The earliest reports of *D. elator* in Oklahoma report the species as not common, but not threatened with extirpation either. However, the changing social adjustments of Oklahoma land allotments, conversion of habitats due to land use practices, massive changes engendered by grazing and other farm management practices, and climate change have led to the extirpation of this species from Oklahoma and a very unlikely prognosis for its return in the foreseeable future.

Acknowledgments

This paper honors Dr. David J. Schmidly for a lifetime's work on mammal research in the United States and Mexico. He published books on Texas mammals, reviewed the life of Vernon Bailey, and co-edited a volume of South American mammalogy. He also published more than 100 papers on mammal systematics, biogeography, conservation, and natural history. As president of several major universities, he was a tireless supporter of mammal research, collections, and museums. We thank the following individuals who aided in the field work; we appreciate greatly their assistance and they are remembered fondly: A. Allen, A. Ciarlante, E. Ellsworth, R. Estrada, D. Glidewell, B. Narr, and C. Zhou. We also thank the individuals that allowed access to their private property. This material is based upon work supported by a grant from the State Wildlife Grants Program, Oklahoma Department of Wildlife Conservation (F14AF01224 (T-78-1). Additional support was provided by the Sam Noble Oklahoma Museum of Natural History, University of Oklahoma. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the Oklahoma Department of Wildlife Conservation.

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Vaquita: beleaguered porpoise of the Gulf of California, México

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The vaquita (*Phocoena sinus*), an endemic porpoise of the Gulf of California, México, was first described scientifically in 1958, from three skulls. It is considered a sister taxon of an ancestor of the Southern Hemisphere Burmeister's porpoise (*P. spinipinnis*) and spectacled porpoise (*P. dioptrica*), a case of antitropical distribution and speciation. Vaquita in modern times seem to have existed largely in waters 10 to 30 m deep of the very northern Gulf of California, and may have already existed in relatively low numbers by the 1950s and 1960s. The external appearance of the vaquita was not described until the late 1970s, and not until the 1980s and 1990s did additional information about ecology and biology emerge. Those studies and more recent shipboard and aerial visual line transect surveys, as well as stationary and boat-towed acoustic arrays, mapped occurrence patterns and approximate numbers in greater detail than before. The first credible estimates of abundance appeared in the 1990s, with numbers in the mid-hundreds and declining. While several reasons for the decline were originally postulated, mortality due to entanglement in nets has been established as the only known cause of decline, especially due to bycatch in large-mesh gillnets set for the endangered croaker fish totoaba (*Totoaba macdonaldi*). This fish is prized in China for human consumption of its swim bladder, generally ground up for purported therapeutic purposes. An extensive, lucrative fishery for totoaba, now illegal for many decades, has existed since at least the 1920s, and has recently increased. Although there have been laudable attempts to stem or halt totoaba fishing, these have largely been unsuccessful, and as of this writing the vaquita is on the brink of extinction. However, rapid concentrated action against illegal fishing with gillnets may yet save the species, and hope (with attendant action) must be kept alive. This overview is followed by an appendix of a previously unpublished popular essay by K.S. Norris describing when, where, and how he first discovered the species, and subsequent early work relative to this newly-described porpoise.

La vaquita marina (*Phocoena sinus*), una marsopa endémica del Golfo de California, México, fue descrita científicamente por primera vez en 1958, a partir de tres cráneos. Se considera un taxón hermano de un ancestro de la marsopa de Burmeister del hemisferio sur (*P. spinipinnis*) y la marsopa de anteojos *(P. dioptrica*), un caso de distribución y especiación antitropical. En los tiempos modernos, la vaquita marina parece haber existido principalmente en aguas de 10 a 30 m de profundidad en el extremo norte del Golfo de California, y es posible que ya existiera en cantidades relativamente bajas en las décadas de 1950 y 1960. La apariencia externa de la vaquita no se describió hasta fines de la década de 1970, y no fue hasta las décadas de 1980 y 1990 cuando surgió información adicional sobre ecología y biología. Esos estudios y los estudios más recientes de transectos de líneas visuales aéreas y a bordo, así como los arreglos acústicos estacionarios y remolcados por bote, cartografiaron patrones de ocurrencia y números aproximados con mayor detalle que antes. Las primeras estimaciones creíbles de abundancia aparecieron en la década de 1990, con cifras de alrededor de cientos y en declive. Si bien originalmente se postularon varias razones para la disminución, la mortalidad debida al enredo en las redes se ha establecido como la única causa conocida de disminución, especialmente debido a la captura incidental en redes de enmalle de malla grande colocadas para la totoaba corvina (*Totoaba macdonaldi*), en peligro de extinción. Este pescado es apreciado en China para el consumo humano de su vejiga natatoria, generalmente molido con supuestos propósitos terapéuticos. Una pesquería extensa y lucrativa de totoaba, ahora ilegal durante muchas décadas, ha existido desde al menos la década de 1920 y ha aumentado recientemente. Aunque ha habido loables intentos de detener o detener la pesca de totoaba, en gran parte no han tenido éxito y, al momento de escribir este artículo, la vaquita está al borde de la extinción. Sin embargo, una acción rápida y concentrada contra la pesca ilegal con redes de enmalle aún puede salvar a la especie, y la esperanza (con la acción correspondiente) debe mantenerse viva. A esta descripción general le sigue un apéndice de un ensayo popular inédito de K.S. Norris describiendo cuándo, dónde y cómo descubrió la especie por primera vez, y los primeros trabajos posteriores relacionados con esta marsopa recién descrita.

Keywords: Conservation efforts; endangered; gillnets entanglement; Gulf of California; harbor porpoise; limited distribution; scientific discovery; totoaba; vaquita.

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Local tales of a small, mysterious porpoise. Although the world's smallest porpoise, the vaquita or Gulf of California harbor porpoise (*Phocoena sinus*), was first scientifically recognized in the 1950s, the species has likely been known to fisherfolk and Indigenous Peoples in parts of the northern Gulf of California since long before then. Interviews with local fisherfolk and long-time residents of the Gulf tell of an animal that is variously called "vaquita" (little cow), "cochito" (little pig; Brownell 1982), or even sometimes "duende" (ghost or spirit; Norris and Prescott 1961), which is likely what we know today as *Phocoena sinus*, although other species might also be referred to by these names. These reports, besides listing the known distribution in the northern Gulf of California, also describe the animals from much further south in the Gulf (*e. g.*, Bahia de Los Angeles, Topolobampo; Norris and McFarland 1958; Norris and Prescott 1961), and even south to the very southern limits at the mouth of the Sea of Cortez (*e. g.,* Islas Tres Marias, Bahia de Banderas; Norris and McFarland 1958). However, reports of vaquita further south than in the northern Gulf are likely to be incorrect (see below).

It is a sad fact that much of the history and lore of Indigenous Peoples of and near the Baja Peninsula appears to have been lost. We can imagine that the Peoples now termed Seri, Paipai, Kumeyaay, Cochimi, Cucapás, Kiliwa, and Guaycura, presently often greatly mixed with other tribes and westerners, would have wonderful recollections of a small porpoise in a vast sea (https://www.houstonculture.org/mexico/baja.html). An early unpublished attempt to investigate the vaquita and attempt to obtain a specimen, suggested that most or all of the southern reports were cases of misidentification and/or inconsistent use of the common names listed above, which are also used by some fishermen for common bottlenose (*Tursiops truncatus*) and common (*Delphinus delphis*) dolphins (Kelly 1975). The unpublished report by Kelly (1975) also suggested that two other assumptions that had been made about vaquita ecology may have been wrong: that 1) there were seasonal movements of the species south to the Midriff Islands area in summer, and 2) the species is mainly distributed in very shallow waters in and near the estuary of the Colorado River. Vaquita currently are restricted to the very upper portion of the Gulf of California, exhibit no significant migratory behavior, and occur mainly in waters about 10 to 30 m deep near submarine ridges often several kilometers from shore (Silber 1990a).

Scientific discovery by Kenneth S. Norris. Professor Kenneth S. Norris collected a porpoise skull in Spring 1950, while a graduate student at Scripps Institution of Oceanography in La Jolla, CA, studying several species of desert lizards. On that trip, he was mapping locations and habitat use of the *Uma* genus of lizards, that "lives on windblown sand" (see Appendix I, a draft by K. S. Norris of the discovery of vaquita in his own words). He found that first (for him) *Phocoena sinus* skull in the habitat of the zebra-tailed lizard (*Calisaurus* genus) among sand dunes occasionally swept by the amazingly forceful tides (and tidal fluxes of up to 5 m) of the upper Gulf of California, north of the little town of San Felipe. Norris (Appendix I) goes on to describe how this skull and two more collected by a colleague were used to establish them as of the *Phocoena* (porpoise) genus, probably most closely related to the Burmeister's porpoise (*Phocoena spinipinnis*) of South America, a wonderful example of anti-tropical distribution, as heralded earlier by Hubbs (1952) and Davies (1963) for some animals and plants of the Gulf of California region. It is also possible that vaquita derived from an ancestor of the present-day Burmeister's and spectacled (*P. dioptrica*) porpoises (Chehida *et al.* 2020; Morin *et al.* 2020). The Norris (Appendix I) draft is a delightful recollection of his discovery, the resulting first scientific trip in 1956 to find vaquita in nature and subsequent early studies. Details describing the vaquita as a new species are in Norris and McFarland (1958).

Curiously, in the initial draft description (Appendix I), Norris does not acknowledge the probable first scientific expedition to document and photograph vaquita in nature in 1979, organized by Norris himself (Wells *et al.* 1981; Figure 1, see also below), but then his recollections are in a never-before published first draft, and he likely would have elaborated as his draft developed. Since in the 1970s he advocated to us that vaquita had not been documented by scientists in nature, he may have in his draft writings accidentally confounded purported discoveries in 1956 with those that actually occurred in 1979 (Norris, Appendix I; Wells *et al.* 1981).

Early expeditions to find vaquita. According to Norris (Appendix I), he and colleagues sighted (probable) vaquita on their trip out of the town of San Felipe, during a short expedition in 1956. This is not represented in the published literature, but is in Appendix I. To the best of our knowledge, the first systematic survey effort dedicated to characterizing the vaquita occurred in 1979, conceived by Norris and Bernardo Villa-Ramírez, and conducted by Norris, Bernd Würsig, Randall Wells, and Benjamin López (Wells *et al.* 1981). Among the objectives of the survey were determining: 1) whether there was an extant population of vaquita in the Gulf, and 2) the present threats to the population. During March 3 - April 1, 1979, a survey was conducted over 1,960 km of transects through the upper Gulf, from the Rio Hardy, a distributary of the Colorado River, southward to the Midriff Islands, using a twin-engine, 7.6 m vessel. The survey recorded 206 sightings of 10 species of marine mammals, but only two were sightings that were probably vaquita (Figure 1). Both of these sightings occurred on March 10th, both involved two to three individuals, and both were in the northern portion of the upper Gulf in a region where most subsequent sightings of vaquita have occurred. The paucity of sightings was consistent with findings from subsequent surveys and indicative of the tenuous status of this species even 40 years ago. The surveys also noted extensive commercial fishing activities in the upper Gulf, and numerous carcasses of small cetaceans on the beaches near nets set perpendicular to shore.

Figure 1. This is possibly the first photo published of a vaquita in nature, on a rather placid sea, taken on 10 March 1979. Photo by R.S. Wells.

Upon reflection, while much is often made of "first discovery of", this in general means "for science", and almost always indicates re-discoveries of animals (or plants or other phenomena) long ago known and appreciated by local people. Vaquita were well known to the local fisherfolk of San Felipe, El Golfo de Santa Clara, and Puerto Peñasco, long before "we" western scientists came along. This kind of "science thinking" is outmoded, it seems to us, much as the "Columbus discovered the Americas" perspective seems ridiculous when one recognizes that the Americas were well-inhabited by humans long before Europeans came along.

Gillnetting for the croaker totoaba threatens the vaquita. The fate of vaquita may ultimately be linked to that of the totoaba (*Totoaba macdonaldi*), a large fish of the croaker family Sciaenidae and relative of the white seabass (*Atractoscion nobilis).* Like the vaquita, the totoaba is endemic to the Gulf of California. Spawners occur in the northern Gulf from December through May, with a peak January through March, sometimes in very large numbers (Flanagan and Hendrickson 1976).

Once exceedingly abundant in the upper Gulf of California during its winter-spring spawning, over-fishing severely depleted the totoaba population. The totoaba is presently listed as Critically Endangered on the IUCN Red List, listed on CITES Appendix 1, and is designated as Endangered under the United States Endangered Species Act. However, the IUCN classification is from 2010, so out of date and merits a new evaluation.

Records of commercial exploitation of the fish extend to at least the early 1920s (Craig 1926; Flanagan and Hendrickson 1976) or 1930s (Brownell 1982). Early totoaba fisheries were limited primarily to the export of dried swim bladders to Asian markets as an ingredient in gourmet soups and other uses (Chute 1928). Exports of the totoaba to the United States -- primarily San Francisco, Los Angeles and San Diego; also for swim bladder exportation to Asia -- were first reported as having occurred in the mid-1920s (see also Cisneros-Mata *et al.* 1995). Fishing villages in the upper Gulf grew rapidly in this period (Berdegue 1955); an estimated 200 fishermen, using mostly hook and line gear, participated in the fishery out of San Felipe alone in the mid-1920s (Craig 1926).

In this period, except for some local consumption of its meat, Craig (1926) reported there were "still many fish left to rot after their swim bladders have been removed, as the primary object of the fishery is still the manufacture of 'buche' (swim bladder)". At that time, swim bladder material was sold in Chinese markets for U.S. $1.50-2.00 per pound. A market for its meat soon developed.

Initially the totoaba was harvested using spears from small boats and hook and line (Berdegue 1955; Flanagan

and Hendrickson 1976). Gillnets were in use in the upper Gulf by the 1940s (Vidal *et al.* 1999), which may have accounted, at least in part, for an explosion of totoaba landing levels in the 1930s and 1940s (Arvizu and Chavez 1972; Flanagan and Hendrickson 1976). More than 1,200 metric tons (mt) were harvested in all but one year from 1935 through 1946; landings exceeded 2,000 mt annually in two of those 12 years (Rojas-Bracho *et al.* 2013; Figure 2). Judging by landing levels for the totoaba and in other fisheries using gillnets, bycatch of vaquita must also have been high in this period.

By the 1960s, the fishery expanded to even more efficient nylon gillnets. These nets consisted of a stretch-mesh size of 25 cm and were generally used at a height of 4.5 m and were 100-200 m long (Flanagan and Hendrickson 1976). These were mesh sizes that are fully capable of entangling dolphins, porpoises, and sea turtles. Such nets are especially dangerous for porpoises in the family Phocoenidae, which often seem to have difficulty avoiding them (Jefferson and Curry 1994). Following the introduction of gillnet fishing, totoaba landings exceeding 500 mt occurred each year from 1960 to 1970 (Flanagan and Hendrickson 1976; Figure 2). But by this time a depletion of totoaba numbers from over-fishing was probably well underway (Arvizu and Chavez 1972). Fishermen working in the community of El Golfo de Santa Clara alone had landings (in March, during peak spawning period) of totoaba that exceeded 50 mt each year from 1964 to 1970. Yields were over 200 mt in March 1964, 1965 and 1968 (Figure 2).

Over-fishing of the species has led to protection measures by the Mexican Government. Among these, as reported by Rojas-Bracho and Reeves (2013; Table 1), was a ban on totoaba fisheries established in 1975 (Flanagan and Hendrickson 1976), followed by another, banning totoaba gillnets in 1993. However, it is widely recognized that fishing in defiance of these restrictions often continued unabated by regulations (Rojas-Bracho and Reeves 2013; Taylor *et al.* 2017). One of us (GKS) observed illegal fishing activities numerous times in the mid-1990s, despite existing prohibitions, and another of us (TAJ) observed multiple cases of illegal fishing in 2006, 2008, 2010, 2013, 2015, and 2019. The IUCN Cetacean Specialist Group regularly summarizes such illegal net setting and results relative to vaquita (https://iucn-csg.org/vaquita/, accessed 13 March, 2021).

To our knowledge, first mention in the scientific literature of the taking of vaquita incidental to upper Gulf fisheries was by Norris and Prescott (1961), as well as by Norris (Appendix I). They described occasions, as reported to them by local fishermen, of vaquita being caught in the totoaba fishery, by trawlers (that were setting gillnets), and in nets set near beaches. Some of these incidents presumably occurred in the 1950s or before. Therefore, we believe substantial vaquita bycatch levels almost certainly accompanied gillnet fisheries for the totoaba and other species in this period before the species was even described scientifically.

Dedicated studies clarify the vaquita's endangered status. Renewed interest in the study and conservation of the vaquita occurred in the mid-1980s. Key among these were

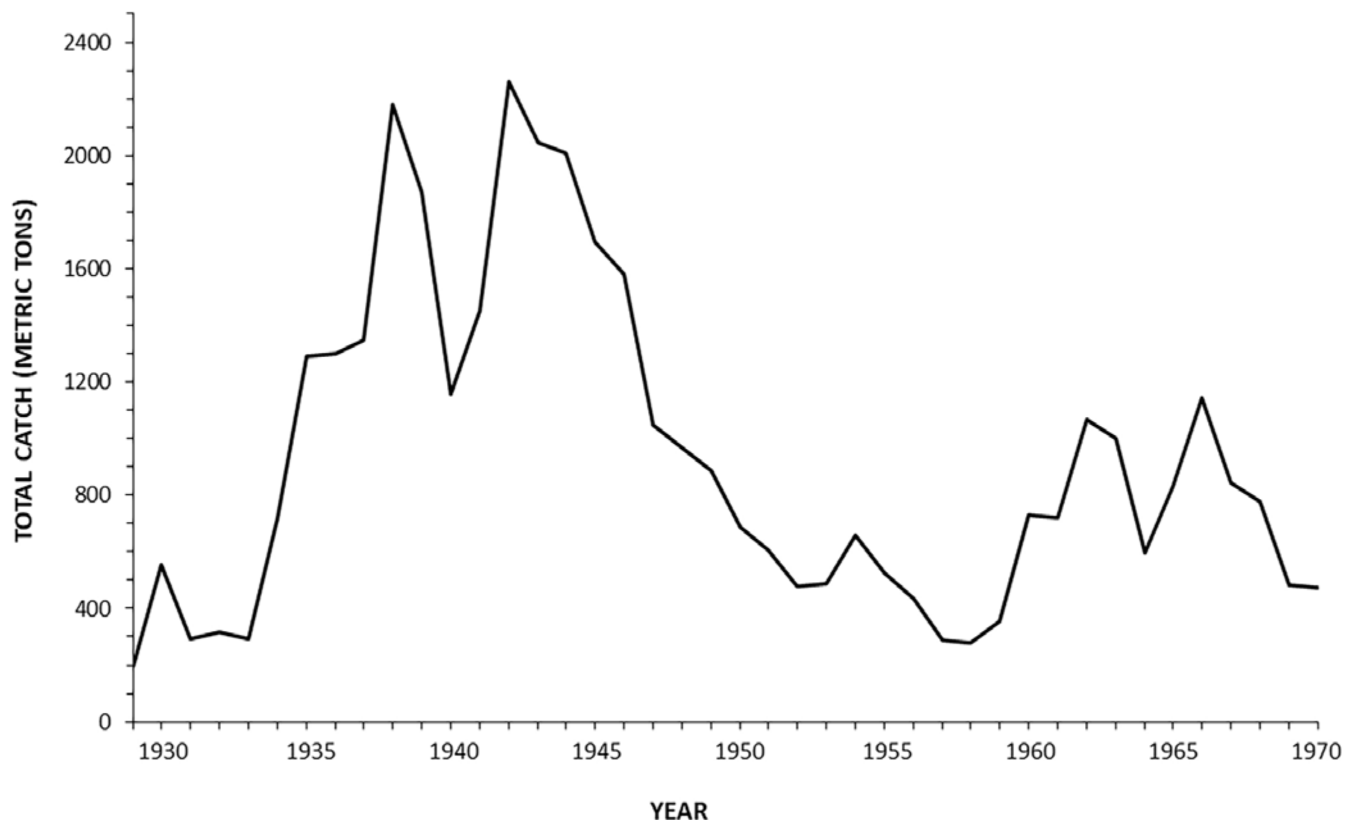

Figure 2. Yield of commercial totoaba (*Totoaba macdonaldi*) fishery, northern Gulf of California, 1929-1970 period. Figure modified from Arvizu and Chávez, 1972.

Figure 3. The sea bass totoaba (*Totoaba macdonaldi*) in a fisher's boat. The white tissue under the knife is of a recently-extracted totoaba swimbladder. Photo by G.K. Silber.

studies by Omar Vidal and Alejandro Robles, then graduate students at the Instituto Tecnológico y de Estudios Superiores de Monterrey-Guaymas. As a result, new information emerged on the totoaba fishery and apparent low vaquita abundance (*e. g*., Findley and Vidal 1985; Vidal 1993). These researchers helped focus attention on vaquita bycatch rates in various upper Gulf fisheries. For example, Robles *et al.* (1987) reported that 14 vaquita (four adults, nine calves, and one neonate) were bycaught in just three months in 1985 and 1986. Concerns were raised about the level of this bycatch and its impact on an already depleted population (Villa-Ramirez 1990, 1993).

In 1986-1989, Silber (1990a, b) conducted more than 1,700 km of surveys from a small boat and more than 1,500 km of surveys from an aircraft. The surveys yielded nearly 60 vaquita sightings representing an estimated total of 110 individuals sighted (Silber 1990a, b; Silber *et al.* 1994). Overall, sighting rates were low (approx. two sightings per 100 km surveyed). Nearly all sightings were concentrated in a relatively small area in the northwestern Gulf (Silber 1990b). No vaquita were seen during aircraft surveys conducted along the eastern Baja California Sur peninsula south to about 29° 34' N latitude (Silber 1990a; Silber and Norris 1991), but then aerial surveys are not optimal for sighting vaquita (Barlow *et al.* 1997). These findings suggested a range that might not have exceeded several thousand square kilometers.

Additional information about the species' ecology, behavior, and affiliation with certain habitat features began to emerge from the 1980 studies. For example, 'conventional wisdom' at the time indicated that vaquita inhabited extremely shallow waters close to shore (see Kelly 1975). In contrast, new findings indicated that vaquita occurred primarily in water depths of 10 to 30 m, at times ten or more kilometers offshore. These were areas characterized by moderate to strong currents (although still high turbidity) driven by strong tidal surges (Silber 1990a).

Habitat partitioning among several odontocete species occurring in the upper Gulf was posited (Silber 1990a). Among other things, bottlenose dolphins and vaquita were never seen in the same vicinity. Competition between or perhaps active exclusion of vaquita by the larger-bodied bottlenose dolphins from certain very shallow habitats appears likely (see Cárdenas-Hinojosa *et al.* 2020). Moreover, bottlenose dolphins were found almost exclusively in (often very) shallow waters close to shore, including in marshes well into the mouth and channels of the Colorado River delta (Silber *et al.* 1994; Silber and Fertl 1995).

In addition, the vaquita appeared (anecdotally) to favor 'surface slicks' (Silber 1990a), a phenomenon resulting from mixing in the water column and water movement accompanying tidal ebb and flow and near submarine ridges. At these times, vaquita may have been feeding along underwater fronts, *i. e.*, 'internal waves', resulting from moving and highly mixed water masses, again driven by substantial tidal flow. A similar use of internal waves has been reported in other cetacean species such as the harbor porpoises (*Phocoena phocoena*) in Monterey Bay, California (Silber and Smultea 1990) and pygmy killer whales (*Feresa attenuata*) in Hawai (Pryor *et al.* 1965).

Also occurring in the 1980s and 1990s, attempts were made to assess vaquita bycatch death rates by accompanying fisherfolks during net sets and retrievals (D'Agrosa *et al.* 2000), rates of recovery of net-caught vaquita (*e. g.*, Vidal 1991), and through interviews with local fishers who were asked to recollect when vaquita were entangled (D'Agrosa *et al.* 2000; Turk-Boyer and Silber 1990). Estimated averages of the number of bycaught vaquita in artisanal gillnet fisheries included 15.3 individuals per year (100 between 1985 and 1990; Vidal *et al*. 1993), 32.3 per year (Turk-Boyer and Silber 1990), 78 per year (D'Agrosa *et al.* 2000), 30 to 40 per year (Vidal 1991), 58 per year (Rojas-Bracho and Taylor 1999), and tens to hundreds per year (in the 1970s; Brownell 1982, 1983). The D'Agrosa *et al.* (2000) estimate is based on statistical analysis, and the Rojas-Bracho and Taylor (1999) estimate is based on an experimental totoaba fishery, and these are likely the most reliable estimates in this list.

Studies of increasing sophistication ring the alarm bell. In the late 1990s and into the 2000s, research on vaquita occurrence and distribution became ever-more sophisticated, with visual sighting by 25-power "big eye" binoculars from large ships, limited hydrophone arrays for acoustic detections towed behind sail boats, and passive acoustic monitoring devices attached to the seafloor near suspected vaquita habitat (summarized in Gerrodette *et al.* 2011; Rojas-Bracho *et al.* 2019). The realization that vaquita could be identified and tracked as individuals from natural markings (Jefferson *et al.* 2009) provided new options for studying their biology. As a result of such photo-identification work, it was discovered that vaquita females were capable of annual calving, something which had previously been thought not possible for this species, although it has been documented for other phocoenid species (Taylor *et al.* 2019; see also below). This gave new hope for the species' survival prospects.

We do not know the historical population size of the vaquita, from the period before gillnet fisheries began taking a toll on the population. The only such information comes from statistical modeling using recent estimates from vessel surveys and acoustic data, and back-calculating by incorporating information on known bycatch levels. This exercise suggests that the pre-exploitation population of the species was likely <5,000 individuals (Jaramillo-Legorreta 2008; Table 1). From analysis of the full genome, around 5,000 for around 200,000 years ago has also been estimated (Morin *et al.* 2020). As might be expected for a species with such a small and confined range, the vaquita has never been an abundant species. The earlier referenced extensive survey in 1979 (Wells *et al.* 1981), for example, yielded very low sighting rates relative to the survey area covered, evidence of a low abundance even at that time.

So, although the vaquita has always been thought to be a species with low abundance, there were no statistically defensible estimates for the species until the late 1980s. Barlow *et al.* (1997) presented the first estimates of abundance made from various methods, but all based on surveys that were somewhat compromised or incomplete (Table 1). The estimates also had high CVs, ranging from 39 to 143 %, and therefore had to be considered very approximate. These estimates did, however, show both that the population was small, in the hundreds (224 to 855), and that the species was declining (Table 1).

In the late 1900s and early 2000s, three ship surveys were conducted, which covered the entire range of the species, and therefore provided complete estimates using consistent state-of-the-art methods. These estimates ranging

Table 1. Numerical population estimates made for the vaquita. Note that the estimates in Barlow *et al*. (1997) are very imprecise and potentially biased and therefore should not be taken as evidence of an increase in numbers.

Time Period	Estimate	Conf. Int.	%CV	Reference
Historical	ca. 5,000	2,088-10,697	nd	Jaramillo-Legorreta 2008
1986-1988	503	163-1,551	63	Barlow *et al*. 1997
1988-1989	855	340-2,149	50	Barlow *et al*. 1997
1991	572	73-4,512	143	Barlow *et al*. 1997
1993	224	106-470	39	Barlow *et al*. 1997
1997	567	177-1,073	51	Jaramillo-Legorreta *et al*. 1999
2008	245	68-884	73	Gerrodette *et al*. 2011
2015	59	22-145	50	Taylor *et al*. 2017
2016	30	8-96	nd	Thomas *et al*. 2017
2018	<19	6-19	nd	Jaramillo-Legorreta *et al*. 2019

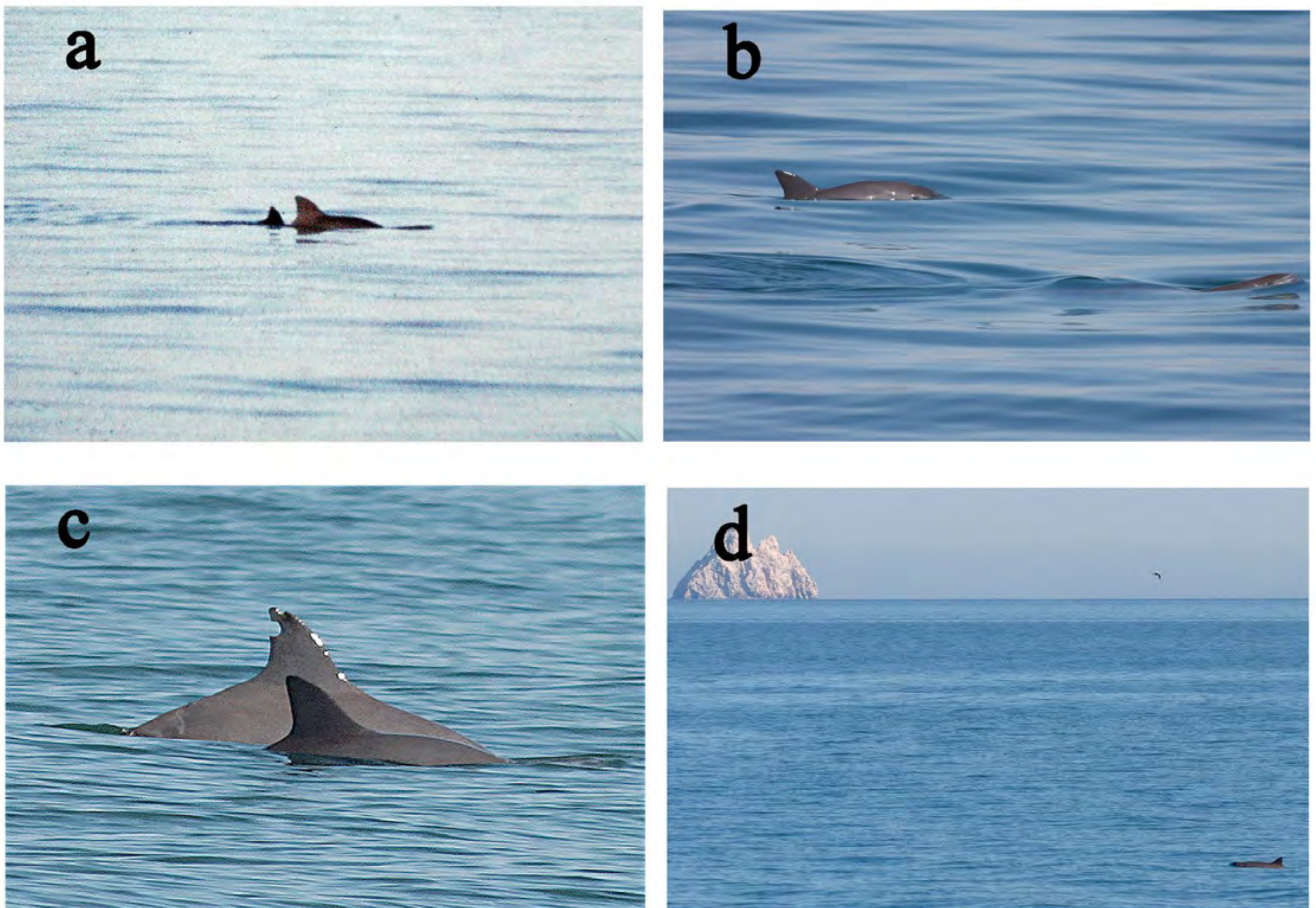

Figure 4. Progression of photographs from the 1980s through the 2000s, with modern digital photography and rapid automatic focusing of images (Figure 4b-d) portraying this cryptic species as never before. The photo with Roca Consag (Figure 4d) is especially iconic for the authors, as this is near where we have seen "the most" vaquita over our lifetimes, over a deeper sea than in surrounding areas, as mentioned by K.S. Norris, Appendix I. Photos by G. K. Silber (Figure 4a) and T. A. Jefferson (Figure 4b-d).

from 567 in 1997 to 59 in 2015, confirmed with high confidence that the vaquita population was in the low hundreds, and was declining at a very rapid rate (see Table1 for statistical summaries).

In recent years, a moored passive acoustic monitoring (PAM) array of hydrophones has been used to determine trends in abundance, and these trends have been compared to abundance "anchor points" from the vessel surveys described above to determine abundance in intervening years. Bayesian statistical methods have been employed to increase precision, thereby reducing the uncertainty of the estimates. Using these methods, vaquita abundance was shown to have declined from an estimated 59 individuals in 2015 to <19 in 2018 (Table 1). A recent estimate suggests that there may have been fewer than 19 individuals surviving by 2018 (Jaramillo-Legorreta *et al.* 2019), but there is at least the possibility that this estimate was biased downwards, especially if there are individuals outside the survey study area.

High bycatch rates have probably persisted for decades; reported declines in the past two decades or so likely are not new phenomena. It is possible that animals outside the survey area were not counted, and that earlier estimates of abundance were simply too low. However, recent molecular studies appear to have confirmed that the vaquita has persisted at relatively low population levels for a very long time (Morin *et al.* 2020). One implication of this finding is that it is possible that harmful genetic alleles have to some degree been purged from the vaquita genome, thereby increasing the species' ability to at least partially avoid "inbreeding depression" problems often associated with very small populations. This means that it may not be too late for the species to make a recovery! However, such a positive scenario is only possible with immediate cessation of gillnet fishing in waters where vaquita occur.

Some conservation attempts. It was early on realized that bycatch in fishing nets was likely the main problem for vaquita (Norris and Prescott 1961), and Rojas-Bracho and Taylor (1999) systematically eliminated worries about large-scale habitat degradation, pollution, and low genetic diversity as major causes of decline (see also Gulland *et al.* 2020). Although it is not our intention here to list all conservation efforts (Rojas-Bracho and Reeves 2013 and other documents provide a thorough treatment of these efforts), a major one was the establishment of a Refuge for the

Protection of the Vaquita in 2005 (Gerrodette and Rojas-Bracho 2011). A large part of this eventually-unsuccessful program relied on paid support to fisherfolk and fishing communities to curtail large-mesh netting for totoaba (Rojas-Bracho and Reeves 2013). In 2008, a Species Conservation Action Plan for the Vaquita: An integrated strategy of management and sustainable use of marine and coastal resources in the upper Gulf of California (shortened to PACE-Vaquita) was promulgated by the Mexican government. Its major aims were to ban gillnet fishing in updated protected areas believed to be vaquita relative "hot-spots", promote alternative fishing techniques, and provide compensation to fisherfolk that abided by fishing bans (Gerrodette and Rojas-Bracho 2011). Although there were some early apparent successes, this too did not halt or reverse declines of vaquita. Taylor and Rojas-Bracho (2017) provided an overview of the species' conservation status in the IUCN Red List of Threatened Species, as also in Taylor and Rojas-Bracho 2020). In 2017, a major international *ex situ* conservation effort, under the direction of the Mexican government, was launched to attempt to remove vaquita from the dangers caused by continued exposure to gillnets, and maintain them under human care until such time as their natural habitat was safe for them to be returned (Rojas-Bracho *et al*. 2019). However, the team decided to suspend capture efforts after catching two porpoises. A juvenile was released as it appeared stressed, and an adult female died of capture myopathy (Rojas-Bracho *et al*. 2019).

The Present Situation. The tenacity of the species to survive should not be underestimated, and this is a very strong incentive for us to not give up hope. But, we must also remember that a beleaguered species can "blink out" very suddenly. Among cetaceans, this may have been the case for the baiji (*Lipotes vexillifer*), which appears to have already become extinct when the first systematic and complete survey of its entire range was conducted in 2006 (Turvey *et al.* 2007).

In mid-2019, the vaquita was approaching extinction, with estimates of abundance of no more than 19 individuals (Jaramillo-Legorreta *et al.* 2019), population decline rate at about 50%/year, and illegal gillnet fishing was still rampant in the species' range. The outlook does not look good, but there is still some glimmer of hope, as indicated by the most recent CIRVA report (CIRVA 2019), and the recent

Figure 5. Two vaquita surface near an illegal gillnet vessel retrieving its net, one of many observed during the most recent survey for the vaquita, in the fall of 2019. Photo by Diego Ruiz, Museo de la Ballena y Ciencias del Mar.

genetic findings (Morin *et al.* 2020). The statement in this report, that extinction for the vaquita is only months to a few short years away (if nothing changes), may be accurate. However, the remaining vaquita appear to spend much of their time in a very small area (a Zero Tolerance Zone for illegal fishing, which, if properly protected, could form an effective refuge), and are still reproducing (a fact, born out by the sighting of multiple newborns in 2019). Some of the remaining individuals bear evidence of surviving previous entanglements, based on dorsal fin scars (Taylor *et al.* 2019). And finally, we have realized that vaquita can give birth annually (Taylor *et al*. 2019), a major discovery, since previously we assumed that all females had a minimum of a 2-year inter-calf interval (see Hohn *et al.* 1996).

There are studies of other small and threatened odontocete cetaceans that may inform our interpretations of how best to help vaquita. One of the best studied is a little dolphin in South America. The franciscana (*Pontoporia blainvillei*), a species ecologically-similar to the vaquita by also occurring in medium depth waters not far from shore, is also threatened by human activities throughout its range, and continues to be taken at rates leading to unsustainable declines in abundance. Franciscanas are found in shallow coastal waters of southern Brazil, Uruguay, and Argentina, where they are exposed to artisanal fishing nets and coastal development. In the absence of empirical data, initial international management schemes suggested the existence of only four large management units across the species range (Bordino *et al.* 2008). In contrast, subsequent telemetry and genetic studies have determined that franciscanas instead live in small, local populations with definable home ranges, and occur in social groupings that may have much bearing on the reproductive potential for the populations (Mendez *et al.* 2010; Wells *et al.* 2013; Wells *et al.* in review). Concentrated removals from these small population units can have dire consequences on their continued existence. It would be good to have similar data on social groupings and behaviors of vaquita to help gauge severity of random killings due to net entanglements on their social structure and reproductive capabilities, but it is likely too late to obtain such information on the few vaquita alive at this writing.

Concluding thoughts… and a potential sign of hope. One truth apparent from the vaquita story is that species can sometimes surprise us with their 'tenacity' to survive even when projections based on best available data may suggest the opposite trend. Jaramillo-Legorreta *et al.* (2007), in their paper in Conservation Biology, justifiably attempted to raise awareness of the severity of the vaquita issue. The authors stated that vaquita were in imminent danger of extinction. They were correct, although the timeline is now extended towards the present, possibly because of elevated recruitment due to recently-recognized annual calving rates (Taylor *et al*. 2019).

Recent reports also highlight the urgent need for action, such as by CIRVA (2017), Taylor *et al.* (2017), Thomas *et al.* (2017), and Taylor and Rojas-Bracho (2020). Concerns expressed in those reports are not unlike those raised decades earlier by Norris and Prescott (1961), Villa-Ramirez (1976, 1993), Robles *et al.* (1987), Vidal (1995) and others. Importantly, the paper by Jaramillo-Legorreta *et al.* (2007) succeeded in catalyzing concern for the species and helping to jump-start needed initiatives, including the 2008 large vessel survey, which provided convincing evidence of the rapid rate of decline that gillnet fishing was causing. See also Jaramillo-Legorreta *et al.* (2019) for an update.

Vaquita are critically endangered by one threat, entanglement in shrimp and finfish gillnets; particularly, in the past 8 to 9 years with bycatch in gillnets due to capture of totoaba. But the human societal hurdles to overcome are several. To switch from gillnets to alternative ways of fishing and perhaps alternative ways of living, requires not only good governance and enforcement of existing laws, but also buy-in by stakeholders at all levels. A lucrative, well-organized criminal element that subjugates the laws with extensive illegal fishing and corruption in societal and political sectors must also be addressed (Bessesen 2018 provides a general overview). What would make the greatest difference now is cessation of all gillnet fishing throughout the vaquita range, but in a manner that does not destroy the livelihood of fisherfolks, their families, and local economies. It is our strong impression that the Mexican government has not adequately enforced existing laws and this has allowed "bad actors" to set nets illegally again and again; see also Rojas-Bracho and Reeves (2013) and Taylor *et al.* (2017). One important avenue besides enforcement of fishing methods, is to cut off the trade of swim bladders at all levels, at towns and cities of the Gulf of California, international borders, and the marketing venues in Asia. Although *ex situ* establishment of a small breeding population might have been a possibility at some point, it is likely too late for that (Taylor and Rojas-Bracho 2020), and debatable whether it would be an appropriate effort to pursue for this species, given what was learned by the attempt in 2017 (Rojas-Bracho *et al.* 2019). See also Brownell *et al.* (2019) for an up-to-date description of bycatch in gillnets relative to endangered cetaceans. The most immediate threat to the continued existence of vaquita is entanglement by gillnets. This threat must be stopped. It is not only the vaquita that are critically endangered. There are many other populations, species, and ecosystems of our oceans. We need to keep hope alive, and have well-thought-out avenues for realizing potential ways to preserve species and their ecosystems (see for example Bearzi 2020; Jefferson 2019; Notarbartolo di Sciara and Hoyt 2020; Safina 2020; Würsig 2020). Gillnets and other fishing gear are the most immediate but not the only threats.

The relentlessly advancing certainty of climate change is likely threatening vaquita (Silber *et al.* 2017; although we do not have direct data for this), fisherfolks' livelihoods, and all of Earth's ecosystems on sea and land. We, as a species, must do a much better job of recognizing and ameliorating these very real threats to our planet's biological diversity, and ultimately to our own survival.

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Appendix 1

Draft Chapter by Kenneth S. Norris, deceased 1998. Ken wrote this in the early 1990's, but did not have an opportunity to finish it as part of a book. We reproduce it here, in first draft, even with minor errors; but Ken's first draft words were much better than many people's final writing. We took the liberty of correcting some typographical errors and have provided footnote annotations, where appropriate, to clarify some passages.

Please read carefully and enjoy. It is a naturalist's journey delight (printed with permission by and with the courtesy of Ken's son, Prof. Richard Norris, Scripps, UCSD).

Signed with love by the authors and dear friends of Ken, BW, TAJ, GKS, and RSW.

Here now from Kenneth S. Norris:

Vaquita

The flame of an era flickers down. Though now it is likely that only a handful of whales and dolphins remain undiscovered, it was a different story only just three or four decades ago. All through the 1800s and well into our century, cetologists did mostly two things. They studied cetacean anatomy, usually by doing painstaking dissections of beached animals. The other activity was biological bookkeeping, or systematics. How many kinds of these animals are there? What should we call them? Where are they found?

It may sound dull, but it wasn't. Imagine discovering a new mammal, one unknown to science. For the first time in the history of the earth this animal had been discovered by man. If you described and named your find, you and the animal became linked forever. "My dolphin", you thought, full of proprietary feelings for this critter swimming out there in the ocean, who hadn't the slightest idea about you.

The beach was the usual source, and the prize frequently reeked with decay and dripped with fetid oil. Someone had phoned, a lifeguard or a policeman. Typically, such calls came just as dinner was put on the table.

"There's a dolphin on the beach. You're the dolphin guy aren't you?" Those who study whales, dolphins and porpoises. I learned to my surprise that I was one.

"Where is it, and what does it look like?" The decision was usually made to check it out before the tide came in again. And off we cetologists went, half loving the possibility of a prize, a rarity, and half wanting to stay home by the fireplace, with a glass of wine.

"Put on your coveralls and rubber boots" said the wife, a voice of experience who had had a pungent, blood-and-oil covered husband burst in the house before, with a heedless "Guess what it was, honey!, as incredibly ugly boots and coveralls were pulled off in the pantry.

Once near the beach the wine and the fireplace were forgotten. All that was swept away by the scene; the wind, the waves purling up on the darkening strand, the birds swooping, and far down the beach, a knot of people standing looking down at something, surely the stranded animal.

I sidle into the crowd, look down and at a glance make out the curious, overhanging snout of a pygmy or dwarf sperm whale- a rarity cast in from the abyssal ocean. I say: *"Kogia*." The knot of people hear the unfamiliar word, and note my boots, clothing and measuring gear. "What is it?" they ask, having found a voice of authority in their midst.

Every once in a while, though, identification is not so simple. It may then take real sleuthing to find out what was lying there on the sand. I'll tell you now about the first such discovery I ever made. It was of the vaquita or Gulf of California Harbor Porpoise . I had collected the first specimen of this little animal well before I began in earnest to learn about either fish or cetaceans.

It was a spring day in 1950. I was savoring the brand new, high-bermed gravel road to San Felipe, Baja California, México. The old road that it had replaced had been a thing of legends. Even though it was the lifeline between the trading center of Mexicali and the fishing village of San Felipe on the upper Gulf of California, the old road was no more than a winding, two-rut track that, against all the dictates of reason, transected the periodically flooded salt pan at the entrance to the Laguna Salada, a huge, soggy lake bed usually glittering with salt. When the surging spring tides of the upper Gulf (some topping 30 feet at the crest) came sweeping across the vast, vegetationless mudflat from the Gulf of California to the east, the road sometimes disappeared utterly and became a miles-long wallow of bottomless mud. That was half of the problem. The torrential rains of summer frequently achieved the same end as they flooded down the barren Sierra Cocopah, sluicing mud and boulders across everything in their path. When one or the other happened the market town of Mexicali and the fishermen of San Felipe were on different planets. It was weeks before the first intrepid truck to Mexicali swam its way through again.

I was nearing the end of a two-week scientific trip alone across the southwestern deserts and could feel the terminal nervous stage coming on. That's when I checked my gas tank three times a morning. I was making a desert-wide map of the dunes on which a certain species of lizard lives.

I had one more dune to check, one just north of Punta San Felipe. The lizard I sought, a beautiful little animal called *Uma*, lives only on windblown sand dunes. I'd wondered how they got between the widely separated dunes if, as they were thought to do, they always stayed on wind-blown sand. I'd plotted the several dozens of dunes where the lizard had been found and discovered that I could link most of them by proposing sand corridors along stream courses or over low divides, and by relating the development of dunes to past climates as the Ice Age had left the desert. But Punta San Felipe was 60 miles south of the last dune on which *Uma* was known to live, and a stony, flood-washed desert intervened.

According to my theory *Uma* shouldn't be there. As my truck jounced along over the newly laid gravel I could see the flat, blue Gulf of California far down the bajada. Then the hazy, purple headland of Punta San Felipe materialized along its shore. I knew that on the south side of that point lay the dusty little fishing village itself.

I rolled my window down to let in the balmy desert air and sang as loud as I could, not because I was thinking of Burma, or lissome maidens, but because I was alone and on the last lap toward home.

"By the old Moulmein pagoda Looking eastward to the sea, There's a Burma Girl a'sittin, And I know she thinks of me."

A few years later when I sang that song in a family-filled station wagon my little daughter Barbara would clasp her hands over her ears and cry: "Daddy, daddy, *too* loud!!"

No volume control this time, though. I could see the dune, a pale pinkish splotch stretching north behind a curving beach. I turned off the gravel berm and onto a jouncy two wheel track.

"Ohh the wi-i-ind is in the-e pa-aim trees."

The trail shied this way and that around the bases of big clumps of creosote bush and palo verde, both alight with the yellow flowers of spring and attended by the loud hum of thousands of bees. The track ran down long stretches of open wash, which I assessed with a practiced eye. My decision was whether or not, on my way back uphill I was likely to sink into the gravel and bog down. The slightly damp sand told me I could make it if I kept my speed constant and didn't spin my wheels.

In half an hour I pulled to a stop at the edge of the flat salt pan behind the dunes, pulled my hat down against the growing power of the sun, and began to walk along the dune margin. The dune was heaped between the sea and me-clean sweeps of immaculate, pale pink sand piled by the wind in lovely arcs. It wasn't long before I had my answer about the sand lizards.

No *Uma*'s here. Their ecological space, up on the open sand, and in the little hollow valleys between dune crests called *blowouts,* was occupied by another lizard, the sometimes extravagantly beautiful *Callisaurus,* or zebra-tailed lizard[1]. I'd learned that *Uma* and *Callisaurus*, in some unknown way compete for habitat, and that *Uma,* when it is present, is the winner on open sand.

The male zebra-tails were posturing when I arrived, probably jockeying for space. I hid behind a creosote and watched one light-footed encounter, two lizards in a circling dance, going almost taster than I could follow, spiraling off across the flat.

As I walked the dune margin, I saw a whitened skull partly protruding from the sand, buried just above the reach of the usual high tide. The flooding sea had coursed around the dune and over the pickleweed flat, filling long ponds back of the dunes. Anything that floated- paper boxes, bits of wood, scrub brushes off of fishing boats, animal carcasses- was borne in, tumbled by the wave swash, and then left when the water receded. This skull was all that remained after the coyotes and mice had cleaned it. I couldn't identify it, except to say that it was obviously a very small porpoise or dolphin.

I picked it up, shook the sand out of the brain case, and carried it back to the truck. That night after dinner I sat on my cot under a circle of light from the hissing Coleman lantern and wrote out a specimen tag for the skull. I tied it on carefully, using a good square knot, and then I wrapped the skull in newspaper and stowed it away in an empty carton. I thought little more of it. It didn't even warrant a line in my journal.

Three years later a paleontologist friend, Dr. Jim Warren, brought me two more skulls from the beach a short distance south of San Felipe. They were of the same little animal from the same general place. There really was a tiny cetacean down there, and so far as I could learn, no scientist knew about it!

By then I was busy in my curator's post, with Mac[2] running the vital little laboratory that attempted to develop answers for the cloud of questions that descended upon us. Why, for example, was the water in the big fish tank cloudy this morning? Why had a trigger fish died? Mac can build anything, knows all sorts of stuff I don't, will bray in indignity when somebody does something stupid, and is first to pick up whatever needs to be picked up. Everything we did together became a shared high adventure, not a boss and employee thing. "Hey Mac, why don't we try this?..."

Mac and I were both finishing our doctoral degrees *in absentia*; he at UCLA and me at Scripps Institution of Oceanography, so under our leadership the Oceanarium's lab took a real scientific turn. It became an exciting place of discovery.

1 KSN footnote: I call the zebra-tails " 'sometimes' extravagantly beautiful." That's because in spring the males become painted with the most beautiful colors, reds, chrome yellow, china blue, mostly on their flanks, bellies and throats, which they display to other lizards by flattening their sides and standing up on the tips of their long toes.

2 We assume that this refers to William N. McFarland, with whom KSN wrote the species description published in 1958.

To my surprise I found that very few careful observations of Pacific dolphins had been made, and here we were working with them daily.

Scientists barely knew what kinds of dolphins and whales lived off the California shore , even a few miles from the Los Angeles Harbor entrance, l*et al*one what they did. The seasonal comings and goings of the various species, how many there were, what they ate, how they swam and dove were all nearly a complete blank. Terra incognita Swiss Family Robinson! Mac and I were in a biologist's version of Hog Heaven!

To instill some order in all this treasure I designed a scientific contribution series for the oceanarium. It was planned as a collection of scientific reports about the best of our discoveries. We'd staple a special cover with a dolphin printed on it to every new report, advertising "Marineland of the Pacific Laboratory Scientific Contribution No. --". By the time I left the oceanarium for a University post we had produced dozens of such papers, and our little dolphin logo graced a good many library collections.

Contribution Number 1 in our series was a collection of behavioral observations of the cetaceans of southern California waters made while we were out collecting. We just edited and arranged our field notes and voila! there was a worthy contribution to the knowledge of California's wildlife.

I showed Mac the Gulf of California cetacean skull. Maybe this little guy would be a good subject for Publication No. 2?

"Where do we start, Mac?" I asked.

"Identify it, of course, dummy."

"I knew that, Mac! I replied, with a bit of pain in my voice.

But 'we put the skull in my car and drove to the University of California at Berkeley, where the major scientific collection of western United States vertebrate animal specimens is housed. One didn't just knock and enter the Museum of Vertebrate Zoology, either, especially two untested graduate students didn't. Both courtship and obsequiousness were involved. Furthermore, we were both from lesser institutions at the south end of the state, a factor that loomed large in 1953.

A suspicious schoolmarmish lady recited the numerous rules of MVZ toward the precious materials in the collection, and then led us to the zinc cabinets where the specimens were housed. Those cabinets were not ours to open, either. We watched while the curator picked out this or that specimen as if it were Dresden, and placed it on a big, open table for us to look at. He watched us while we compared our skull with others in their collection, and then antiseptically replaced the specimen in its vault.

An older man, foppish and dapper, slid behind us. Peering over our shoulders as he went by.

"What's the genus of your skull?" He queried, as if he was giving a final exam.

"That's what we are here to find out." I replied, a little cowed. After a long, almost theatrical pause, he said:*"Phocoena."*

"What does the Latin name mean?" He then asked. I hadn't the slightest idea, nor did Mac. "It's Greek for Porpoise" he replied, detaching his thumbs from his weskit and moving on.

It was Dr. Seth Benson, who was reputed to know nearly everything. Undaunted, as good graduate students often are, we turned back to our skulls. Mac's and my social sense at the time scarcely recognized that while we had written ahead for permission to examine the collection, we still remained two graduate students who didn't know the genus of one of the best-known of cetaceans in the United States!

Benson was right. The little skull was from a specimen of *Phocoena.* In the following days Mac and I dug out everything we could find on the genus.

Phocoena. We found that *Phocoena* was the name of the common porpoise, and that England's Queen Elizabeth was reputed to have considered it a delicacy. *Phocoena* ranged widely around the shores and bays of the temperate and subarctic Northern Hemisphere. In some places it was called the puffing pig, in others the herring hog. We learned that one can tell modern porpoises from dolphins because they usually have very low triangular dorsal fins, little cusped teeth (instead of conical ones), blunt snouts, and more easily movable cervical vertebrae than the dolphins do.

A lot of wonderful history emerged from our sleuthings. The Swedish botanist, Carl von Linne in his 1758 *Systema Natura,* his great attempt to classify all living things, had named the little cetacean *"Delphinus phocoena*." Since everything he wrote was in Latin he changed his own name to *Linnaeus*, which is what most people still call him today.[3]

In 1817[4] Baron Jean Léopold Nicolas Frédéric Cuvier (how lovely it must be to have all those names and all those accent marks in one's name!) recognized the genus *Phocoena* as different from the dolphins and there it remains today.

The more we searched the more we became convinced that our little critter was not the common porpoise of the Northern Hemisphere but an unrecognized species. Even though our three skulls were from adults (you can usually

3 This is not correct. He was born Carl Linnaeus, and the name Carl von Linné is how he came to be known after his ennoblement. Some people later 'latinized' his name as *Carolus Linnaeus*.

4 The correct date is 1816. Cuvier's full name has been corrected from that of KSN's draft.

tell an adult mammal by its skull because the sutures between the skull bones are fused) the porpoises were tiny compared to other cetaceans. We later learned that this little cetacean is, in fact, one of the world's two smallest-its nearest rival is a spinner dolphin that lives in the Gulf of Thailand.

What convinced us that we had a new species were several features of their skulls-little details that were different from the general run of puffing pigs. Proving such a contention involves description, precise measurements of all the specimens one can muster, and statistical analysis of the results. It's not work for the flighty and it took us months.

We began to realise that our little animal was closest to a South American *Phocoena*, a curious almost totally black animal with a cut-off sloping dorsal fin studded with a double row of little horn-like prickles. It is called Burmeister's Porpoise, *or Phocoena spinipinnis*.

Aha! Mac and I suddenly realized that somehow, sometime, the ancestors of *Phocoena* had crossed the equator! How, when? Which way?

We didn't have to look far. My professor, Carl Hubbs at Scripps Institution had been there before us. In a seminal paper on the subject he had written *of antitropical* species, and there were many.

These are creatures that had somehow jumped the equator and now occur on both sides of the tropics, either as the same or closely related species. He wrote of anchovies, kelps, jack mackerel, dolphins, and a good many other fish and invertebrates that obviously had done this. There was even the huge sleeper shark, *Somniosus*, that today is distributed *under* the warm surface waters of the tropics. This ancient creature is seen among the ice floes of the Arctic, and there has even been reported attacking walruses, but it has also been identified from photographs taken in deep water beneath the warm surface waters of the tropics, where it never ventures to the surface.

Mac and I asked next: "When and how had all these animals made the trek across the equator?"

The most likely time for this porpoise ancestor to have swum across the Equator, it seemed, was during the recent Ice Age, which placed the crossing from a few thousand to perhaps two million years ago. Judging from fossils, most modern species of dolphins and porpoises have been around for several million years, and the skulls of the two species from opposite sides of the equator are pretty similar. The "feel" was of a fairly recent separation.

"What happened to the tropics during the Ice Age? I asked Mac, while I thumbed through a text on ocean history. "Hey, look at this!"

Down near the Equator, in the general latitude of the Panamanian Isthmus, the Caribbean and eastern Pacific waters are very different. On the Caribbean side at latitude 0°, one finds coral reefs and clear, warm water with many tropical species swarming about. On the Pacific side, however, things are very different. The water tends to be a bit murky and on average it is much cooler. The animals that live in the Pacific tropics are a very different collection of species, too. A cool water corridor all but exists today across the tropics! The cause of all this lies in the different ocean currents of the two oceans.

The enormously long ocean currents that rim the eastern Pacific, the California Current of the Northern Hemisphere, which sweeps south along the west coast of the United States and the Humboldt Current of South America, which flows northward along the shores of Chile and Peru- both, as they near the tropics, begin to bend due westward across the mid Pacific toward Asia. A thousand miles off the Central American shore they come to run parallel, to stream clear across the ocean side-by side, two "rivers" going the same way but separated by a countercurrent coming back toward the Western Hemisphere. Before these currents bend west, one north and one south, they trap between their conjoining arms a triangle of cool water that lies against the shoreline of Central America. It is this water that supports all those cool water species I just mentioned.

Maybe this cool triangle of shore water, made even colder during the Ice Age, was the corridor that our little porpoise required to cross the new world tropics. Burmeister's porpoise lives today as if poised for such a swim, since it is found in the chilly Humboldt Current right up to the Equator in northern Peru.

Finally, Mac and I decided to name the little porpoise *Phocoena sinus,* the Gulf of California Harbor Porpoise." *Sinus* is Latin for "Gulf." A nice, neat comprehensible little name, we thought.

"O.K., Mac?" I said, let's go see a live one! We wanted to talk to fishermen who doubtless knew about the little porpoise, and we wanted to keep our own watch looking for fins. On our collecting schedule were trips to the upper Gulf of California to collect some of the many interesting fishes that live there, especially the giant six foot white sea bass, the *totoaba*. With a little luck we might see our porpoise at the same time.

Our plan was to hire totoaba fishermen out of San Felipe to catch three or four specimen fish. We planned to hold these big fish in a "live car," a specially built decked over skiff with doors in the side, which we could tow behind a fishing vessel and through whose side door any big fish could be led. Then we would attempt to haul our huge catch back to the oceanarium at Los Angeles. For this job Mac and I had designed, and Frank and Boots had built, a special fish transport tank that we could

load on the oceanarium's biggest truck for the drive to México and back; its 26,000 pounds of circulating sea water being pumped through aerators to keep the fish alive.

So, one spring day in 1956, the four of us, our truck, fish tank and live car hauled into San Felipe. The first stop was to find a base camp for the expedition. Frank began a process that he called "snootin' around" among the local fishermen. Soon he located a roofless cinder block building that we were able to rent.

"If you are in a foreign place, buy into the local economy if you want to be taken care of", was Frank's wise rule. Though the building had doors that we could lock, providing the illusion of security, it was the owner from whom we rented that mattered. He, as Frank predicted, made sure that no one tampered with his chance to earn money from the gringos.

We christened the tumble down edifice "The St. Frances Hotel", swept out the considerable trash, and moved in. Soon the spaghetti water was steaming on the gasoline stove and a squadron of local dogs began circling- a formation that would last as long as we were in occupancy. After our meal, gear was laid out for the next day's fishing and then we crawled into sleeping bags under a starry sky that shone down where a roof ought to be.

Before dawn Frank was up making a pot of coffee. We four drank it standing up, and then made our way down to the dark strand flashing with star flicker. The spring air of San Felipe, even that early in the season, can be soft and warm enough to bathe in. Offshore, a short row away, lay a venerable fishing vessel *San Luis*, rocking in the low swell, a rust bucket whose hull next season would part peacefully and sink at anchor to the bottom of San Felipe Bay. We all knew it was coming. One had only to lean on a rail and have a piece of it come off in your hand to know that. But it was what these fishermen had and everyone took their chances, we included.

Captain Fortunato Valencia was already on board, warming up the engine, and Olaya the cook took up his position straddling the gaping hole created by the missing engine room hatch, bare feet gripping the hatch combing on either side, and began cooking breakfast- potatoes and bacon that we had provided. Down, six feet below his crotch the rocker arms of the old diesel had begun to click methodically. This arrangement had something to do with providing air both for the engine and the engineer.

In addition to breakfast makings, Frank left Olayo a tinned ham to work on for the evening meal. Later that day I came by Olayo's deckless galley to find him gripping the slippery jelly covered ham with his toes. Those toes, I could see, were crucial organs for a person with his job. He had the ham pinioned in an angle of the galley deck, and there he sliced off pieces with a long butcher knife. These were dexterously retrieved before they fell on the diesel engine. Olayo seemed to regard all this as ordinary.

Thus splayed across the galley he spoke to me of his twelve children ashore. The San *Luis,* he implied, was a peaceful place by comparison, and he was happy to be with us. The big screw swirled and we left the harbor, the sea as calm as if it had been oiled. We headed for the totoaba grounds off Punta San Felipe. An hour or so later the vessel slowed to a stop. I looked to the west and could see the pale dunes where I had pulled the type specimen of *Phocoena sinus* from the sand six years before.

A small group of tiny cetaceans- the largest about five feet long- broke water. I asked Fortunato to take us closer. Soon I could hear the puffs of their breaths, and could make out the blunt head of one. Such tiny clues, which we had learned from our reading, all suggested that they were porpoises, not dolphins.

The little mammals didn't school tightly together as dolphins usually do, but dove raggedly and headed in different directions underwater.

"Those are our porpoises, Mac!" "For sure," replied Mac.

Another curious thing struck us, and has since been seen by other more recent observers. The Northern Hemisphere common porpoise can be identified at once by its low, broad-based dorsal fin, shaped like a flattened triangle. If you see a cetacean with such a fin in North American waters, you can be pretty sure it is attached to a porpoise. Yet one of the little Gulf animals that swam off our beam had a tall fin, almost like a little killer whale.

Later I proposed that this has to do with the very warm water of the Gulf in summer. The shallow upper Gulf may turn to warm soup under the implacable desert sun, the water pushing into the 90° F range. This means that a little mammal, immersed in such a tepid sea with no way to seek shade, has at most about ten degrees difference between its body and the water to work with should it seek to escape a predator or catch a fish.

That translates to lassitude, or to a very small "*scope for activity*" as the physiologists call it. That tall fin is a porpoise's heat exchanger. If the porpoise exerts itself blood pours into special vessels just beneath the skin of the fin to dump the excess heat into the passing water. The bigger the porpoise the larger fin surface it requires to cool itself; so, reasoning backward, a large fin must also mean a large porpoise. Parenthetically, I think this is also why old bull killer whales have huge, disproportionately large fins compared with their much smaller female consorts. Scientists frown on these circles of logic, and rightly so, but they can be wonderfully useful to pose conundrums for someone else to prove, or to toss in the junk heap of discarded ideas.

Fortunato knew the little porpoise well. "Vaquita", he called out. In English that translates as "little cow." He told us some fishermen called them "duende" or "ghost," a fitting name I thought for a creature so elusive!

"We sometimes catch vaquitas in our gillnets," he told us, but vaquitas aren't worth much in the market." In later years it became clear that such chance captures of the vaquita were so common throughout the heavily fished upper Gulf that, even at the time we first saw them the species was on a precipitous slide toward extinction. That first skull that I had pulled from the sand dune was most likely from a *vaquita* that died in a fisherman's net.

The general area where we met our first vaquitas, offshore and a bit to the north of San Felipe, is, remarkably enough, even to this day the place where more than 90% of all the sightings of live vaquitas have been made. No one is totally sure why. Fishermen net them widely over the murky shallow waters of the upper Gulf of California, but that one small area of water is the place where one can almost guarantee actually seeing this rare little porpoise.

What's so special about this vaquita headquarters? Its major feature is a submarine canyon cutting north-south into the otherwise shallow undulating muddy bottom of the upper Gulf of California. Some scientists speculate that this canyon is a remnant of the old channel of the Colorado River, while others say it is a trace of the huge San Andreas Fault that splits off Baja California and much of western Alta California from the North American continent. Probably it's both.

This submarine channel zig-zags past an incongruous, rocky pinnacle, Roca Consag, that juts like a jumbled finger almost vertically out of the otherwise featureless Gulf. The gathering dark found us without fish to tend, so Fortunato maneuvered the San Luis south of the pinnacle. With a seaman's care he dropped the anchor, testing it again and again. The ship stood away to the south, pulling the hauser into a shallow catenary. In the last light we could see the water stream past us, alive and swirling. The hollow barking of a colony of sea lions on the dark rock never ceased.

Emissaries arrived to greet us: an old bull[5] and three calves circled us, leaving rocket trails of cold bioluminescence in the water off the rail of the San Luis. Seabirds swirled by the thousands in the last light, coming to the rock to roost -flights of brown boobies and gulls who settled in silhouette up among the rocks.

Before the tide could turn north and swing us toward the rock we were gone. It was just enough time for Olayo to spraddle across the open hatch and do his stuff, and for the four of us to share a cup with the captain. We watched the bond between the two skippers, Frank and Fortunato, grow. It was built on subtle assessments of each other's competence, and then of respect. Both could read the weather and the currents, and both knew when and where to anchor, and took no chances they could avoid.

Frank's usual admonition was: "Always be afraid of the sea".

Fortunato told us matter-of-factly of his coming to San Felipe a dozen years before. If Mac or I had made the trip he described it would have been a saga to be talked about with wonder from then on. For Fortunato it was just life as he had to live it.

Born of Yaqui Indian parentage in the mountains of Sonora, he had made his way down to the sea as a young man, and then when word of jobs on the rich fishing grounds of San Felipe had reached him, he and a handful of comrades had taken their sole possession of worth, a dugout canoe, and made their way almost 400 miles up and across the Gulf of California at its most treacherous point, the midriff islands, to San Felipe. On charts of those islands one finds rocky promontories with names such as Sal si puedes or "Get out if you can." But Fortunato and his friends made it safely past those waterless desert islands to the promise of jobs at San Felipe.

At San Felipe, Fortunato's calm skill won him his captaincy with the fisherman's cooperative, an organization built around cast-off vessels such as the San Luis.

"What did you do for food and water, Fortunato?" I asked.

"We had tortillas and beans and some bottles of water."

"Did you paddle?"

"Yes, and we used a sail when it took us the right direction," was his reply.

The San Luis's anchor came up and we stood off Roca Consag as the current began to swing around, part of the great sluicing that tips the entire water mass of the Gulf as if on a teeter-totter, with its fulcrum at Guaymas, 300 miles south. This tipping produces enormous tides in the upper Gulf, and boiling currents past Roca Consag.

I began to suspect that the submarine canyon off Roca Consag, the currents, the sea lions, and the birds with their guano, produce a rich piece of water where the little vaquita can find food, and perhaps even a place of surcease from the incessant scraping of the sea bottom by shrimp trawlers. We were told that nearly every inch of the northern Gulf was, at that time, scraped by a trawl about seven times a year. Submarine canyons are no places for nets though; the big spreader boards of otter trawls tend to jam in mud banks, and as surely as if the anchor had been dropped, stop a vessel in its tracks. Worse, if one of the outlying rocks of Roca Consag was to be snagged the net might never surface again.

5 We presume KSN is referring to California sea lions, *Zalophus californianus*, as they are common in the Upper Gulf.

Once the word was out that there might be a new cetacean in the Gulf several biologists from both México and the United States began to report possible sightings; an unidentified fin cutting the swift water in an estuary at Guaymas, a group of cetaceans wriggling on a mud bank in shallow water of Topolobampo Bay, fins seen in Bahia La Ventana, down near the tip of Baja California; any or all might have been the little *Phocoena*. Where *did* the vaquita live? It took a long time to find out.

In México the focal person was, and still is a remarkable man, Dr. Bernardo Villa Ramirez[6], long the head of the Department of Mammalogy at the University of México, in México City, and by extension, his many students. I call him Bernardo, because he has become a treasured friend. Bernardo is the heart and soul for the conservation of Mexican wildlands and their life. More than anyone else he has fought to protect every wild thing, from the bats, to the Mexican wolves of the Sierra Madre Occidental, to the whales, seals, manatees, and even the islands of the Gulf of California themselves. To me he is one of the saints in the human struggle to save the wild world, and he includes in his reach the vaquita.

Two U.S. scientists: Dr. Greg Silber, who was a student in my own laboratory at the University of California at Santa Cruz, and who is now with the U.S. Marine Mammal Commission[7], and Dr. Robert Brownell, Chief of Marine Mammalogy for the U.S. Fish and Wildlife Service[8] have led the U.S. effort. Silber and his Mexican colleagues traveled the Gulf widely by boat and plane in order to define the range of *Phocoena sinus*.

They interviewed fishermen along the length of the Gulf. They sought to discover if these men had dealt with the vaquita or if it was unknown to them. A somber, and to me surprising pattern emerged. The vaquita, they concluded, occupied perhaps the smallest geographic range of any living cetacean. If on the day I discovered that first skull of *Phocoena sinus* I had turned and clambered up the thousand feet or so to the top of Punta San Felipe I could have looked out across the Gulf of California to Sonora, and north to the delta of the Colorado River, and southeast 50 miles beyond Roca Consag, and I could have seen it all.

Greg and his colleagues defined a range limit across the shallow uppermost Gulf. South of that line almost no one knew about the vaquita, north of it just about every fisherman had seen the little mammal pulled on deck, drowned in the meshes of a net. That's all there is to vaquita country. Their home, Silber his colleagues said, limited to the murky water uppermost Gulf of California.

Silber discovered another thing about the little porpoise that convinced me he was right. He listened to them underwater and recorded their voices. They proved to have what I think of as murky water voices, and, with such an adaptation the only real place for them was where Greg said they were, the murky upper Gulf. To the south of Greg's line the Gulf becomes more and more transparent, and the species of many kinds of life change.

The only sound Greg heard from the vaquita was clicking, given in creaks and long trains, like a rusty hinge. The clicks were incredibly high in frequency- up about nine times as high as an adult human can hear.

How I envision these sounds working goes like this. In muddy water the little porpoise can search for fish with its click trains. The fish can't hear the high frequency sounds of the porpoise. Apparently the primitive hearing mechanisms of most of them aren't good for sounds even a tenth that high. The water is too dirty for the porpoise to be seen by eye for more than a couple of yards, at best. By the time the porpoise comes that close a fish has no time to react. Lunch.

Because no other sea animal, except some other cetaceans, make sounds of such high frequencies it might also be that the little porpoises can locate each other and carry on their society without fear of detection.

Greg and I also hoped that this acoustic circumstance might provide a way for biologists to detect the presence of porpoises that couldn't be seen at the surface. We might then try to listen throughout the upper Gulf with gear sensitive to such high frequencies. By plotting where each sound contact it might be possible to make an accurate acoustic count of vaquitas. If the method worked it could provide an accurate assessment of how many vaquitas remain alive, and where, exactly, they ranged.

While our attempts at plotting their occurrence by listening were not successful due to the high cost of obtaining the proper equipment, another scientist, Jonathan Gordon, of Great Britain has made the method work for the Queen's porpoises, and I hope it will soon be applied to the vaquita to tell us just how many survive.

So, rare the vaquita certainly is. Only forty-two years after I put that first skull in its cardboard carton the vaquita is now sometimes given the dubious distinction of being called the world's most endangered cetacean. It vies for this unwelcome crown with two river dolphins, the Indus River Dolphin of Pakistan, and the Baiji of the Chang Jiang (Yangtze) River of China, each species likely comprised of less than 400 living individuals[9].

The vaquita has been classed as "endangered" by the International Union for the Conservation of Nature[10], the organization who maintains the authoritative "Red Data Book," that keeps track of the world's endangered wildlife.

6 Villa Ramirez passed away in 2006, after an amazingly productive career.

7 Silber was later with the NOAA Fisheries, and is now retired.

8 Brownell is now with the NOAA Fisheries' Southwest Fisheries Science Center.

9 Unfortunately, the baiji is considered to have gone extinct in around 2006. The Indus River dolphin, however, is doing better than KSN thought, currently numbering about 1,800 individuals.

10 The IUCN currently classifies the vaquita as Critically Endangered.

In truth, no one knows how many vaquitas there are. They are shy little animals, and only when the sea is calm is there much hope of seeing them. Yet, the upper Gulf is a very small piece of not very remote ocean. The thirty or so that are caught each year in fishing nets seem clearly more than the species can stand for verylong.

I have yet to stand looking at a fresh-caught vaquita; yet I know what the species looks like from the few photographs that have been taken, some by Flip Nicklin. It is a roly-poly little animal, the tall fin and big pectoral flippers are there, alright. It looks as if it wears a pair of dark sunglasses; a big round blotch of black skin surrounds each eye. It has a wide-eyed look about it, as if it easily placed its fate in our hands. Its tiny little babies are marked with vertical bands of light grey from the time when they lay curled in their mother's uterus, and like many baby mammals they are all dependence and innocence. The fate of the vaquita, adults and young is, in fact, wholly in our hands. As I wrote this, word came from Greg Silber that the government of México, through the good grace of President Salinas himself, has established a preserve on behalf of the vaquita and the totoaba including the entire upper Gulf of California. See the fine hands of my various colleagues in this.

Hang in there, vaquita. It might just work...

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Seasonal use of bridges as day-roosts by bats in the Trans-Pecos of Texas

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Bats commonly use highway infrastructure as day- or night-roosts. Nonetheless, little is known regarding how regularly bats use these structures or whether they do so only on a seasonal basis. We surveyed 13 parallel box beam bridges along 15 km of State Highway 17 in Jeff Davis County, Texas monthly for 12 months to examine seasonality of day-roost use. Bats using bridges, ranked based on abundance, were: *Tadarida brasiliensis*, *Myotis velifer*, *M. californicus/ciliolabrum*, *M. yumanensis*, *Antrozous pallidus,* and *M. thysanodes*. *Myotis velifer*, *M. californicus/ciliolabrum*, and *M. yumanensis* exhibited significant differences among bridges and significant seasonality in roost use. *Tadarida brasiliensis* exhibited significant differences among bridges but no significant seasonality of bridge use. Seasonality of use of bridges as day-roosts likely reflects seasonal patterns of distribution of species in the Trans-Pecos. Moreover, these results suggest that surveys of bats roosting in highway infrastructure should be planned carefully and consider the seasonal nature of roost use.

Generalmente los murciélagos utilizan la infraestructura de carreteras como perchas durante el día y la noche. No obstante, se conoce muy poco con que regularidad los murciélagos utilizan dichas estructuras, o si las utilizan de manera estacional. Durante12 meses, hemos examinado 13 puentes de vigas cuadradas y paralelas a lo largo de 15 Km de la Carretera Estatal 17 en el condado de Jeff Davis, Texas, para examinar la estacionalidad en el uso diurno de las perchas. Los murciélagos que utilizan los puentes fueron clasificados en base a su abundancia, en el siguiente orden: *Tadarida brasiliensis*, *Myotis velifer*, *M. californicus/ciliolabrum*, *M. yumanensis*, *Antrozous pallidus* and *M. thysanodes*. *Myotis velifer*, *M. californicus/ciliolabrum* and *M. yumanensis* exhiben diferencias significativas entre puentes y también estacionalidad significativa en el uso de perchas. *Tadarida brasiliensis* exhibe diferencias significativas entre puentes pero no muestra estacionalidad significativa en el uso de los puentes. La estacionalidad en la utilización de puentes como perchas diurnas probablemente refleja los patrones estacionales de distribución de la especie en el Trans-Pecos. Por otra parte, los resultados sugieren que estudios de murciélagos basados en la infraestructura de carreteras deben de ser planeados cuidadosamente considerando la naturaleza estacional del uso de las perchas.

Keywords: Chiroptera; *Myotis velifer;* seasonality; *Tadarida brasiliensis*; transportation infrastructure.

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Introduction

Many organisms, especially higher vertebrates, use daytime or nighttime resting places (*e. g.*, burrows, dens, nests, roosts) to sleep, rest, or escape the elements. Bats may spend more than 50 % of their time in day-roosts (Kunz and Pierson 1994). Not only do roosts provide a place to rest and protection from weather, they protect bats from predators, as well as facilitate day-to-day activities such as mating, raising of young, and torpor/hibernation (Kalcounis-Ruppell *et al.* 2005; Mering and Chambers 2014). As a result, decisions as to where and what kind of roost to use have fitness consequences (Kunz 1982).

Bats are highly variable in their use of day-roosts (Kunz and Lumsden 2003). Natural roosts include foliage (Constantine 1958), tree cavities (Pierson 1998), rock crevices (Vaughan and O'Shea 1976), and caves (Davis *et al.* 1963), among others. Bats also use a variety of manmade structures as roosts including buildings (Brigham and Fenton 1986), attics (Humphrey and Cope 1976), mines (Fenton 1970), bridges (Davis and Cockrum 1963), and culverts (Bender *et al.* 2010).

Twenty-four species of bats in North America frequently use highway infrastructure as day-roosts (Keeley and Tuttle 1999). The most frequently used structures are large and long box culverts or parallel box beam bridges (Keeley and Tuttle 1999). In a study by Keeley and Tuttle (1999) spanning 25 of the states of the United States, approximately 9 % of bridges and culverts were used as day roosts by bats. In North Carolina, three species of bats roosted under 15 of 23 bridges (Felts and Webster 2003). Similar patterns have been described in more focused studies from central New Mexico (Geluso and Mink 2009), southern Illinois (Feldhamer *et al.* 2003), and Texas (Meierhofer *et al.* 2018).

The Trans-Pecos region of west Texas is home to 27 species of bats and represents the ecoregion with the greatest bat diversity in Texas, which consists of 34 species (Schmidly and Bradley 2016; Krejsa *et al.* 2020). Most bats occur in this region seasonally, being most numerous during summer and migrating south to México and other locales to overwinter (Higginbotham and Ammerman 2002). Our objectives were to characterize bridge-specific and season-specific use of bridges as day-roosts by bats in the Trans-Pecos region of Texas.

Methods

Study area and sampling of bats. Thirteen bridges were examined monthly along an approximately 15 km stretch of Texas State Highway (SH) 17 in Jeff Davis County, Texas between June 2018 and May 2019 (Figure 1). All bridges were parallel box beam bridges, typically with six parallel box beams with expansion joints up to 15 cm in width. Bridges were distributed across the riparian corridor of Limpia Creek and typically more than 2 m and always less than 4 m in height off the ground. Joints were inspected for bats with the aid of a spotlight. Individuals of each species except *T. brasiliensis* were enumerated. Quantities of *T. brasiliensis* were recorded into six bins (0, 1-10, 11-100, 101-1000, 1001-5000, 5000). Some bats were captured periodically using 24 inch forceps, identified and sexed.

Myotis ciliolabrum and *M. californicus* are difficult to distinguish in the field (Ammerman *et al.* 2012). Accordingly, we did not distinguish between these two species while surveying bridges. Prior to this study, several specimens from this stretch of Texas SH 17 were collected and identified as *M. ciliolabrum* based on the key to the skulls in Ammerman *et al.* (2012). These are housed in the Mammal Collection of the Natural Science Research Laboratory, Museum of Texas Tech University. *Myotis ciliolabrum* tends to occur at higher elevations than does *M. californicus* (Constantine 1998; Holloway and Barclay 2001), including elevations where it was observed in this study. It is likely that these individuals were *M. ciliolabrum*, but we did not collect any museum vouchers and cannot be sure. Consequently, we herein refer to these two species collectively as *M. californicus/ciliolabrum*. Keely and Tuttle (1999) listed only *M.*

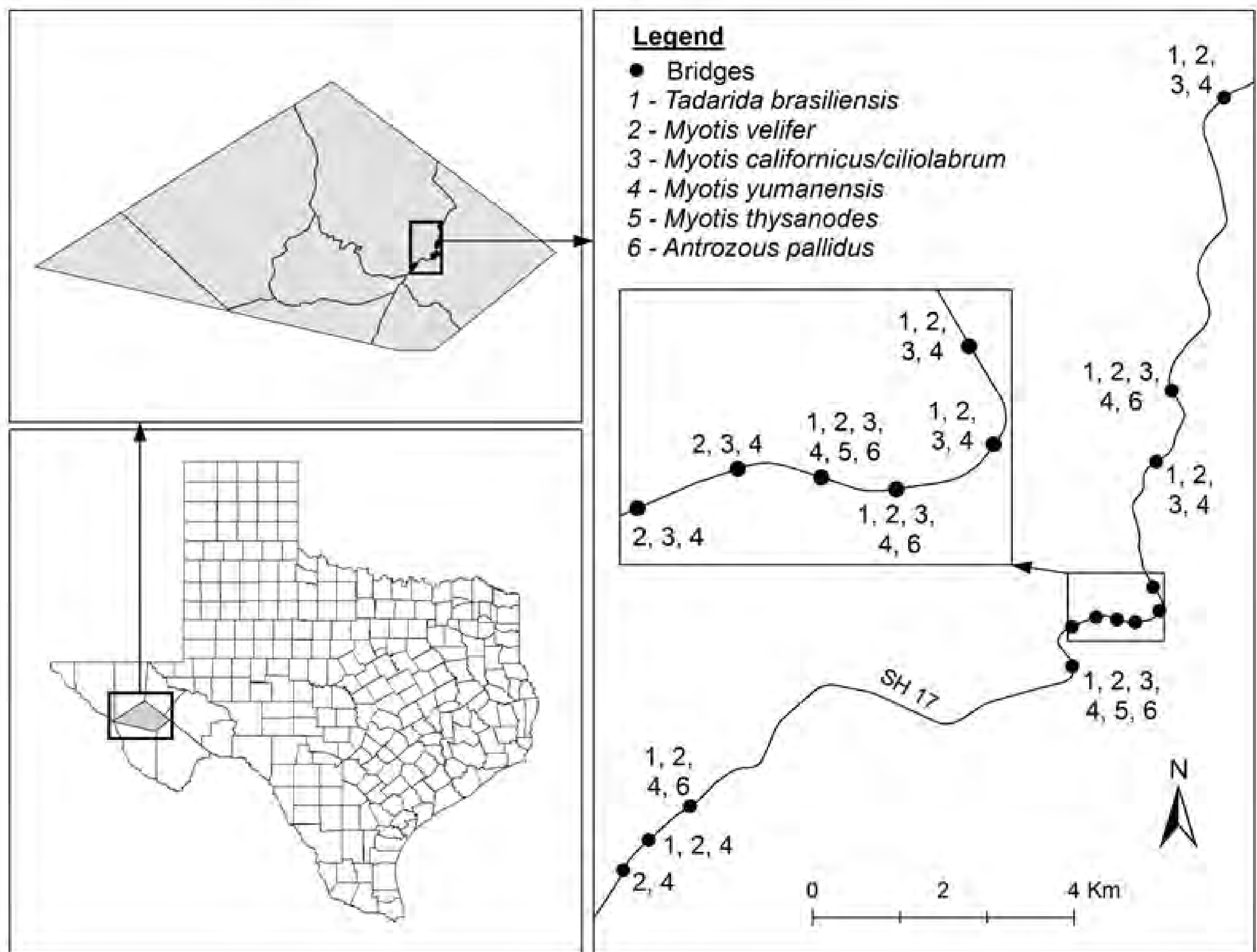

Figure 1. Thirteen bridges surveyed along 15 km of Texas SH 17 to examine seasonality of day-roost use by bats in Jeff Davis County, Texas. Black lines are highways. Black dots are bridges. Numbers to the side of black dots correspond to bat species found roosting in each bridge.

ciliolabrum as roosting in highway infrastructure, but *M. californicus* was reported using a bridge in central New Mexico (Geluso and Mink 2009).

Statistical analysis. We examined seasonality for the most common taxa (*T. brasiliensis*, *M. velifer*, *M. californicus/ciliolabrum*, and *M. yumanensis*) based on Generalized Linear Models (Hoffmann 2004). We used a negative binomial model with a log link function to evaluate effects of bridge, month, and month^2 on number of individuals (or abundance class for *T. brasiliensis*). The bridge factor was included to account for non-independence of sampling the same bridges month after month. Month and month^2 terms were used to evaluate if there was a quadratic (hump-shaped) relationship between month and number of bats that would indicate seasonality. In some situations, correlations between bridges (measured across months) were so high that the Hessian matrix was singular. In these cases, we removed one or more bridges causing singularity.

Results

Day-roosting bats were observed at all 13 bridges examined in the Trans-Pecos region of western Texas. Descending rank order for number of observations of bat species were *T. brasiliensis* (n > 20,000), *M. velifer* (n = 1,112), *M. californicus/ciliolabrum* (n = 147), *M. yumanensis* (n = 100), *Antrozous pallidus* (n = 37), and *M. thysanodes* (n = 3). All species with sufficient sample sizes for analysis (*T. brasiliensis*, *M. velifer*, *M. californicus/ciliolaburm*, and *M. yumanensis*) exhibited significant differences among bridges (Table 1)

Table 1. Results of Generalized Linear Models evaluating effects of bridge and month on numbers of bats per bridge along Texas SH 17. A significant bridge effect suggests bats are selecting particular bridges as day-roosts. A significant quadratic month term (*i. e.*, month^2) indicates seasonality of bridge use by bats.

Species	Independent Variable	Wald Chi^2	df	P-Value
Tadarida brasiliensis	Bridge	45.96	12	<0.001
	Month	0.25	1	0.616
	Month^2	0.19	1	0.667
Myotis velifer	Bridge	50.41	11	<0.001
	Month	105.71	1	<0.001
	Month^2	104.76	1	<0.001
M. c./ciliolabrum	Bridge	26.57	8	0.001
	Month	22.58	1	<0.001
	Month^2	23.58	1	<0.001
Myotis yumanensis	Bridge	23.48	10	0.009
	Month	21.78	1	<0.001
	Month^2	22.99	1	<0.001

suggesting that some bridges contained structural features or were located in areas more conducive for bats as day-roosts. Significant seasonality was exhibited by *M. velifer*, *M. californicus/ciliolabrum* and *M. yumanensis* but not for *T. brasiliensis* (Table 1) whereby bats used bridges at higher frequencies during warmer months (Figure 2).

Tadarida brasiliensis. Brazilian free-tailed bats roosted in bridges on Texas SH 17 in the greatest numbers. These bats colonized the northern four and one extreme southern bridges in large numbers, often >5,000 individuals per bridge. These northern and southern sites were separated by eight bridges and approximately 10 km. It is likely that these represent at least two discrete colonies, one on the north side and one on the south side. *Tadarida brasiliensis* was encountered in small numbers (<10) in five other bridges, whereas three bridges were not occupied at any time. In the five bridges that were lightly occupied, when we encountered *T. brasiliensis,* individuals were roosting singly or in small groups of two to three. *Tadarida brasiliensis* used bridges in high numbers in all months and did not demonstrate seasonality of bridge use (Table; Figure 2).

Myotis velifer. The cave myotis was the second most common bat encountered in bridges. This species was observed using every bridge at least twice during our 12-month observation period. *Myotis velifer* often roosted singly or doubly but was also occasionally found roosting in groups of 15 to 20 individuals. *Myotis velifer* roosted across the entire range of widths between expansion joints. This species often roosted alongside individuals of or even within large groups of *T. brasiliensis*. Because of its high abundance relative to other species of *Myotis*, *M. velifer* exhibited conspicuous seasonality (Table 1; Figure 2), being completely absent from bridges from December to February and represented by less than 15 individuals across all 13 bridges during the months of October, November and March.

Myotis californicus/ciliolabrum. This species complex was common and was encountered in eight bridges more than once, two bridges only once, and never in the three that were to the south near Fort Davis. Individuals almost always roosted singly in bridges. Often individuals roosted within one meter of another member of the complex, and when this occurred it was typically an individual of the opposite sex. Individuals almost exclusively roosted in the narrowest expansion grooves, just wide enough for the bats to fit. This species complex exhibited significant seasonality (Table 1; Figure 2). Due to its lower abundance, differences among months were less pronounced. During the months of December through February, individuals never used bridges as roosts. The greatest number of observations were in the months of May (n = 44), June (n = 25), and July (n = 37).

Myotis yumanensis. The Yuma myotis was encountered across all 13 bridges, but in low numbers. This species often roosted singly where the width between expansion joints was small and just large enough for them to fit. It was not encountered in bridges during the months of November through February.

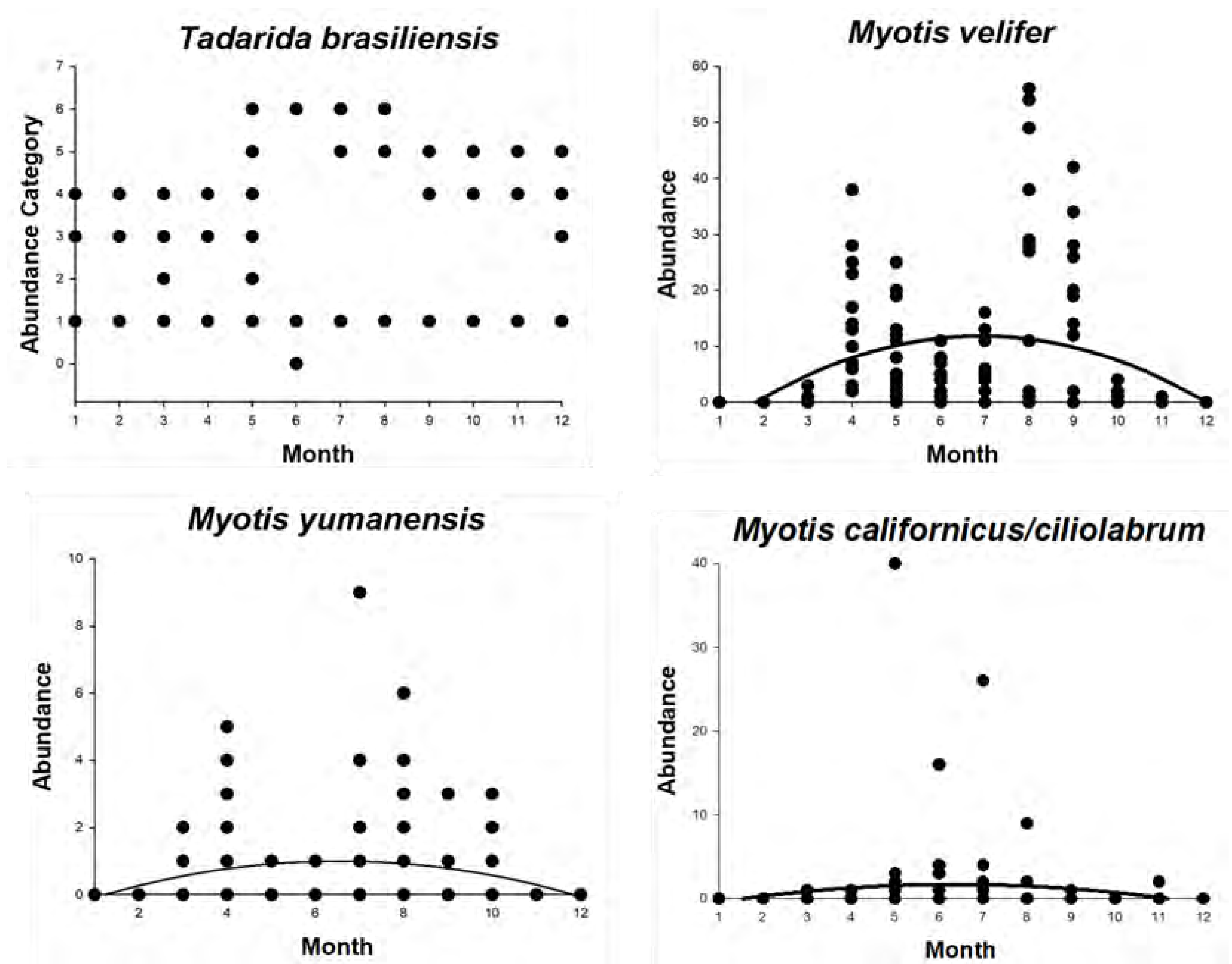

Figure 2. Abundance of bats per month measured across 13 bridges along 15 km of Texas SH 17 in Jeff Davis County.

Myotis thysanodes and *Antrozous pallidus*. These two species, while using bridges as day-roosts, were observed only rarely. *Myotis thysanodes* roosted singly the three times we observed it and was observed only in spring and fall during the months of May, September and October. *Antrozous pallidus* roosted singly and in groups. We encountered a maternity colony of 10 individuals of mixed ages (*i. e.*, juveniles and adults) in late May and June, but this species also used bridges as day-roosts during the months of March, September and October. *Antrozous pallidus* always roosted in wide expansion joints that were closest to the exterior edge of the bridge.

Discussion

We documented bridges used as day-roosts by six bat species in the Trans-Pecos region of Texas. Substantive variation among bridges, as well as months, existed regarding numbers of bats roosting in bridges. Only *T. brasiliensis* used bridges as day-roosts across all seasons. No other taxa roosted in bridges from December to February, the coldest months in the Trans-Pecos. Significant differences among bridges regarding number of bats roosting in them suggest that bats were selecting particular bridge attributes such as length or height off the ground or perhaps something within the habitat where the bridge occurs.

Despite the high species richness of bats in the Trans-Pecos, a limited number of species appear to inhabit bridges. However, those that do, do so with regularity. *Corynorhinus townsendii*, *Eptesicus fuscus*, *Lasionycteris noctivagans*, *M. volans*, *Parastrellus hesperus*, and *Nyctinomops macrotis* were all documented by Keely and Tuttle (1999) to occupy highway infrastructure and their geographic distributions overlap Jeff Davis County. However, we did not observe these species during our survey. Keely and Tuttle (1999) did not distinguish between bridge-use and culvert-use, so one possibility is that those species that went undetected by us roost in culverts and not bridges. Another possible explanation for absence of these taxa is differences in spatial extent between our and the Keeley and Tuttle (1999) studies. Keeley and Tuttle (1999) examined bridges and

culverts across 25 different states, whereas we examined bridges within a single county. Most of the species listed in Keeley and Tuttle (1999) are relatively rare and are rarely encountered in our survey area (DeBaca 2008). Moreover, *P. hesperus*, *N. macrotis*, and *C. townsendii* are lowland species (DeBaca 2008) and may not occur in large numbers at the relatively high elevations (1550 m) around Fort Davis. *Eptesicus fuscus*, *L. noctivagans*, and *M, volans* are higher elevation species and would be expected to inhabit this region. In fact, *E. fuscus* and *M. volans* were two of the most commonly mist-netted bats in the Davis Mountains (DeBaca 2008), approximately 30 km from our survey area. Other possible explanations for why we did not encounter these species under bridges are that species are geographically variable in their tendencies to use highway infrastructure as day-roosts and they choose not to do so in this region, or these bridges did not possess characteristics that promote day-roosting by these species.

Of the bats we did encounter under bridges, rank abundances were consistent with other places in the Trans-Pecos where bats were mist-netted to determine species composition. For example, rank abundance of *T. brasiliensis* > *M. velifer* > *M. californicus/ciliolabrum* > *M. yumanensis* > *M. thysanodes* is similar to that at Davis Mountains State Park (DeBaca 2008) and Big Bend Ranch State Park (Yancey 1997). Such agreement suggests that these bats are roosting in bridges at frequencies similar to their abundances in the region.

A notable observation was the year-round use of bridges as roosts by *T. brasiliensis* and their consistently high roosting frequency. *Tadarida brasiliensis mexicana* traditionally has been thought to migrate out of the Trans-Pecos, and much of Texas, during winter (Schmidly 1977). A number of scattered records suggest that a small number of individuals remain in Texas to overwinter (Spenrath and LaVal 1974; Tuttle 2003; Keeley and Keeley 2004; Scales and Wilkins 2007; Weaver *et al.* 2015). A similar observation has been made in neighboring New Mexico (Geluso and Mink 2009). In 2018, Kasper and Yancey reported year-round roosting in large numbers in a single bridge in Big Bend Ranch State Park. Considering our results and these other studies, *T. b. mexicana* may overwinter in the Trans-Pecos at much higher frequency than originally thought. Moreover, global climate change is making winters milder (Sherwin *et al.* 2012), which may be contributing to *T. b. mexicana* overwintering in the Trans-Pecos. Migration has an associated risk (Fleming and Eby 2003; Popa-Lisseanu and Voigt 2009), and in a warming climate risk may outweigh the benefits of migration. Climate change may be contributing to an increase in *T. b. mexicana* populations in Texas during winter (Kasper and Yancey 2018; Weaver *et al.* 2015).

Seasonality of use of highway infrastructure by bats also has methodological implications. Bats may not use highway infrastructure as roosts during all seasons and surveys should be conducted during seasons when bats actively use these structures. Bats exhibit species-specific seasonality of use of bridges as roosting sites as witnessed here by comparing *T. brasiliensis* to the other species examined. Other species of bats, especially those found in the southeastern portion of the United States including *P. subflavus*, *M. austroriparius*, and *C. rafinesquii*, may use highway infrastructure with greater frequency in winter (Rice 1957; Stevens *et al.* 2017; Lutsch 2019; Meierhofer *et al.* 2019) than summer. A dynamic interplay exists regarding use of anthropogenic structures as roosting sites by bats and this is likely dictated by species-specific, season-specific, and habitat-specific influences that are deserving of future study.

Acknowledgments

We dedicate this publication to Dr. David J. Schmidly. Dr. Schmidly has been *the* leader of Texas Mammalogy for decades. His many contributions, either from his own research or through facilitation of that of others through administration, collaboration, or coordination with state and federal agencies will be highly regarded for decades to come. We thank Heidi Amarilla-Stevens for translating the abstract to Spanish.

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An overview of the mammals of the Gila region, New Mexico

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A study of the mammals of the Gila region of New Mexico was conducted from 2012 through 2020, with 2,919 voucher specimens collected through fieldwork and collaborations with commercial trappers, in addition to data from camera traps, review of major holdings at 46 museums (*n* = 12,505 georeferenced specimens), and literature review. Specimens cover a 170-year span, dating back to 1850 and were unevenly distributed spatially and temporally across the Gila region. Most areas were very poorly represented and when summed across all mammal species, ranged from 0.02 to 3.7 specimens per km^2. The survey documented 108 species (104 now extant) for the region. High species richness, greater than that reported for 38 states in the United States, is likely due to the juxtaposition of multiple biomes in the Gila, including the Sonoran, Chihuahuan, and Great Basin deserts, the Rocky Mountains and Sierra Madre Occidental, and nearby "sky islands" of the Southwest. Two species, *Leptonycteris yerbabuenae* and *Zapus luteus,* are documented for the first time from the study area. Expansions of the known range of these species, and *Sciurus arizonensis* are described from specimen and camera data. Preliminary phylogeographic studies of four species (*Notiosorex crawfordi, Neotoma albigula, Perognathus flavus,* and *Thomomys bottae*) using the mitochondrial cytochrome-*b* gene reveal the dynamic biogeographic history of the region and reinforce how landscape complexity and climate change have jointly contributed to diversification and thus high mammalian diversity in the region.

Se condujo estudio de los mamíferos de la región Gila en Nuevo México desde 2012 hasta 2020, con 2,919 vouchers de especímenes recolectados a través de trabajo de campo y colaboraciones con cazadores comerciales, además de datos de trampas cámara, revisión de las principales colecciones en museos (*n* = 12,505 especímenes georeferenciadas) y revisión de literatura. Los especímenes cubren un lapso de 170 años, se remontan a 1850 y se distribuyeron de manera desigual en la región de Gila. La mayoría de las áreas estaban muy mal representadas, y sobre todo las especies oscilando entre 0.02 a 3.7 especímenes por km^2. En este estudio se documentaron 108 especies (104 existentes ahora) a la región. Alta riqueza de especies, más que la diversidad reportada para 38 estados en los Estados Unidos, se debe probablemente a la yuxtaposición de múltiples biomas en la región Gila, incluido los desiertos de Sonora, Chihuahua, y la Gran Cuenca, las Montañas Rocosas y la Sierra Madre Occidental, y las cercanas islas del cielo ("sky islands") del suroeste Estados Unidos. Dos especies, *Leptonycteris yerbabuenae* y *Zapus luteus*, se documentaron por primera vez en el área de estudio. Las expansiones de área de estas especies y *Sciurus arizonensis* se describen a partir de especímenes colectados y de cámaras trampa. Los estudios filogeográficos de cuatro especies (*Notiosorex crawfordi*, *Neotoma albigula*, *Perognathus flavus* y *Thomomys bottae*) utilizando el gen mitocondrial citocromo-*b* revelan la historia biogeográfica dinámica de la región y refuerzan cómo la complejidad del paisaje y el cambio climático han contribuido a la alta diversidad de mamíferos en la región.

Keywords: biodiversity; conservation; distribution; Mammalia; Southwest; taxonomy.

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Introduction

Together with adjacent southeastern Arizona, the Gila Region of southwestern New Mexico (Figure 1) supports high biotic diversity, likely due to a dynamic geologic history, topographic complexity, considerable elevational relief, and the confluence of multiple, distinctive biomes. This diversity and an abundance of archaeological and paleontological sites have long generated interest in the fauna and flora of the region, including a series of mammalogists (*e. g.*, Jones 1965; Hayward and Hunt 1972; Frey *et al.* 2008; Geluso and Geluso 2020). In a seminal paper on patterns of mammalian diversity across North America, Simpson (1945) noted that this region supported among the highest species richness north of the Mexican border (Figure 2). As a young forest ranger, Aldo Leopold spent his formative years in the region, gaining valuable insights in wildlife ecology and later proposing and pushing through Congress the creation of the renowned Gila Wilderness of New Mexico. This was the first federally designated wilderness in the United States (designated in June 1924) and, together with the adjoining Aldo Leopold Wilderness to the east (designated in 1980), these untrammeled wilderness areas encompass more than 309,000 ha and provide valuable habitat for wild mammals.

In recent decades, the Gila Region has been the focus of conservation efforts and controversy for several imperiled taxa (*e. g.*, Hibbitts *et al.* 2009; National Academies of Sciences, Engineering and Medicine 2019; Propst *et al.* 2020): the Mexican spotted owl (*Strix occidentalis lucida*), Mexican gray wolf (*Canis lupus baileyi),* Chiricahua leopard frog (*Lithobates chiricahuensis*), Mexican garter snake (*Thamnophis eques*), and a series of threatened fishes (Gila trout,

Oncorhynchus gilae, headwater chub, *Gila nigra,* loach minnow, *Tiaroga cobitis,* and spikedace, *Meda fulgida*). Despite this long history tied to conservation and the wealth of interest in the Gila, relatively few specimen-based biotic surveys (with the notable exception of fishes) have been conducted and no comprehensive review of the mammals of the region has been completed.

Herein we provide the first overview of the mammals of the Gila Region based on a synthesis of historical information (*e. g*., museum specimens), updated taxonomy and distributional data, the addition of 2,919 new specimens, and observational camera trap data for the region.

Materials and Methods

Study area. The Gila Project Area (hereafter the Gila), as defined in this study, encompasses 24,383 km^2 in Catron, Grant, and Sierra counties of New Mexico (Figure 1). The western boundary is the border of New Mexico and Arizona, and the eastern border is the Rio Grande. The northern boundary is the southern end of the Plains of San Agustin, but extends north into the higher elevations of the Mangas, Escondido, and Gallo mountains and Jones Peak. The Gila is roughly bounded by the Deming Plains in the South. The region includes both public (primarily the Gila National Forest with 1,335,462 ha, Bureau of Land Management with 1,042,386 ha, and New Mexico State Land Office with 676,864 ha) and private lands (*e. g*., small communities or ranches throughout the region).

Physiography and habitat. The Gila is located near the confluence of multiple major biomes, including three regional deserts: the northeastern Sonoran Desert, the northwestern Chihuahuan Desert, and the southern edge of the Great Basin Desert (Riddle and Hafner 2006). The Gila straddles the Continental Divide, which extends from the southern Rocky Mountains to the northern Sierra Madre Oriental. Regional desertification resulted from uplift of the Continental Divide during the Pliocene-Pleistocene (~2 Ma; Wilson and Pitts 2010). As the eastern extension of the Mogollon Rim (Mogollon Plateau) of Arizona, the Gila is situated at the southern boundary of the Colorado Plateau and Basin and Range provinces (Julyan 2006), and is characterized by extensive topographic complexity that is compounded by relatively recent volcanism (Eocene, ca. 40 Ma). Regional basin and range block faulting and volcanism also resulted in "sky islands," isolated mountains surrounded by radically different lowland environments. Elevation

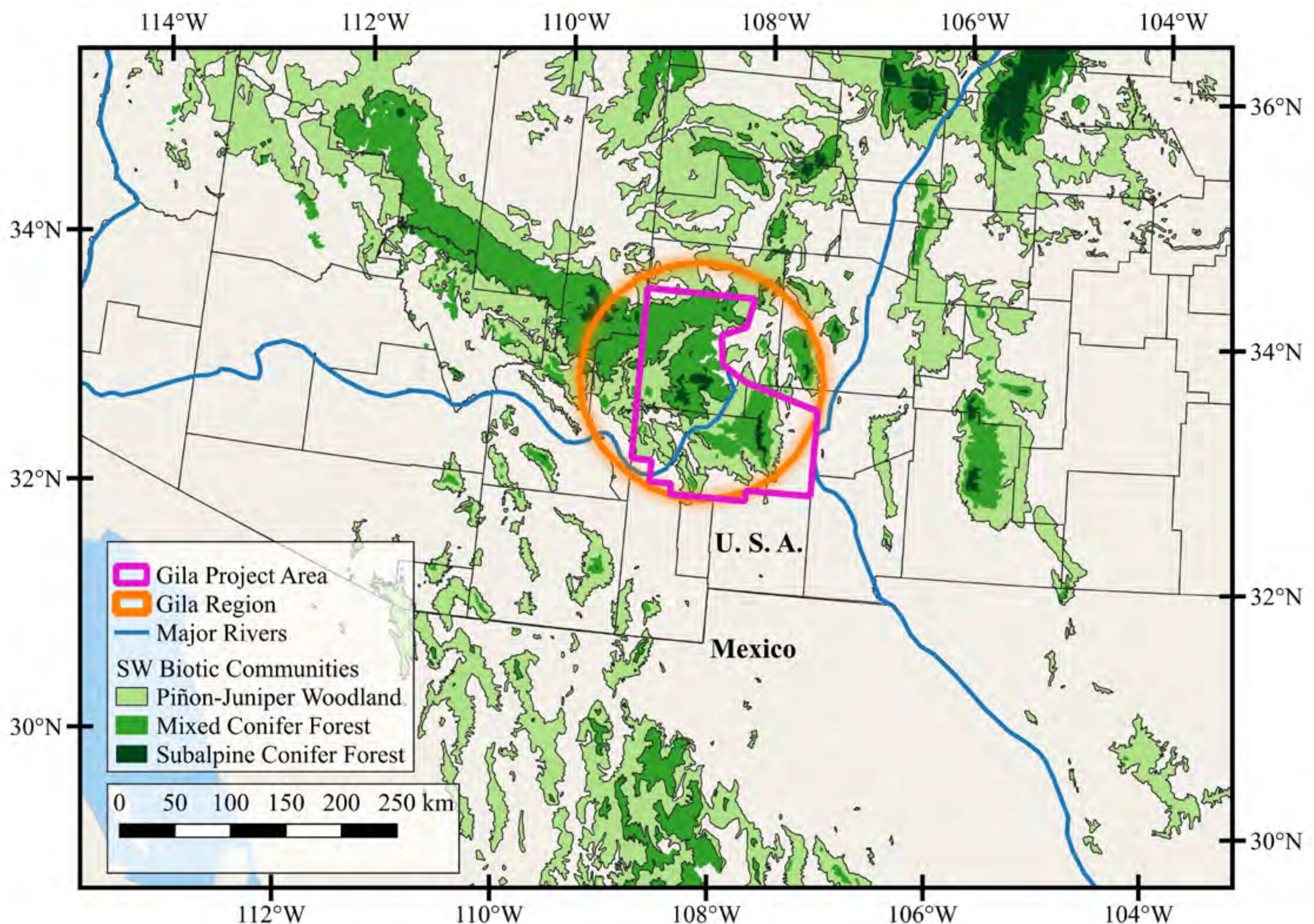


Figure 1. Location of the Gila Project Area and distribution of woodland and forest montane communities within the Sky Islands of the Southwest.

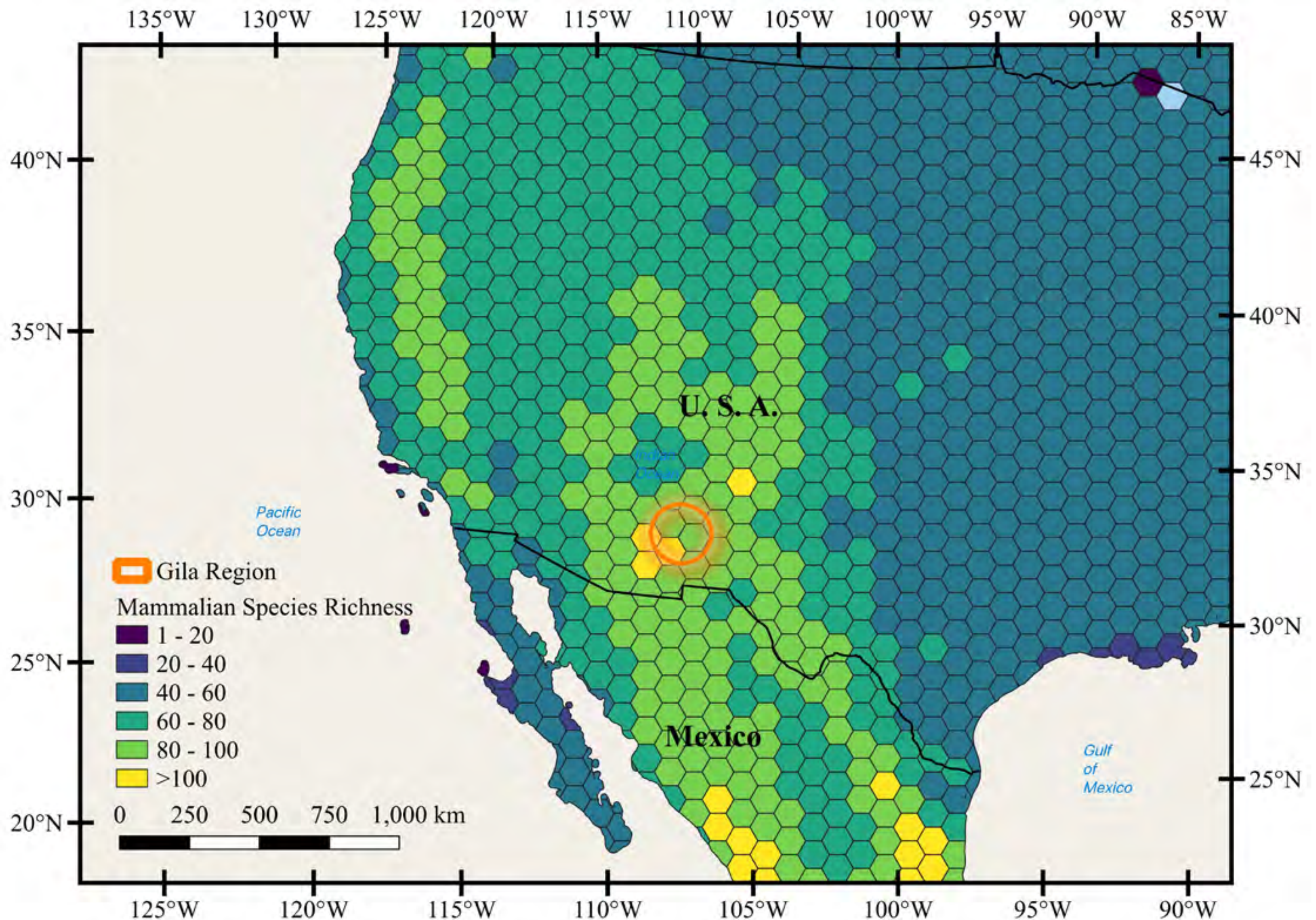


Figure 2. Mammalian species richness across the southern borderlands of western North America indicates that the Gila is among the most diverse terrestrial mammal regions on the continent. Each pixel represents an area of 100 km^2 and the orange circle denotes the location of the Gila.

within the Gila ranges from 1,008 m where the San Francisco and Gila rivers meet to 3,322 m at Whitewater Baldy in the Mogollon Mountains. This substantial elevational relief and topographic complexity supports high habitat diversity and associated high mammalian diversity.

This generally arid region is transected by an array of river drainages and riparian systems. Most of the area is drained by the Gila River and numerous tributaries including the San Francisco River, whereas other creeks drain the area east of the Continental Divide into the Río Grande watershed. The Mimbres River drains the southcentral Gila into an endorheic basin on the Deming Plain (formerly Pleistocene Lake Animas). Vegetation in the Gila ranges from desert grassland at the lowest elevations, to desert scrubland, piñon-juniper woodland, ponderosa pine forest, and mixed coniferous forest and montane grassland at increasingly higher elevations (Dick-Peddie *et al.* 1993).

Collection survey. Four museum collections containing either the largest or most important historical series of mammal specimens for the Gila were visited, including the Museum of Southwestern Biology, University of New Mexico (MSB; *n* = 7,255), Western New Mexico University (WNMU; *n* = 2,474), the National Museum of Natural History (USNM; *n* = 310), and the American Museum of Natural History (AMNH; *n* = 262). These museums hold important historical specimens from the Gila, including species that were extirpated from the Gila, rarely occur in the Gila, or that may have expanded their distribution into the region. In order to identify all other records for the region, we performed a Global Biodiversity Information Facility (GBIF) search using the following parameters: mammal, from Catron, Grant, and Sierra Counties. In addition to the holdings from the four primary collections, specimen records from 42 other museums were downloaded (16,278 total; https://doi.org/10.15468/dl.vddbz5; accessed 20 November 2020). After culling county records which fell outside the delineated Gila region, a total of 12,505 georeferenced specimen records remained for further analyses (Figure 3). Of these, 3,773 specimen records were newly georeferenced using GeoLocate Web Application (https://www.geo-locate.org/).

Specimen collection. Field work (48 expeditions plus ancillary salvaged material from a total of 194 localities; Figure 4) was conducted from October 2012 to August 2020 throughout the region, with emphasis on the rela-

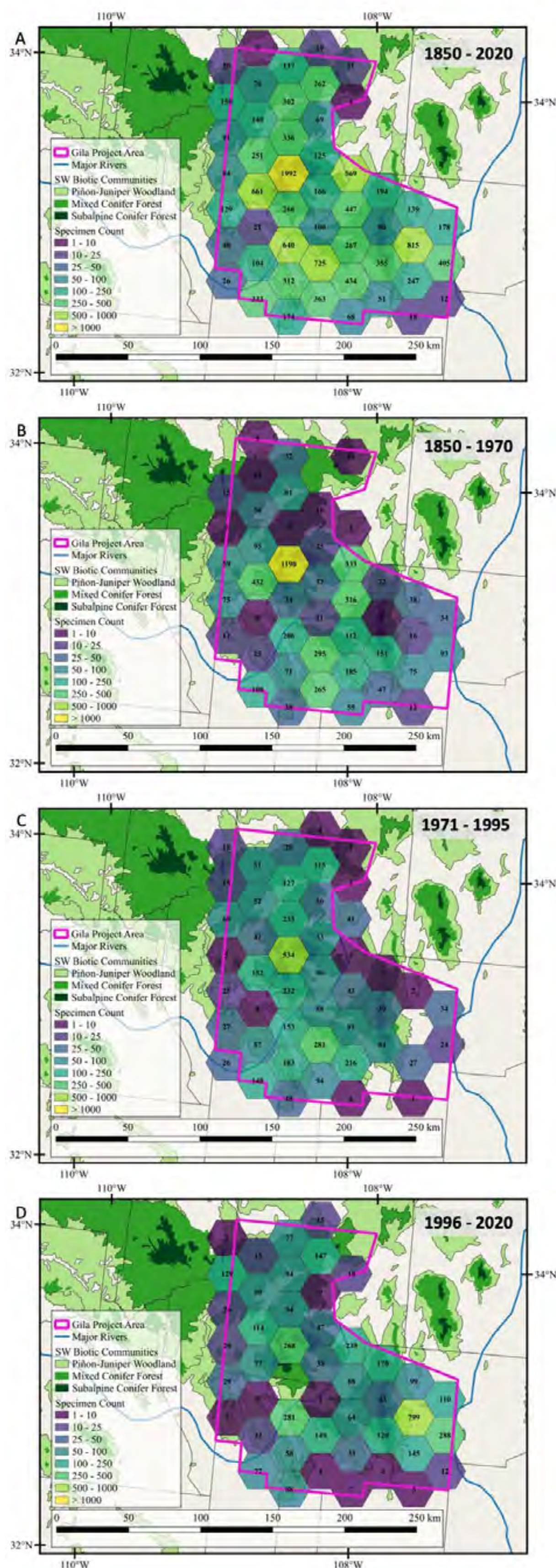


Figure 3. Temporal and spatial distribution of mammal specimens preserved from the Gila (1850-2020). These are compiled across all years (3a), but also broken into three time periods (3b, 1850-1970; 3c, 1971-1995; 3d, 1996-2020) to examine specimen density across time and especially in relation to the most recent period of accelerating environmental change. Hexagons=541 km^2.

tively poorly documented eastern extent of the Gila in Sierra County, which includes the 636 km^2 Ladder Ranch. This ranch has focused on privately-funded habitat and threatened species restoration efforts. Specimens were collected under a scientific collecting permit from the New Mexico Department of Game and Fish issued to Joseph Cook (NMDGF Cook #3300), using standard trapping methods approved by the American Society of Mammalogists (Sikes *et al.* 2016) and the Institutional Animal Care and Use Committees at the University of New Mexico. Collecting efforts focused on producing holistic specimens (Cook *et al.* 2016; Schindel and Cook 2018) of rodents, bats, shrews, small carnivores, and their associated parasites from multiple sites representative of all major habitats in the Gila. Trapping was conducted primarily with Sherman© live traps and museum special snap traps, augmented by Tomahawk© live traps for small- to medium-sized mammals, Macabee© traps for pocket gophers, and pitfall traps for shrews. Small mammal surveys were conducted by MSB field crews, some of which included UNM mammalogy field classes. Trap lines typically consisted of 50 live traps and 50 snap traps, with up to a total of 600 traps per night. Bats were collected with mist nets over streams, ponds, and stock tanks (Kunz and Parsons 2009), including sporadic netting during winter months, particularly the "buffer" months of November and March (Geluso 2007). GPS locations were recorded for all specimens along with standard voucher information. Specimens were preserved as either skin-plus-skeleton (Hafner *et al.* 1984) or fluid-preserved (95% ethanol) along with multiple ultra frozen tissues (typically heart, lung, liver, kidney, spleen, and muscle) and ecto- and endoparasites (either preserved in 70 % EtOH or frozen in liquid nitrogen; Yates *et al.* 1996; Galbreath *et al.* 2019). All specimens were deposited in the collections of the Museum of Southwestern Biology (MSB) and available on the Arctos database (https://arctos.database.museum) along with historical records from MSB and WNMU.

Physical records of larger mammals were obtained through salvaged specimens archived at the MSB by the New Mexico Department of Game & Fish, U. S. Department of Agriculture Wildlife Services, and local commercial fur trappers. Photographic data on medium and large mammals were collected from a grid of camera traps situated on the southern portion of the Ladder Ranch near Animas Creek (Figure 5) from April 2008 to 30 December 2019 for a total of 86,061 camera nights. The Ladder Ranch Headquarters is 14 km NNE of Hillsboro. Initial surveys (2008 to 2009) included 16 cameras (64 km^2 coverage); an additional nine cameras (44 km^2 coverage) were added in 2010, for a total of 25 cameras and approximately 100 km^2 of coverage. Additional records (*e. g.*, annual harvests) and distributional information were obtained from the website of the New Mexico Department of Game and Fish (NMDGF; https://www.wildlife.state.nm.us/hunting/harvest-reporting-information).

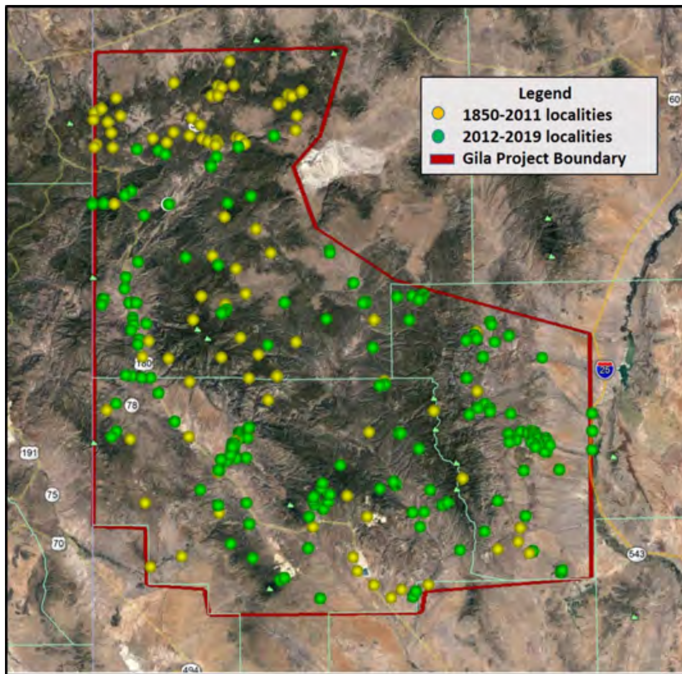
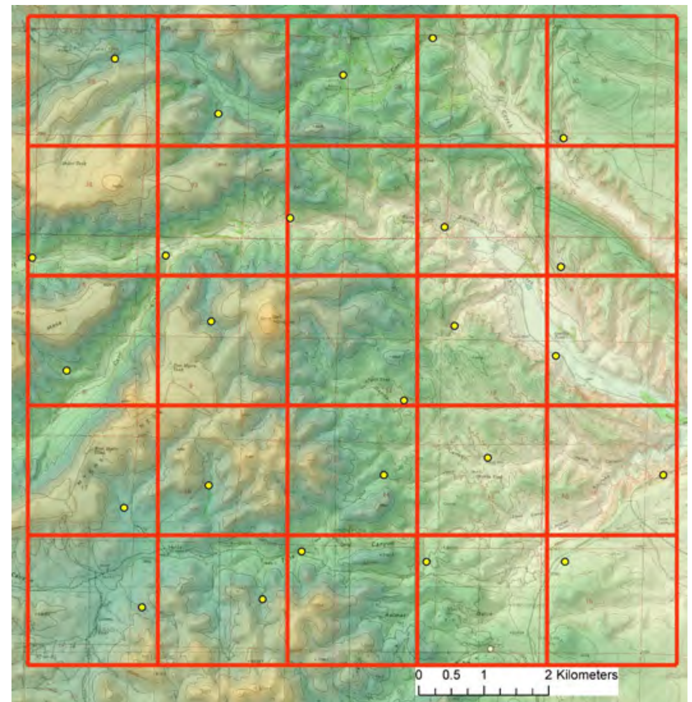


Figure 4. Sampling localities in the Gila. Yellow dots represent historical mammal collecting localities prior to our fieldwork (1851-2011) and green dots are collecting localities sampled during this study (2012-2020).

Over the last seven decades, a series of graduate studies that included Gila mammals were conducted, primarily at the University of New Mexico and New Mexico State University. We avoided unpublished reports on Gila mammals, particularly those that failed to produce data validated by specimens, due to the citation requirements of Therya. We acknowledge that there have been multiple bat, rodent, carnivore, or ungulate focused studies in the Gila that have produced non-peer reviewed reports and encourage their eventual publication. The one exception is that we include the specimen-based field studies of Bruce Hayward, who is now deceased. In particular, Hayward and Hunt (1972) summarizes a survey of remote sites in the Gila Wilderness (specimens deposited at WNMU) conducted in 1972.

Phylogeographic analyses. We compared sequences of the mitochondrial (mtDNA) cytochrome-*b* gene (*cytb*) for four select species to begin to place specimens from the Gila into phylogeographic context and improve insights about the evolutionary history of these species. The species studied include representatives from the Gila, surrounding areas, and appropriate outgroups. For packrats, specimens include: *Neotoma albigula* (n = 10 individuals from three localities in the Gila), Arizona (n = 7), and Chihuahua (n=1), *N. leucodon* (n=3), *N. micropus* (n = 2) including one from the Gila), *N. stephensi* (n = 4 including one from the Gila), and *N. mexicana* (n = 4). Outgroups used to root this tree were *Hodomys alleni* and *Neotoma cinerea*. For pocket gophers, these include *Thomomys bottae* (n = 11 individuals from five localities in the Gila), elsewhere in New Mexico (n = 7), Arizona (n= 4), and Texas (n =4). Outgroups used to root this tree was *Thomomys talpoides*. For desert shrews, specimens include *Notiosorex crawfordi* (n = 5 individuals



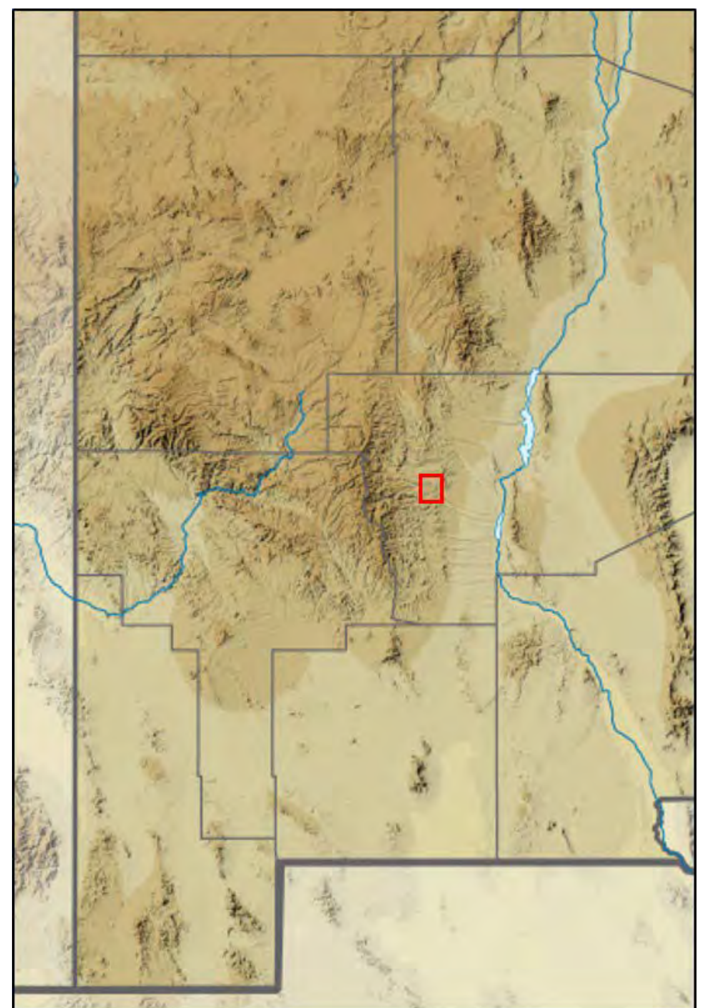


Figure 5. Camera-trap localities (n = 25) established across 100 km² (10 X 10 km grid) on the Ladder Ranch (2008-2012). Red outline indicates Ladder Ranch location within New Mexico. The New Mexico map is modified from original (USA New Mexico relief location map.svg).

from four localities in the Gila), Arizona (n = 1), and Texas (n = 6). N*otiosorex cockrumi*, *N. tatticuli* and *Sorex cinereus* were used to root the tree. For pocket mice, *Perognathus flavus* (n = 17 individuals from 15 localities), with *P. flavescens* as the outgroup. Lab procedures followed standard salt extraction methods (Fleming and Cook 2002), and amplification and Sanger sequencing methods for the entire *cytb* gene (1,140 base pairs; Hope *et al.* 2010), using the MSB05/MSB14 primer set. Representative *cytb* sequences were downloaded from GenBank (https://www.ncbi.nlm.nih.gov/genbank/) to assess phylogeographic placement of Gila specimens (Appendix 1). To graphically examine geographic variation, phylogenetic trees were inferred under a Bayesian framework with Mrbayes 3.2.6 (Ronquist and Huelsenbeck 2003). A mixed model was used to sample all possible model space with rates set to a gamma distribution run for 1,000,000 generations sampling every 1,000 with two runs and four chains. Convergence of runs was determined by verifying that the standard deviation of split frequencies was below 0.01 after which trees were summarized to produce a 50 % consensus tree following a 25 % burn-in and then manually rooted with the outgroup in FigTree 1.4.4 (Rambaut 2014).

Conservation status. We assess conservation status by compiling risk assessments across geographic scales, from global sources through the International Union for Conservation of Nature-IUCN (www.iucn.org) and Convention on International Trade on Endangered Species-CITES (https://www.cites.org), to national through the Endangered Species Act-ESA (https://www.fws.gov/endangered/laws-policies), and regional through the New Mexico Department of Game and Fish-NMDGF (https://www.wildlife.state.nm.us/conservation/wildlife-species-information/threatened-and-endangered-species) following MacDonald and Cook (2007). We then assess species identified as being imperiled based on distributions or relative abundances found during this survey.

Results

Faunal composition. We documented 108 native mammal species in the Gila (104 extant; Table 1). This diversity of species is documented by 12,505 specimens held in 46 museum collections that span the period 1851 to 2020. The majority of these specimens (7,312; 58 %) are archived at the MSB, including 2,919 specimens collected during this study (https://dx.doi.org/10.7299/X73B60G6). Rodents (74 %) and bats (20 %) make up most of these specimens, and most of the rodents are in the families Cricetidae (69 %), Sciuridae (13 %) or Heteromyidae (11 %; Figure 6). Medium (beaver-sized) and larger game mammals continue to be poorly represented in museum collections (323 specimens across 15 species), although many are harvested (*i. e.*, fur trapping, hunting) at high annual rates. Photographic data (Figure 7) from the camera-trap grid yielded data on medium and large mammals (Figure 8). Six species have been extirpated from the region including: *Cynomys gunnisoni* (last recorded by a museum voucher in 1972),

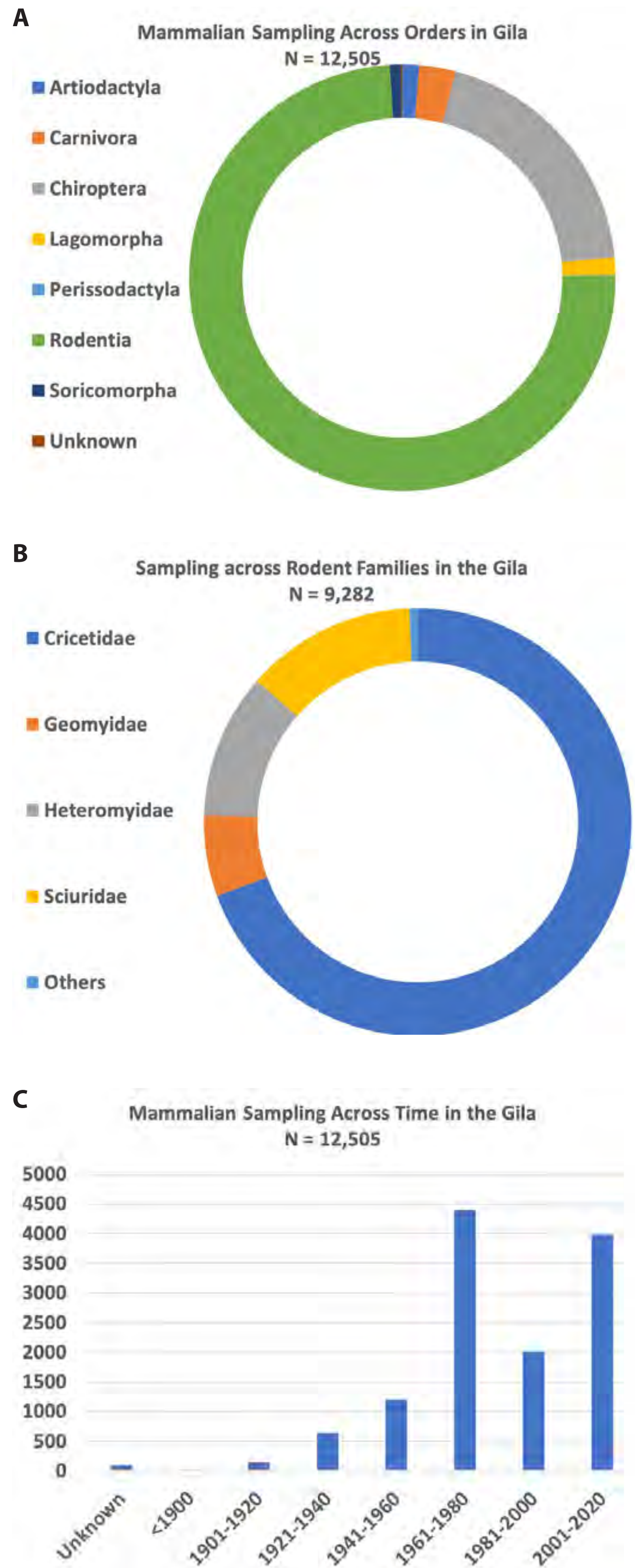


Figure 6. A) Concentration of taxonomic sampling (number of specimens) in the Gila across Mammalian orders. The vast majority of specimens represent the Order Rodentia and Order Chiroptera. B) Within the Order Rodentia, Cricetidae is the most commonly sampled family. C) Comparison of mammalian specimen acquisition across 20-year intervals shows that the periods 1961-1980 and 2001-2020 were the most intensively sampled.

Table 1. Mammal species of the Gila. Of 108 species, 104 are extant. Nine of the 108 were extirpated (†), but four of those have been reintroduced (*) and another non-native species has been introduced (**). Prior taxonomic names, revised since Wilson and Reeder (2005), are provided (in parentheses).

Order	Family	Species
Artiodactyla (6)		
	Tayassuidae (1)	
		Pecari tajacu (Linnaeus, 1758)
	Cervidae (3)	
		**Cervus canadensis* Shaw, 1804
		Odocoileus hemionus (Rafinesque, 1817)
		Odocoileus virginianus (Zimmerman,1780)
	Antilocapridae (1)	
		Antilocapra americana (Ord, 1815)
	Bovidae (1)	
		**Ovis canadensis* Shaw, 1804
Primates (1)		
	Hominidae (1)	
		Homo sapiens Linnaeus, 1758
Rodentia (51)		
	Sciuridae (12)	
		Ammospermophilus harrisii (Audubon & Bachman, 1854)
		Callospermophilus (*Spermophilus*) *lateralis* Say, 1823
		†*Cynomys gunnisoni* (Baird, 1855)
		**Cynomys ludovicianus* (Ord, 1815)
		Ictidomys (*Spermophilus*) *tridecemlineatus* (Erxleben,1777)
		Neotamias (*Tamias*) *cinereicollis* (J.A. Allen, 1890)
		Neotamias (Tamias) dorsalis (Baird, 1855)
		Otospermophilus (*Spermophilus*) *variegatus* (Erxleben, 1777)
		Sciurus aberti Woodhouse, 1852
		Sciurus arizonensis Coues, 1867
		Tamiasciurus fremonti (*hudsonicus*) (Audubon &Bachman, 1853)
		Xerospermophilus (*Spermophilus*) *spilosoma* (Bennett, 1833)
	Castoridae (1)	
		Castor canadensis Kuhl, 1820
	Heteromyidae (9)	
		Chaetodipus baileyi (Merriam, 1889)
		Chaetodipus hispidus (Baird, 1858)
		Chaetodipus intermedius (Merriam, 1889)
		Chaetodipus penicillatus (Woodhouse, 1852)
		Dipodomys merriami Mearns, 1890
		Dipodomys ordii Woodhouse, 1853
		Dipodomys spectabilis Merriam, 1890
		Perognathus apache (*flavescens*) Merriam, 1889
		Perognathus flavus Baird, 1855
	Geomyidae (1)	
		Thomomys bottae Eydoux & Gervais, 1836
	Dipodidae (1)	
		Zapus luteus (*hudsonius*) Miller, 1911
	Cricetidae (25)	
		Baiomys taylori (Thomas, 1887)
		†*Microtus drummondii* (*pennsylvanicus*) (Ord, 1815)
		Microtus longicaudus (Merriam, 1888)
		Microtus mogollonensis (*mexicanus*) (Mearns, 1890)
		Microtus montanus (Peale, 1848)
		Myodes gapperi (Vigors, 1830)
		Neotoma albigula Hartley, 1894
		Neotoma mexicana Baird, 1855
		Neotoma micropus Baird, 1855
		Neotoma stephensi Goldman, 1905
		Ondatra zibethicus (Linnaeus, 1776)
		Onychomys arenicola Mearns, 1896
		Onychomys leucogaster (Wied-Neuwied, 1841)
		Onychomys torridus (Coues, 1874)
		Peromyscus boylii (Baird, 1855)
		Peromyscus eremicus (Baird, 1857)
		Peromyscus gratus Merriam, 1898
		Peromyscus leucopus (Rafinesque, 1818)
		Peromyscus sonoriensis (*maniculatus*) (Wagner, 1845)
		Peromyscus nasutus (J.A. Allen 1891)
		Peromyscus truei (Schufedlt, 1885)
		Reithrodontomys megalotis (Baird, 1857)
		Sigmodon fulviventer J.A. Allen, 1889
		Sigmodon hispidus Say & Ord, 1825
		Sigmodon ochrognathus V. Bailey, 1902
	Muridae (1)	
		***Mus musculus* (Linnaeus, 1758)
	Erethizontidae (1)	
		Erethizon dorsatum (Linnaeus, 1758)
Lagomorpha (3)		
	Leporidae (3)	
		Lepus californicus Gray, 1837
		Sylvilagus audubonii (Baird,1857)
		Sylvilagus floridanus (J.A. Allen, 1890)
Eulipotyphla (Soricomorpha) (3)		
	Soricidae (3)	
		Notiosorex crawfordi (Coues, 1877)
		Sorex merriami (Dobson, 1890)
		Sorex monticola Merriam 1890
Chiroptera (23)		
	Phyllostomidae (1)	
		Leptonycteris yerbabuenae Miller, 1900
	Molossidae (2)	
		Nyctinomops macrotis (Gray, 1840)
		Tadarida brasiliensis (I. Geoffroy,1824)
	Vespertilionidae (20)	
		Aeorestes cinereus Palisot de Beauvois, 1796
		Antrozous pallidus (Le Conte, 1856)
		Corynorhinus townsendii (Cooper, 1837)
		Eptesicus fuscus (Beauvois, 1796)
		Euderma maculatum (J.A. Allen, 1891)
		Idionycteris phyllotis (G.M. Allen, 1891)
		Lasionycteris noctivagans (Le Conte, 1831)
		Lasiurus blossevillii (Lesson & Garnot, 1826)

Table 1. Continuation....

Order	Family	Species
		Lasiurus borealis (Müller, 1776)
		Myotis auriculus (Baker & Stains, 1955)
		Myotis californicus (Audubon & Bachman, 1842)
		Myotis carissima Thomas, 1904
		Myotis ciliolabrum (Merriam, 1886)
		Myotis evotis (H. Allen, 1864)
		Myotis occultus Hollister, 1909
		Myotis thysanodes Miller, 1897
		Myotis velifer (J.A. Allen, 1890)
		Myotis volans (H. Allen, 1866)
		Myotis yumanensis (H. Allen, 1864)
		Nycticeius humeralis (Rafinesque, 1818)
		Parastrellus (*Pipistrellus*) *hesperus* H. Allen, 1864
Carnivora (19)		
	Felidae (3)	
		Lynx rufus (Schreber, 1777)
		Puma concolor (Linnaeus, 1771)
		†*Panthera onca* (Linnaeus, 1758)
	Canidae (4)	
		Canis latrans Say, 1823
		**Canis lupus* Linnaeus, 1758
		Urocyon cinereoargenteus (Schreber, 1775)
		Vulpes macrotis Merriam, 1888
	Ursidae (2)	
		Ursus americanus Pallas, 1780
		†*Ursus arctos* Linnaeus, 1758
	Mustelidae (3)	
		†*Lontra canadensis* (Schreber, 1777)
		Mustela frenata Lichtenstein, 1831
		†*Mustela nigripes* (Audubon & Bachman, 1851)
		Taxidea taxus (Schreber, 1777)
	Mephitidae (4)	
		Conepatus leuconotus (Lichtenstein, 1832)
		Mephitis macroura Lichtenstein, 1832
		Mephitis mephitis (Schreber, 1776)
		Spilogale leucoparia (*gracilis*) Merriam, 1890
	Procyonidae (3)	
		Bassariscus astutus (Lichtenstein, 1830)
		Nasua narica (Linnaeus, 1766)
		Procyon lotor (Linnaeus, 1758)

Microtus drummondii (last voucher from 1915), *Panthera onca* (no voucher specimen), *Ursus arctos* (no voucher specimen), *Lontra canadensis* (last voucher from 1933), and *Mustela nigripes* (last voucher from 1915). Four other species were extirpated and subsequently reintroduced (*Cynomys ludovicianus,* last recorded by a museum voucher specimen in 1936, reintroduced in 1997; *Canis lupus baileyi,* last voucher from 1925, reintroduced in 1998; *Cervus canadensis,* last voucher from 1900, reintroduced 1910; and *Ovis canadensis*, no voucher, reintroduced 1964).

Figure 7. Photos from Ladder Ranch camera trap array documenting species occurrences as well as interactions among species. a) Grey fox (*Urocyon cinereoargenteus*) and spotted skunk (*Spilogale leucoparia*) were documented travelling together on many occasions; b) bobcat (*Lynx rufus*) carrying woodrat (*Neotoma* sp.) prey (other prey items documented included black-tailed jackrabbits, cottontail rabbits, kangaroo rats, rock squirrels; c) puma (*Puma concolor*) are one of the apex predators of the Gila.

Dates of extirpation based on museum vouchers represent a minimum estimate, as wild but uncollected populations may have persisted later. A single introduced species, *Mus musculus*, persists in the wild. We did not directly address several domesticated species that now have significant ecological roles in some ecosystems (*e. g*., cattle, *Bos taurus*).

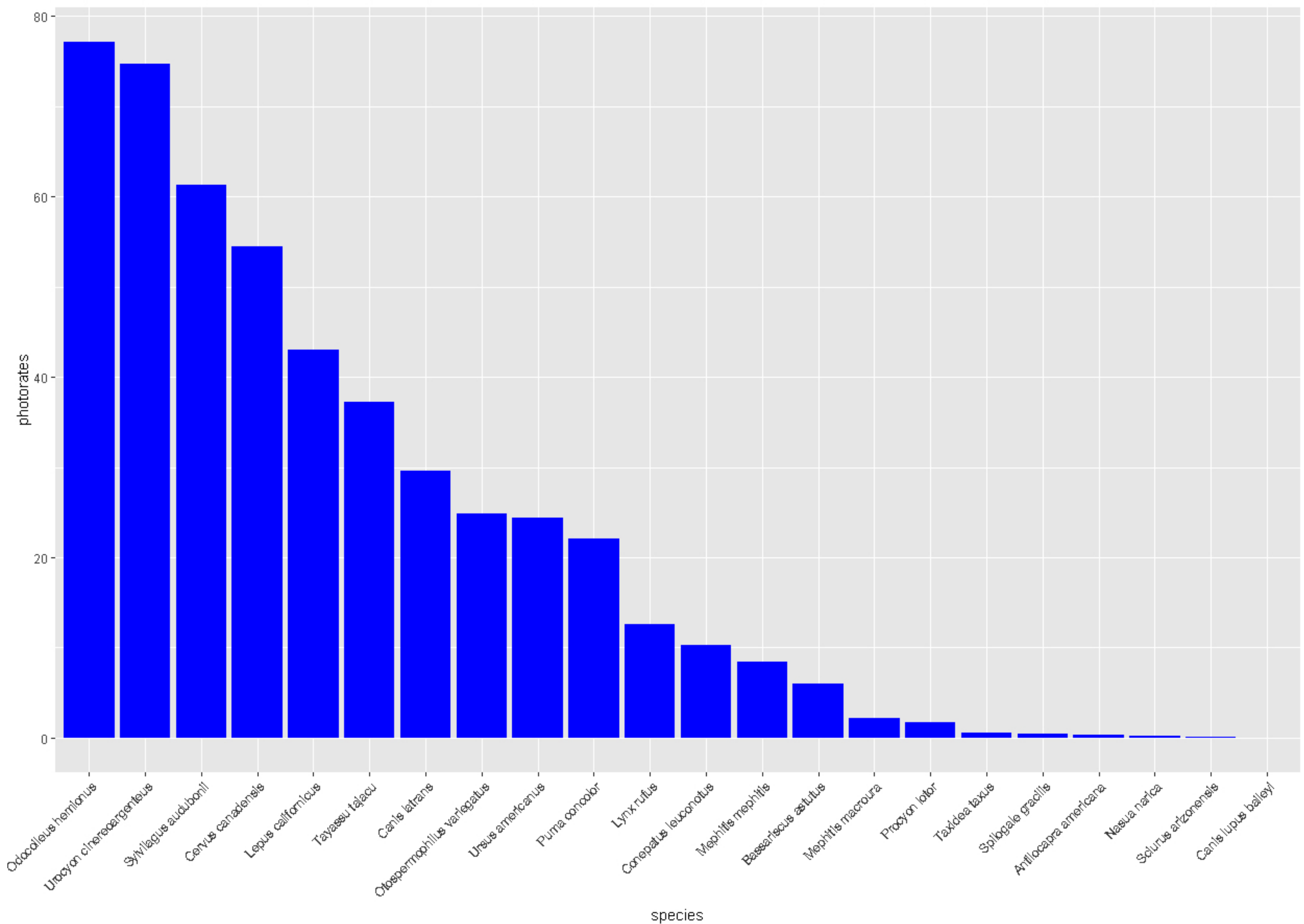


Figure 8. Photo capture rate of large and medium mammal species recorded from 25 cameras covering 100 sq km at a density of 1 per 4 sq km. A 16-camera grid was established in 2008 and expanded to 25 cameras in 2009. Cameras have been operated continuously since that time, for a total sampling effort of 81,293 camera nights through 2020. Photo rates in the figure are per 1,000 camera nights.

Phylogeographic analyses. We examined four species groups to detect potential endemism in the Gila or the possibility that multiple clades converged in this region. For white-throated woodrats, two major clades within *N. albigula* appear to converge in the Gila. A northeastern clade includes specimens from McKinley, Socorro, and Otero counties and the northern end of Sierra County in New Mexico, and a southwestern clade includes all specimens from Arizona, Chihuahua, and from the southern end of Sierra County (Figure 9).

For desert shrews, *Notiosorex crawfordi* and *N. cockrumi* both occur in sympatry in nearby Pima County, Arizona (adjacent to the Gila; Figure 10), but we did not detect the latter in the Gila. These two species, along with *N. tataticuli*, form an unresolved trichotomy (although similarity is slightly higher between the two most distant species, *N. crawfordi* and *N. tataticuli*, at *PP* = 0.87). In this case, there appears to be little phylogeographic structure within *N. crawfordi* across Arizona, New Mexico, and Texas.

For pocket gophers, we obtained multiple unresolved clades that form a polytomy within *T. bottae*. Most of the Gila specimens clustered with a specimen from the nearby Graham Mountains in Arizona and this distinctive clade was the only grouping found in the Gila to date (Figure 11), although more extensive geographic sampling of this polymorphic species is needed to better understand evolutionary relationships and biogeographic history of this ecologically important clade of mammals.

With regard to silky pocket mice, there appear to be two clades of *P. flavus* that converge in the Gila region (Figure 12). Representatives of these clades apparently are syntopic in Sierra and Catron counties. Specimens from those counties and Grant County form a southwestern clade along with specimens from southern Arizona and northern Chihuahua. Other specimens examined from Sierra and Catron counties are united in a northeastern clade with specimens from central Arizona, west Texas, and Oklahoma. This latter clade is weakly united (*PP* = 0.87) with specimens from southeastern New Mexico and west Texas.

Discussion

Faunal composition. With 104 extant species, the Gila supports one of the most diverse mammalian faunas in North America north of the USA-Mexico border. It includes nearly

two-thirds of all the mammalian species known for New Mexico (Malaney *et al.* 2021), and exceeds state-wide totals for 38 states in the United States (https://www.mammalogy.org/mammals-list). The addition of six more species to the Gila inventory in the last decade suggests the area harbors even higher mammalian diversity than documented here. The paucity of sampling and uneven distribution of specimens (Figure 3a) suggests more site intensive and broad spatial sampling has the potential to reveal further unrecorded taxa. Only six areas (hexagonal grids = 541 km^2) within the region contain more than 500 specimens summed across all species. The most well sampled region in the Gila (Willow Creek area with 1,992 specimens) equates to fewer than four specimens per km^2 summed for all species. The majority contain far less, ranging from 0.02 to 0.87 specimens per km^2 documenting the entire temporal span of the past 170 years (Figure 3a). Most areas did not have sufficient specimens across a temporal scale for any single species to effectively assess environmental change through time (Figure 3b-d).

More than a third (35) of the 104 mammal species recorded in the Gila reach their distributional range limit within the Gila (Figure 13), reflecting the position of the Gila at the confluence of distinct physiographic provinces and biomes. A number of other species have distributions that are peripheral to the Gila region and may be recorded there in the future. For example, two heteromyid rodents (*Chaetodipus eremicus, Perognathus flavescens*) and two cricetid rodents (*Reithrodontomys montanus, Sigmodon arizonae*) occur in the surrounding lowlands. Intensive inventories for shrews are lacking for the Gila and some taxa may yet to be recorded, including the western water shrew, *Sorex navigator,* which has been collected from the adjacent White Mountains in Arizona. Fossil records of *Vulpes vulpes* exist from the Gila and recent specimens have been collected just west of the region, near Dusty, New Mexico. The northern limits of a number of bat species (*Eumops perotis*, *Nyctinomops femorosaccus*, *Choeronycteris mexicana*, *Leptonycteris nivalis*, and *Dasypterus xanthinus*) occur in the Bootheel region of New Mexico (Hidalgo County) within 100 km of the Gila. The cottontails collected in ponderosa pine forest in the Gila were tentatively referred to *S. floridanus*, following Hoffmeister (1986) and Findley *et al.* (1975); however, we emphasize that the definitive identity of these higher elevation lagomorphs in the Gila awaits detailed taxonomic investigation. Whether they are this species, or *Sylvilagus cognatus, S. nuttallii, S. holzneri,* or a new species remains unknown (Hoffmeister and Lee 1986; Ruedas 1998). *Panthera onca* has been sighted in several nearby mountain ranges along the border and may well be found again in the Gila in the near future.

Regional historical biogeography. Although the geographic position, complex topography, and dynamic geological and climatic history of the Gila have played the primary roles in assembling this diverse fauna, additional phylogeographic (*e. g.*, Duran *et al.* 2012; Malaney *et al.* 2012) and paleontological analyses in the future likely will yield new insights into this dynamic biogeographic

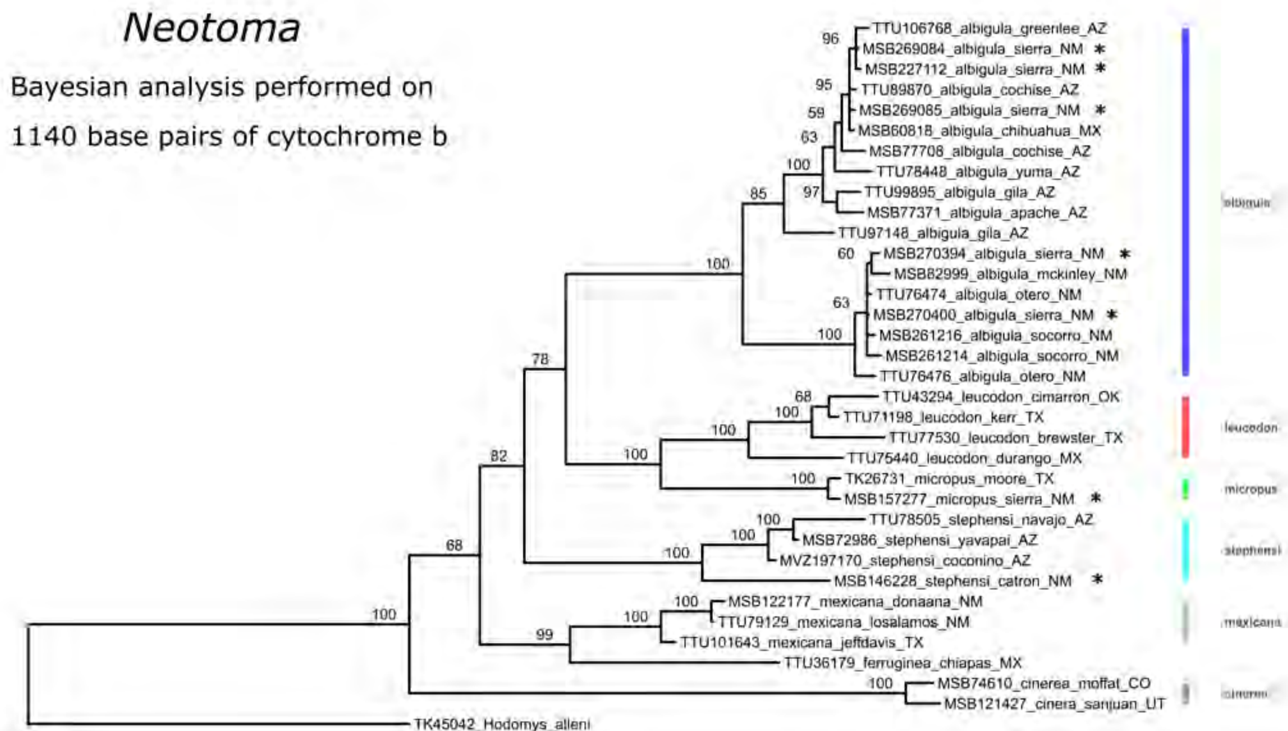


Figure 9. Phylogenetic placement of *Neotoma albigula* from southwestern New Mexico inferred under a Bayesian framework using 1140 base pairs of the mitochondrial cytochrome b gene. Node labels represent the posterior support probabilities with values of 50% or greater shown, but only values >95% are considered well supported. Specimens from the Gila region are indicated with an asterisk.

history. Desertification in the Southwest began in the Tertiary and continued into the Neogene with mountain uplift, creating rain shadows and deserts (Riddle 1995). During the Oligocene, dry tropical habitats were replaced with seasonal dry woodlands and savannas (Riddle 1995). With the uplift of the Sierra Madre Occidental and Colorado Plateau, and more local block-faulting of the Basin and Range province during the middle Neogene, diversity and provinciality of these faunas increased in western North America (Riddle *et al.* 2000). During the Miocene and Pliocene, three provinces that meet near the Gila (Basin and Range, Colorado Plateau, and Mexican Plateau/Chihuahuan desert) and their corresponding habitats (semi-desert/woodland, grassland/savannah, semi-desert/sub-tropical, respectively) were dynamically assembled and evolving (Riddle 1995). Episodic events during glacial-interglacial cycles of the Pleistocene set the stage for contraction and expansion of populations that led to divergence of many lineages of southwestern mammals (Conroy and Cook 2000; Jezkova *et al.* 2009; Andersen and Light 2012).

High biotic diversity in both montane and desert habitats of the Gila is likely the result of vicariance that fragmented species distributions and led to subsequent divergence of populations during shifting pluvial-interpluvial periods of the Pleistocene (Findley 1969; Patterson 1980; Riddle and Hafner 2006). This cyclic history of expansion and contraction, followed by isolation and divergence can be seen today in the disjunct distributions of high-elevation, montane populations of species such as *Myodes gapperi*, *Callospermophilus lateralis*, *Tamiasciurus fremonti*, and *Sciurus aberti* (Findley *et al.* 1975).

Fossil record. Nineteen paleontological sites in the Gila that span a period from the Eocene to the Pleistocene (https://www.utep.edu/leb/pleistNM/default.htm) illustrate both the rich fossil history of the region as well as distributional shifts. Schollmeyer and MacDonald (2020) added records of faunal remains from an additional 90 (of their total 105) archaeological sites from this region that range in age from 2000 BC to AD 1450. From all these sites, 137 mammal specimens representing nine mammalian orders have been identified including: Eulipotyphla (*n* = 4), Pilosa and Cingulata (*n* = 3), Chiroptera (*n* = 1), Carnivora (*n* = 18), Artiodactyla (*n* = 29), Perissodactyla (*n* = 24), Proboscidea (*n* = 14), Rodentia (*n* = 35), and Lagomorpha (*n* = 9), representing 28 extant and 51 extinct taxa (Stearns 1942; Cosgrove 1947; Wills 1988; Morgan 2015; Morgan *et al.* 2011; Schollmeyer and MacDonald 2020). Specimens of extant mammals not currently found in the Gila include *Marmota flaviventris, Urocitellus elegans, Cynomys gunnisoni, Cratogeomys castanops, Geomys arenarius, Thomomys talpoides, Neotoma cinerea, Microtus drummondii* (extirpated in the last *ca.* 50 years), *Sylvilagus nuttallii, Vulpes vulpes,* and *Vulpes velox,* and some of these document changes that have occurred since the Last Glacial Maximum.

Phylogeographic analyses. For the four selected species that we examined using cytochrome-*b* sequences, we found variable phylogeographic patterns. One species shows a single phylogeographic clade largely centered on the Gila (*T. bottae*), another (*N. crawfordi*) was shown to be closely related to populations to both the east and west of the Gila, and two species (*N. albigula* and *P. flavus*) were shown to have multiple clades that converged in the Gila.

Edwards *et al.* (2001) recognized *N. leucodon* to represent *N. albigula* east of the Río Grande, based on sequence analysis of the mtDNA *cytb* gene. Bradley and Mauldin (2016) suggested that populations from McKinley and Otero counties in New Mexico (spanning the Río Grande) may represent a third species, based on *cytb* divergence of 6.2 %. Derieg *et al.* (2021) reexamined the two species using multiple nuclear loci in addition to *cytb*, and instead concluded that the distinction between the two taxa was the result of mitochondrial introgression from an unsampled, or perhaps a now-extinct, lineage related to *N. micropus* into the eastern form of *N. albigula* (nominally *N. leucodon*). *Neotoma leucodon* is minimally differentiated from *N. albigula* across nuclear loci and therefore represents either a recent divergence or is conspecific. The two clades that we recovered within *N. albigula* (Figure 9) do not conform with clades recovered by Edwards *et al.* (2001), but instead sort into northeastern and southwestern clades that roughly are parallel to, but north of, the Mogollon Rim.

There is no phylogeographic structure within specimens of *N. crawfordi* across the Gila (Figure 10). Similarly, the distinctive phylogeographic clade of *T. bottae* of the Gila (Figure 11) suggests the possibility of finding other endemic taxa from the region. Patton and Smith (1990) and Smith and Patton (1988) found that southwestern New Mexico populations ultimately related to the widespread Basin and Range genetic group that extends from southern California to southeastern Coahuila. The boundary between the Basin and Range and Great Basin clades of *T. bottae* (Smith and Patton 1988) also approximates the boundary between two clades of *N. albigula* we identified. The two paraphyletic clades recovered within *P. flavus* (Figure 12) in the Gila are consistent with an east-west split although representatives of each clade are found in both Catron and Sierra counties. The northeastern and southeastern clades represent the Southern Rockies/Colorado Plateau clade and the Northern Chihuahuan clades, respectively, of Neiswenter and Riddle (2010).

Relatively few recent phylogeographic studies of Southwestern mammals have included representative specimens from the Gila, likely because the region is marginal to the various biomes that converge there. However, this edge dynamic makes the Gila a critical region for the examination of interactions among expanding lineages and species. Surveys of the literature reveal only 11 published phylogeographic studies of mammals to date that we are aware of that include samples from the Gila. These studies are: *Lynx rufus* (Reding *et al.* 2012), *Ursus americanus* (Van den Bussche *et al.* 2009), *Microtus mogollonensis* (Crawford *et al.* 2011), *Onychomys arenicola* (Riddle 1995), *Peromyscus maniculatus* (Dragoo *et al.* 2006), *Chaetodipus hispidus*

(Andersen and Light 2012), *Chaetodipus penicillatus* (Jezkova *et al.* 2009), *Perognathus flavus* (Neiswenter and Riddle 2010), *Neotamias cinereicollis* and *N. dorsalis* (Sullivan *et al.* 2014), *Tamiasciurus fremonti* (Hope *et al.* 2016), and *Sciurus aberti* (Lamb *et al.* 1997). Our analyses of *Notiosorex crawfordi, Neotoma albigula, Perognathus flavus*, and *Thomomys bottae* expand the spatial and temporal views of biotic variation in the Gila and highlight the region's potential for more detailed examination of contact zone dynamics. Further, the possibility of endemic populations in this region requires further focus and investigation.

Conservation status. Frey (2010) identified mammalian species of potential concern in the Gila and included mammals from Apache and Greenlee counties in Arizona and Luna and Hidalgo counties in New Mexico. Modifying the methods of Yu and Dobson (2000), she assessed aspects of rarity, concluding that > 90 % of the mammal species of the Gila should be classified at some level of rarity, with 50 % at risk for habitat loss (Frey 2010). This preliminary approach has merit, but only when based on robust documentation of the distribution and status of species in the Gila. Lacking such documentation, the relatively high levels of apparent rarity may primarily reflect insufficient field work (Malaney and Cook 2018). For example, we recorded three of the 11 species considered by Frey (2010) as "extremely rare" at multiple localities: *Myotis evotis* (five localities), *Myotis auriculus* (three localities), and *Peromyscus nasutus* (two localities, restricted to rocky substrate).

Using conservation criteria across formal international to statewide risk assessments and new data gathered in this study, we concluded that nine extant and one extirpated species should be considered for immediate (or reinvigorated) conservation assessment and monitoring (see beyond). These represent about 10 % of the species identified in this study, but we acknowledge that many species remain data deficient.

Euderma maculatum was designated as "Threatened" by NMDGF in 1988, although Geluso (2017) reported the persistence of this species at many sites in his 2006 resurvey of historic sites of occurrence in New Mexico. He found this species at seven sites in the Gila based on audible calls. Hayward and Hunt (1972) stated that *E. maculatum* is a late-night flyer (after midnight), which could conceal detection because nets are often closed before midnight. The spotted bat is a mid-elevation bat in the Gila (1,850 to 2,450 m), usually occurring in ponderosa pine and mixed conifer forests, and usually within 1.5 km of rocky outcroppings and cliffs where they likely roost (Findley *et al.* 1975). In Arizona, spotted bats are often captured in riparian areas near cliffs and rocks (Hoffmeister 1986). Hayward and Hunt (1972) caught one specimen in their 1972 survey of the Gila Wilderness and Jones (1965) reported that he captured one during his survey of the Mogollon Mountains, in ponderosa pine forest near the town of Mogollon. One of the few Gila specimens (WNMU: Mamm:1842) was found on a screen door in May at Lake Roberts. The spotted bat is listed on

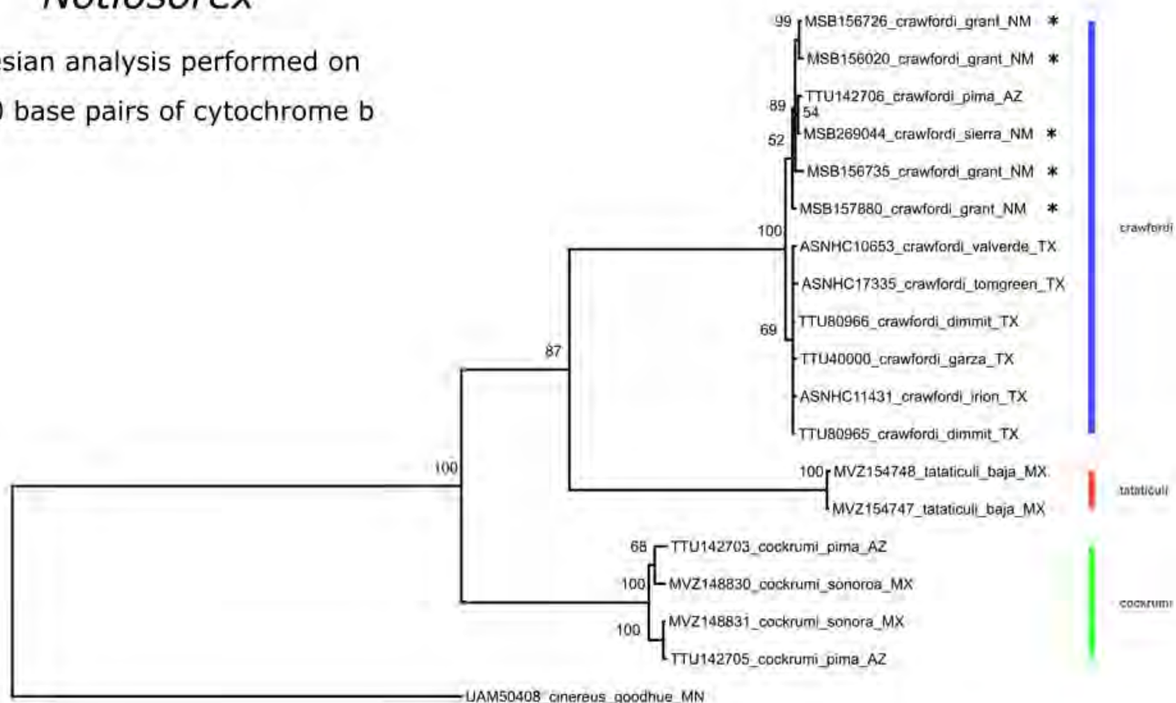


Figure 10. Phylogenetic placement of *Notiosorex crawfordi* from southwestern New Mexico inferred under a Bayesian framework using 1140 base pairs of the mitochondrial cytochrome b gene. Node labels represent the posterior support probabilities with values of 50% or greater shown, but only values >95% are considered well supported. Specimens from the Gila region are indicated with an asterisk.

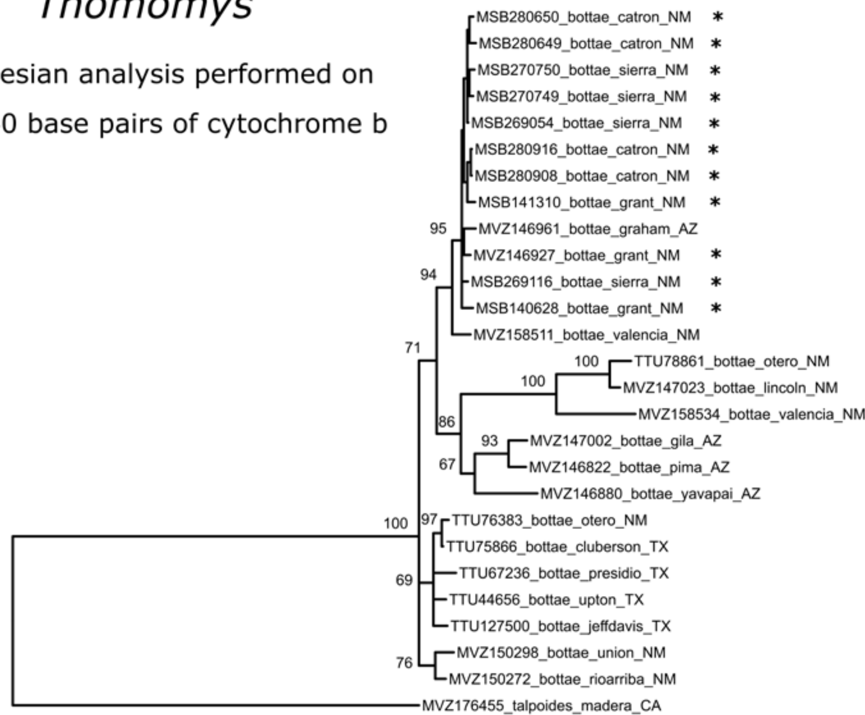


Figure 11. Phylogenetic placement of *Thomomys bottae* from southwestern New Mexico inferred under a Bayesian framework using 1140 base pairs of the mitochondrial cytochrome b gene. Node labels represent the posterior support probabilities with values of 50% or greater shown, but only values >95% are considered well supported. Specimens from the Gila region are indicated with an asterisk.

the US Fish and Wildlife Service's Species of Concern List (O'Shea *et al* .2018), as Threatened by the NMDGF, but Least Concern by the IUCN.

The migratory *Lasiurus blossevillii* is recommended for additional conservation study, although it has not been mentioned previously in conservation assessments. Although other migratory tree bats are found in moderate numbers (*Aeorestes cinereus*, *n* = 20, and *Lasionycteris noctivagans*, *n* = 40), only five *L. blossevillii* have been captured in the Gila. The two taken in 2013 were a male and a female captured in early April (MSB: Mamm: 267123, MSB: Mamm: 267125) and likely early migrants, as they represent the earliest annual record in New Mexico. Western red bats are usually found in riparian areas (Ammerman *et al.* 2012), consistent with the two we captured in cottonwood riparian areas on Animas Creek, just east of Ladder Ranch Headquarters. Hayward captured a female (WNMU: Mamm: 6615) in Reserve in ponderosa pine habitat.

Leptonycteris yerbabuenae is reported for the first time from the Gila. Cook (1986) reported *L. yerbabuenae* in the Animas Mountains. Hoyt *et al.* (1994) summarized records for *Leptonycteris* in New Mexico, noting that *L. yerbabuenae* and *L. nivalis* were found in the Peloncillo Mountains and Animas Mountains. Bogan *et al.* (2017) reported *L. yerbabuenae* from the Big Hatchet Mountains farther east in Hidalgo County. In September 2016, a resident living near Bill Evans Lake southeast of Cliff recorded a video of nectar bats swarming her hummingbird feeder (K. Beckenbach, pers. comm.) and in October 2018 recovered a specimen (MSB: Mamm: 333977) that died of unknown causes at her home. In September 2019, Keith Geluso documented a lesser long-nosed bat just east of the Gila Post Office (MSB: Mamm: 328326). These newer records may indicate an ongoing distributional expansion or this species simply was not previously detected, but given Bruce Hayward's long-term monitoring of bats in this area, the former hypothesis seems more likely. Lesser long-nosed bats were recently removed from the Endangered Species List (USFWS 2019), but populations should continue to be monitored and documented.

Zapus luteus is reported for the first time from the Gila and this new record (Malaney *et al.,* submitted) may indicate a range expansion from the nearest source populations in the White and Mogollon mountains of Arizona (Hoffmeister 1986) as close as 15 km away, however limited sampling in the western Gila may also explain why this species was not previously detected. Hoffmeister (1986) reported 63 specimens from ten localities in the White and Mogollon Mountains on the nearby Mogollon Plateau. Malaney *et al.* (2012) predicted that the jumping mouse could occur in the Mogollon Mountains and Black Range of New Mexico based on species distribution modeling. This species occurs in riparian, mesic habitats at both high and low elevations (Malaney *et al.* 2012). Given these discoveries, a more thorough sampling effort to determine the geographic distribution of the New Mexico jumping mouse in the Gila is warranted. The seasonal habits of *Zapus* restrict fieldwork because they go into hibernation in late September or early October, seeking hibernacula in higher ground above their usual streamside habitat, and remain in hibernation until

April or May. We and others have found jumping mice to be more readily captured in Museum Special traps than in Sherman live traps, so live traps set in the wrong season, or in the wrong habitat after they have begun moving towards hibernacula, are likely to be unsuccessful. Currently listed as federally Endangered, a more thorough sampling of their geographic distribution in the Gila is warranted (Malaney *et al.* submitted).

Canis lupus is listed by CITES as a species that may not currently be threatened with extinction but could become so (https://www.cites.org/eng/app/index.php). The Mexican wolf (*Canis lupus baileyi*) is listed as Endangered by NMDGF and the ESA. Mexican gray wolves were extirpated from the Gila, the region of their greatest historical abundance (Robinson 2005) by 1925 with the last recorded specimen from the Gila from 24 km SE Reserve on 11 May 1925 (USNM:245841). Plans to recover Mexican gray wolves began in 1977 and were executed in 1998 with the release of 11 wolves into the Blue Range of the Gila Wilderness as an experimental population (Hedrick and Fredrickson 2008; USFWS 2010, 2015, 2017). According to a recent quarterly update (October-December 2020, USFWS 2020), there are at least 87 wolves that primarily roam the Gila in New Mexico, but they also venture into the Cibola National Forest and the San Mateo Mountains of Socorro County.

Another large carnivore native to the Gila, *Panthera onca*, was extirpated from the region in the last century, but could potentially recolonize from the south with an ever-increasing population in northern México and an increasing number of camera trap records from the Animas, Peloncillo, Chiricahua and other sky island mountain ranges just south of the Gila. There are jaguar petroglyphs present on rock faces within the Gila and numerous historic records (photos, accounts, and specimens) document jaguar occurrence far north of US Interstate 10 (I-10) from the late 1800s through the 1960s. One specimen held in the Smithsonian (USNM 289015) was collected by a U.S. Biological Survey hunter from the White Mountain Apache Indian Reservation in Arizona just west of the Gila. Others include animals killed near the Grand Canyon. USFWS (1994) acknowledged that a minimum of 64 jaguars have been killed in Arizona since 1900. Camera trap data have recorded regular use of the mountains in SE Arizona and SW New Mexico in the past two decades (McCain and Childs 2008). To date, those observed were males.

USFWS (2018) restricted the northern edge of the Northwestern Jaguar Recovery Unit to areas south of US I-10 and suggested the carrying capacity for jaguars within the US was only six animals. Sanderson *et al.* (2021) reevaluated previous models and assessments and concluded that ample suitable habitat extends northward into central Arizona and New Mexico, including the Gila region. They suggested that the carrying capacity within the US is from 90-151 animals, which could be sufficient for maintaining a viable population north of the international border wall. Their findings suggest that conservation efforts should not only focus on connectivity between Mexican and US populations but also

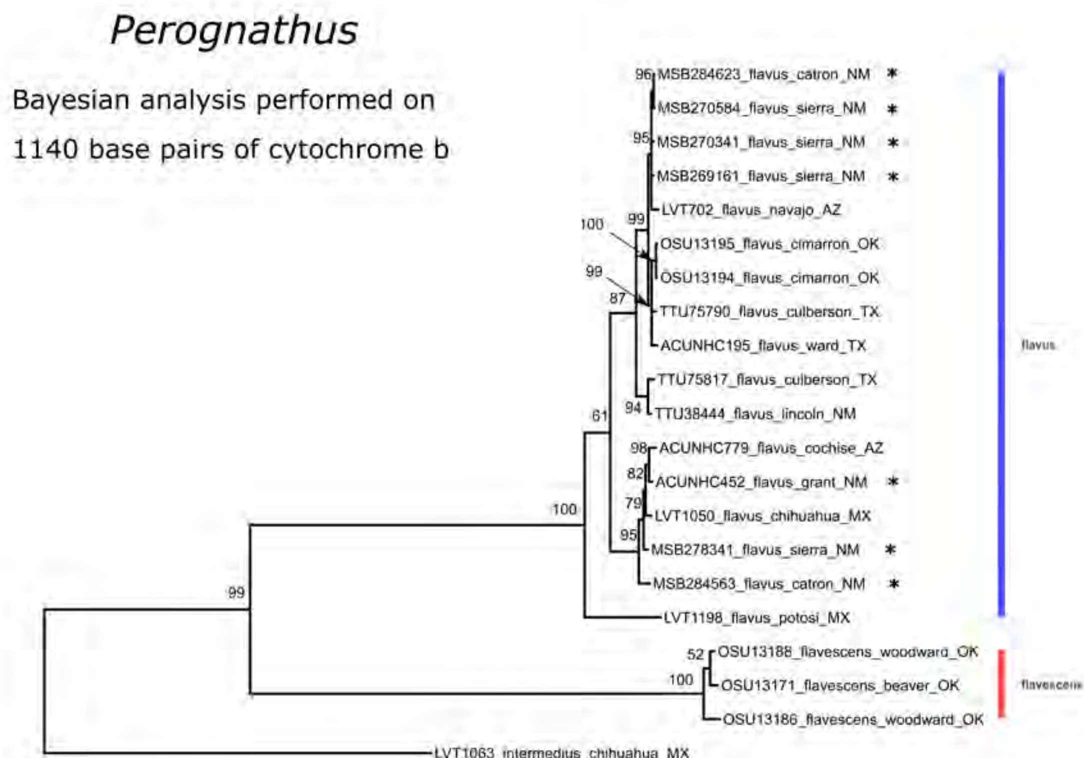


Figure 12. Phylogenetic placement of *Perognathus flavus* from southwestern New Mexico inferred under a Bayesian framework using 1140 base pairs of the mitochondrial cytochrome b gene. Node labels represent the posterior support probabilities with values of 50% or greater shown, but only values >95% are considered well supported. Specimens from the Gila region are indicated with an asterisk.

in the re-establishing a self-sustaining population within a proposed Central Arizona/New Mexico Recovery Area (Sanderson *et al.* 2021) including the Gila region.

Microtus montanus arizonensis was listed as Endangered by NMDGF in 1979 and in 2008 NMDGF attempted to implement a recovery plan for *M. montanus*, but those plans were not formally approved. The threat is listed in terms of pressures on an already small, isolated population including anthropogenic activities (*e. g*., grazing, water diversion, and wetland conversion) as well as projected diminished habitat due to climate change (NMDGF 2020). There were 11 records from Catron County (two localities) before 1994, and 15 from Catron County (five localities) from 1994 to 2008 (including nine records from the Apache National Forest of Catron County). In 2020, one was captured at Romero Creek in Catron County (NMDGF 2020), but more work on the status and distribution of the montane vole in the Gila is needed.

In November 2014, we failed to find the meadow vole, *M. drummondii*, at two previously known localities near Aragon in the Gila (recorded in 1915). We also failed to detect the species about 140 km N of the Gila, near San Rafael in Cibola County. Riparian habitat around Aragon is severely over-grazed, reducing the riparian grass-sedge habitat of *M. drummondii* and potentially causing the local extirpation of this species (Anderson 1961; Anderson and Hubbard 1971; Findley *et al.* 1975; Jackson and Cook 2020). Southern peripheral populations of this species are genetically distinctive and important components of overall diversity within this species (List *et al.* 2010; Jackson and Cook 2020). Populations in northern México recently also have been lost due to conflicts with agriculture (List *et al.* 2010).

Dipodomys spectabilis is listed as Near Threatened by IUCN throughout its range due to loss of desert grassland habitat to encroaching mesquite and creosote (List *et al.* 2010; Linzey *et al.* 2013) and a history of federally funded poisoning campaigns. Mitigation of anthropogenic impacts that directly impact kangaroo rats, or lead to shrub encroachment, should be implemented to protect *D. spectabilis*. We captured 11 specimens during the 2012 to 2019 survey at two localities on the Ladder Ranch and saw extensive mounding activity of this species northwest of Winston, New Mexico in 2014. Their mounds are seen along Highway 180 south to Deming and near Faywood in Mimbres Valley.

We include *Sciurus arizonensis* because of its IUCN Data Deficient status and note that the species is listed as Threatened in México (Coronel-Arellano *et al.* 2016). Bailey (1931) reported that the Arizona gray squirrel is common along the San Francisco River, and Findley *et al.* (1975) recounted that it is "limited to the deciduous riparian forest of the San Francisco drainage in Catron Co." However, as Hayward and Hunt (1972) observed, Arizona gray squirrels have been extending their range up the Gila and San Francisco drainages and east towards the Rio Grande. We examined two specimens from 1937 captured near the Sacaton Landing Strip (AMNH M-127032-127033) and one from 1928 captured in the Mogollon Mountains near Big Dry Creek (AMNH M-127260). Findley *et al.* (1975) reported that Arizona gray squirrels are usually found in ponderosa pine forest, and Hayward's captures came from low-elevation riparian habitat and piñon-juniper woodland. Hayward also recovered a road-killed Arizona gray squirrel near his home 6 km north of Silver City in 1985 (WNMU: Mamm: 4781). Frey *et al.* (2008) reported evidence of range expansion of Arizona gray squirrels eastward into Sierra County of the Gila, including several personal observations, two photographs, and one specimen (MSB: Mamm: 124820) from Sierra County. Camera data from the Ladder Ranch (Figure 8) show Arizona gray squirrels in January, June, July, and September of 2009 providing additional evidence (time-stamped and geo-tagged photographs) of their range extension eastward and persistence. Frey *et al.* (2008) assert that lack of historic records from the Ladder Ranch belies a range expansion. The distribution of the Arizona gray squirrel in the Gila requires continued monitoring.

Nine other bat species from the Gila (*Corynorhinus townsendii, Idionycteris phyllotis, Myotis evotis, M. occultus, M. thysanodes, M. velifer, M. volans, M. yumanensis,* and *Nyctinomops macrotis*) are listed on the US Fish and Wildlife Services Bats of Concern List (O'Shea *et al.* 2018). Some of these are also listed as Species of Concern by the New Mexico Department of Game and Fish. Of these, *Myotis evotis* was the least common bat encountered in this survey. *Idionycteris phyllotis* is listed at Imperiled by New Mexico Game and Fish (O'Shea *et al.* 2018) and was the second least common bat captured in this survey. *Corynorhinus townsendii* also was among the least often encountered species in mist net surveys in the Gila (O'Shea *et al.* 2018, this study). A renewed focus on the status of bats of the Gila is needed. In addition to these species, there are a series of other species listed as Data Deficient that should be reviewed. Broad specimen-based surveys, such as conducted here, are the most efficient approach to gather the sampling necessary to stimulate the study of these species.

Habitats of Concern. It is most cost efficient to focus limited conservation and rehabilitation resources on habitats that support multiple threatened species. Increased protection of riparian habitats should be considered a top conservation priority, most importantly along the Mimbres, Gila, and San Francisco rivers and tributaries. More robust exclusion of cattle from these drainages would address the habitat requirements of associated species such as *L. blossevillii*, *M. drummondii*, *Z. luteus*, and *S. arizonensis*. Over the past five decades, the Gila River in southwestern New Mexico has been proposed for damming and diversion multiple times and efforts will likely continue with a warming and drying climate coupled with increased water demands from major metropolitan areas. The most recent effort to impound the Gila River was rejected by the Interstate Stream Commission in June 2020. Flow variability across different seasons characterizes the Gila River and helps maintain diverse habitat types (riparian forest, wet-

lands, and floodplains) that elevate mammalian diversity. Proposed diversions would decrease both mid-size flows and negatively impact persistence of riparian forest (*e. g*., affect roosting sites for bats and habitat for Arizona gray squirrels); lessen the connection of the river to the floodplains (affecting habitat for hydroseric species); diminish aquatic habitat (fish are prey for multiple carnivorous mammals); lower reproduction and emergence of aquatic and aquatic-associated invertebrates (decreased food supply for riparian-associated bats; Fukui 2006; Valdez and O'Shea 2014); and decrease vegetation productivity, potentially important for multiple mammalian species such as the Arizona squirrel, beavers, and muskrats.

Desert-grasslands are in decline regionally. The nearby Jornada Experimental Range found that shrub cover increased by > 12 % and grassland decreased by > 16 % from 1937 to 2003 (Laliberte *et al.* 2004). Desert-grassland obligate species, like *Dipodomys* and potentially *Onychomys, Chaetodipus*, and *Perognathus,* may be at risk if open grasslands continue to decline. Studies on mammalian population responses to shrub encroachment in the Southwest remain few, but studies elsewhere show dramatic mammalian declines with increased shrub cover (Blaum *et al.* 2007).

For both riparian and desert-grassland habitats, overgrazing poses a severe threat. The Gila has a long history of heavy grazing pressure, beginning in the late 1880s when cattlemen and sheepherders moved into the area. Shortly thereafter the Stock Raising Homestead Act of 1916 allowed for fencing of grazing allotments and encouraged cattle growers to make water improvements. After the designation of the Gila Wilderness and the Aldo Leopold Wilderness, some of these allotments were reduced and allotments generally decreased from 1928 to 2007 (from 83,499 to 18,772 ha); however, large numbers of livestock continue to graze on federal lands. Livestock grazing generally decreases biodiversity (Fleischner 2002; Jones 2000; Milchunas and Lauenroth 1993), with especially heavy impacts in riparian zones in the arid Southwest. Hayward *et al.* (1997) performed small mammal surveys over 10 years at San Simon Cienega in southeastern Arizona (about 90 km SW of the Gila) and found total abundance of small mammals was about 50 % less in grazed plots, with *Sigmodon hispidus* and *Reithrodontomys megalotis* especially sensitive to grazing effects (Hayward *et al.* 1997). Similarly, Moser and Whitmore (2000) found significantly greater abundance of small mammals, species richness, and diversity on sites that were ungrazed when compared with grazed sites and shrews were only captured on ungrazed sites.

Fire. Fires are a natural part of the ecosystems of the Southwest. Historically, national forests of this region averaged more fires annually than other regions stemming from diverse sources, including one of the largest concentrations of lightning-caused fires worldwide (Pyne 1982). In recent years, however, large catastrophic fires and subsequent flooding have become more common with two of the largest fires in New Mexico's history occurring in the Gila in the last decade (*e. g*., Whitewater-Baldy in 2012, Silver in 2013). Snowmelt earlier in the spring, increasing temperatures, and high fuel loads due to historic fire suppression have elevated the severity of wildfires in the region (Hurteau *et al.* 2014). Of particular note for forest and riparian-associated mammal communities is the prediction that many of these forests will not return to prefire forested conditions due to having crossed climatic thresholds for regeneration related to ongoing warming and drought conditions (Davis *et al.* 2019). Post-fire burned sites of Douglas-fir (*Pseudotsuga menziesii*) and ponderosa pine (*Pinus ponderosa*), in particular, have decreased potential to return to their pre-fire forest structure (Haffey *et al.* 2018; Rodman *et al.* 2019). Those changes may have negative consequences for a wide variety of both riparian-associated (*e. g*., Whitney *et al.* 2015) and forest-associated species of the Gila, such as tree-roosting bats, *L. blossevillii, A. cinereus,* and *L. noctivagans* (Kunz 1982; Shump and Shump 1982). Nonetheless, analyses of the impact of fires of different intensities on bat communities in the Sierra Nevadas of California are now revealing the role for forest fires in bat ecology, with some species showing increased occupancy of burned areas (Blakey *et al.* 2019; Buchalski *et al.* 2013; Steel *et al.* 2019). Sciurids, including *Sciurus aberti, S. arizonensis, Neotamias cinereicollis*, and *Tamiasciurus fremonti*, also may be impacted by large, catastrophic fires (Koprowski *et al.* 2006). *Tamiasciurus fremonti,* for example, has been shown to no longer inhabit areas that have experienced stand-replacing fires (Ream 1981). Although lower intensity fires do not appear to have lasting impact (Blount and Koprowski 2012) on red squirrels in nearby Pinaleño Mountains of southeastern Arizona, the cumulative and synergistic impacts of severe fires, climate warming, drought, insect-invasion and other forest habitat disturbances have resulted in reduced food availability, survival, and reproductive output for this endemic squirrel (*Tamiasciurus fremonti grahamensis*). Allard-Duchene *et al.* (2014) reported that *T. fremonti* will inhabit a manually thinned area about 20 years sooner than a burned area. Other studies found that prescribed burns were negatively correlated with occurrence of *Neotamias cinereicollis* (Converse *et al.* 2006), but relatively little is known about impacts of these events on biodiversity in the Gila (Whitney *et al.* 2015).

Climate Change. Although climate variability is natural, recent warming and drying trends in southwestern New Mexico have been accentuated through increased production of CO_2 by humans. Data from local weather stations show temperatures in the Gila National Forest, Aldo Leopold National Forest, Ladder Ranch, and surrounding areas have increased while precipitation has decreased since the early 1900s (Girvetz *et al.* 2009), and these trends have accelerated recently (Jones and Gutzler 2016, O'Connor *et al.* 2020). Using museum specimens, Moritz *et al.* (2008), Rubidge *et al.* (2012), and McCain *et al.* (2021) have each shown that climate change over the past century can affect the elevational distributions of some mammals.

Figure 13. At least 35 species of mammals have their distributional limit in the Gila.

Through the Grinnell Resurvey Project, Moritz et *al.* (2008) investigated mammalian range shifts in the Sierra Nevada Mountains and generally found that low elevation species expanded their ranges upslope, while the ranges of higher elevation montane species contracted. Some species showed idiosyncratic movements, hypothetically due to competitive release when congeners were displaced. Similarly, we hypothesize potential range shifts of species along elevational gradients in the Gila Region with possible range expansion of lower elevation desert, grassland, and woodland-associated species and range contraction in montane and riparian species. Such changes may increase the risk of extirpation of high-elevation species as their ranges decline (McDonald and Brown 1992; Parmesan 2006).

Bats are also at risk due to disrupted climate regimes. Adams (2010) showed a significant decrease in reproduction of insectivorous bats in years with lower precipitation in the Front Range of Colorado. Rebelo *et al.* (2010) hypothesized that many bat species will be at risk of extinction due to rising temperatures and declining precipitation; thus, some populations in the Gila may be locally extirpated in the future with increasing drought conditions. Several New Mexico bat species roost communally, where dehydration presents significant challenges due to the high temperature and low humidity of the microclimates within roosts. Communal maternity roosts may be challenged by the increased physiological stress on nursing mothers due to milk production (Adams 2008). Therefore, the ability of female bats to survive and raise young hinges on readily accessible open water. Given projected trends for warming and drying across the Southwest, reproductive success of bats in New Mexico may be severely reduced. Sherwin *et al.* (2012) reviewed potential risk factors for bats with changing climate and classified food, roosts, reproduction, and distribution as potential risks. Reduced precipitation may decrease food availability for insectivorous and frugivorous bats. Habitat availability also may be decreased for tree-roosting bats. In addition, problems associated with ability to reproduce as outlined by Adams (2008, 2010) are likely, and species with small ranges or higher elevation associations may be differentially affected by dramatic environmental warming and aridification (Sherwin *et al.* 2012). As most bat species migrate or hibernate, their phenology should be carefully monitored in relation to climate trends to understand whether they are impacted. Georeferenced specimens not only unequivocally document the time and place of species occurrence, but they also can provide insights into physiological status related to hibernation, migration, and reproduction.

Specimen-based research. The lack of repeated faunal surveys documented by holistic specimens continues to hamper the study of Gila biodiversity, suggesting an urgent need to build natural history collections, which can serve as primary infrastructure or libraries of biodiversity for the region (Malaney and Cook 2018). Natural history specimens not only provide the necessary retrospective materials (*i. e*., historical baselines; Suarez and Tsutsui 2004; McLean *et al.* 2016; Cook and Light 2019) to understand how changing conditions are impacting organisms, but they also provide a foundation for forecasting how future changing conditions may impact aspects of biodiversity (Schindel and Cook 2018; Funk 2018). Recent evolution of the concept of voucher specimens has resulted in collections of holistic specimens that include traditional vouchers (*e. g*., fluid, skin and skeleton) that are now associated with diverse ancillary materials (*e. g*., multiple ultra frozen tissues), and preserved endoparasites and ectoparasites (Dunnum and Cook 2012; Galbreath *et al.* 2019). Parasitism plays a large role in structuring biotic communities, yet this aspect of mammalian biology has been minimally evaluated throughout the Southwest. Parasites can also yield historical and biogeographic insights not revealed by examination of host-history alone (Galbreath *et al.* 2020). Holistic collections of mammals, such as exemplified in this study, provide a sampling infrastructure that will facilitate integrated insights into the history and future trajectory of natural communities (Gardner and Campbell 1992; Galbreath and Hoberg 2015; Brooks *et al.* 2019). This broad array of material allows for more intensive and integrated investigations of the biology of these organisms (Dunnum *et al.* 2020), taking advantage of recent advances in technology (*e. g*., genomics, stable isotopes, microCT scans), analyses (*e. g*., ecological niche modeling; Cook and Light 2019), and immediate on-line access to these ever-growing data

streams through vast data repositories (*e. g*., GenBank, IsoBank, MorphoBank).

Our ability to investigate and more completely understand aspects of these complex systems, including the impact of changing environmental conditions in the future, will primarily be limited by the extent of the sampling that we preserve now for future generations of scientists, managers, planners, policy makers, and educators (Malaney and Cook 2018). With regard to educators, biological collections that are accessible on-line have proven to be novel resources for K-16 educators and citizen scientists to more deeply learn about the natural world (Powers *et al.* 2014; National Academies of Sciences, Engineering, and Medicine 2020). When associated with active research programs at academic institutions, collections have long been key to the development of the next generation of naturalists (Cook *et al.* 2014, 2016; Lacey *et al.* 2017). Educators can draw on the rich biodiversity datasets now available for the Gila, and through specimen-based activities students can learn how to integrate across disciplines as they are drawn into a diverse and growing network of scientists that are producing fascinating insights into biology, chemistry, public health, and an array of other fields (Schmidly 2001, 2005).

We recognize that a key finding emerging from this overview of taxonomy, distribution, phylogeographic history, and conservation concerns for Gila mammals is that our understanding of this fauna remains incomplete. By emphasizing information principally backed by specimens, there is now a durable sampling platform that will stimulate future integrative studies (*e. g*., Cook *et al.* 2017; Funk 2018; Thompson *et al.* 2021), investigations that will substantially extend these preliminary results as they unravel the complex role of mammals and their symbionts in Gila ecosystem dynamics.

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Appendix 1

Thomomys		*Neotoma*		*Notiosorex*		*Perognathus*	
Catalog #	**GenBank Accession #**	**Catalog #**	**GenBank Accession #**	**Catalog #**	**GenBank Accession #**	**Catalog #**	**GenBank Accession #**
MSB140628	MW752461	MSB121427	AF186799	MSB156020	MW156020	ACUNHC195	FJ514864
MSB141310	MW752462	MSB74610	AF186800	MSB156726	MW156021	ACUNHC452	FJ514880
MSB269054	MW752463	TTU71198	AF186806	MSB156735	MW156022	ACUNHC779	FJ514882
MSB269116	MW752464	TTU75440	AF186809	MSB157880	MW156023	LVT702	AY926405
MSB270749	MW752465	TTU43294	AF186815	MSB269044	MW156024	LVT1050	FJ514881
MSB270750	MW752466	TTU79129	AF294345	TTU142705	AY611574	LVT1063	AY926389
MSB280649	MW752467	TTU101643	AF294346	TTU142706	AY611573	LVT1198	FJ514887
MSB280650	MW752468	TTU36179	AF298840	MVZ148831	AY611572	LVT3610	AY926383
MSB280908	MW752469	MSB60818	KF267873	MVZ154747	AY611571	MSB269161	MW752455
MSB280916	MW752470	MSB77371	AF186808	MVZ154748	AY611570	MSB270341	MW752456
MVZ146822	EU240745	MSB77708	AF186811	TTU80965	AY611569	MSB270584	MW752457
MVZ146880	TBU65269	MSB82999	EU141962	ASNHC11431	AY611568	MSB278341	MW752458
MVZ146927	TBU65271	MSB122177	MW752445	MVZ148830	AY611567	MSB284563	MW752459
MVZ146961	TBU65268	MSB146228	MW752446	TTU40000	AY611566	MSB284623	MW752460
MVZ147002	EU240741	MSB157277	MW752447	TTU142703	AY611565	OSU13171	FJ514890
MVZ147023	TBU65272	MSB227112	MW752448	TTU80966	AY611563	OSU13186	FJ514897
MVZ150272	TBU64979	MSB261214	MW752449	ASNHC17335	AY611562	OSU13188	FJ514899
MVZ150298	TBU64980	MSB261216	MW752450	ASNHC10653	AY611564	OSU13194	FJ514871
MVZ158511	EU240739	MSB269084	MW752451	UAM50408	AY014952	OSU13195	FJ514869
MVZ158534	TBU65270	MSB269085	MW752452			TTU38444	FJ514888
TTU127500	AF445059	MSB270394	MW752453			TTU75790	FJ514865
TTU44656	AF445047	MSB270400	MW752454			TTU75817	FJ514889
TTU67236	AF445052	MSB72986	AF307834				
TTU75866	AF445055	TTU78505	AF308867				
TTU76383	AF445053	MVZ197170	DQ781305				
TTU78861	AF445061	TTU76474	DQ179817				
MVZ176455	U65291	TTU76476	AF376472				
		TTU77530	AF186828				
		TTU78448	AF186816				
		TTU89870	KM488337				
		TTU97148	EU141961				
		TTU99895	EU141964				
		TTU106768	KM488338				
		TTU42833	EU286808				
		TK45042	DQ179810				

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Diversity and activity patterns of medium- and large-sized terrestrial mammals at the Los Tuxtlas Biosphere Reserve, México

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Studies on diversity of animal communities allow determination of their species richness and composition. This information is particularly relevant to establish sound conservation programs in biosphere reserves, where human activities should be focused on the sustainable use of natural resources and ensure biodiversity protection. This study estimated the diversity and species richness and determined the species composition and activity patterns of medium- and large-sized terrestrial mammals in the Los Tuxtlas Biosphere Reserve (LTBR) located in Veracruz, Mexico. We set 18 camera traps to record medium and large-sized terrestrial mammals from August 2016 to January 2017. We calculated the trapping rate, guilds, and activity patterns of species. Diversity was estimated with Hill numbers. We compared our estimates with other studies in tropical forest in Mexico. We obtained 308 independent captures of 13 species; *Cuniculus paca* and *Dasyprocta mexicana* were the species with the highest trapping rate. Order-0, order-1, and order-2 diversity values were 13.99, 6.50, and 4.75 effective species, respectively, which ranks LTBR the fourth-most diverse reserve of medium- and large-sized terrestrial mammals compared to six other tropical rainforest sites in southern Mexico. We recorded mammals representing five trophic guilds, of which frugivore-folivores (five species) and omnivores (three species) ranked highest. All recorded species were primarily nocturnal (six species) or diurnal (six species). *Tamandua mexicana*, *Leopardus pardalis*, *L. wiedii*, and *Eira barbara* are listed as endangered in the Mexican Official Standard Norm NOM-059-2019, and *L. wiedii* is listed as near threatened in the IUCN Red List of Threatened Species. We were able to record 40.6 % of the terrestrial mammal species known to inhabit the LTBR. The absence of large-sized species such as large predators and herbivores was notable. Comparison of medium and large-sized mammal diversity of camera trapping studies in Mexico show that landscape degradation is impoverishing terrestrial mammal communities.

Los estudios de diversidad de las comunidades animales permiten determinar la riqueza de especies y su composición. Esta información es particularmente relevante para establecer programas de conservación en reservas de la biosfera, donde las actividades humanas deben ser enfocadas en el uso sustentable de los recursos naturales y asegurar la protección de la biodiversidad. Este estudio estimó la diversidad y riqueza de especies, y determinó la composición de especies y patrones de actividad de mamíferos terrestres de talla mediana y grande de la Reserva de la Biosfera Los Tuxtlas (RBLT), en Veracruz, México. Entre agosto de 2016 y enero de 2017, se colocaron 18 trampas cámara para registrar mamíferos terrestres de talla mediana y grande. Calculamos la tasa de captura, gremios, y patrones de actividad de las especies. La diversidad la estimamos con los números de Hill. Se compararon los valores estimados con estudios en otros bosques tropicales húmedos de México. Se registraron 308 capturas independientes de 13 especies; *Cuniculus paca* y *Dasyprocta mexicana* fueron las especies con la tasa de captura más alta. Los valores de diversidad del orden-0, orden-1, y orden-2 fueron 13.99, 6.50, y 4.75 especies efectivas, respectivamente, los cuales colocan a la RBLT en el cuarto lugar en diversidad de mamíferos terrestres medianos y grandes, de seis bosques tropicales húmedos del sur de México. Se registraron cinco gremios, de los cuales el de los frugívoros-folívoros (cinco especies) y el de los omnívoros (tres especies) fueron los mejor representados. Las especies fueron principalmente nocturnas (seis especies) y diurnas (seis especies). *Tamandua mexicana*, *Leopardus pardalis*, *L. wiedii* y *Eira barbara* están enlistadas como en peligro de extinción en la Norma Oficial Mexicana NOM-059-2019 y, *L. wiedii,* está enlistada como cercanamente amenazada en la Lista Roja de Especies Amenazadas de la UICN. Se detectaron el 40.6 % de las especies de mamíferos terrestres conocidos que potencialmente habitan en la RBLT. La ausencia de especies de talla grande, como grandes depredadores y herbívoros, fue notable. La comparación de la diversidad de mamíferos terrestres de talla mediana y grande de estudios con foto-trampeo en México, muestran que la degradación del paisaje está empobreciendo estas comunidades.

Keywords: Camera traps; community-based monitoring; defaunation; Hill numbers; species richness; trapping rate.

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Introduction

Studies on the diversity of animal communities provide a direct method to determine their species richness and composition, and the relative abundance of individual species (Magurran 2004; Robinson 1999; Sigel *et al.* 2006; Laurance *et al.* 2008). This information is of particular relevance in protected areas such as biosphere reserves, to establish sound conservation and management programs involving their inhabitants, where human activities should be focused on a sustainable use of natural resources and ensure biodiversity protection (Sigel *et al.* 2006; Negrões *et al.* 2011).

Medium- and large-sized terrestrial mammals frequently are used, for various reasons, as a faunistic group to identify potential impacts of habitat loss and fragmentation due to human-induced activities on ecosystems (O'Connell *et al.* 2010; Monroy-Vilchis *et al.* 2011; Canale *et al.* 2012). First,

this group of terrestrial mammals occupies a high trophic level in food webs and, thus, their occurrence and abundance have cascade effects at lower trophic levels (Roemer *et al.* 2009). They also comprise a wide range of guilds and have diverse spatial and habitat requirements, and include both generalist and specialist species. This wide range of ecological characteristics likely results in a differential species-by-species response to the impact of habitat loss and fragmentation. For example, species with large size, habitat and diet specialists, and/or requiring large home ranges are more likely to experience local population extirpations than medium-sized species, and/or habitat and diet generalist species, and those holding smaller home ranges (Ferguson and Larivière 2002; Michalski and Peres 2007).

The Mexican State of Veracruz is well-recognized for its high biodiversity, species richness, and endemicity (González-Christen and Delfín-Alfonso 2016). It is the third-richest State in the country in number of terrestrial vertebrate species, only after the States of Oaxaca and Chiapas (Flores-Villela and García-Vázquez 2014; Navarro-Sigüenza *et al.* 2014; Parra-Olea *et al.* 2014; Sánchez-Cordero *et al.* 2014). A total of 195 terrestrial mammal species — 39.3 % of the species known to inhabit Mexico — have been recorded in Veracruz to date (Ramírez-Pulido *et al.* 2014). However, about one-third (53 species; 27 %) of those species currently are listed in some category of risk in the Mexican Official Standard Norm NOM-059-2010 (González-Christen and Delfín-Alfonso 2016).

Veracruz has suffered from rampant deforestation over the past decades, causing wide areas of habitat loss and fragmentation as the land is transformed for agricultural and livestock uses (Challenger and Dirzo 2009; Mas *et al.* 2009; Sánchez-Colón *et al.* 2009; Gerez-Fernández and Pineda-López 2011; Von Thaden *et al.* 2020). According to the Instituto Nacional de Estadística y Geografia (INEGI 2015), only 18 % of the State remains covered by natural vegetation, and 64 % is secondary vegetation. The tropical rainforest is an ecosystem that particularly has been affected by deforestation, with less than 15 % of its original area remaining (Velázquez *et al.* 2002). For example, what once was an almost continuous area across the lowlands of Veracruz, large fragments of tropical rainforest can be found only in a few regions such as the Sierra de Zongolica mountain range, Las Choapas, Uxpanapa, and Los Tuxtlas (Gerez-Fernández and Pineda-López 2011). Los Tuxtlas region harbors the northernmost remnants of tropical rainforest in the Americas (Morrone 2019), and has been decreed as a biosphere reserve in the national system of protected areas to conserve biodiversity (Comisión Nacional de Áreas Naturales Protegidas; CONANP 2006).

Los Tuxtlas Biosphere Reserve (LTBR) possesses an exceptional biodiversity including a high species richness and endemicity, and includes a significant human population distributed in many widespread and small, local communities (González-Soriano *et al.* 1997; CONANP 2006; Gutiérrez-García and Ricker 2011). Furthermore, this region shows a high degree of habitat loss and fragmentation; over 50 % of the original vegetation has been transformed into areas for agriculture and livestock (Vega-Vela *et al.* 2018; Von Thaden *et al.* 2020). Habitat loss and fragmentation at large scales have profound negative effects on species richness and composition of terrestrial mammals in the tropical forests of southern Mexico. For instance, in Los Tuxtlas, Estrada *et al.* (1994) found that of 30 species detected in undisturbed forests, only 14 were found in disturbed forests, 11 in old second growths, and eight in young second-growth habitats. They also observed a negative relationship of species richness and individual species abundance concerning isolating distance of fragments, and a lower species richness in smaller fragments. Larger species were found only in larger fragments and in undisturbed forests. In the Lacandona tropical forest, Chiapas, Garmendia *et al.* (2013) found similar results; species richness increased with larger remnant habitat fragments, and large-sized species were only present in continuous forests.

At the landscape level, studies provide insights on the local conservation status of species, and provide a better understanding of patterns on the local effects of habitat loss and fragmentation on biodiversity (Bogoni *et al.* 2020). In this context, it is important to gather information on the status of the terrestrial mammal community to establish adequate conservation and management programs to promote a sustainable use of the natural resources by local inhabitants and to ensure the protection of biodiversity. This study aims to estimate the diversity, species richness, and composition of medium- and large-sized terrestrial mammals at the LTBR, and describe their activity patterns in a mosaic of forests and secondary vegetation landscapes (locally known as *acahuales*).

Material and Methods

Study Area. The LTBR is part of the Los Tuxtlas region, located in the central-southern part of Veracruz, Mexico (18° 13' and 18° 42' N, -94° 40' and -95° 20' W) with an area of 1,551 km^2 (Figure 1). A distinctive feature of the Los Tuxtlas region is its extensive fluvial network, part of the drainage basin of the Papaloapan River, which is one of the major basins in the country in terms of water volume discharged into the Gulf of Mexico (SEMARNAT 2016). From a biogeographic perspective, Los Tuxtlas is regarded as a district of the Veracruzana physiographic province, which stands out for its ecological and physiographic identity as well as for being an area of high endemism dominated by tropical rainforest, and it is related to the Chiapas Highlands physiographic province (Morrone 2019). The prevailing climate belongs to the group of warm and semi-warm, according to the Köppen classification (García 2004); annual precipitation ranges from 1,500 to 4,500 mm, and temperature ranges from 21.5 to 27.3 °C. Nine different vegetation types have been reported for the region, the most important being high- and medium-stature tropical rainforest, low-stature seasonally-flooded tropical rainfor-

est forest, and mountain cloud forest (Von Thaden *et al.* (2020). According to Von Thaden *et al.* (2020), 50 % of the LTBR is covered by pastures, 20 % by tropical rainforest, 9 % by riparian vegetation, 7 % by mountain cloud forest, 4.5 % by secondary vegetation derived from tropical rainforest, 4.5 % by agriculture, and other land-uses, each covering less than 5 % of the area.

Sampling protocol. We followed the community-based framework for camera-trap studies proposed by Lavariega *et al.* (2020). This approach involves engaging a number of actors, including government staff of the protected-area system, community monitors (local people previously trained in biodiversity studies), non-governmental organizations, and academic institutions, aiming to exchange knowledge and experience, make decisions on sampling design, participate in data collection, and discuss and communicate the results.

Maps on vegetation and land use, roads, human settlements, rivers, and elevation were used to select and locate sampling sites. A grid of 40 contiguous 9-km^2 cells was overlaid on maps using the geographic information system Quantum Geographic Informatic Systems (QGIS Development Team 2017). This cell size corresponded to the minimum home range size reported for jaguars in Mexico (Ceballos *et al.* 2016) — the largest species known to (historically) occur in the region (González-Christen and Delfín-Alfonso 2016). A total of 18 grid cells were selected within the reserve and its surrounding area of influence based on vegetation cover, accessibility, the experience of community monitors, and security.

One camera-trap station was set on each cell between late August and early December 2016. Each camera was securely fastened to a tree trunk 40 cm above the ground and approximately 1-2 m from an animal trail. Cameras were set to operate 24 hours a day, shooting photographs every 30 s. The geographic location (datum WGS84) and elevation of each camera trap were recorded using a GPS (Figure 1). Each camera trap was tested before leaving the site to confirm its correct operation. All camera traps were checked 30 days after installation to download photographs, replace batteries, and monitor operating conditions. Cameras operated for 62.5 days on average (minimum 58 and maximum 85 days) and were removed between late October 2016 and late January 2017.

Statistical analyses. The sampling effort was calculated as the total number of camera-traps set multiplied by the number of days sampled. All photographs of a given species captured within a 24-hour cycle were regarded as a single independent capture. In those cases where groups of individuals were photographed, each individual was counted as an independent capture (Pérez-Irineo and Santos-Moreno 2010). Community diversity was evaluated according to four components: species richness, camera-trapping rate, diversity indices, and trophic guilds (Magurran 2004). Species richness was estimated as the total number of species recorded by camera-traps. The camera-trapping rate was estimated as the total number of independent captures divided by the sampling effort, and multiplied by 100 (Jenks *et al.* 2011; Lira-Torres and Briones-Salas 2012).

We evaluated diversity in terms of Hill numbers, ${}^{q}D = (\sum^{S}_{(i=1)} p_i^{q})^{1/(1-q)}$), where: S is the number of species, p_i is the abundance of the ith species, and q is the order of diversity. The value of q controls the degree of influence of rare or common species on diversity (Jost 2006; Jost and González-Oreja 2012). Order-0 diversity is the effective species richness, regardless of the abundance of individual species; order-1 diversity takes into account the relative abundance of species without favoring any, and is equivalent to the exponential of Shannon's diversity index; order-2 diversity gives a greater weight to the most common species, and numerically is equivalent to the inverse of Simpson concentration index (Jost 2006; Gotelli and Chao 2013; Chao and Jost 2015). Diversity values can be interpreted as "effective number of species" or "equivalent species", and denoted the number of equally common or equally abundant species composing a hypothetical community (Jost 2006; Jost and González-Oreja 2012). Diversity indices were computed using the software SPADE (Chao *et al.* 2016). Order-0 diversity indices were calculated using the abundance-based coverage estimator; the maximum likelihood estimation was used for order-1 and order-2 diversity indices. The respective 95 % confidence intervals were constructed with the same software using the bootstrap method with 1,000 iterations, to evaluate the sampling uncertainty and allow comparisons between areas (see below).

All species recorded were assigned to the trophic categories considered by Pérez-Irineo and Santos-Moreno (2013) and González-Salazar *et al.* (2014), as follows: 1) Small-prey carnivores, species consuming prey smaller than 1 kg in body size; 2) Small- and medium-sized-prey carnivores, consuming prey whose body size ranges between 1 and 10 kg; 3) Large-prey carnivores, consuming prey larger than 10 kg; 4) Frugivores, consuming mostly fruits; 5) Folivores, consuming mostly leaves; 6) Granivores, consuming mostly seeds; 7) Scavengers, consuming mostly carrion; 8) Insectivores, consuming mostly insects; and 9) omnivores, species that show no preference for a particular food type.

The activity patterns of each species were classified according to the categories proposed by Cortés-Marcial and Briones-Salas (2014) and Buenrostro *et al.* (2020): diurnal (8:00 h-18:00 h), crepuscular (6:00 h-8:00 h and 18:00 h-20:00 h), and nocturnal (20:00 h-6:00 h). We quantified the daily activity levels of species with at least 18 independent captures, by fitting a smoothed circular Kernel density model (Meredith 2018; Sollmann 2018), using the package Overlap (Meredith 2018). Trapping rates were standardized by rescaling them to the total number of records, divided by sampling effort and multiplied by 100. Systematics and taxonomy followed Ramírez-Pulido *et al.* (2014). All photographs recorded were deposited in the official archives of the LTBR office at the *Comisión Nacional de Áreas Naturales Protegidas.*

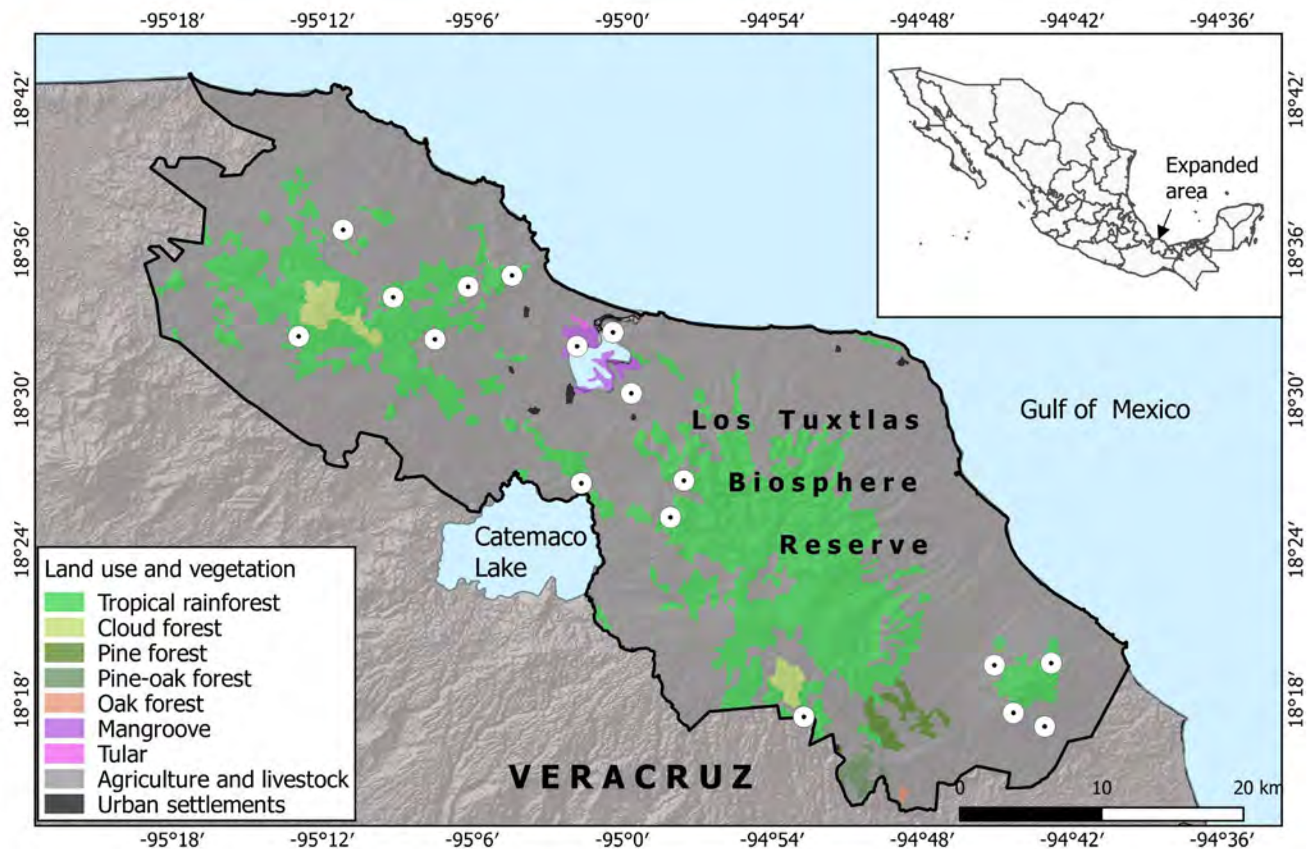

Figure 1. Location, land use, and vegetation types in Los Tuxtlas Biosphere Reserve (LTBR), Veracruz. The locations of the camera-trapping stations set for recording medium- and large-sized terrestrial mammals are indicated by white circles.

The diversity-index and trapping-rate values obtained for LTBR were compared with those obtained in other camera-trap studies conducted in tropical forests in southeast Mexico. Studies were selected based on the following criteria: use of digital camera traps, individuals of the same species recorded with a separation of at least 24 h were regarded as separate captures, the study provided information for the dry season of the year, and no baits were used for sampling. The selected studies were from: 1) Los Chimalapas, Oaxaca, 3,240 camera-days during the dry season in tropical rainforests and *acahuales* (Lira-Torres and Briones-Salas 2011); 2) Tolistoque, Oaxaca, 2,592 camera-days during the dry season in tropical dry forest (Cortés-Marcial and Briones-Salas 2014); 3) Los Petenes, Yucatán, 1,884 camera-days during the dry season in *peten* vegetation within mangrove swamps (Hernández-Pérez *et al.* 2015); 4) La Encrucijada, Chiapas, 2,700 camera-days during the dry season in mangrove swamps, medium-stature sub-evergreen tropical rainforests, tropical dry forests, *zapotonales* (*Pachira aquatica* swamps), and *tulares* (*Typha dominguensis* swamps; Hernández-Hernández *et al.* 2018); and 5) El Gavilán, Oaxaca, 7,235 camera-days, in tropical dry forest (Buenrostro *et al.* 2020). All the independent captures of species recorded during the dry season of the year in each of these studies were compiled. These data were used to calculate the Hill numbers and the respective 95 % confidence intervals (*CI*) with the software SPADE, to identify significant differences between the studies. The trapping rates of species recorded in at least three of the camera-trap study sites from southeast Mexico were compared.

Results

A total sampling effort of 1,110 camera-days yielded 308 independent captures of medium- and large-sized terrestrial mammals. Species richness included 13 species in 12 genera, 12 families, and six orders (Table 1). Two species accounted for 60 % of all records: *Cuniculus paca* and *Dasyprocta mexicana,* with 105 and 81 records, respectively. In contrast, seven other species were recorded less than ten times each (*Didelphis* spp., *Sciurus aureogaster*, *Eira barbara*, *Canis latrans*, *Mazama temama*, and *Tamandua mexicana*). *Leopardus pardalis* and *L. wiedii* were recorded only once. Most (65 % of total) captures were in the medium-stature tropical rainforest, 27 % in high-stature tropical forest, and 8 % in acahuales. *C. latrans*, *E. barbara*, *L. pardalis*, and *S. aureogaster* were recorded only in medium-stature tropical rainforests, *L. wiedii* was recorded only in *acahuales*, and the remaining species were recorded in at least two different vegetation types (Table 1). *C. paca* and *D. mexicana* had the highest trapping rates, with 9.46 and 7.30, respectively. Three other species (*Nasua narica*, *Dicotyles angulatus*, and *Dasypus novemcinctus*) showed intermediate trapping rates (1.71 - 3.06), and five others had low values (< 1.0); *L. pardalis* and *L. wiedii* occurred very rarely and had the lowest trapping rates (Figure 2).

Table 1. Number of records and trapping rate of medium- and large-sized terrestrial mammals at the LTBR. For each species, the trophic category, daily activity pattern, and conservation status as per the Mexican Official Standard NOM-059-SEMARNAT-2019 (NOM) and the International Union for the Conservation of Nature (IUCN) are provided. D = Diurnal, N = Nocturnal, C = Crepuscular, PE = Endangered, NT = Near Threatened, and DD = Data Deficient.

Order	Family	Species	Trophic guild	D	C	N	NOM	IUCN
Didelphimorphia	Didelphidae	*Didelphis* spp.	Omnivore		37.5	62.5		
Cingulata	Dasypodidae	*Dasypus novemcinctus*	Insectivore		26.3	73.7		
Pilosa	Myrmecophagidae	*Tamandua mexicana*	Insectivore	33.3		66.7	PE	
Rodentia	Sciuridae	*Sciurus aureogaster*	Frugivore-Folivore	37.5	62.5			
	Agutidae	*Dasyprocta mexicana*	Frugivore-Folivore	67.1	25.6	7.3		
	Cuniculidae	*Cuniculus paca*	Frugivore-Folivore	7.6	17.1	75.2		
Carnivora	Felidae	*Leopardus pardalis*	Carnivore of small and medium-sized vertebrates			100.0	PE	
		Leopardus wiedii	Carnivore of small-sized vertebrates			100.0	PE	NT
	Canidae	*Canis latrans*	Carnivore of small and medium-sized vertebrates	83.3	16.7			
	Mustelidae	*Eira barbara*	Omnivore	57.1	42.9		PE	
	Procyonidae	*Nasua narica*	Omnivore	63.6	15.2	21.2		
Artiodactyla	Tayassuidae	*Dicotyles angulatus*	Frugivore-Folivore	48.3	17.2	34.5		
	Cervidae	*Mazama temama*	Frugivore-Folivore	66.7	16.7	16.7		DD

Trophic guilds. We identified five trophic guilds: small-sized prey carnivores, small and medium-sized prey carnivores, frugivore-folivores, insectivores, and omnivores. The frugivore-folivore guild was the most species-rich, with five species, followed by the omnivore guild with three species (Table 1).

Activity patterns. *Dasyprocta mexicana*, *C. latrans*, and *M. americana* were largely diurnal (> 66.6 % of the records in this category), while *D. novemcinctus*, *T. mexicana*, *C. paca*, *L. pardalis*, and *L. wiedii*, were primarily nocturnal (> 66.6 % of the records in this category). Other species, such as *D. marsupialis*, were mainly nocturnal (62.5 %) but exhibited crepuscular activity (37.5 %). *S. aureogaster* was crepuscular (62.5 %) and diurnal (37.5 %). *E. barbara* was diurnal and crepuscular. Records of *N. narica* and *D. angulatus* were mostly diurnal, but also showed crepuscular and nocturnal activities (Table 1).

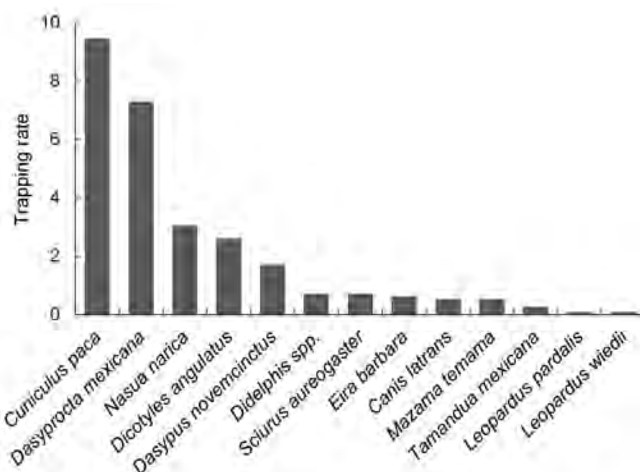

Figure 2. Trapping rates of medium- and large-sized terrestrial mammals recorded from camera-traps at the LTBR, calculated with the number of independent records, divided by the sampling effort and multiplied by 100. See Methods for details.

Five species had sufficient records (> 18) to fit Kernel models and describe their daily activity patterns. *D. mexicana* and *N. narica* were typically diurnal, with two activity peaks, one before noon and the second, most active period, at dusk. *D. angulatus* showed both diurnal and crepuscular activity, but its activity peaked before noon. *C. paca* and *D. novemcinctus* exhibited crepuscular and nocturnal activity; their activity peaked early in the evening and showed a secondary peak before dawn (Figure 3A-E). As for the other species, *C. latrans*, *E. barbara*, and *S. aureogaster* were recorded mainly in the daytime and, less frequently, at crepuscular or nocturnal periods; *Didelphis* spp. and *D. novemcinctus* were nocturnal and crepuscular, and *T. mexicana* was nocturnal and diurnal. *L. pardalis* and *L. wiedii* were both recorded only at night.

The highest species richness was recorded in Los Chimalapas (21 species) and La Encrucijada (18 species); intermediate species richness was recorded in Tolistoque (14 species) and Los Petenes and LTBR (13 species each). The lowest species richness was recorded at El Gavilán (Figure 4). Order-0 diversity was 13.80 effective species (CI: 13.10 to 22.70), order-1 diversity was 6.47 (CI: 5.79 to 7.15), and order-2 diversity was 4.69 (CI: 4.15 to 5.24). Order-0 diversity in the other study sites conducted in the dry season in tropical forests from southeastern Mexico was highest in Tolistoque (25.6), followed by Los Chimalapas (22.8), and La Encrucijada (20.0). Except for El Gavilán, where the lowest order-0 diversity (10.00) was recorded, the 95 % confidence intervals of the other localities overlap with each other. In contrast, when the relative species abundance was taken into account, El Gavilán showed the highest diversity value, with 9.39 effective species, followed by Los Chimalapas (8.22), La Encrucijada (7.97), and Tolistoque (6.22), and most of these differences were statistically significant, except for Los Chimalapas vs. La Encrucijada, and LTBR vs. Tolistoque,

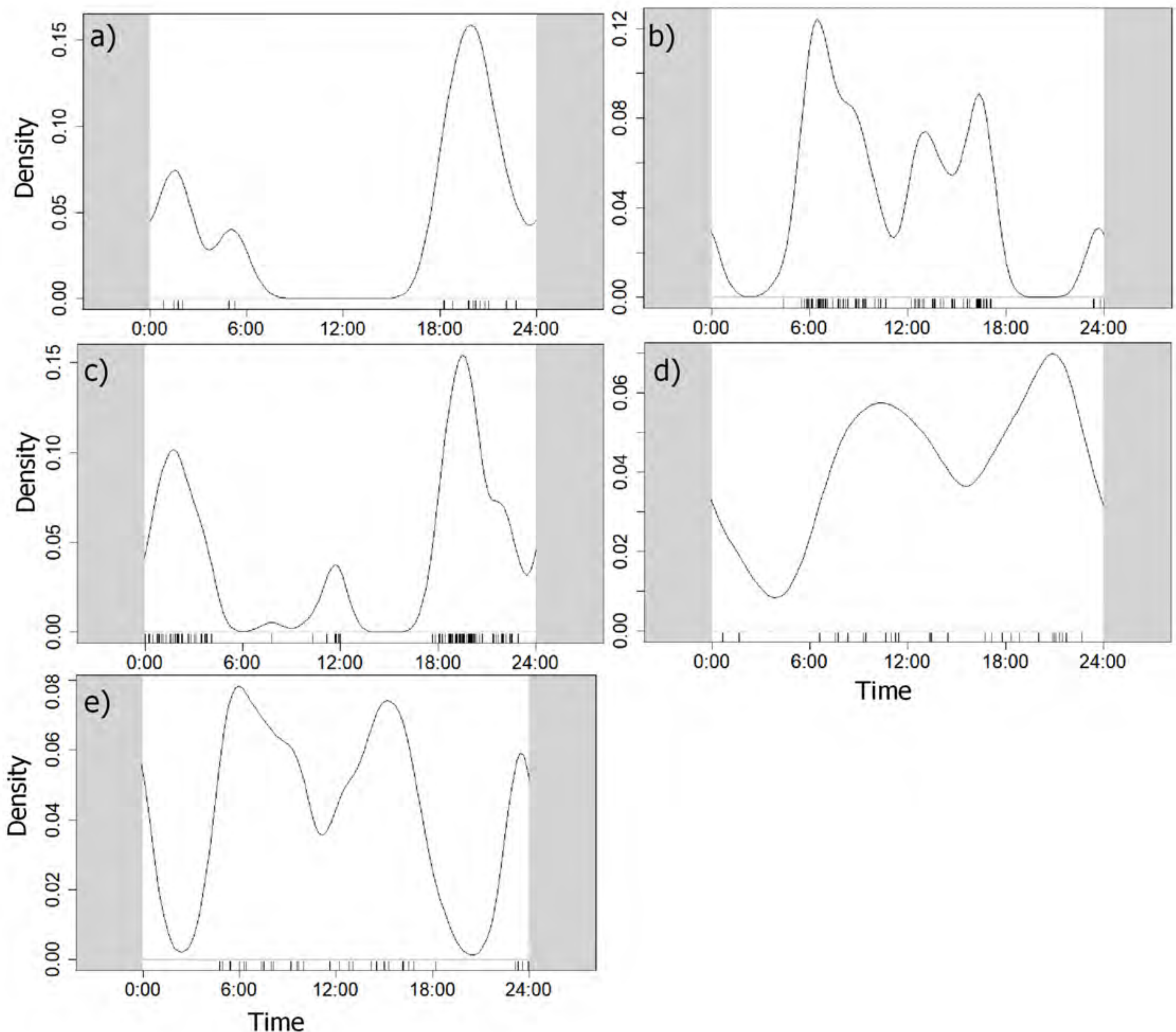

Figure 3. Circular models showing density daily activity patterns of medium- and large-sized terrestrial mammals at the LTBR. Armadillo, *Dasypus novemcinctus* (A); Mexican agouti, *Dasyprocta mexicana* (B); Agouti, *Cuniculus paca* (C); Collared peccary, *Dicotyles angulatus* (D), and Coati, *Nasua narica* (E).

whose 95 % CI overlapped. Finally, when the most common species were weighted more heavily, El Gavilán had the highest diversity (8.91 effective species), followed by La Encrucijada (6.12), Los Chimalapas (5.90), LTBR (4.69), Tolistoque (4.44), and Los Petenes (3.61). Only the order-2 diversity of El Gavilán was significantly different from those of the other areas; there were no significant differences between LTBR, Tolistoque, and Los Petenes, nor between Los Chimalapas and La Encrucijada (Figure 4).

Trapping rates. The comparison of trapping rates across the different study areas showed that the trapping rates of *C. paca* and *D. mexicana* were noticeably higher in the LTBR than in the other study sites. These rates almost doubled those observed at Los Chimalapas and Los Petenes — the areas where these species had the second-highest trapping rates. The trapping rates of *D. novemcinctus* and *T. mexicana* were also highest in the LTBR, although these were only slightly higher than in the other study sites. The trapping rates of *D. angulatus* and *S. aureogaster* in the LTBR were the second-highest among the other study sites (Figure 5).

Discussion

The 13 species recorded in our study had all been reported previously for the LTBR (Flores-Martínez *et al.* 2014; González-Christen and Coates 2019). González-Christen and Coates (2019) recently concluded, based on a review of the relevant literature, that 32 species of medium- and large-sized terrestrial mammals (*i.e.,* excluding mice, shrews, and bats) have been recorded in the region. Thus, our study recorded 41.6 % of all species in this group reported previously for LTBR.

The species richness recorded in our study at LTBR was lower than a previous study, which recorded 17 species using the same methods (Flores-Martínez *et al.* (2014). The species not recorded in our study were *Sciurus deppei*, *Herpailurus yagouaroundi*, *Galictis vittata*, *Procyon lotor*, *Conepatus semistriatus,* and *Philander opossum*. Further, Flores-Martínez *et al.* (2014) did not record *S. aureogaster* nor *M. temama*. The differences in the species number and identities between our study and Flores-Martínez *et al.* (2014) may result from the fact that the latter was conducted exclusively in the Los Tuxtlas Biological Station. It is likely that the Los Tuxtlas Biological Station serves as a refuge for medium and large-sized terrestrial mammals of the region and harbors a higher number of animal species than other areas in the region (Laurance *et al.* 2012; Rodríguez and Domínguez 2017). For example, some rare or cryptic mammal species were recorded just once or twice by Flores-Martínez *et al.* (2014; *e. g.*, *G. vittata* and *P. opossum*) or only occasionally (*e. g.*, *H. yagouaroundi* and *P. lotor*, eight records each). On the other hand, we were unable to record some of the species frequently recorded by Flores-Martínez *et al.* (2014), such as *S. deppei* (188 records) and *C. semistriatus* (27 records). *S. aureogaster* and *M. temama*, which were not recorded by Flores-Martínez *et al.* (2014), occurred in low abundance and were recorded only occasionally in our study. It is important to highlight the absence of large-sized terrestrial mammals such as *Panthera onca*, *Puma concolor*, *Odocoileus virginianus*, and *Tayassu pecari,* which are known to (historically) occur in the region (Estrada *et al.* 1994; Dirzo and Mendoza 2007).

Cuniculus paca, *D. mexicana*, *N. narica*, and *D. angulatus* were the species with the highest trapping rate in our study (Figure 2). This is consistent with previous studies documenting *D. mexicana*, *N. narica*, and *C. paca* as the species most frequently recorded in this region (Flores-Martínez *et al.* 2014). Further, in other regions of the State of Veracruz, the species showing the highest trapping rates were *D. marsupialis*, *D. novemcinctus*, *C. paca*, and *D. mexicana* (Gallina and González-Romero 2018). The low trapping rates of another species observed in our study can be explained by the fact that species such as *M. temama* and *L. wiedii* have become locally rare due to habitat loss and fragmentation. These species have shown higher trapping rates in other regions that are better conserved or have suffered less anthropogenic impact (Muñoz-Vazquez and Gallina-Tessaro 2016; Pérez-Irineo and Santos-Moreno 2016 a, b). Other species, such as *L. pardalis*, require sufficient prey availability and areas for dispersal (Pérez-Irineo and Santos-Moreno 2014).

Diversity indices. The estimated order-0 diversity (species richness) for LTBR was slightly above the observed number of species (13.99 vs. 13 species, respectively). Order-0 diversity for LTBR was lower compared to values reported for other study sites in southeast Mexico; it was only higher than order-0 diversity at El Gavilán. Although the lowest order-0 diversity was recorded at El Gavilán, this

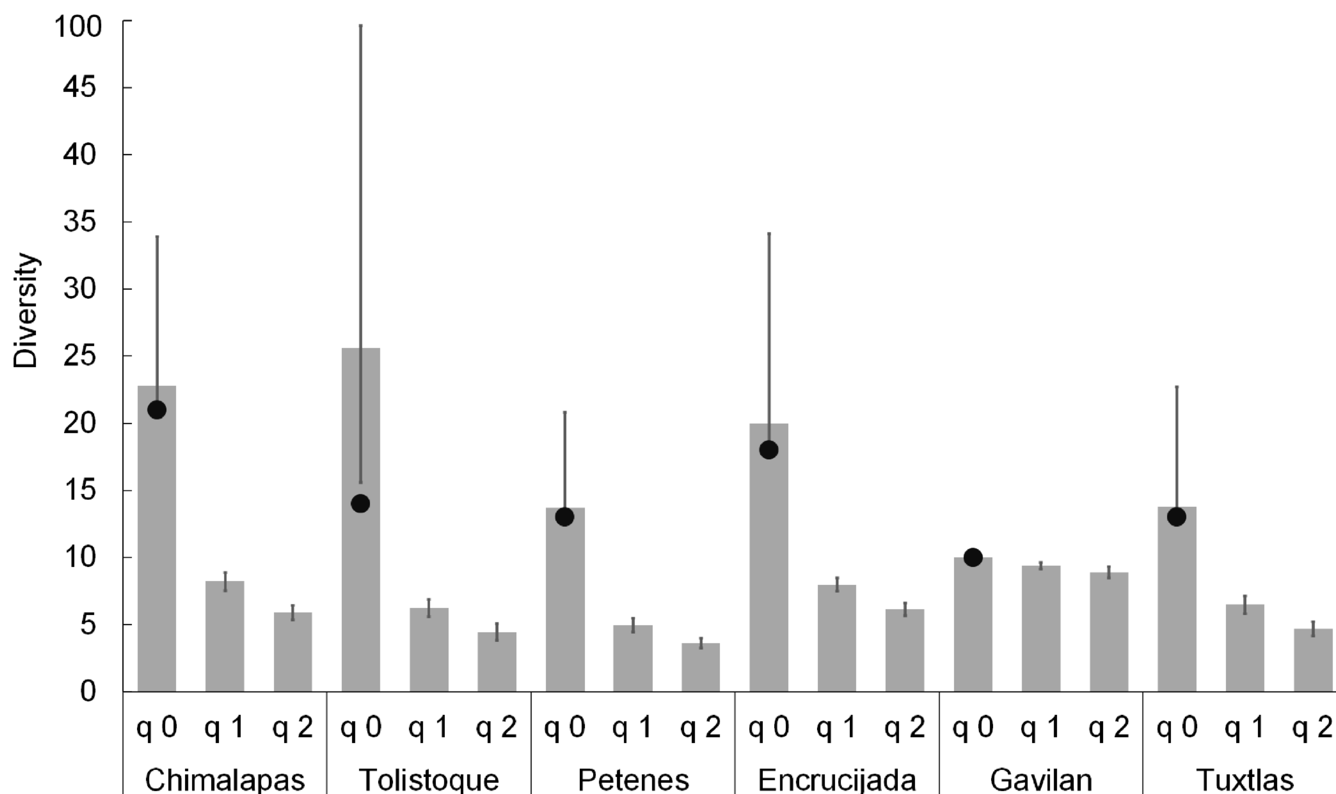

Figure 4. Estimated orders of diversity values (grey bars) for camera-trapping studies conducted during the dry season in different tropical forests located in southeast Mexico. Orders of diversity for the Hill numbers: only species richness (q0), taking in count the relative abundance of species (q1), and a greater weight to the most common species (q2). Black dots indicate the observed species richness; 95% confidence intervals are indicated by vertical lines.

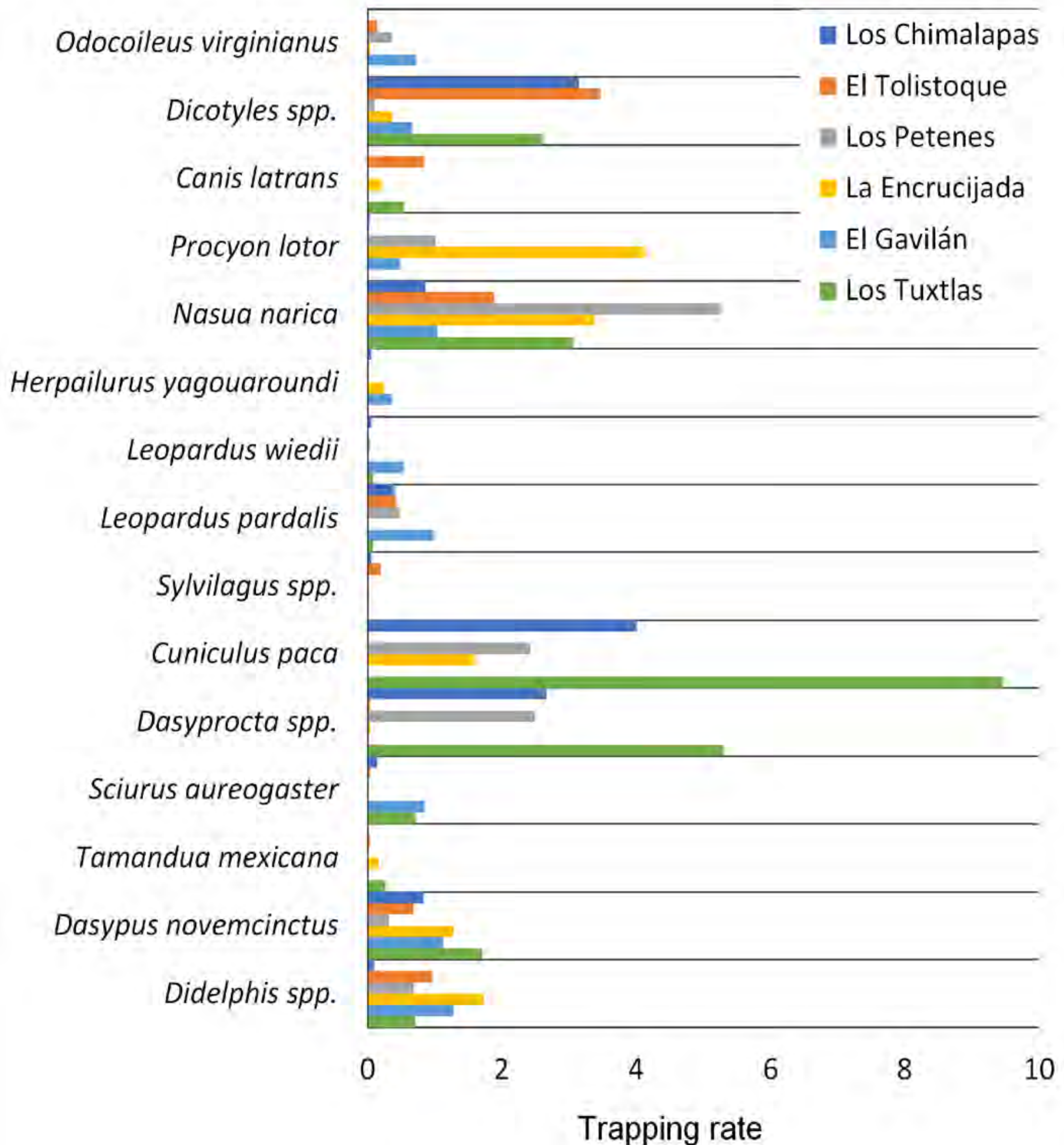

Figure 5. Frequency of trapping rates for medium- and large-sized terrestrial mammals recorded at six study sites located in southeastern Mexico. See Methods for details.

site had higher values for the other diversity values, which include the relative abundance of species into account (order 1), or place a higher weight on the dominant species (order 2). The high order-1 and order-2 diversity values at El Gavilán reflect the existence of a more diverse community with a more even distribution of abundances (Moreno *et al.* 2011). For instance, the difference in recording frequency between the species most and least frequently recorded at El Gavilán was 28.9 %, whereas this difference was 0.4 % at Los Chimalapas, 1.1 % at Tolistoque, 1.0 % at Los Petenes, 0.5 % at La Encrucijada, and 0.9 % at Los Tuxtlas (Figure 4). The protection status of El Gavilán as an area voluntarily dedicated to conservation might have contributed to these high diversity values (Buenrostro-Silva *et al.* 2020).

Los Chimalapas and La Encrucijada study sites also showed high diversity values. Although Los Chimalapas is not included in any formal protection scheme, this area comprises a vast extension of almost continuous tropical rainfor-

est of difficult access, and holds a low human population density, which favors the persistence of medium and large-sized terrestrial mammals (Lira-Torres and Briones-Salas 2011). In comparison, La Encrucijada is a federally protected area; its protection status and diverse vegetation cover, ranging from mangrove swamps to deciduous tropical forests, favors the presence, abundance, and diversity of mid-sized and large mammals (Hernández-Hernández *et al*. 2018).

The lowest order-1 and order-2 diversity indices were recorded at El Tolistoque and Los Petenes study sites (Figure 4). El Tolistoque is an area that, out of a social initiative, has been voluntarily dedicated to conservation, thus favoring the preservation of habitats and persistence of the local fauna (Cortés-Marcial and Briones-Salas 2014). Nevertheless, hunting is a common practice around this area, thus undermining its aim of conserving biodiversity (Cortés-Marcial 2009). Los Petenes are composed mainly of *Petenes*, which are plant formations that are key for mammals in the Yucatán Peninsula because they supply water and food, and which might be limited in the contiguous tropical dry forest. However, their restricted area might explain the lack of records and the low trapping rate of medium and large-sized terrestrial mammals that are common in the Yucatan Peninsula (Hernández-Pérez *et al.* 2015). Although LTBR is a federally protected area, it has historically undergone deforestation resulting in habitat loss and fragmentation, undermining the diversity of medium- and large-sized terrestrial mammals (Dirzo and Mendoza 2007), which was evident in the recorded intermediate values of the order-1 and order-2 diversity indices (Figure 4).

Further, Ruiz-Gutiérrez *et al.* (2020) studied medium and large-sized terrestrial mammals in eight landscapes on the Sierra Madre del Sur mountain range in the State of Guerrero, Mexico. They estimated species richness ranging from 13 to 19 species, and an order-1 diversity index from 6 to 12 effective species, for the eight landscapes. These ranges are consistent with those reported in the studies reviewed herein. Ruiz-Gutiérrez *et al.* (2020) found a positive relationship between the order-1 diversity index and variations in elevation and ecological integrity of the landscape. These findings agree with the close relationship found by Galindo-Aguilar *et al*. (pers. comm.) between the ecological integrity of the landscape and defaunation rates of medium- and large-sized terrestrial mammals in Mexican tropical forests. Both analyses explain why well-preserved areas such as Los Chimalapas and La Encrucijada show higher diversity values relative to more degraded sites.

Activity patterns. The activity patterns exhibited by five species from the LTBR are consistent with those reported elsewhere. A diurnal activity pattern has been described for *N. narica* and *D. mexicana* (Lira-Torres and Briones-Salas 2011; Hernández-SaintMartín *et al.* 2013; Hernández-Hernández *et al.* 2018; Buenrostro *et al.* 2020), a nocturnal and crepuscular pattern for *D. novemcinctus* and *C. paca* (Harmsen *et al.* 2011; Lira-Torres and Briones-Salas 2011; Cortés-Marcial and Briones-Salas 2014; Arroyo-Arce *et al.* 2017; Hernández-Hernández *et al.* 2018), and diurnal and crepuscular with some nocturnal activity for *Dicotyles* spp. (Harmsen *et al.* 2011; Lira-Torres and Briones-Salas 2011; Hernández-SaintMartín *et al.* 2013; Cortés-Marcial and Briones-Salas 2014; Buenrostro *et al.* 2020). These results show that species retain their overall circadian rhythm regardless of the type of habitat or location and suggest a conservatism of this trait (de Oliveira *et al.* 2016). However, other detailed studies have shown that species can vary their activity patterns in response to environmental changes, such as natural vs. artificial lighting conditions (Harmsen *et al.* 2011; Michalski and Norris 2011; Mendes *et al.* 2020) or the landscape configuration (Norris *et al.* 2010). Further studies across the landscapes within a disturbance gradient in Mexican forests are needed to investigate potential impacts on circadian rhythms of terrestrial mammals.

Conservation implications. Neotropical forests are ecosystems harboring a high number of species (Sánchez-Colón *et al.* 2009; Reynoso *et al.* 2017). However, these ecosystems have suffered from rampant deforestation over the last decades with a significant negative impact on biodiversity. Several studies have shown the adverse effects on vertebrate diversity, including terrestrial mammals (Dirzo and Mendoza 2007; Laurance *et al*. 2012). Protected areas and biological field stations have served as refuges for numerous plant and animal species (Laurance *et al*. 2012; Flores-Martínez *et al.* 2014; Rodríguez and Domínguez 2017). Los Tuxtlas Biological Station has been the focus of an impressive research effect on studies of biodiversity for several decades (Estrada *et al.* 1994; González-Soriano *et al.* 1997; Reynoso *et al.* 2017; Gallina and González-Romero 2018; González-Christen and Coates 2019). Further studies aimed at inventorying the flora and fauna of Mexican tropical forests are of high relevance to produce basic information on their conservation status.

The loss of medium- and large-sized terrestrial mammals has major negative consequences on ecosystem dynamics as it might lead to an increase of small mammal population densities with a potential change in the rates of seed predation and seedling recruitment in tropical forest (Sánchez-Cordero and Fleming 1993; Kurten 2013; Galetti *et al.* 2015; Carreira *et al.* 2020). In the case of the LTBR, we were unable to record large-sized mammals such as *P. concolor*, *P. onca*, or *T. pecari*. Restoring the continuity of this tropical forest is necessary to facilitate the movement of individuals of these species away from areas inhabited by human populations.

Our study showed that protected areas created as a result of community-based (Los Chimalapas, El Tolistoque, and El Gavilán) or government (La Encrucijada, Los Petenes, and LTBR) initiatives are key for conserving medium- and large-sized terrestrial mammals. Community-based conservation initiatives are promoted by local communities with support from non-governmental organizations, aiming to conserve biodiversity by adopting a respectful and inclusive approach (Briones-Salas *et al.* 2016). Therefore, community-based conservation initiatives should be

encouraged and supported to further advance biodiversity conservation in southern Mexico.

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Sometimes I see spots: patterns of abundance and distribution of the bobcat (*Lynx rufus*) in different regions of México

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Bobcats (*Lynx rufus*) are distributed throughout North America, but bobcat densities have been well-documented primarily only for the United States. The highest known density of this species is in the southern United States, and from there their density decreases northward to southern Canada. Despite the fact that México contains about 35 % of the species range, there are no data on their abundance or density in México. The objectives of this study were to document the density of bobcats from northwestern to southern México and to understand the abundance patterns of this species. Camera traps were used in combination with capture-recapture models to estimate densities. Using the MaxEnt ecological niche model, the potential distribution of the bobcat was obtained. With an effort of 2,070 camera trap days, we obtained 41 bobcat photographs in five localities from 2005 to 2007. Our estimated bobcat densities varied from 0.05 to 0.53 bobcat/km^2. Using MaxEnt, we estimated the available habitat in each site and extrapolated the density data to obtain a range of bobcat population estimates per site. The areas estimated were between 1,861 to 16,663 km^2, with a population from 592 to 2,161 bobcats. Bobcat density in México is highest in the north and decreases to the south of its range. Probably the optimal habitat for this species is found in the southern United States and northern México where the highest densities of its entire distribution occur.

El lince (*Lynx rufus*) se distribuye a lo largo de América del Norte. Sin embargo, sus densidades solo han sido documentadas principalmente en los Estados Unidos. La densidad más alta conocida para esta especie se encuentra en el sur de los Estados Unidos. Desde allí su densidad disminuye hacia el norte hasta el sur de Canadá. A pesar de que en México se estima que se encuentra el 35% de su distribución no hay datos sobre su abundancia ni densidad. Los objetivos de este proyecto fueron documentar las densidades del lince desde el noroeste hasta el sur de México y comprender los patrones de abundancia de esta especie. Se utilizaron trampas cámaras en combinación de modelos de captura-recaptura para estimar las densidades. Utilizando el modelo de nicho ecológico MaxEnt, obtuvimos la distribución potencial del lince. Con un esfuerzo de 2,070 días trampa obtuvimos 41 fotografías en 5 localidades de 2005 a 2007. Nuestras densidades estimadas de lince variaron de 0.05 a 0.53 linces/km^2. Mediante el uso de MaxEnt estimamos el hábitat disponible en cada sitio y extrapolamos los datos de densidad para obtener un rango sobre la estimación del tamaño de la población del lince por sitio. Las áreas estimadas variaron entre los 1,861 a 16,663 km^2 con poblaciones de 592 a 2,161 linces. La densidad de linces en México es más alta en el norte y disminuye hacia el sur de su distribución. Probablemente el hábitat óptimo para esta especie se encuentra en el sur de los Estados Unidos y el norte de México, donde se encuentra las densidades más altas de toda su distribución.

Keywords: Abundance; bobcat; densities; *Lynx rufus*; México.

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Introduction

The bobcat (*Lynx rufus*) is distributed from southern Canada through the United States of America (U.S.A.) to southern México. This felid is present in about 80 % of the Mexican territory, with records from 27 of México's 32 states. The bobcat inhabits many ecosystems including desert, mesquite-grassland, thorn forest, tropical deciduous forest, and pine-oak forest (Hall 1981; Barcenas and Romero 2014). Estimating densities and population sizes is crucial to understanding the ecology and conservation needs of wildlife. Bobcat densities reported for the U.S.A. vary from 0.05 ind/km^2 in southeastern Idaho to 0.58 ind/km^2 in South Carolina (Rolley 1985; Heilbrun *et al.* 2003). In México, the bobcat is considered the most abundant wild felid (Leopold 1959), and about 35 % of the bobcat's distribution lies in México (Hall 1981). However, there is no published information available about densities of this wild cat in México. The aim of this study was to document bobcat density throughout México to determine whether a similar abundance pattern occurs there as in the U.S. We hypothesize that bobcat densities will vary along its distribution, with higher densities in northern México and lower densities in southern México. The results of this study will be useful to inform conservation and management policy and will help secure the long-term survival and sustainable management of the species.

Methods

This study was carried out at six sites in México. Sites are listed in a north-south gradient, as shown in Figure 1. 1) Sierra Seri, Sonora, in the Sonoran Desert, is characterized by columnar cacti, microphyllous shrub, succulent

scrub, scarce annual precipitation of around 400 mm, and extreme temperatures with a very arid climate. Average temperature is 21 ºC and summer temperatures may reach 48 °C. Elevation is 100 to 300 masl. 2) Janos, Chihuahua, is covered by mesquite-grassland and small patches of riparian vegetation, annual precipitation is 500 mm, and climate is arid-dry (García 1981). Average temperature is 16 ºC and elevation is 900 to 1,200 masl. 3) San Ignacio, Sinaloa, on the pacific coastal plain, is characterized by tropical dry forest, with an annual temperature of 21 to 24 °C, dry climate, and elevation 0 to 100 masl. 4) Monte Grande, in Sierra Fria, Aguascalientes, is characterized by a mixed forest dominated by *Quercus* spp. and *Arctostaphylos pungens*. Annual temperature is 11 to 18 °C, climate is humid temperate, and elevation is 1,800 to 2,200 masl. 5) San Miguel Topilejo, México City, is a region covered by pine-oak and pine-tufted grassland, with an annual temperature of 4.5 to 11 °C, precipitation averaging 1,200 mm, and elevation 2,700 to 3,000 masl. 6) Acatlán de Osorio, Puebla, is characterized by tropical thorn forest mixed with disturbed tropical deciduous forest, and elevation is 1,000-1,300 masl. The climate is arid-dry, with an average annual rainfall of 652 mm and annual temperature of 22 °C (García 1981).

Visits to each site were carried out between 2005 and 2007. The first visit was to confirm the presence of bobcats at each site by recording tracks and feces. The next two visits were carried out in the dry season (March-June) and in the rainy season (July-October). Camera traps were active for 15 to 20 days during each site visit. Sinaloa and Puebla were visited only once each during the dry season. We added these two sites at the end of the study to increase representation of the southernmost extreme of the species' range and to sample two additional habitats: tropical deciduous forest and tropical thorn forest mixed with tropical deciduous forest. At each site, 20 camera-trap stations were activated for a period of 15 to 20 days per season (wet and dry), for a total of 30 to 40 days at each site (except Sinaloa and Puebla). The cameras were deployed at one site and then moved sequentially to all other sites. Twelve CamTrakker® Ranger and 8 Stealth Cam® TM analog 35 mm cameras were used; each had a white flash and used film with only 36 images per roll. Camera traps were checked every 4 to 8 days to replace film and batteries. Half of the trapping stations were set with double cameras (to capture both flanks of the animals) and half were set with single cameras. Trapping stations were 800 to 1,000 m apart, thereby covering an area of approximately 10 km^2 at each site.

For individual identification of the bobcats, we used a combination of distinguishing characters including the patterns of rosettes, spots, and stripes on flanks, banding patterns of tails, marks on their faces, and sex, as recommended in Heilbrun *et al.* (2003). We estimated densities using the number of photographed and re-photographed individual bobcats using the software CAPTURE (Otis *et al.* 1978). CAPTURE estimates the size of monitored populations through the following steps: 1) tests that capture

Figure 1. Location of the 6 sites where bobcat density was estimated. Gray shading represents the known bobcat distribution in México (Hall, 1981).

and recapture assumptions were not violated, including whether the monitored population behaved as a closed population; 2) checks the capture history (data) with various statistical tests (null model, catch heterogeneity model, catch response model, temporal variation model in catch probability, and the combination of all these models), to determine which model is the most appropriate for those data; and 3) estimates the probability of capture and the population size or the absolute abundance (N), with standard error and a confidence interval. The size of the effective sampling area was calculated by two methods: the first method considered a circular buffer around each camera trap station, with radius of half the mean maximum distance moved (1/2 MMDM) among multiple captures of individual bobcats during the sampling period (Wilson and Anderson 1985); the second method considered a circular buffer around each camera trap site but the radius was the mean maximum distance moved (MMDM) among multiple captures of individual bobcats during the sample period (Soisalo and Cavalcanti 2006).

In combination with the density estimates obtained at each site and MaxEnt (Phillips *et al.* 2006), the potential habitat available was calculated for each site. First, an ecological model for the entire bobcat range in México was constructed with 530 GBIF bobcat records plus our own observations, and 23 continuous variables (19 of worldclim, plus vegetation of México, slope, topography, and elevation). The cells were 0.01 km^2 for each grid. We used 50 % of the records to construct the model and 50 % were used for validation of the model. Also, the proportional contribution of each variable was calculated with a Pearson correlation analysis using program R to calculate their weight in the bobcat distribution model (R Core TEAM 2015). Using the best model, the size of each polygon, and estimated bobcat density, we predicted the number of bobcats present in each studied area.

Results

With an effort of 2,070 camera trap days, we obtained 41 bobcat photographs. Bobcats, like other spotted or striped cats, can be identified individually by their coat pattern (Figure 4). Density analysis was made using 35 photographs; six photographs were only a tail or a foot and thus not useful to identify individuals (Table 1). Densities varied from 0.053 to 0.523 bobcat/km^2 in the rainy season to 0.174 to 0.536 bobcats/km^2 in the dry season (Table 2). The most robust model in most cases was model heterogeneity (M_h; Table 1). Janos, Chihuahua, had the highest reported density at 0.536 bobcat/km^2, and San Miguel Topilejo, Distrito Federal, had the lowest reported density at 0.053 bobcats/km^2 (Table 2). In Monte Grande, Sierra Fria, Aguascalientes, in 540 trap-days we did not obtain any bobcat records. Instead, we obtained 21 photographs of mountain lions (*P. concolor*) and the first record of ocelot (*Leopardus pardalis*) for the state of Aguascalientes (Barcenas and Medellin 2010).

The MaxEnt ecological niche model obtained for all the distribution is depicted Figure 2. The model showed an area under the curve (AUC) of 0.843. Correlations were calculated using cor.test in R package stats (R Core TEAM 2015) and show that five variables contributed approximately 60 % of the total variance: vegetation type (21.6 %), precipitation of the driest trimester (11.8 %), annual range in temperature seasonality (11.1 %), isothermality (8.7 %), and mean temperature of the coldest quarter 5.7 %; www.worldclim.org). After the ecological niche was defined, the calculated abundances were extrapolated to estimate the population size in each area (Figure 3). The largest area estimated by MaxEnt was San Miguel Topilejo with 16,663 km^2 and 883 to 2,066 bobcats, and the smallest area but with the highest density was in San Ignacio, Sinaloa, with only 1,861 km^2 and an estimated population of 592 to 890 bobcats (Table 3).

Discussion and Conclusions

The densities reported in this study suggest the density patterns in the general bobcat range (Table 4). We speculate that the highest densities of this species are found in northern México, close to the middle of the species range, and densities decline towards the southern end of the species range. This can be related to the optimal ecological niche hypothesis (Hutchinson 1958), in this case apparently located in the area between northern México and the southern U.S.A. The México-U.S.A. border wall will very likely disrupt bobcat dispersal and movements, because the gap between the bollards (steel beams) is 100 mm and the bobcat zygomatic breadth is 84.2 to 107.1 mm (Hall 1981). Only the smallest bobcats would be able to squeeze between bollards, and adult bobcats probably will not be able to cross the border wall. Given our result of the high density in northern México, this restriction in movements will reduce bobcat genetic flow between México and the U.S.A.

Prior to this study, there were no data on bobcat density or abundance in México. In the U.S., bobcat densities vary from 0.05 to 0.58 bobcat/km^2. Our data from five Mexican locations show a very similar density variation, between 0.05 to 0.53 bobcat/km^2. In fact, our highest density esti-

Table 1. Number of captures and recaptures of bobcats identified in each season and site, the best-fit model selected by CAPTURE, and the probability of capture in each sample. M (h) is the heterogeneity model, M (bh) is the behavior/heterogeneity model, and M(0) is the null model.

Site	Season	Model selected	Estimated probability of capture	Bobcats_ID	Capture/recaptures
Janos, Chihuahua	Rainy	M (h) 1.00	0.0771	Chi_1	4
				Chi_2	2
				Chi_3	1
	Dry	M (h) 1.00	0.0606	Chi_1	3
				Chi_4	1
				Chi_5	1
Sierra Seri, Sonora	Rainy	M (bh) 1.00	0.1000	Son_1	2
				Son_2	1
	Dry	M (h) 1.00	0.0426	Son_3	4
				Son_4	1
				Son_5	1
				Son_6	1
Topilejo, D. F.	Rainy	M (h) 1.00	0.1250	Top_1	2
				Top_2	1
				Top_3	1
Acatlán, Puebla	Rainy	M (h) 1.00	0.1333	Pue_1	3
San Ignacio, Sinaloa	Rainy	M (0) 1.00	0.1074	Sin_1	4
				Sin_2	1
				Sin_3	1

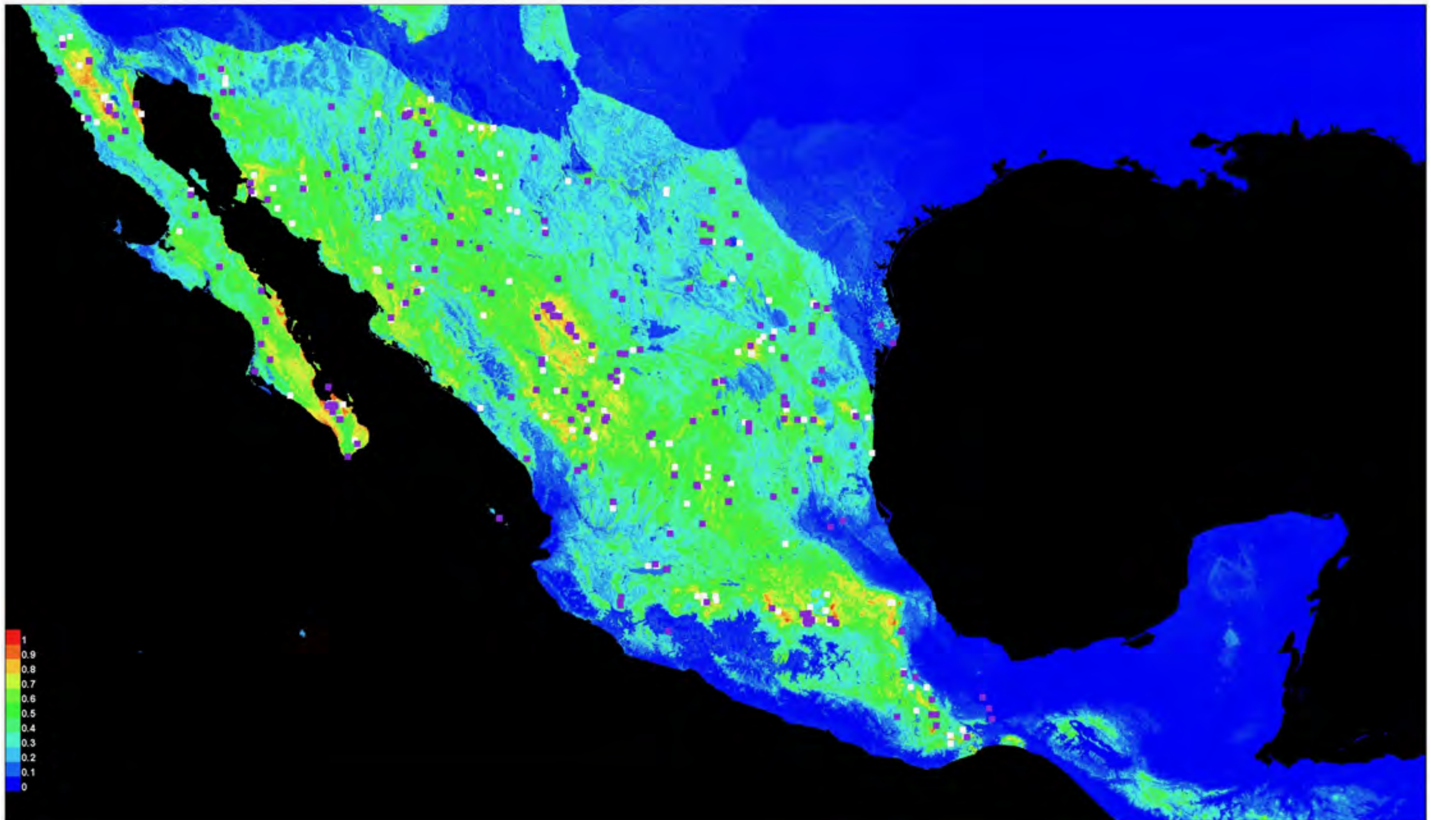

Figure 2. Potential distribution of bobcats in Mexico as predicted by Maxent model based on 530 GBIF records plus our own observations. White squares represent 50 % of the 530 GBIF records and were used to build the model, and purple squares are the other 50 % that were used to validate the model.

mates in Janos, Chihuahua, in dry (0.309 to 0.536 bobcat/km^2) and rainy (0.303 to 0.523 bobcat/km^2) season, and in San Ignacio, Sinaloa, in wet season (0.478 bobcat/km^2) are within the five maximum densities reported for bobcats in the U.S. and second only to the density reported from South Carolina (0.318 to 0.478 bobcat/km^2; Marshall 1969). It is important to highlight that the highest bobcat densities reported in the southern U.S.A. are similar to the highest densities reported from northern México, indicating likely suitable conditions for this species and providing robustness to our data (Table 4).

San Ignacio, Sinaloa, is a tropical dry forest in the coastal plain of the Mexican Pacific. Before this study, there was only one report of the presence of bobcat in this habitat (Lopez-Gonzalez *et al.* 1998) and our results show that this region supports one of the highest densities reported in México for the species (0.318 to 0.478 bobcat/km^2), contrasting with other studies (Jones and Smith 1979; Larrucea *et al.* 2007) that bobcats prefer sites with rocks and accidental orography (Larivière and Walton 1997).

We also found a very low density (0.05 to 0.12 bobcat/km^2) in Topilejo Distrito Federal (now state Ciudad de México) that is comparable to those obtained in Idaho (0.05 to 0.09 bobcat/km^2). Idaho and Topilejo are close to the northern and southern extremes of the species range. However, it is striking that Topilejo is less than 10 km from one of the largest cities in the world (México City), so it is very surprising that bobcats can continue to survive and

Table 2. Bobcat (*Lynx rufus*) density estimates by site and season in México.

Site	Season	Density	Effective area (km²)		Density (bobcats/km²)	
		CAPTURE	Wilson and Anderson (1985)	Soisalo and Cavalcanti (2006)	Wilson and Anderson (1985)	Soisalo and Cavalcanti (2006)
Janos, Chihuahua	Rainy	5 (se ± 1.96)	9,558	16,448	0.523 (se ± 0.205)	0.303 (se ± 0.119)
	Dry	5 (se ± 1.99)	9,328	16,156	0.536 (se ± 0.123)	0.309 (se ± 0.213)
Sierra Seri, Sonora	Rainy	2 (se ± 0.0004)	13,858	26,316	0.144 (se ± 0.00002)	0.075 (se ± 0.00001)
	Dry	4 (se ± 2.51)	12,515	22,932	0.319 (se ± 0.200)	0.174 (se ± 0.109)
Topilejo, D. F.	Rainy	3 (se ± 1.38)	24,070	56,564	0.124 (se ± 0.057)	0.053 (se ± 0.024)
San Ignacio, Sinaloa	Rainy	3 (se ± 1.07)	6,575	11,428	0.478 (se ± 0.162)	0.318 (se ± 0.093)
Acatlán, Puebla	Rainy	1 (se ± 0.00)	8,160	15,289	0.122 (se ± 0.000)	0.065 (se ± 0.000)

Table 3. Minimum and maximum bobcat population size in each area as estimated by MaxEnt.

Site	*Density estimating bobcats /km²		Estimation area by MaxEnt (km²)	Number of bobcats in the estimate area	
	Minimum	Maximum		Minimum	Maximum
Janos, Chihuahua	0.309 (se ± 0.213)	0.536 (se ± 0.123)	4,033	1,246 (se ± 859)	2,161 (se ± 496)
Sierra Seri, Sonora	0.174 (se ± 0.109)	0.319 (se ± 0.200)	8,849	1,540 (se ± 964)	2,823 (se ± 1,779)
San Ignacio, Sinaloa	0.318 (se ± 0.093)	0.478 (se ± 0.162)	1,861	592 (se ± 173)	890 (se ± 144)
San Miguel Topilejo, D.F.	0.053 (se ± 0.024)	0.124 (se ± 0.057)	16,663	883 (se ± 399)	2,066 (se ± 949)
Acatlán de Osorio, Puebla	0.065 (se ± 0.000)	0.122 (se ± 0.000)	10,446	679 (se ± 0.00)	1,274 (se ± 0.00)

are still present in densities comparable to other regions in North America (Table 4).

In some sites and seasons, we did not record any bobcat photographs. For example, there were no records from Topilejo during the dry season. One possible cause may be the persistent and abundant presence of illegal hunters during the monitoring period; six of our camera traps were stolen. We also did not document any bobcats in the Sierra Fria, Aguascalientes. One possible explanation for its absence is the high relative abundance of mountain lions, with 21 photographs corresponding to at least three different individuals in a very small area (around 10 km²) and few days. At sites where mountain lions are abundant, bobcats tend to be less abundant and vice versa (Leopold 1959). In addition, other studies show that mountain lions can prey on bobcats; in some areas the bobcat can be part of the mountain lion diet in occurrence of 1.6 to 3.0 % (Hass 2009; Lindzey 1987).

Our study sets the stage for the first time for the authorities of México to make decisions and implement policies that are scientifically informed. The bobcat is included in CITES Appendix II (CITES 2021), and exports of bobcat parts and products is legal if non-detriment finding reports are filed. There is an important international trade in bobcat pelts from the U.S. and México issues legal hunting permits every year. Bobcats are a surprisingly resilient species, surviving in areas very close to México City, and its conservation and science-based management can become an important example of conservation success.

One of the most important features of our study is that we were able to compare our data across a great spatiotemporal scale containing drastically different habitats used by bobcats. In México, the Ley General del Equilibrio Ecologico y la Protección al Ambiente (LGEEPA) is the main legal instrument for conservation, recovery, and preservation of natural resources and for sustainable use of natural

Table 4. Comparison of bobcat abundance estimates from the United States and those reported in this study.

Site	Bobcats/ km²	Methods	References
South Carolina	0.58	Telemetry	Marshall 1969
Northeastern California	0.5	Telemetry	Zezulak 1998
Welder Wildlife Foundation Refuge in southern Texas	0.43	Camera trap	Heilbrun *et al.* 2003
Coast Range, California	0.39	Camera trap	Larrucea *et al.* 2007
Reservation Creek in California	0.35	Camera trap	Larrucea *et al.* 2007
San Ignacio, Sinaloa (dry)	0.318-0.478*	Camera trap	This study
Janos, Chihuahua (dry)	0.309-0.536*	Camera trap	This study
Janos, Chihuahua (wet)	0.303-0.523*	Camera trap	This study
Sacramento Valley in California	0.27	Camera trap	Larrucea *et al.* 2007
Southeastern Illinois	0.27-34	Telemetry	Nielsen and Woolf 2001
Three Bar Wildlife in Arizona	0.24-0.27	Capture/Recapture	Jones and Smith 1979
Three Bar Wildlife in Arizona	0.25	Telemetry	Lawhead 1984
Sierra Seri, Sonora (dry)	0.174-0.319*	Camera trap	This study
Sierra Seri, Sonora (wet)	0.075-0.144*	Camera trap	This study
Southeastern Oklahoma	0.09	Telemetry	Rolley 1985
Southeastern Idaho	0.05	Telemetry	Bailey 1974
Topilejo, CDMX (wet)	0.053-0.124*	Camera trap	This study
Acatlán de Osorio (dry)	0.065-0.122*	Camera trap	This study
Topilejo, CDMX (dry)	0	Camera trap	This study

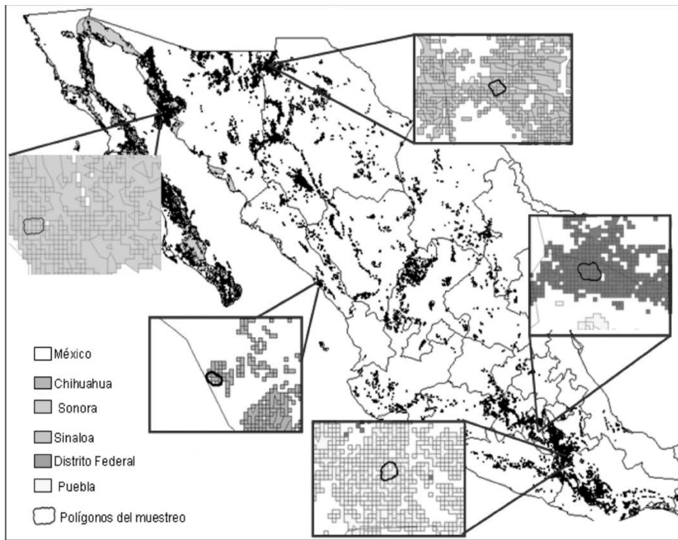

Figure 3. Potential distribution of bobcats in Mexico according to MaxEnt. The polygons in each box are the study areas and the shading around the areas represent potential distribution as calculated with MaxEnt.

resources. Under this idea, in 1997 the Unidades de Manejo para la Conservación de Vida Silvestre (UMA) were created. These are sites where alternative schemes of sustainable use of biodiversity compatible with conservation of wildlife in a determined privately-owned area can be implemented, provided a management plan is submitted for approval (Organ *et al.* 2012). There are two types of UMA, one considered as extractive (hunting, for pet trade or ornamentals, arts and crafts, etc.), and the other not extractive (research, photography, ecotourism, exhibition, environmental education, etc.). On the ground, in UMAs, focal species monitoring for sustainable use in UMAs use indirect methods (tracks, scats, etc.) to estimate population levels. Our study confirms that camera trapping is likely the best and easiest method to estimate populations of animals that may be subjected to a sustainable use program, in particular the bobcat in México. Our study also confirms that bobcats can be individually identified by the patterns on their skins. We strongly recommend that the Mexican government implements a similar method to allocate adequate harvest quotas of bobcat in extractive UMAs.

Figure 4. For individual bobcat identification, we compared distinct markings on the bobcat coat that were easily detected in different photographs. The two photographs in this figure show the same individual.

Acknowledgments

This paper is dedicated to David J. Schmidly, a formidable scientist, extraordinary mentor and friend, and a legendary personality in the field of mammalogy in the United States, in México, and beyond. The second author of this paper had the incredible luck to meet David back in the 1980s, and since then he became a fast friend and an absolutely key element in my professional development, and of course in the inception and launching of AMMAC, the Mexican Mammal Society. We also thank two anonymous reviewers and Lisa and Robert Bradley for their careful, detailed editorial handling. The authors wish to thank the U.S. Fish and Wildlife Service, Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO), and Dirección General de Vida Silvestre, SEMARNAT for the funds received to carry out this project and for the necessary permits to carry out our fieldwork. Thanks are extended to CONACYT for the scholarship received by the first author. We also acknowledge and thank E. Najera, A. Araujo, A. de la Torre, G. Castellanos, K. Quiroz, I. Sanchez, S. Barcenas, J. Velazquez and V. García, J. Rosas, A. Rosas, J. Flores, C. Flores, G. Ruiz, L. F. Lozano, M. Romo, S. Romo, Y. Rubio, C. Ayala, G. Ceballos, J. Pacheco, R. List, E. Ponce, R. Sierra, F. Rodríguez, T. Ptister and J. Cruzado, and finally The Seri people from Punta Chueca, Sonora, especially to Cmiique "El Indio" F. Molina, "Archie" E. Molina, for their help in the field work.

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Morphological and genetic variation of black-tailed jackrabbit (*Lepus californicus*) populations separated by rivers

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Two rivers in the hot desert of northwestern México have been considered as filter barriers in the distribution of mammals: Río Conchos in Chihuahua and Río Nazas in Durango. Between both rivers, the black-tailed jackrabbit, *Lepus californicus*, shows significant differences in external morphological traits. We investigated if these differences are supported by phylogenetical signals and compared them with populations living at similar latitudes in the Baja California Peninsula to determine the importance of the genetic variation caused by the rivers. An external mophology, and a cranial geometric morphometric analysis were performed using the dorsal, ventral, and lateral views of the skull; and a genetic analysis of cytochrome *b* gene. Measurements and fur color patterns of specimens from two continental groups, north of Río Conchos (NRC) and south of Río Nazas (SRN), were compared to four groups (A-D) inhabiting different latitudes of the Baja California Peninsula (BCP). The parietal region, zygomatic arch, and auditory bullae were identified as the main cranial structures related to skull shape; however, no differences were observed in size and shape between groups. The phylogenetic reconstruction of *L. californicus* showed that it is a monophyletic species, with high branch support values (100). It is represented by two polyphyletic subclades, one with haplotypes of the SRN and NRC populations and the other with haplotypes of the BCP populations. The average genetic distance (p-distance) and genetic differentiation (F_{ST}) between SRN and NRC were low (0.8 % and 0.09, respectively), with higher mean values between the BCP groups (1.23 % and 0.30, respectively). The statistical parsimony network of Cyt *b* did not identify a clear geographic genetic structure between haplotypes of SRN and NRC and they did not share haplotypes with the BCP populations. There are neither cranial geometric morphometric nor genetic differences between *L. californicus* populations related to either the rios Conchos or Nazas; thus, these rivers cannot be considered geographic barriers. However, there are morphological differences between the populations in Chihuahua and Durango and the populations inhabiting Baja California Peninsula, which may be associated with evolutionary distance and local habitat characteristics.

Dos ríos en el desierto cálido del noroeste de México se han considerado como barreras de filtro en la distribución de mamíferos, el Río Conchos en Chihuahua y el Río Nazas en Durango. Entre ambos ríos, la liebre cola negra, *Lepus californicus*, muestra diferencias significativas en los rasgos morfológicos externos. Investigamos si estas diferencias están respaldadas por señales filogenéticas y las comparamos con poblaciones que viven en latitudes similares en la Península de Baja California para determinar la importancia de la variación genética ocasionada por los ríos. Realizamos un análisis mofológico externo, morfométrico geométrico craneal usando las vistas dorsal, ventral y lateral del cráneo y genético con el gen citocrormo *b*. Las medidas y los patrones de color del pelaje de los especímenes de dos grupos continentales, al norte de Río Conchos (NRC) y al sur de Río Nazas (SRN), se compararon con cuatro grupos (A-D) que habitan en diferentes latitudes de la Península de Baja California (BCP). La región parietal, el arco cigomático y las bullas auditivas fueron las principales estructuras craneales relacionadas con la forma craneal; sin embargo, no se observaron diferencias en tamaño y forma entre los grupos. La reconstrucción filogenética de *L. californicus* mostró que es una especie monofilética con valores altos de soporte de ramas (100). Está representada por dos subclados polifiléticos, uno con haplotipos de poblaciones de SRN y NRC y otro con haplotipos de poblaciones de BCP. La distancia genética promedio (p-distancia) y diferenciación genética (F_{ST}) entre SRN y NRC fueron bajas (0.8 % y 0.09, respectivamente), con valores promedio mayores entre los grupos de BCP (1.23 %, 0.30, respectivamente). La red de parsimonia estadística de Cyt *b* no identificó una estructura genética geográfica clara entre los haplotipos de SRN y NRC y no comparten haplotipos con las poblaciones de BCP. No existen diferencias morfométricas geométricas craneales ni genéticas entre las poblaciones de *L. californicus* relacionadas con los ríos Conchos o Nazas; por tanto, estos ríos no se pueden considerar como barreras geográficas. Sin embargo, existen diferencias morfológicas entre las poblaciones de Chihuahua y Durango y las poblaciones que habitan en la Península de Baja California, que pueden estar asociadas con la distancia evolutiva y las características del hábitat local.

Keywords: Baja California; black-tailed jackrabbit; genetic break; geometric morphometrics; México; phylogeny; Rio Conchos; Rio Nazas.

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Introduction

Physical barriers and climatic variation within the distributional range of species are factors that influence the speciation process, and fluvial barriers have been considered to limit the dispersal of mammal species. This has been observed in many cryptic species inhabiting both sides of the Chihuahuan Desert and the drainage basins of the *Altiplano Central* (Mexico's central highlands), including the rodent genera *Chaetodipus*, *Geomys*, *Neotoma*, and *Peromyscus* (Patton 1969; Walpole *et al.* 1997; Riddle *et al.* 2000a, 2000b; Edwards *et al.* 2001; Riddle and Hafner 2006; Patton *et al.* 2007; Neiswenter and Riddle 2010; Cornejo-Latorre *et al.* 2017; Neiswenter *et al.* 2019; Camargo and Álvarez-Castañeda 2020). In addition, this river seems to be a factor in the divergence between *Perognathus flavus* phylogroups within the Chihuahuan Desert, related to the expansion grasslands in the late Miocene and the Basin and Range geomorphology of the Miocene-Pliocene (Neiswenter and Riddle 2010).

The Chihuahuan Desert consists of portions divided by the Río Bravo (or Río Grande) and Río Conchos rivers (Figure 1). In addition, these rivers act as physical barriers in a climatic transition zone; the north encompasses the temperate Chihuahuan Desert, covered primarily by grasslands and *Larrea*, and the south includes the Mexican Plateau, a warm desert area with a predominance of cacti species (Baker 1977). The Río Conchos is 910 km long, rising in the Sierra Madre Occidental in Chihuahua (in the Sierra Tarahumara region), and emptying into the Río Bravo. It appears to be a major barrier restricting gene flow between ancestral populations of mammal species in the *Altiplano Central*.

The Río Nazas is south of the Río Conchos and flows across the main axis of the *Altiplano Central* (Figure 1). Both the rivers Conchos and Nazas act as physical barriers that limit the north-south dispersal of mammals, and the Río Nazas has been considered as part of the Southern Coahuila filter barrier (Baker 1956; Hafner *et al.* 2008) that separates the north and south *Altiplano Central* (Arriaga *et al.* 1997). The 322 km long Río Nazas has carved a canyon about 506 m deep and 33 km wide over the first two-thirds of its course (Petersen 1976), rising in north-central Durango, on the eastern slopes of the Sierra Madre Occidental where it represents a major geographic barrier (Tocchio *et al.* 2014). A molecular analysis of pocket mice showed that the two rivers serve as boundaries of the sister species *Chaetodipus nelsoni* south of Río Nazas, *C. collis* between the two rivers, and *C. intermedius* north of Río Conchos (Neiswenter *et al.* 2019).

For some species, both rivers can be considered as permeable barriers (Hafner and Riddle 2011). This assumption is consistent with information from natural history collections, spatial environmental analyses, and ecological niche modeling based on environmental parameters (Anderson and Gaunt 1962: Soberón and Peterson 2005; Peterson *et al.* 2011).

The black-tailed jackrabbit, *Lepus californicus*, is widely distributed across México and the United States (Flinders and Chapman 2003; Beever *et al.* 2018; Brown *et al.* 2019), is capable of inhabiting many types of habitat, including grazing by domestic livestock. Its diet (grasses, forbs, shrubs) is variable dependent upon vegetation availability (Brown *et al.* 2019). Originally, 17 subspecies were recognized; currently 18 subspecies are recognized based on morphological and genetic traits (Álvarez-Castañeda and Lorenzo 2017; Lorenzo *et al.* 2018).

The distribution of *L. californicus* stretches beyond several filter barriers that effectively restrain the range of other species. These barriers include both rivers and mountain ranges (*i. e.*, Río Colorado, Río Bravo, Río Conchos, Río Nazas, and the Sierra Madre Oriental; Petersen 1976). Several geological events in the area also have produced changes in the pluvial regime, plant community structure, floristic composition, appearance of vicariance or dispersal events at various temporal scales (*e. g.*, Miocene to Last Glacial Maximum), leading to the evolutionary divergence of various taxonomic groups (Hafner and Riddle 2011). However, the Río Nazas and its canyon (hereafter called Nazas canyon), although considered a physical barrier for subspecies of the desert cottontail (*Sylvilagus audubonii*) and the white-sided jackrabbit (*L. callotis*), appears to have no effect on populations of *L. californicus* (Petersen 1976; Hall 1981; Brown *et al.* 2018).

The combination of the Nazas canyon and Río Conchos may nonetheless act as a major barrier limiting the north-south dispersal of individual animals within the Chihuahuan Desert and, more specifically, in the *Altiplano Central*. It is assumed that the dispersal of *L. californicus* is in the north-south direction since it has been postulated that the first expansion of Leporidae occurred in North America during the Miocene (Dawson 1981). Further, it has been suggested that there is a North American origin for the family Leporidae based on fossil discoveries (Matthee *et al.* 2004). In addition, these rivers run across a climatic transition zone that harbors various vegetation types. Therefore, it is expected there would be an important area of discontinuity between populations of black-tailed jackrabbits in the Chihuahuan Desert and those in the *Altiplano Central*. Under these circumstances, these rivers would influence species distribution and genetic flow resulting in genetic breaks from the combined effect of physical barriers, climate, and ecological differences. This study evaluated the degree of genetic and morphological variation between populations on both sides of the Nazas-Conchos barrier and examined if variation is a consequence of the interruption or delay of gene flow caused by this barrier or only isolation by distance. Genetic and morphological variation of *L. californicus* in the Chihuahuan Desert was compared with that of other populations at the same latitude, separated from each other by approximately the same north-south distance, and associated with similar vegetation and with no current physical barriers to limit gene flow.

Materials and Methods

Sample Collection. Specimens from 30 geographic localities in México were collected and examined (Appendix 1; Figure 1). Four trips were made to four localities in the State of Durango (group south of Río Nazas, or SRN; between 24.0242°, -104.2808°, and 25.1902°, -104.0998°) and three trips to three localities in the State of Chihuahua (group north of Río Conchos, or NRC; between 28.7468°, -106.0882°, and 29.3827°, -106.3504; Appendix 1). The two localities were separated by approximately 600 km. Specimens were collected under the scientific collection permit number FAUT-0143 of CL (official letter No. SGPA/DGVS/002779/18) and were handled following the recommendations of the American Society of Mammalogists (Sikes *et al.* 2016). Voucher specimens from Durango and Chihuahua were deposited in the Mammals Collection at El Colegio de la Frontera Sur (ECO-SC-M).

Morphological Comparison. Morphological comparisons included visual differences in fur color variation, as well as somatic measurements between specimens of *L. californicus* from south Río Nazas (SRN) and from north Río Conchos (NRC). Those two groups were then compared to four groups from the Baja California Peninsula (BCP), grouped for comparative purposes according to different latitudes from north to south. The four groups were: group A (29.9342° to 28.7323°; Cataviña, Calamajue, 83 km N Guerrero Negro, Valle de la Trinidad); group B (28.0786° to 27.0742°; Vizcaíno, Sierra de San Francisco, Guerrero Negro, Santa Rosalía, San Ignacio, Bahía Asunción, San Zacarías); group C (25.5593° to 25.1800°; Última Agua, María Auxiliadora, Ley Federal de Agua No. 4, Insurgentes); and group D (24.1581° to 23.5747°: La Paz, Reforma Agraria, Los Planes; Todos Santos, Carretera Transpeninsular, Santa Anita) (Figure 1; Appendix 1). The mean linear distance between the

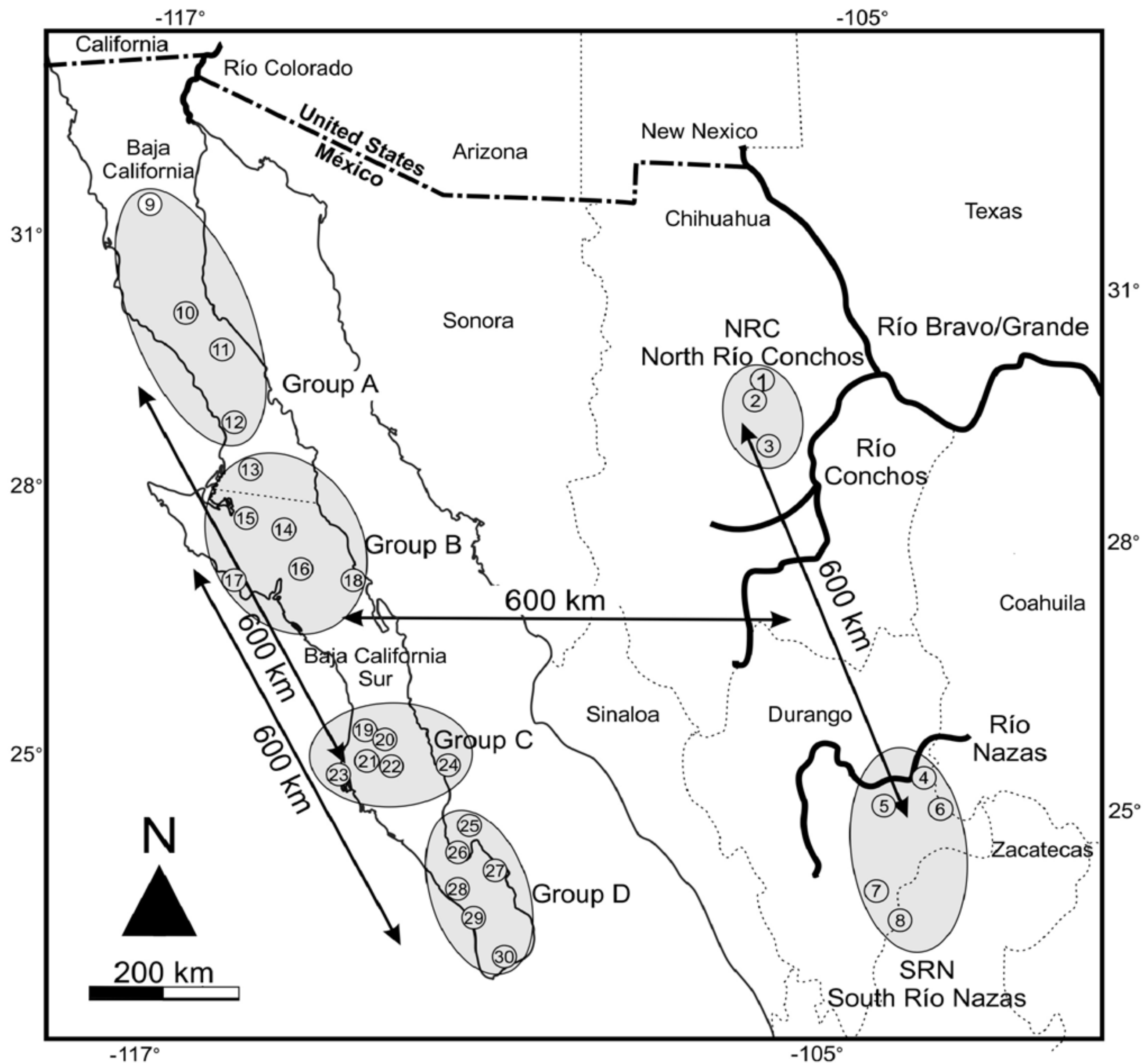

Figure 1. Location of black-tailed jackrabbit (*Lepus californicus*) specimens from north Río Conchos (1–3; Chihuahua), south Río Nazas (4–8; Durango), and four groups (9–30; A-D) from the Baja California Peninsula, México. Geographic group numbers as per Appendix 1.

four BCP groups was *c. a.* 250 km. Voucher specimens from BCP were deposited in the mammal collections of El Colegio de la Frontera Sur (ECO-SC-M) and Centro de Investigaciones Biológicas del Noroeste (CIB).

The somatic measurements were taken from the labels of voucher specimens and compared using descriptive statistics and a *t*-test between all pairs of groups using the software STATISTICA (ver. 8.0; StatSoft, Inc. 2007). Samples sizes (Appendix 1) were: SRN (n = 7), NRC (n = 7), group A (n = 12), group B (n = 13), group C (n =18), and group D (n= 59).

Geometric Morphometrics Analysis. This analysis included specimens of the same groups as the morphological analysis. In addition, samples from Isla Magdalena, Isla Margarita, and Isla Carmen were included in group C, and samples from Isla Espíritu Santo were included in group D (Figure 1; Appendix 1). We used 85 adult specimens: 4 males, 3 females in SRN; 1 male, 6 females in NRC; 3 males, 9 females in group A; 4 males, 12 females, 2 not determined in group B; 11 males, 13 females, 2 not determined in group C; and 8 males, 6 females, 1 not determined in group D. Adult specimens were identified by the fusion of the cranial suture and the eruption of the last molar (Hoffmeister and Zimmerman 1967). Three cranial views were analyzed (sample size in parentheses): dorsal (n = 83; NRC = 7, SRN = 7, A = 12, B = 17; C = 25, D = 15), ventral (n = 79; NRC = 7, SRN = 6, A = 12, B = 17; C = 24, D = 13), and lateral (n = 77; NRC = 7, SRN = 7, A = 11, B = 16; C = 24, D = 12; Appendix 1 for details). Photographs (n = 239) were made with a Nikon D500 fitted with a macro-focusing lens; a 1 cm scale was included in each photograph, and the position, distance, and photographic plane were standardized. All photographs were saved as JPEG files.

The coordinates *X* and *Y* of the shape of each cranial view were recorded from photographs using the programs tpsUtil v 1.78 (Rohlf 2019) and tpsDig v. 2.12 (Rohlf 2017). The selection of landmarks for dorsal and ventral cranial views was based on the configuration proposed by Ge *et al.* (2015). Fourteen landmarks were set for the dorsal view, 27 for the ventral view, and 14 for the lateral view (Figure 2; Appendix 2).

Statistical Analysis of Shape Variation. The MorphoJ 1.07a program (Klingenberg 2011) was used to perform a superimposition by generalized Procrustes analysis. The effects of size, position, and scale were eliminated to obtain only shape variation in the data (Rohlf and Slice 1990). No outliers were found in the three cranial views. A Procrustes ANOVA between sexes was performed to evaluate the effect of sex on the size and shape of the cranium. To remove the effect of size on shape (allometric effect) between groups, a multivariate regression of Procrustes coordinates (as shape variables) was used against the log-transformed centroid size (as size variables; Klingenberg and Maruga-Lobon 2013).

To analyze the variation between groups of *L. californicus*, a principal component analysis (PCA) and a canonical variate analysis (CVA) were performed with the residuals obtained from the regression in the program MorphoJ 1.07 (Klingenberg 2011). These analyses included a significance test with a permutation test for pairwise distance run with 10,000 iterations, using Procrustes distances for the *a-priori* groups visualized in the morphometric space of the canonical variables. To analyze the variation in shape, the average shape per group was estimated from the regression residuals and two PCAs were performed with the mean data, 1) between SRN and NRC, and 2) with all groups. Additionally, a broken-stick test was performed to estimate the number of statistically significant principal components (Frontier 1976; Jackson 1993) using regression residuals with a variance-covariance matrix with PAST v. 2.17 (Hammer *et al.* 2008). Correct assignment between pairs of groups was performed by discriminant function analysis and cross-validation (to test the predictive capacity of the discriminant function).

Genetic Analysis. DNA was extracted from 13 muscle samples. Genomic DNA was extracted from muscle (kept at -20ºC in 70 % ethanol) by immersion in cell lysis solution (EDTA, Tris HCl, and Proteinase K) followed by purification with phenol/chloroform-alcohol-isoamyl organic solvent protocols (adapted from Hamilton *et al.* 1999). The cytochrome *b* (Cyt *b*) gene was amplified in fragments of *c. a.* 800 bp with the primer pairs MVZ05 (CGA AGC TTG ATA TGA AAA ACC ATC GTT G-3') and MVZ16 (5'-AAA TAG GAA ATA TCA TTC TGG TTT AAT-3'; Smith and Patton 1993; Smith 1998). The following quantities were used for initial double-strand amplifications: 12 µl Master Mix (Promega) solution, 10 µl nuclease-free water, 2 µl of each primer (10 nM), and 2–3 µl DNA, to a final volume of 28 µl. The amplification conditions consisted of an initial 3-min denaturation at 94°C followed by 37 denaturation cycles, each at 94°C for 45 s. Samples were annealed for 60 s at 50°C, followed by an extension step at 72°C for 60 s for mitochondrial DNA. The products of the PCR reactions were visualized by electrophoresis on 2 % agarose gel. Subsequently, the purification and sequencing of each amplified sample were performed overseas at Macrogen Inc, in Seoul, Korea.

Sequences were aligned (first part of the gene, 625 bp) with Clustal X ver. 2.1 (Thompson *et al.* 1997) and a visual examination in Chromas ver. 2.4.4 (McCarthy 1998, 2016). Sequences were translated into amino acids to confirm the alignment. Missing data were coded with a question mark. Non-redundant haplotypes were identified using the DNASP software ver. 5.10 (Librado and Rozas 2009). The null distribution to test for the significance of variance components and pairwise F-statistic equivalents (F_{ST}) was constructed from 10,000 permutations with Arlequin ver. 3.1 (Excoffier *et al.* 2005). A minimum spanning network was performed based on the 625 bp fragment of Cyt *b* from 27 specimens. The genealogical relationships of the haplotypes were determined from the construction of a haplotype network, through the Median-Joining method according to the criteria of Bandelt *et al.* (1999) and maximum parsimony implemented in the Network program

version 5.0.1.1. (Fluxus Technology Ltd. 2004-2020). The parameters used were: 0 epsilon, 1/1 transitions-transversions weight, 5/10 characters weight and the connection cost criterion.

Genetic variation levels according with the number of haplotypes (*H*), unique haplotypes per group (*UH*), number of polymorphic sites (*P*), number of observed sites with transitions (*Tt*), number of observed sites with transversions (*Tv*), mean number of pairwise differences (*NP*), and nucleotide diversity (π) between the 3 groups (SRN, NRC, and BCP) were examined using the Cyt *b* gene in Arlequin ver. 3.1 (Excoffier *et al.* 2005). Non-redundant haplotypes were deposited in GenBank under the following accession numbers: Cyt *b* – MW940630 to MW940636; MZ055403 to MZ055408. The genetic distances between groups were calculated with MEGA ver. 7.0.26 (Kumar *et al.* 2015) with the p-distance. A Mantel test was used to evaluate the correlation between geographic distance and genetic distance.

Phylogenetic reconstructions based on distance, maximum likelihood (ML), and Bayesian inference (BI) were performed with non-redundant haplotypes. ML algorithm (Felsenstein 1981) reconstructions were conducted using PAUP ver. 4.0b10 (Swofford 2000) with a heuristic search of 1,000 replicates and swapping with the TBR (Tree-Bisection-Reconnection) algorithm. BI trees were constructed using MrBayes ver. 3.1.2 (Huelsenbeck and Ronquist 2001).

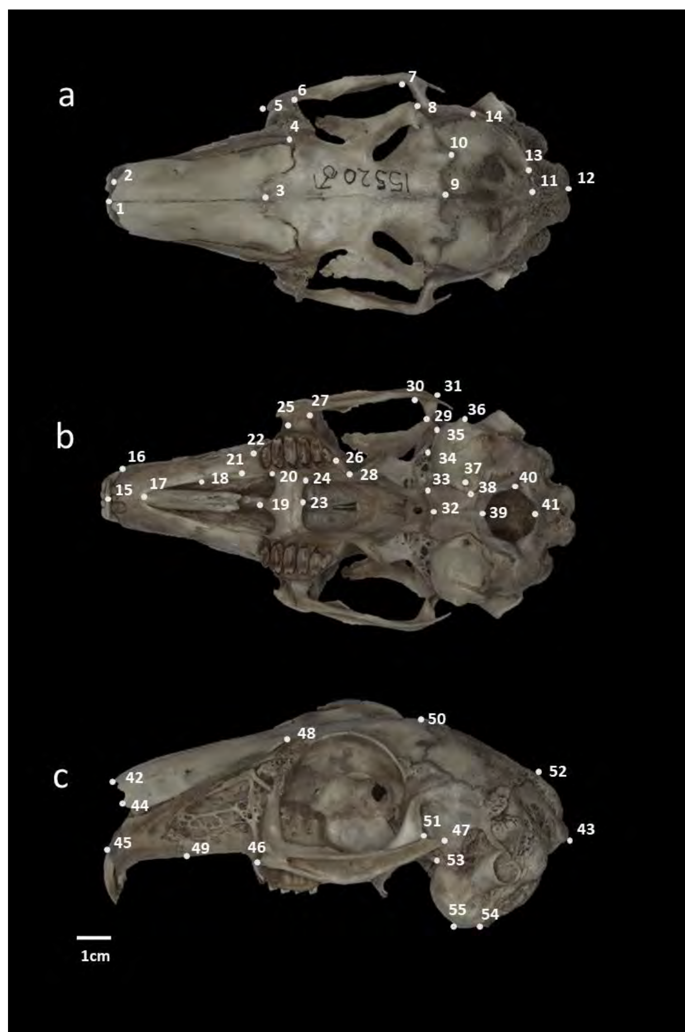

Figure 2. Location of cranial landmarks. a = dorsal view, b = ventral view, and c = lateral view. Voucher specimen the black-tailed jackrabbit (*Lepus californicus;* CIBNOR 15520) from Todos Santos, Baja California Sur, México, corresponding to group D.

Two separate analyses were conducted using BI. Metropolis-coupled Markov chain Monte Carlo (MCMC) sampling was performed with four chains run for 5 million iterations using default model parameters as baseline values. The sequence evolution model that best fitted each of our sequence datasets was determined using jModeltest ver. 2.1.10 (Darriba *et al.* 2012) with the Akaike Information Criterion (AIC). Trees were sampled every 1,000th iteration after examining the output files for convergence using the online software AWTY (Wilgenbusch *et al.* 2004). Majority-rule consensus trees were obtained by summarizing all trees after a burn-in period of 2 million generations. Bayesian probabilities and the frequency of a nodal resolution were taken from the 50 % majority-rule consensus of the trees sampled.

The ingroup included 49 specimens of *L. californicus* obtained from GenBank (Álvarez-Castañeda and Lorenzo 2017; see Appendix 1) representing the same groups as the geometric morphometrics analysis (Figure 1). Outgroup comparisons used sequences from *Sylvilagus audubonii* (GenBank accession number KU759759) and *S. floridanus* (GenBank accession number KU759758).

Results

Morphological Comparison. Specimens from south of Río Nazas (SRN) and north of Río Conchos (NRC) differed in some external morphological characteristics despite having been collected in the same season (Figure 3). SRN specimens had short fur, and the back was whitish-gray; belly was white; a black nape patch extended towards the base of the ears; the tip of the ears had a black patch on the back that extended to the distal edge of the ear; ears were light yellowish-brown on the front with a white outer border (Figure 3). On the other hand, NRC specimens had longer fur and lacked any black stripe on the nape; back was whitish-brown; belly was white; nape patch was light gray; the tip of the ears had a black patch on the back that extended to the distal edge; ears were light brown with whitish hairs on the front of the ears and white outer border (Figure 3). The specimens in the BCP groups A-D show no noticeable differences in pelage coloration; all had medium fur, and the back was blackish-brown mixed with white; belly was yellowish (slightly darker brown in group D); the nape patch was blackish brown and extended towards the base of the ears; the tip of the ears had a black patch on the back that extended to the distal edge of the ear; ears were gray mixed with white on the front with a white outer border (Figure 3).

Somatic measurements are displayed in Table 1. Ear length was slightly shorter in specimens in BCP Groups B, C, and D; hindfoot length also was shorter in Groups C and D. In general, NRC and SRN specimens were larger in body

Table 1. Average and ranges (in parenthesis) of somatic measurements of specimens of *Lepus californicus* from the *Altiplano Central* (SRN = South Río Nazas; NRC = North Río Conchos) and Baja California Peninsula (Groups A-D), México. *n* = sample size; lowercase letters represent different sample sizes by group: a (*n* = 2), b (*n* = 12), c (*n* = 10), d (*n* = 62).

Group	n	Body length (mm)	Tail length (mm)	Hind foot length (mm)	Ear length (mm)	Weight (g)
SRN	7	620.1 (590-678)	88.6 (77-95)	121.1 (111-135)	146.3 (140-160)	2,435.7 (2,200-2,600)
NRC	7	600.4 (563-650)	79 (70-89)	127.6 (115-150)	147 (138-160)	2,414.3 (2,100-2,700)
Group A	12	521.9 (460-570)	89.2 (80-102)	115.3 (102-130)	153.2 (104-185)	1,900.0 (1,700-2,100)[a]
Group B	13	528.5 (445-630)	73.4 (55-100)[b]	114.5 (90-150)	120.5 (70-151)	2,110.1 (1,800-2,750)[c]
Group C	19	515.1 (480-544)	89.7 (75-115)	101.7 (88-110)	111.6 (90-128)	1,876.3 (1,350-2,300)
Group D	63	500.3 (390-795)[d]	81.6 (50-130)	106.8 (88-125)	120.3 (102-157)[d]	1,876.6 (1,200-4,400)

length, hindfoot length, and ear length, relative to BCP specimens (except group A). There were significant differences between NRC and SRN groups in tail length (*t*-value 2.44, d. f. = 12, $P = 0.03$). Significant differences ($P < 0.05$) by t-test values were observed between NRC-SRN and A-D groups in all somatic measurements. In addition, significant differences ($P < 0.05$) were observed between groups A-D in tail length, hindfoot length, and ear length.

Geometric Morphometrics Analyses. The skulls of *L. californicus* specimens from the *Altiplano Central* and the Baja California Peninsula were not significantly different in size between sexes (ANOVA of log centroid size, $P > 0.05$; Appendix 3); therefore, data from both sexes were combined in subsequent analyses. The allometric correction (changes in shape correlated with changes in size) between groups showed a highly significant relationship between skull size and shape in dorsal, ventral, and lateral views of the skull, explaining 6.94 %, 11.39 %, and 8.25 % of the variation, respectively. The main cranial structures related to cranial shape were the parietal region (dorsal view), zygomatic arch (dorsal, ventral, and lateral views), and auditory bullae (ventral view); however, no difference was observed in size and shape between any of the groups analyzed. The first two principal components (PC1 and PC2) of the three cranial

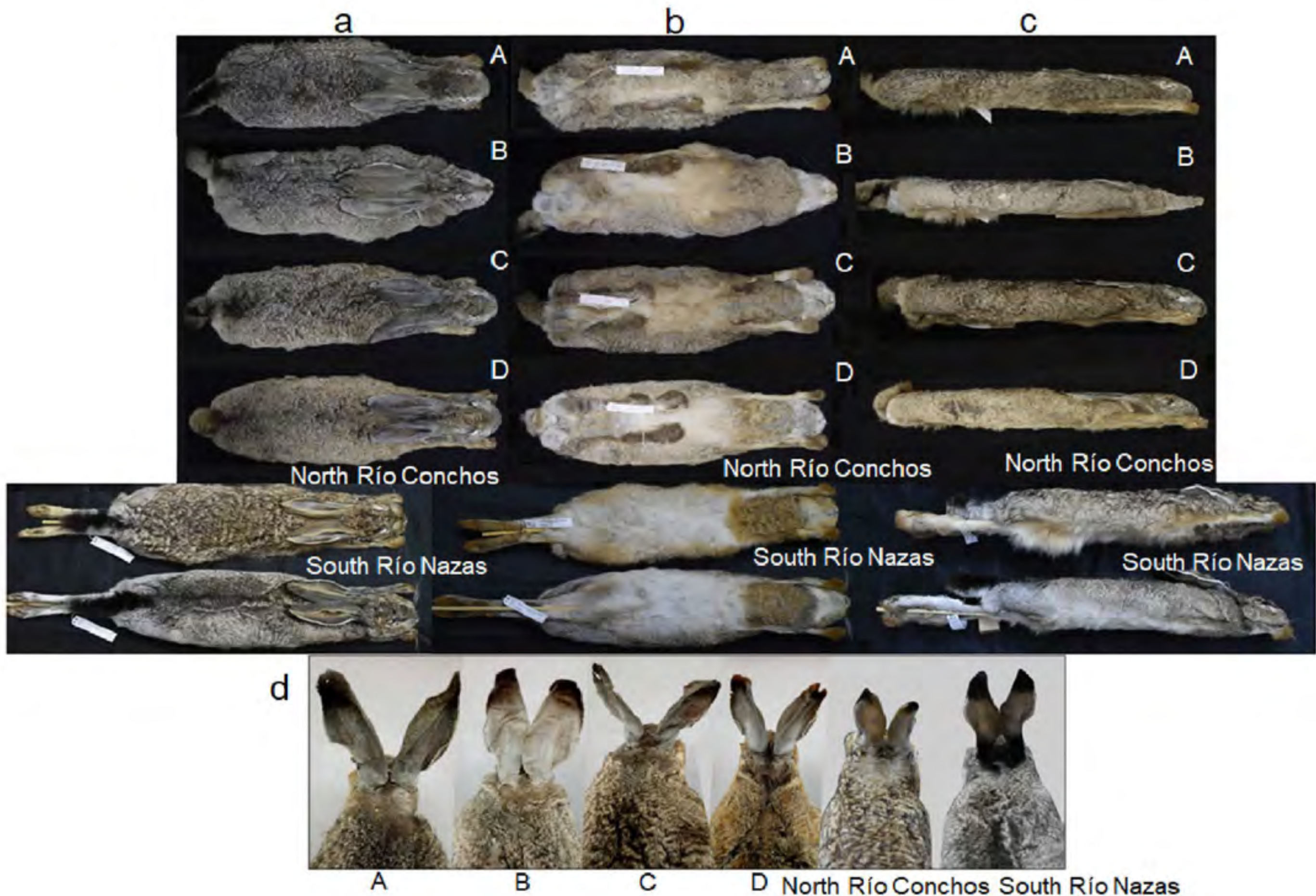

Figure 3. Comparison of external morphology among black-tailed jackrabbit (*Lepus californicus*) specimens from north Río Conchos (ECO-SC-M 9500), south Río Nazas (ECO-SC-M 9490), and four groups from the Baja California Peninsula (BCP), México: A (CIBNOR 16459); B (CIBNOR 2870); C (CIBNOR 15200); and D (CIBNOR 15508). See Appendix 1 for details of specimens. A-D = groups from the Baja California Peninsula. a = Dorsal view. b = Ventral view. c = Lateral view. d = Nape view.

views failed to identify the different groups (Figure 4a-c); therefore, the variation in the average shape by group was analyzed. This was confirmed by the broken-stick model that indicated that none of the first five eigenvalues obtained for the three views are significant, indicating that each explains less than the minimal variation of the analysis (Appendix 4).

Relative to the canonical variate analysis (CVA) for the three cranial views, the canonical variate 1 (CV1) was the most useful variate for differentiating between *L. californicus* from PBC vs. SRN-NRC. The canonical variate 2 (CV2) of the dorsal and lateral views distinguished between NRC and SRN; however, SRN and NRC were not differentiated based on the ventral view (Figure 5a-c).

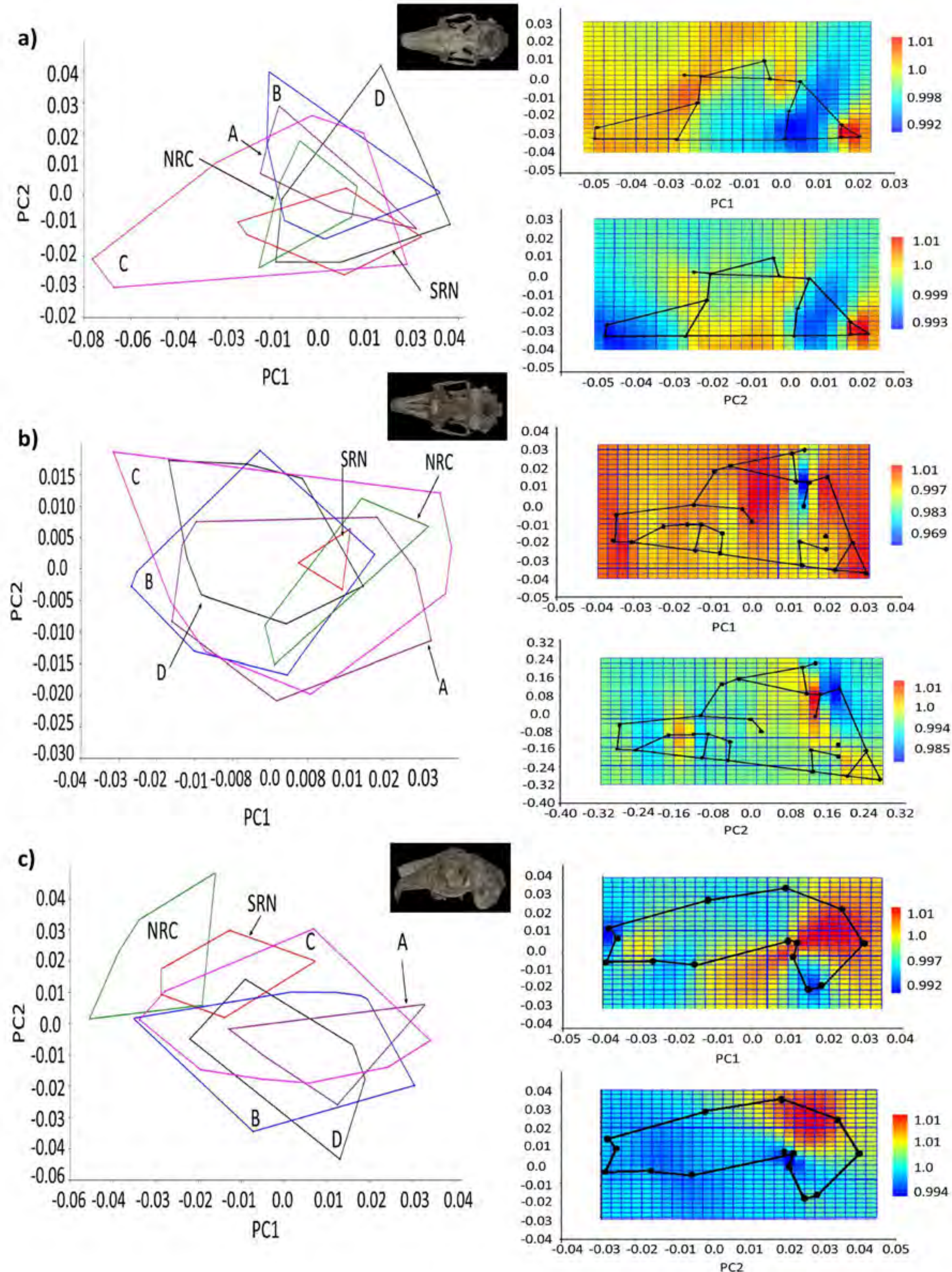

Figure 4. Principal component analysis (PCA) graph, displaying the first two principal components (PC1 and PC2), and deformation grids for PC1 (above) and PC2 (below) for each cranial view of black-tailed jackrabbits (*Lepus californicus*) specimens: a) dorsal, b) ventral, c) lateral. NRC = north Río Conchos. SRN = south Río Nazas. A-D = groups from the Baja California Peninsula.

Skull Dorsal View. The PCA between NRC and SRN indicated that the variation in shape was related primarily to the parietal region, which was higher in NRC and lower in SRN specimens. In contrast, for the BCP group the main differences in the skull concerned the zygomatic arch. The analysis of the continental and peninsular populations combined in PC1 was related primarily to the anterior extension of the zygomatic process, which was larger in NRC-SRN specimens and smaller in the BCP group; PC2 was related to the parietal region, differentiating between NRC (expansion) and SRN (contraction).

The first three canonical variates of the CVA explained 87.7 % of the total variation (Appendix 5). CV1 separated the BCP group from SRN-NRC due to the relative length of the nasals (shorter for SRN-NRC) and the anterior end of the zygomatic process (longer for SRN-NRC). CV2 partially separated SRN from NRC due to the broader inner edge of the orbit, being broader for NRC specimens. The Procrustes distances showed statistically significant differences between NRC and SRN, and between the BCP (except C) and the SRN-NRC groups.

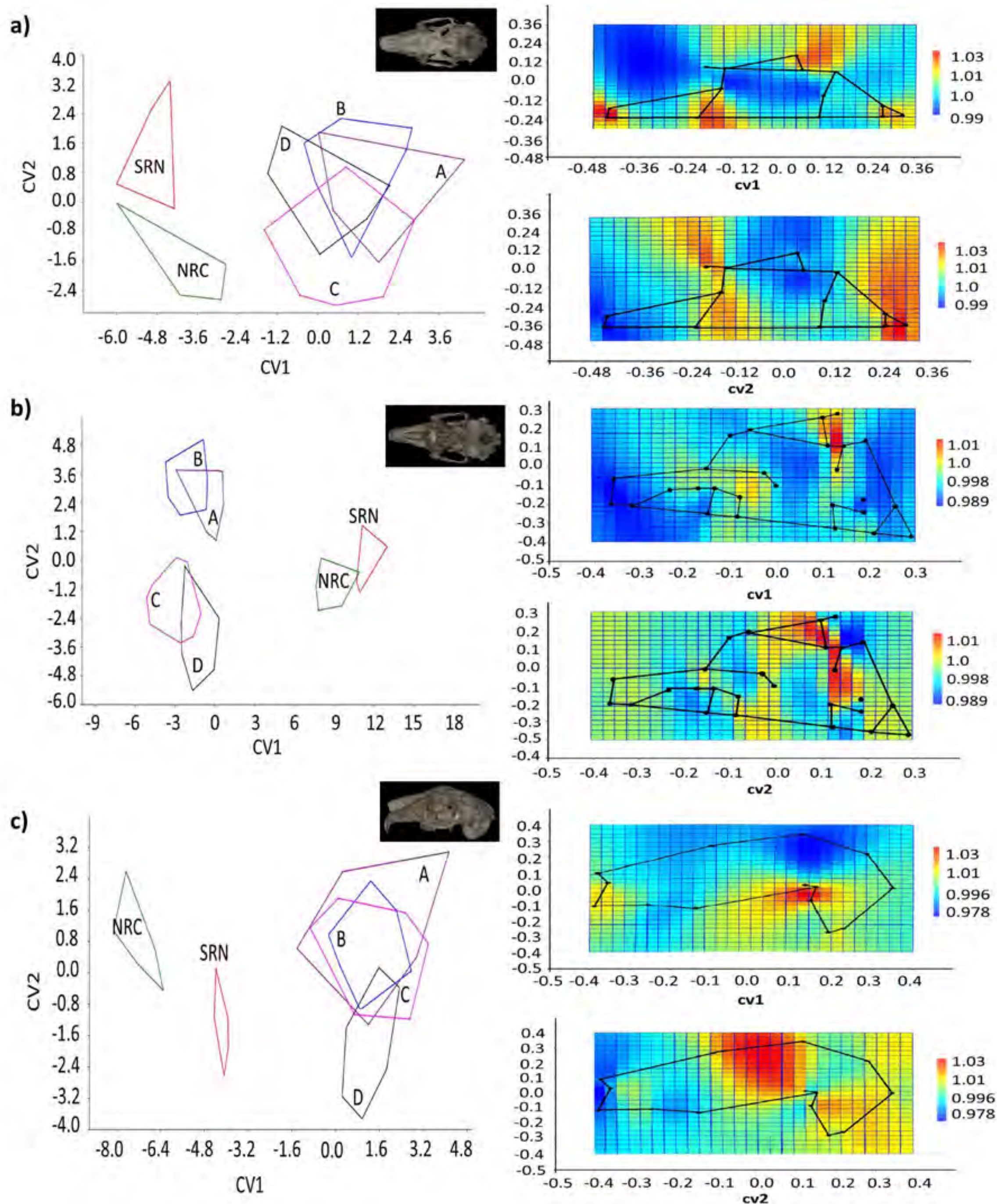

Figure 5. Canonical analysis of variance (CVA) graph, displaying the first two canonical variables (CV1 and CV2), and deformation grids for CV1 (above) and CV2 (below) for each cranial view of black-tailed jackrabbits (*Lepus californicus*) specimens: a) dorsal, b) ventral, c) lateral. NRC = north Río Conchos. SRN = south Río Nazas. A-D = groups from the Baja California Peninsula.

Skull Ventral View. The PCA between NRC and SRN indicated that the differences were related to the posterior expansion of the zygomatic arch and the auditory bullae (larger in NRC). The variation in shape was minimal between BCP groups A-D, mainly related to the zygomatic arch and auditory bullae; among the four groups, group C showed the lowest variation. The average variation in shape between continental and peninsular populations associated with PC1 was related to the zygomatic arch and the auditory bullae. SRN-NRC differed from BCP in a smaller zygomatic arch and lateral expansion of the bullae. PC2 discriminated between NRC and SRN due to the smaller bullae in SRN.

In the CVA, the first three factors explained 92.4 % of the total variation (Appendix 5). CV1 (65.2 %) discriminated between SRN-NRC and BCP due to the zygomatic arch and auditory bullae; in CV2 (17.8 %), SRN and NRC overlapped, and no differences were observed. A similar result was detected for the BCP groups A-D. The *P* values for the Procrustes distances indicated statistically significant differences between SRN-NRC and BCP groups A-D.

Skull Lateral View. The PCA between SRN and NRC indicated that the variation in shape was due to the posterior extension of the zygomatic arch (larger in SRN) and auditory bullae (larger in NRC). The variation in shape in BCP was lower compared to NRC-SRN and was related to the zygomatic arch. The average variation in the shape of the continental and peninsular populations associated with PC1 was related to zygomatic arch length. SRN and NRC had a dorsoventrally shorter zygomatic arch compared to the BCP group. PC2 indicates slight differences between SRN and NRC associated with the relative length of the nasal, which was smaller in NRC.

The first three canonical variates explained 90.2 % of the variation (Appendix 5), CV1 (63.3 %) discriminated between SRN-NRC and BCP due to the dorsoventrally smaller zygomatic arch in SRN-NRC. CV2 (14.4 %) showed a slight overlap between SRN and NRC due to the larger auditory bullae in NRC. The Procrustes distances did not show significant differences between NRC and SRN, but did so between NRC-SRN and BCP (except for group B).

Discriminant Function Analysis. Assessment of correct classification between pairs of groups by the discriminant function resulted in high allocation percentages (> 92 %) between SRN and NRC and between the different BCP populations for the three cranial views (Table 2). The cross-validation analysis indicated high percentages (83 to 100 %) of correct classification between SRN-NRC and the BCP groups for the ventral and lateral views and low allocation percentages for the dorsal view (45 to 100 %). Regarding the dorsal and lateral views, the incorrect classification of NRC and SRN was 14 % ($n = 1$) and vice versa, whereas for the ventral view it was 28.57 % ($n = 2$) and 33.33% ($n = 2$), respectively. Between NRC and the BCP group, only the ventral view of

Table 2. Percentage of correct assignment for the *Lepus californicus* groups according to the discriminant function analysis/cross-validation (DFA/CV). See Figure 1 for allocation of localities by group.

Dorsal cranial view DFA/CV	Group A	Group B	Group C	Group D	North Río Conchos	South Río Nazas
Group A	---	100/35	100/65	100/67	100/100	100/86
Group B	100/44	---	88/58	93/40	100/71	100/86
Group C	100/67	95/50	---	87/47	100/71	100/86
Group D	100/44	95/60	85/61	---	100/43	100/86
North Río Conchos	100/100	100/45	100/88	100/80	---	86/43
South Río Nazas	100/78	100/95	100/85	100/87	86/43	---
Ventral cranial view DFA/CV						
Group A	---	100/55	100/76	100/61	86/57	100/83
Group B	100/55	---	100/72	100/77	100/71	100/100
Group C	100/89	100/70	---	92/38	100/100	100/100
Group D	89/78	100/75	92/48	---	100/100	100/83
North Río Conchos	100/100	100/90	92/96	100/100	---	67/50
South Río Nazas	100/100	100/100	100/100	100/100	71/43	---
Lateral cranial view DFA/CV						
A	---	100/53	100/72	100/75	100/100	100/100
B	100/62	----	100/72	100/75	100/100	100/100
C	100/62	100/74	----	92/75	100/86	100/100
D	100/62	100/68	100/56	---	100/86	100/86
SRC	100/100	100/100	100/92	100/100	---	86/57
SRN	100/100	100/95	100/96	100/83	86/57	---

the skull misclassified 14 % of cases (n = 1). No incorrect classifications were identified between SRN and BCP.

Genetic Analysis. In total, 27 haplotypes for the Cyt *b* gene were found in 49 sequences (5 SRN, 5 NRC, and 19 BCP; Appendix 1). Eighteen haplotypes were unique: 2 from SRN, 2 from NRC, and 16 from BCP (Table 3). In the *Altiplano Central*, four haplotypes were shared: two across the rivers between SRN and NRC (haplotypes 4 and 8), one within SRN (haplotype 3), and one within NRC (haplotype 5). The statistical parsimony network of Cyt *b* (Figure 6b) did not show a clear geographic genetic structure between haplotypes of SRN and NRC. The localities are separated from each other between 1 and 2 mutational steps and they do not share haplotypes with those of BCP group. The haplotypes of BCP group were separated from SRN and NRC by 1 mutational step. The most frequent haplotype was haplotype 9 in seven individuals between groups A and B. In the BCP, five haplotypes were shared among all groups (A to D; haplotypes 9, 17, 19, 24, 26). The Mantel test shows a significant correlation ($P < 0.05$) between geographic distance and genetic distance between SRN-NRC and for the BCP between A-C and B-D.

The population with the greatest genetic diversity within-variation was the BCP group D with higher values of H (10), UH (6), P (10), NP (3.25), and π (0.13), followed by BCP group C and SRN (Table 3). Values for the genetic variation parameters of Cyt *b* are given in Table 3. The average p-distance between individuals from SRN-NRC is 0.8 %, and among all BCP groups was 1.23 % (0.5 to 1.6; Table 4). For populations BCP and NRC, the p-distance was 1.52 % (1.0 to 2.0), and between BCP and SRN, 1.5 % (1.1 to 2.0). The pairwise F_{ST} value between NRC and SRN was 0.09; within BCP groups, 0.14 to 0.43; between NRC and BCP groups, 0.43 to 0.58; and between SRN and BCP, 0.39 to 0.56 (Table 4). Statistically significant differences ($P < 0.05$) were found between specimens from NRC-SRN and BCP (groups A to B).

The phylogenetic reconstructions used the sequence evolution model TIM3+I+G that best fit our sequence data set. In the BI tree, the Cyt *b* gene converged on tree topologies that were virtually identical to those of distance and ML (data not shown) analyses. *Lepus californicus* was found to be monophyletic in the Cyt *b* gene, with higher bootstrap support (100), and is represented by two subclades (A and B). Subclade A is polyphyletic, including haplotypes of SRN and NRC populations, and subclade B is polyphyletic, including haplotypes of BCP populations (Figure 6a; Appendix 1).

Discussion

Groups of *L. californicus* from SRN and NRC did not show statistical differences in size and shape versus any of the other groups analyzed in cranial views. Only minor differences were found in four specific skull regions: 1) parietal (dorsal and ventral views), 2) nasal (dorsal and lateral views), 3) zygomatic arch (dorsal, ventral, and lateral views), and 4) auditory bullae (ventral view). Compared to SRN, the NRC population has an expanded parietal region, reduced nasals, wider zygomatic arch, and more prominent auditory bullae. SRN has a contracted parietal region, wider nasals, narrower zygomatic arch, and less prominent auditory bullae.

It has been postulated that skull variation can be associated with ecological and biological aspects and results from the adaptation to specific environmental, dietary, and physiological pressures (Bowers and Brown 1982; Cox *et al.* 2012; Klingenberg 2013). The variations in morphology in *L. californicus* may be related to physiological and nutritional adaptations, making populations capable of inhabiting many types of habitats with diverse ecological and climatic characteristics (Brown *et al.* 2019). The absence of significant differences between SRN and NRC populations could be considered as a similar adaptation process to the different environments associated with dietary and physiological pressures. Therefore, the genetic differences found between SRN and NRC (0.8 %) are similar or lower than those within BCP populations (0.5 to 1.6 %) without a physical barrier that can restrain the dispersal across populations. On the other hand, the genetic differences between east and west across the Gulf of California are far greater (1.0 to 2.0 %). These findings suggest that the east-west analysis across the Gulf of California-Colorado River reveals a very strong effect, with a greater genetic distance; however, the genetic distance between north and south across the Conchos-Nazas rivers is lower than in the BCP, which lacks a fluvial barrier.

Table 3. Parameters used for assessing genetic variation of Cytochrome *b* among groups of *Lepus californicus*. Abbreviations as follows: total sample size (N); number of haplotypes (H); unique haplotypes per group (UH), number of polymorphic sites (P); number of observed sites with transitions (Tt); number of observed sites with transversions (Tv); mean number of pairwise differences (NP); nucleotide diversity (π); and Fu's Fs (F). *Indicates significance at $P < 0.001$.

	N	*H*	*UH*	*P*	*Tt*	*Tv*	*NP*	*π*	*F*
	49	27		25	16	9	4.110 ± 2.082	0.164 ± 0.092	-17.057
Group									
North Río Conchos	7	5	2	4	3	1	2.000 ± 1.276	0.000 ± 0.058	-1.547
South Río Nazas	7	5	2	6	4	2	2.190 ± 1.320	0.087 ± 0.062	-1.352
Group A	3	1	0	0	0	0	0.0 ± 0.0	0.0 ± 0.0	0*
Group B	8	5	4	8	5	3	2.000 ± 1.256	0.080 ± 0.057	-1.151*
Group C	7	5	4	8	8	0	3.238 ± 1.895	0.129 ± 0.086	-0.552*
Group D	17	10	6	10	5	5	3.250 ± 1.761	0.130 ± 0.078	-3.125*

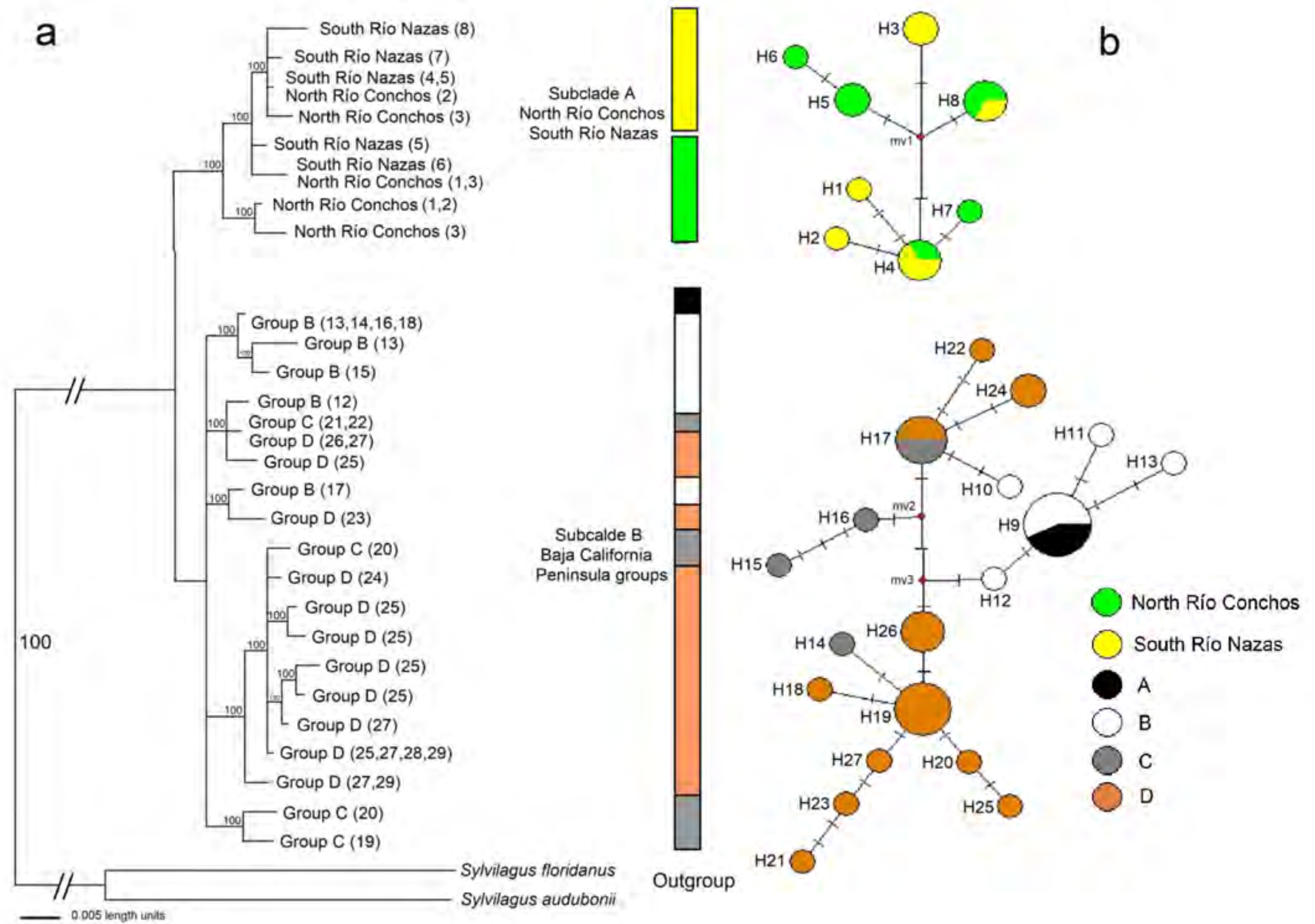

Figure 6. a) Bayesian inference (BI) tree generated by the consensus tree with the 50% majority-rule algorithm of *Lepus californicus* from north Río Conchos, south Río Nazas and groups of the Baja California Peninsula, based on Cyt *b* haplotypes. The tip of each branch includes the group and its location number (in parenthesis; see Appendix 1 and Figure 1 for details). Values of branch support are indicated on the phylogenetic tree. b) Haplotype network of 27 non-redundant haplotypes (H) recovered from the Cyt *b* (625 bp) dataset that included all populations of *L. californicus*. Transverse lines crossing the lines connecting haplotypes represent the number of base-substitution differences. The size of each circle represents the frequency of the haplotype in the gene.

The morphological differences found between SRN and NRC could be more closely related to selective traits that can increase or decrease the predation rate of individuals and as a plastic response to ecological differences (as in small rodents; Neiswenter and Riddle 2010; Neiswenter *et al.* 2019), such as color pattern variations of the body. Each group was found in a different habitat: SRN in thorn scrubs and savannas and NRC in grasslands. The BCP populations possessed differences in coloration, total size (approximately 20 %), and leg and ear sizes. These characters are associated with semidesert grasslands and scrub vegetation growing in sandy habitats with coastal vegetation (Lorenzo *et al.* 2010). Therefore, CVA and DFA show differences in the skull between the mainland and peninsular populations, but not within either the mainland or the peninsula.

Table 4. Average genetic p-distances estimated from Cyt *b* sequences between groups of *Lepus californicus* (above diagonal) and F_{ST} values (below diagonal) for pairs of populations.

	North Río Conchos	South Río Nazas	Group A	Group B	Group C	Group D
North Río Conchos	-	0.8	1.0	1.3	1.7	2.0
South Río Nazas	0.09	-	1.1	1.4	1.5	2.0
Group A	0.58	0.56	-	0.5	1.1	1.5
Group B	0.49	0.48	-0.17	-	1.4	1.6
Group C	0.43	0.39	0.35	0.32	-	1.3
Group D	0.52	0.53	0.43	0.41	0.14	-

Also, data from mitochondrial DNA support two distinct genetic groups in the distance, ML, and BI methods. The first includes SRN-NRC specimens with no phylogenetic break associated with barriers such as Río Conchos and Río Nazas; the second comprises BCP specimens and multiple ramifications suggesting a cryptic structure and genetic diversity (Neiswenter *et al.* 2019) within *L. californicus*.

Among the specimens of different groups collected throughout BCP (at similar latitudes and with no geographic barriers), the genetic p-distance between their populations was greater (1.23 % on average), as well as the genetic differentiation between groups (0.14 to 0.43; Table 4). The results of the Mantel Test were similar for the population with the presence of the rivers as possible barriers (SRN-NRC) that those which do not have a physical barrier (BCP: A-C and B-D). In the three cases, the genetic distances were significantly correlated with the geographical distance in the same geographical distance (*c. a.* 600 km) among groups (SRN-NRC, A-C and B-D). It is probable that the evolutionary process in *L. californicus* has occurred differentially. It may be slower in the Río Conchos and Río Nazas area, so that it is not reflected as a vicariant process. In contrast, evolutionary rates may be faster in BCP, leading to morphological and genetic differences among its populations (Álvarez-Castañeda and Lorenzo 2017).

Four conclusions that can be taken from this study are as follows. A) The canyon system that separates SRN and NRC can limit the gene flow of many species and the dispersal of small mammals (mostly small rodents) and lead to subspeciation, as in *S. audubonii* and *L. callotis*; however, it does not seem to be an effective barrier for *L. californicus*. B) The canyon system in the mainland can be equivalent to the ecological barrier in the BCP (as gene flow between populations is seemingly limited in both cases and the Gulf of California-Rio Colorado barrier is far stronger than that of the Ríos Nazas and Conchos. C) The phenotypic differences between populations may be due to the unique selection pressures in each one, according to their particular distribution occupying different habitats, probably leading to expeditious local adaptations associated with vigorous demographic expansion and probably rapid radiation (Melo-Ferreira and Alves 2018), as suggested by the Fu's Fs test values for Cyt *b*. D) The high vagility of *L. californicus*, favored by changes in land use in addition to its broad distribution range, have probably induced the conditions for a continuous gene flow throughout the *Altiplano Central* driven by dispersal across the canyon system.

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Appendix 1

Localities of *Lepus californicus* from north Río Conchos (Chihuahua), south Río Nazas (Durango), and four groups of *L. californicus* from Baja California Peninsula, México. Haplotype numbers (H) and GenBank accession numbers are provided. Museum acronyms as follows: ECO-SC-M = Mammal Collection at El Colegio de la Frontera Sur; CIB = Mammal Collection at Centro de Investigaciones Biológicas del Noroeste; CNMA = National Mammal Collection at Instituto de Biología. The nomenclature and classification of the subspecies of the Baja California Peninsula is according to Álvarez-Castañeda and Lorenzo (2017). Subscripts indicate genetic $_{(g)}$, geometric morphometrics $_{(a)}$, and morphological $_{(m)}$ analyses.

North Río Conchos

Lepus californicus texianus (*n* = 7$_{(g, a, m)}$). Chihuahua: **Group 1**: Ejido Colaguna, km 100 Carretera a Cd. Juárez 1,620 msnm, 29.3827°, -106.3504°, ECO-SC-M 9503$_{(g, a, m)}$ GenBank MW940634, H8; 9504$_{(g, a, m)}$ GenBank MW940636, H5. **Group 2**: Rancho Experimental La Campana INIFAP, Km 82 Carretera Chihuahua-Cd Juárez, 1,560 msnm, 29.2717°, -106.3468°, ECO-SC-M 9498$_{(g, a, m)}$ GenBank MW940635, H5; 9499$_{(g, a, m)}$ GenBank MW940633, H4. **Group 3**: Ejido la Flor, 5 km NW de la Cd. de Chihuahua 1,460 msnm, 28.7675°, -106.1068°, ECO-SC-M 9500$_{(g, a, m)}$ GenBank MZ055407, H6; 9501$_{(g, a, m)}$ GenBank MZ055408, H7; 9502$_{(g, a, m)}$ GenBank MZ055406, H8.

South Río Nazas

Lepus californicus texianus (*n* = 7$_{(g, a),}$ 6$_{(m)}$). **Group 4**: Durango: Nazas, Cerro de la Cruz. 1.5 km SW del Poblado de Nazas, 1,276 msnm, 25.1902°, -104.0998°, ECO-SC-M 9497$_{(g, a, m)}$ GenBank MZ055403, H4. **Group 5**: Peñón Blanco, Ranchería Los Jacales 21 km por terracería al NW de Peñón Blanco, 1,544 msnm, 24.9404°, -104.1310°, ECO-SC-M 9494$_{(g, a, m)}$ GenBank MW940630, H3; 9495$_{(g, a, m)}$ GenBank MZ055405, H3; 9496$_{(g, a, m)}$ GenBank MW940632, H4. **Group 6**: Mapimí, 140 km E Mapimí, 26.6751°, -103.7499°, GenBank KP735419$_{(g)}$, H8. **Group 7**: Nombre de Dios, Brecha Saca Cosecha. 3.65 km NW de Tuitan 1,881 msnm, 24.0242°, -104.2808°, ECO-SC-M 9491$_{(g, a, m)}$ GenBank MW940631, H2; 9492$_{(a)}$. **Group 8**: Suchil, La Laguna 3.8 km N Suchil 1,979 msnm, 23.6553°, -103.9174°, ECO-SC-M 9490$_{(g, a, m)}$ GenBank MZ055404, H1.

Baja California Peninsula

Group A

Lepus californicus martirensis (*n* = 3$_{(g)}$, 12$_{(a, m)}$). **Group 9**: Valle de la Trinidad, 31.3556°, -115.6250°, CIB 2868$_{(a)}$, 18630$_{(m)}$, 18631$_{(a, m)}$, 18632$_{(a)}$. **Group 10**: Baja California: Cataviña, 26 km N, 14 km W Cataviña, 29.9342°, -114.8983°, CIB 2352$_{(g, a, m)}$ GenBank KP735407, H9. **Group 11**: Calamajue, 12.5 km S, 19 km W Calamajue, 484 msnm, 29.5294°, -114.3055°, CIB 18638$_{(g, a, m)}$ GenBank KP735409, H9; CIB 18635$_{(a, m)}$, 18636$_{(a, m)}$, 18637$_{(m)}$, 18639$_{(a, m)}$, 18640$_{(a, m)}$. **Group 12**: Guerrero Negro, 83 km N Guerrero Negro, 129 msnm, 28.7323°, -114.1080°, CIB 12570$_{(g)}$ GenBank KP735410, H9. Guerrero Negro, 28.0786°, -113.9187°, CIB 16459 to 16461$_{(a, m)}$.

Group B

Lepus californicus martirensis (*n* = 8$_{(g)}$, 18$_{(a)}$, 13$_{(m)}$). **Group 13**: Baja California: Vizcaíno, 14.5 km N, 14.5 km W Guerrero Negro, 52 msnm, 28.0786°, -113.9187°, CIB 16461$_{(g)}$ GenBank KP735411, H10. Baja California Sur: **Group 14**: Palo de Rayo, Sierra de San Francisco, 1,187 msnm, 27.6106°, -113.0277°, CIB 17359$_{(g)}$ GenBank KP735413, H11; CIB 16463$_{(g)}$ GenBank KP735414, H9; CIB 2869$_{(m)}$, 8670$_{(m)}$, 8671$_{(m)}$, 16462 to 16464$_{(a, m)}$, 16465 to 16467$_{(a)}$, 17357 to 17359$_{(a)}$. San Francisco de la Sierra, 1,187 msnm, 27.5899°, -113.0923°, CIB 2870$_{(a, m)}$, CIB 8670$_{(g)}$ GenBank KP735403, H9; CIB 10906$_{(a)}$, 11657$_{(a)}$. **Group 15**: Guerrero Negro, Corral de Berrendos, 61 km S, 5 km W Guerrero Negro. 27.4017°, -114.0192°, CIB 18641$_{(a, m)}$, CIB 18642$_{(g, m)}$ GenBank KP735415, H12. **Group 16**: San Ignacio, 17 km S, 5 km W San Ignacio, 48 msnm, 27.1183°, -112.9675°, CIB 8422$_{(g ,a, m)}$ GenBank KP735417, H13; 8668$_{(a, m)}$, 8669$_{(a, m),}$ 11656$_{(m)}$. **Group 17**: San Zacarías, 27.1419°, -112.9130°, CIB 12489$_{(a)}$, 12490$_{(a)}$. Bahía Asunción, 9 km S, 24.5 km E Bahía Asunción, 14 msnm, 27.0742°, -114.0750°, CIB 10894$_{(g)}$ GenBank KP735412, H9. **Group 18**: Baja California Sur: Santa Rosalía, 2 km SE Santa Rosalía. 27.38102°, -112.4400°**,** CNMA 40823$_{(g)}$ GenBank KP735416, H9.

Group C

Lepus californicus xanti (*n* = 3$_{(g)}$, 9$_{(a)}$, 8$_{(m)}$). **Group 19**: Baja California Sur: Ultima Agua, 265 msnm, 25.5593°, -111.2708°, CIB 15188$_{(g)}$ GenBank KP735423, H16; CIB 15187$_{(a, m)}$, 15188$_{(a, m)}$, 15189$_{(a)}$,15190$_{(a, m)}$, 15191$_{(a, m)}$. **Group 20**: María Auxiliadora, 7 msnm, 25.4463°, -111.9509°, CIB 15193$_{(g)}$ GenBank KP735421, H14, CIB 15195$_{(g)}$ KP735422, H15; CIB 15192 to 15195$_{(a, m)}$.

Lepus californicus magdalenae (*n* = 2$_{(g)}$ 8$_{(a),}$ 11$_{(m)}$). Baja California Sur: **Group 21**: Ley Federal de Agua No. 4, 78 msnm, 25.1904°, -111.5381°, CIB 15200$_{(g)}$ GenBank KP735425, H17; CIB 15196 to 15201$_{(a, m)}$, 15202 to 15206$_{(m)}$. **Group 22**: Insurgentes, 9 km S, 3.37 km E Ciudad Insurgentes, 47 msnm, 25.1800°, -111.7417°, CIB 718$_{(g)}$, GenBank KP735424, H17. **Group 23**: Isla Magdalena, 24.6607°, -112.1521°, CIB 15184$_{(a)}$, 19141$_{(a)}$, 19142$_{(a).}$ Isla Margarita, 24.4604°, -111.8441°, CIB 18883 to 18885$_{(a)}$, 18981$_{(a)}$. ***Lepus californicus sheldoni*** (*n* = 5$_{(a)}$). Baja California Sur: **Group 24**: Isla Carmen, 25.8259°, -111.2139°, CIB 15185$_{(a)}$, 15186$_{(a)}$, 15222$_{(a)}$, 15224$_{(a)}$, 21328$_{(a)}$.

Group D

Lepus californicus insularis (*n* = 2$_{(a)}$). **Group 25**: Baja California Sur: Isla Espíritu Santo, 24.4563°, -110.3069°; CIBNOR 21322$_{(a)}$, 21335$_{(a)}$.

Lepus californicus xanti (*n* = 19$_{(g)}$ 14$_{(a),}$ 63$_{(m)}$). Baja California Sur: **Group 26**: La Paz, 11 km S, 28 km W La Paz, 187 msnm, 24.0244°, -110.6625°, CIB 17679$_{(g)}$ GenBank KP735442, H24; CIB 17680$_{(g)}$ GenBank KP735443, H24. La Paz, El Mogote, 4 km N, 9 km E La Paz, 5 msnm, 24.1581°, -110.3482°, CIB 15506$_{(g)}$ GenBank KP735434, H17; CIB 15505 to 15510$_{(m)}$. Baja California Sur: Carretera Transpeninsular, 25.2569°, -111.7732°, CIB 6603$_{(m)}$, 6604$_{(m)}$, 8423$_{(a, m)}$. El Centenario, 14.5 km W La Paz, 24.1496°, -110.4343°, CIB 892 to 894$_{(m)}$. Brisamar, 25 km W La Paz, 24.1490°, -110.5430°, CIB 4907$_{(m)}$, 4908$_{(m)}$, 13442$_{(m)}$, 13443$_{(m)}$. 2.5 km S, 12.2 km W La Paz, 24.1392°, -110.4365°, CIB 15212$_{(m)}$, 15213$_{(m)}$. El Comitán, 17.5 km W La Paz, 24.1376°, -110.4670°, CIB 891$_{(m)}$. 3.18 km S, 1.43 km E La Paz, 24.1101°, -110.2922°, CIB 15214$_{(m)}$. 11 km S, 28 km W La Paz, 24.0244°, -110.6625°, CIB 17678$_{(m)}$, 17679$_{(m)}$, 17680$_{(m)}$. **Group 27**: Los Planes, km 7 carretera Los Planes, 24.1529°, -110.4869°, CIB 15511$_{(g, m)}$ GenBank KP735435, H18. 1 km N, 6 km E Los Planes, 23.9665°, -109.9362°, CIB 15215$_{(m)}$. Los Planes, 4.24 km S, 400 m W Los Planes, 45 msnm, 23.9326°, -109.9467°, CIB 15218$_{(g)}$ GenBank KP735432, H19; ECO-SC-M 2869$_{(g)}$ KP735445, H26; CIB 15217$_{(g)}$ KP735446, H26; CIB 15221$_{(g)}$ KP735447, H27; CIB 15219$_{(g)}$ KP735448, H19; ECO-SC-M 2870$_{(g)}$ KP735449, H17. 4.24 km S, 400 mts W Los Planes, 23.9326°, -109.9467°, CIB 15216 to 15221$_{(m)}$, 18643 to 18646$_{(m)}$. **Group 28**: 7 km S, 6 km W Reforma Agraria, 24.0545°, -110.9761°, CIB 17667 to 17677$_{(m)}$, 17691 to 17694$_{(m)}$. Reforma Agraria, 7 km S, 6 km W Reforma Agraria, 10 msnm, 24.0545°, -110.9761° CIB 17661$_{(g)}$ GenBank KP735436, H20; CIB 17674$_{(g)}$ KP735437, H21; CIB 17667$_{(g)}$ KP735438, H22; CIB 17691$_{(g)}$ KP735440, H19; CIB 17670$_{(g)}$ KP735441, H23; CIB 17676$_{(g)}$ KP735444, H25. **Group 29**: Todos Santos, 13.5 km N, 10 km W Todos Santos. 23.5867°, -110.3441°, CIB 15520$_{(g)}$ GenBank KP735450, H19. Todos Santos, 16 km N, 12.5 km W Todos Santos. 23.5747°, -110.3257°, CIB 15517$_{(g)}$ GenBank KP735451, H26, CIB 15518$_{(g)}$ KP735452, H19. 13.5 km N, 10 km W Todos Santos, 23.5867°, -110.3441°, CIB 15519$_{(m)}$, 15520$_{(m)}$. 18 km N, 11.5 km W Todos Santos, 23.5747°, -110.3257°, CIB 15516$_{(a, m)}$, 15517 to 15520$_{(a)}$.16 km N, 12.5 km W Todos Santos, 23.5717°, -110.3257°, CIB 15517$_{(m)}$, 15518$_{(m)}$. **Group 30**: Santa Anita, 23.1902°, -109.7610°, CIB 17681 to 17688$_{(a, m)}$.

Appendix 2

Definition of landmarks in three cranial views of the black-tailed jackrabbit (*Lepus californicus*).

Dorsal cranial view. 1. Tip of nasal bone. 2. Point of tangency along anterior lateral margin of nasal bone. 3. Intersection point of the nasals with frontal bone. 4. Posterior tip of suture between nasal and frontal bones. 5. Anterior tip of zygomatic arch in the lateral edge. 6. Anterior meeting point between zygomatic arch and frontal bone in the interior edge of the orbit. 7. Most lateral point along the interior edge of orbit. 8. Most posterior point along the interior edge of the zygomatic process. 9. Middle point between the frontal and the two interparietal bones. 10. Lateral posterior middle point between frontal y parietal bones. 11. Meeting point of parietal bone and supraoccipital bone along longitudinal axial of cranium. 12. Most posterior point of the supraoccipital bone along longitudinal axial of cranium. 13. Most posterior point of parietal bone. 14. Most lateral point of parietal bone.

Ventral cranial view. 15. Meeting point of incisor tooth. 16. Lateral contact point of incisor and premaxilla. 17. Most extreme anterior point of the incisive foramen. 18. Most extreme anterior and lateral point of the suture of the premaxilla and maxilla. 19. Most extreme anterior point of palatal bridge along the longitudinal axial of cranium. 20. Posterior end of the incisive and palatal foramen. 21. Most lateral tangent point of the incisive and palatal foramen. 22. Most extreme anterior point of the first premolar. 23. Most extreme posterior point of the palatal bridge. 24. Anterior end of palatal bridge along the lateral margin of entopterygoid crest. 25. Anterior concave point of the zygomatic process along the lateral margin. 26. Most extreme posterior point of the last molar. 27. Most anterior end of orbit. 28. Most interior lateral end of orbit. 29. Most posterior end of orbit. 30. Tangent point where the posterior lateral margin of zygomatic arch expanded in the internal edge of orbit. 31. Tangent point where the posterior lateral margin of zygomatic arch expanded in the lateral edge of orbit. 32. Most anterior point of basioccipital bone along the longitudinal axial of cranium. 33. Lateral end of basioccipital bone meeting with basisphenoid bone. 34. Most extreme anterior point of the tympanic bullae. 35. Lateral meeting point of tympanic bullae and basisphenoid bone. 36. Apophysis of the bullae. 37. Carotid foramen. 38. Most internal contraction of basioccipital bone along lateral margin. 39. Most extreme anterior point of the *foramen magnum*. 40. Most extreme lateral point of the *foramen magnun*. 41. Most extreme posterior point of the *foramen magnum*.

Lateral cranial view. 42. Most extreme anterior point of the nasal bone. 43. Most extreme posterior point of the skull. 44. Most extreme anterior and lateral point of the nasal bone. 45. Most extreme anterior point of the maxilla. 46. Most extreme anterior point of the zygomatic arch. 47. Most extreme posterior point of the zygomatic arch. 48. Nasal bone suture with frontal bone at the middle part of the skull. 49. Ventral suture between premaxilla and maxilla bones. 50. Parietal bone suture with frontal bone at the middle part of the skull. 51. Posterior dorsal part of the jugal bone. 52. Inteparietal and occipital bones suture at the middle part of the skull. 53. Most extreme lateral superior point meeting the tympanic bullae and the squamous temporal bone. 54. Most extreme lateral posterior point between the bullae and the styloid process. 55. Most extreme inferior point of the bullae.

Appendix 3

ANOVA of log centroid size and Procrustes shape in groups of the black-tailed jackrabbit (*Lepus californicus*) between sexes in different cranial views. SS = sums of squares, MS = mean squares, df = degrees of freedom, F = F statistics, *P* = parametric P-values.

	SS	MS	df	F	P
Dorsal cranial view					
Size	125.19	65.60	2	1.48	0.2341
Shape	0.0062	0.0001	48	1.78	0.001
Ventral cranial view					
Size	54.10	27.05	2	0.31	0.7375
Shape	0.0016	0.0001	100	0.59	0.999
Lateral cranial view					
Size	176.06	88.03	2	1.69	0.1919
Shape	0.0035	0.0001	48	0.85	0.760

Appendix 4

Principal Component Analysis (PCA) of cranial shape between cranial views of *Lepus californicus*. Only PCs that are informative according to the broken-stick test are presented. PC = Principal Component.

PC	Eigenvalues	% Total variance	% Cumulative
Dorsal cranial view			
1	0.0004	27.57	27.57
2	0.0002	13.76	41.33
3	0.0002	10.74	52.06
4	0.0001	8.33	60.40
5	0.0001	6.22	66.62
Ventral cranial view			
1	0.0003	28.04	28.04
2	0.0001	8.86	36.90
3	0.0001	6.62	43.53
4	0.0001	6.16	49.69
5	0.0001	5.55	55.24
6	0.0001	4.80	60.05
7	0.0001	4.06	64.11
8	0.0001	3.50	67.61
Lateral cranial view			
1	0.0003	22.12	22.12
2	0.0002	14.72	36.84
3	0.0002	11.61	48.45
4	0.0001	8.45	56.90
5	0.0001	6.80	63.70
6	0.0001	5.63	69.33

Appendix 5

Canonical Variate Analysis (CVA) of variations in cranial shape between groups of *Lepus californicus*. CV = Canonical variate.

CV	Eigenvalues	% Total variance	% Cumulative
Dorsal cranial view			
1	4.45303	63.26	63.26
2	1.01223	14.38	77.65
3	0.70594	10.03	87.67
4	0.55458	7.88	95.55
5	0.31292	4.45	100.00
Ventral cranial view			
1	21.66371	65.16	65.16
2	5. 93506	17.85	83.01
3	3.13309	9.42	92.43
4	1.48733	4.47	96.90
5	1.02966	3.10	100.00
Lateral cranial view			
1	8.01007	73.47	73.47
2	1.00460	9.21	82.68
3	0.82288	7.55	90.23
4	0.59524	5.46	95.69
5	0.47030	4.31	100.00

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Revision of moles in the genus *Scapanus*

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Scapanus latimanus is a species with many morphological differences among its populations. This variation is associated with multiple taxonomic changes at the species or subspecies level. This study incorporates genetic analyses and comparisons with previous morphological studies to propose a better understanding of the *latimanus* complex. Mitochondrial markers (cytochrome *b*; cytochrome *c* oxidase subunit I; and cytochrome *c* oxidase subunit III) were sequenced to construct a phylogeny for the subfamily Scalopinae in North America. Genetic distances ranged from 2.49 to 10.50 % among geographic areas. Results identified three monophyletic clades with high bootstrap support values. Based on our phylogenetic analysis and previous morphological analyses, we confirm *S. anthonyi* from San Pedro Mártir as a valid species and propose that *S. occultus* from southern California and northern Baja California peninsula be considered as a species.

Scapanus latimanus es una especie con muchas diferencias morfológicas entre sus poblaciones. Esta variación está asociada con múltiples cambios taxonómicos a nivel de especie o subespecie. Para proponer una mejor comprensión del complejo *latimanus*, en este estudio se incorpora la información genética a los estudios previos de morfología. Se secuenciaron genes de origen mitocondrial (citocromo b; citocromo c oxidasa subunidad I y III) para construir la filogenia para la subfamilia Scalopinae en Norteamérica. Se obtuvieron distancias genéticas con un intervalo entre 2.49 a 10.50 % entre áreas geográficas. Los resultados identifican tres clados monofiléticos con altos valores de soporte. Con base en la comparación del análisis filogenético e información morfológica previa conocida, se confirma como una especie válida a *S. anthonyi* de San Pedro Mártir y proponemos que *S. occultus* del sur de California y norte de la península de Baja California también sea considerada como una especie.

Keywords: Molecular markers; moles; North America; phylogeny; Scalopinae; Talpidae; taxonomy.

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Introduction

The family Talpidae includes three subfamilies, Scalopinae, Talpinae, and Uropsilinae, with Scalopinae being restricted to America and containing four genera *Condylura, Parascalops, Scalopus,* and *Scapanus* (Shinohara *et al.* 2003; Hutterer 2005). *Scapanus* is the only genus including more than one species; *S. latimanus, S. orarius,* and *S. townsendii* (Hutterer 2005). A fourth species, *S. anthonyi,* has been considered, although it has undergone many taxonomic changes. *S. anthonyi* was described as a full species by Allen (1893), and later was considered to be a subspecies of *S. latimanus* (Palmer 1937). Palmer (1937) argued that morphometric characteristics of *S. anthonyi*, such as its smaller size and fewer number of upper premolars, also were present in *S. l. occultus*, and consequently, *S. anthonyi* should be considered a subspecies of *S. latimanus* (see: Palmer 1937; Hutchinson 1987).

In his review of American moles, Jackson (1915) recognized *S. anthonyi* as a species because *S. anthonyi* has a projection in the braincase between the interparietal and the mastoid, which was absent in *S. l. occultus* (Jackson 1915). However, Palmer (1937) did not acknowledge these characteristics in the specimens that he examined, and therefore did not consider *S. anthonyi* a valid species. Huey (1936) suggested an additional difference between *S. l. occultus* and *S. anthonyi*; specifically, the manus (part of the pentadactyl limb that includes the metacarpals and phalanges) in *S. anthonyi* is squarer and smaller, with broader and heavier phalanges and with tips of the pterygoids parallel. In an alternative view, Hutchinson (1987) suggested that *S. anthonyi* shared characteristics with *S. orarius*; however, he continued to recognize *S. anthonyi* as a subspecies of *S. latimanus*. Populations of *S. anthonyi* in San Pedro Mártir, Baja California and those of *S. l. grinnelli* and *S. l. occultus* in southern California and northern Baja California peninsula are smaller in size and the skull is wider in relation to all other subspecies of *S. latimanus* from central and northern California (Yates and Salazar-Bravo 2005). Differences in skull morphology also occur between these two groups. *S. anthonyi* has only two or three upper premolars, and the temporal fossae is larger (Allen 1893; Jackson 1915; Huey 1936; Yates and Salazar-Bravo 2005).

Previous morphological analyses of all subspecies of *S. latimanus* indicated that some subspecies should be junior synonyms (Yates and Salazar-Bravo 2005) of other subspecies. For example, *S. l. grinnelli* (Jackson 1914) of *S. l. occultus* (Grinnell and Storer 1916); *S. dilatus* (True 1894), *S. alpinus* (Merriam 1897), and *S. l. caurinus* (Palmer 1937) of *S. l. latimanus* (Bachman 1842); *S. l. sericatus* (Jackson 1914), *S. l. campi* (Grinnell and Storer 1916), and *S. l. monoensis* (Grinnell 1918) of *S. l. minusculus* (Bangs 1899). However, *S. l. insularis* (Palmer 1937) and *S. l. parvus* (Palmer 1937) were not subjected to taxonomic changes (Yates and Salazar-Bravo 2005).

Based on these previous studies, the taxonomic status of species within the *S. latimanus* group has revealed several inconsistencies. The goal of this study is to better define the phylogenetic relationships of populations within *Scapanus* and combine these relationships with known morphological characteristics to evaluate the potential number of species. To achieve this goal, three mitochondrial genes were sequenced: cytochrome *b* (*Cytb*; $n = 23$); cytochrome *c* oxidase subunit I (*CoI*, $n = 29$); and cytochrome *c* oxidase subunit III (*Co3*; $n = 29$).

Materials and Methods

Sample collection. The dataset included specimens of the genus *Scapanus* ($n = 31$) represented by the species *S. orarius*, *S. townsendi*, *S. latimanus,* and outgroup specimens of *Condylura*, *Neurotrichus,* and *Scalopus* ($n = 6$). Tissue samples were obtained from the Collection of Mammalian tissues at Centro de Investigaciones Biológicas del Noroeste (CIB), Field Museum of Natural History (FMNH), Museum of Southwestern Biology at the University of New Mexico (MBS), and Museum of Vertebrate Zoology at the University of California (MVZ). Information for localities and museum catalog numbers are provided in Table 1. All capture and handling methods followed the animal care and use guidelines of the American Society of Mammalogists (Sikes *et al.* 2016). For all analyses, we grouped specimens from these localities into three species, *S. orarius*, *S. townsendi,* and *S. latimanus,* with *S. latimanus* further subdivided into three geographic units: 1) central and northern California (Group A, localities 7-14); 2) southern California and northern Baja California peninsula (Group B, localities 15-17); and 3) Sierra de San Pedro Mártir (Group C, locality 18; Figure 1; Table 1). This resulted in 31 geographic samples of Scalopinae.

DNA extraction and PCR conditions. Genomic DNA was extracted from muscle tissue preserved in 95 % ethanol (archived at -20 °C) or frozen (archived at -80 °C) using the DNAeasy Kit (QIAGEN Inc., Valencia, CA) protocols.

The following conditions were used for the initial double-strand amplification: 12.5 µl of (10 ng) template, 4.4 µl ddH2O, 2.5 µl of each primer pair (10 nM concentration), 0.474 µl (0.4 nM) dNTPs, 0.5 µl (3 mM) $MgCl_2$, 0.125 µl Taq polymerase (platinum, Invitrogen, Carlsbad, CA), and 1× Taq buffer, to a final volume of 25 µl. The amplification conditions consisted of an initial denaturation at 94 °C for 3 min followed by 37 denaturation cycles at 94 °C for 45 s each; 60 s annealing at 50 °C (*Cytb*), 51 °C (*Co1*), 55 °C (*Co3*); and extension at 72 °C for 60 s; the products of the PCR amplification were verified in agarose gel, purified and sequenced both ways using the sequencing service of Macrogen Inc, Korea. The first part of the cytochrome b (*Cytb,* ~800 bp) gene was amplified using the primers MVZ05/MVZ16 (primer sequences given in Smith and Patton 1993; Smith 1998), the 658-bp fragment of cytochrome c oxidase subunit I (*Co1*) was amplified with the primers LCO1490/HCO2198 (Ivanova *et al.* 2007), and the 717-bp fragment of cytochrome c oxidase subunit III (*Co3*) was amplified with the primers L8618/H9323 (Riddle 1995). We aligned nucleotide sequences in Sequencher ver. 3.1 (Gene Codes Corp., Ann Arbor, Michigan), verified alignments visually, and translated them into amino acids for alignment confirmation. The haplotypes generated and used were deposited in GenBank (Table 1).

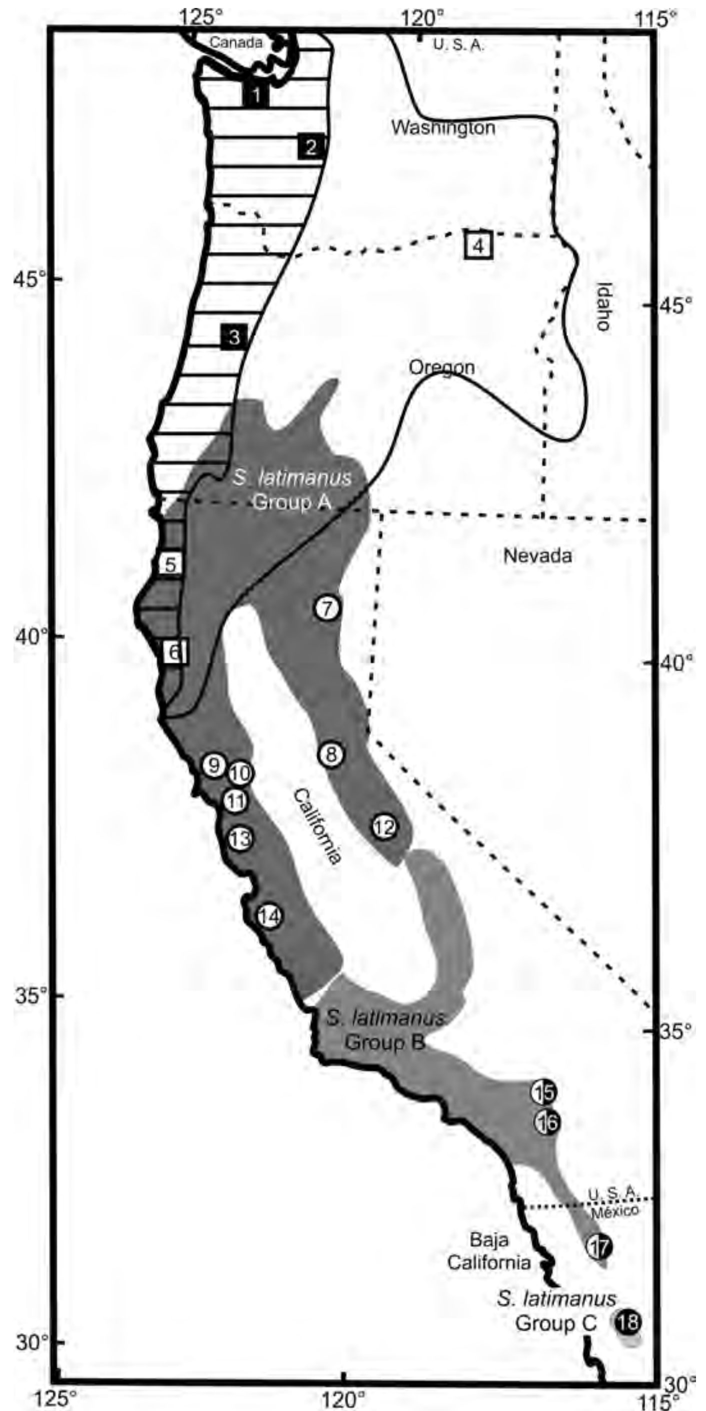

Figure 1. Distribution map of the species of the subfamily Scalopinae in North America. *Scapanus townsendi* (solid squares 1-3), *S. orarius* (open squares 4-6), and *S. latimanus* (circles). *S. latimanus* is split into three geographic groups: A) Central and northern California (localities 7-14, light gray circle); B) Southern California and northern Baja California peninsula (localities 15-17, half light/half dark circles); and C) Sierra de San Pedro Mártir (locality 18, dark gray circle).

Table 1. List of specimens examined, locations according to Figure 1. #Catalog = museum catalog number of the reference collection. GenBank accession number for mitochondrial marker. Group (Gr), Number of map (M), State (ST), * Zhao and Jian 2015. ** Mouchaty *et al.* 2000.

Gr	M	Species	#Catalog	St	Locality	Lat	Long	Co1	CO3	Cytb
								GenBank accession numbers		
	1	*Scapanus t. olympicus*	MSB 43550	WA	9.2 Mi S, 2.7 Mi W Port Angeles	47.9851	-123.4878	MZ150455	MZ217155	MZ217129
	1	*Scapanus t. olympicus*	MSB 43552	WA	9.2 Mi S, 2.7 Mi W Port Angeles	47.9851	-123.4878	MZ150456	MZ217156	MZ217130
	2	*Scapanus t. towsendii*	MVZ 220251	WA	24303 Se 468th Street, Enumclaw	47.1809	-122.0175	MZ150457	MZ217157	
	2	*Scapanus t. towsendii*	MVZ 220252	WA	24303 Se 468th Street, Enumclaw	47.1809	-122.0175	MZ150458	MZ217158	
	4	*Scapanus t. townsendii*	MSB 40780	OR	9 Mi E Alsea	44.3817	-123.4135	MZ150459	MZ217159	MZ217131
	4	*Scapanus t. townsendii*	MSB 40781	OR	9 Mi E Alsea	44.3817	-123.4135	MZ150460	MZ217160	MZ217132
	3	*Scapanus o. schefferi*	MSB 54620	WA	2 Mi W Walla Walla	46.0647	-118.3835	MZ150461	MZ217161	MZ217133
	3	*Scapanus o. schefferi*	MSB 54621	WA	Country Club, Walla Walla	46.0389	-118.3503	MZ150462	MZ217162	MZ217134
	5	*Scapanus o. orarius*	MSB 43626	CA	3.8 Mi S, 2.7 Mi E Trinidad	41.004	-124.0916		MZ217163	MZ217135
	5	*Scapanus o. orarius*	MSB 43627	CA	3.8 Mi S, 2.7 Mi E Trinidad; T7n, R1e, Sec 8	41.004	-124.0916	MZ150463	MZ217164	MZ217136
	5	*Scapanus o. orarius*	MSB 43628	CA	3.8 Mi S, 2.7 Mi E Trinidad; T7n, R1e, Sec 8	41.004	-124.0916	MZ150464		
	7	*Scapanus o. orarius*	MVZ 224399	CA	11 Mi N Westport On Hwy 1.	39.7506	-123.819	MZ150465	MZ217165	
A	6	*Scapanus l. dilatus*	MVZ 217713	CA	Eagle Lake Road (Lassen Co. A1), Eagle Lake.	40.6235	-120.8399	MZ150466	MZ217166	MZ217137
	8	*Scapanus l. dilatus*	MSB 47919	CA	1 Mi S, 4.5 Mi E Somerset, 2850	38.6334	-120.5984	MZ150467	MZ217167	MZ217138
	9	*Scapanus l. caurinus*	MVZ 216930	CA	Easy Sweet Farm, Sebastapol	38.472	-122.8544	MZ150468	MZ217168	
	10	*Scapanus l. caurinus*	MVZ 199506	CA	2930 Redwood Road, Napa	38.3167	-122.3385	MZ150469	MZ217169	MZ217139
	11	*Scapanus l. latimanus*	MVZ 218027	CA	103 Aldarado Rd., Berkeley	37.8579	-122.2396	MZ150470		
	12	*Scapanus l. latimanus*	MVZ 201320	CA	Forest S of Chapel, Yosemite Valley	37.7408	-119.5907		MZ217170	
	13	*Scapanus l. latimanus*	MSB 48532	CA	Palo Alto, Stanford University Campus	37.429	-122.1695	MZ150471	MZ217171	MZ217140
	14	*Scapanus l. latimanus*	MVZ 222251	CA	Hastings Natural History Reservation	36.3785	-121.5568	MZ150472	MZ217172	
	14	*Scapanus l. latimanus*	MVZ 228295	CA	Haystack Hill, Hastings Natural History Reservation	36.3847	-121.5627	MZ150473	MZ217173	MZ217141
B	15	*Scapanus l. occultus*	MSB 47311	CA	10 Mi Se Big Bear City, Heart Bar campground	34.1586	-116.786	MZ150474	MZ217174	MZ217142
	16	*Scapanus l. occultus*	MSB 47317	CA	3.6 Mi N, 9.8 Mi E Hemet, Lake Fulmor	33.8052	-116.7785	MZ150475	MZ217175	MZ217143
	17	*Scapanus l. occultus*	MSB 43120	BC	Laguna Hanson	32.0489	-115.9056	MZ150476	MZ217176	MZ217144
	17	Scapanus l. occultus	MSB 40343	BC	Laguna Hanson	32.0489	-115.9056	MZ150477	MZ217177	MZ217145
	17	*Scapanus l. occultus*	MSB 40344	BC	Laguna Hanson	32.0489	-115.9056	MZ150478	MZ217178	MZ217146
	17	*Scapanus l. occultus*	MSB 40345	BC	Laguna Hanson	32.0489	-115.9056	MZ150479	MZ217179	MZ217147
	17	*Scapanus l. occultus*	MSB 47308	BC	Sierra Juárez, Laguna Hanson	32.0489	-115.9056	MZ150480	MZ217180	MZ217148
C	18	*Scapanus l. anthonyi*	MSB 47306	BC	Sierra San Pedro Mártir, 3.9 Mi by Road W Vallecitos	31.0167	-115.5333	MZ150481	MZ217181	MZ217149
	18	*Scapanus l. anthonyi*	CIB 32000	BC	Sierra San Pedro Mártir	31.0167	-115.5333	MZ150482	MZ217182	MZ217150
	18	*Scapanus l. anthonyi*	MSB 47307	BC	Sierra San Pedro Mártir, 20 Mi S, 10.9 Mi E Vallecitos	31.0167	-115.5333	MZ150483	MZ217183	MZ217151
Out-group		*Condylura cristata*	KU144678 *					KU144678		
		Condylura cristata	NC029762 *					NC_029762		
		Neurotrichus g. hyacinthinus	MVZ 200061	CA	Headwaters of Big Austin Creek, N of Cazadero	38.6138	-123.1315	MZ150484	MZ217184	MZ217152
		Scalopus a. machrinus	FMNH 167212	MI	Fennville	42.5939	-86.1017	MZ150485	MZ217185	MZ217153
		Scalopus a. machrinus	FMNH 167213	MI	Fennville	42.5939	-86.1017	MZ150486	MZ217186	MZ217154
		Talpa europaea	Y19192 **					Y19192		

Phylogenetics analysis. The methodology for phylogenetic analysis was similar to that used by Camargo and Álvarez-Castañeda (2020). The most appropriate substitution model for the dataset for each of the three gene regions, as well as for the concatenated series, was determined using the Akaike information criterion (AIC) as implemented in MrAIC (Nylander 2004). Four separate Bayesian inference and maximum-likelihood analyses were conducted on the three genes independently; the concatenated series had three partitions with one per gene (*Cytb*, *Co1*, and *Co3*).

Bayesian analyses were implemented in (MrBayes ver. 3.0b4; Huelsenbeck and Ronquist 2001) with four separate runs with Markov-chain Monte Carlo simulations starting from a random tree. Each run was conducted for 20 million generations and sampled at intervals of 1,000 generations. Of the samples trees, the first 50 % were discarded as burn-in and all remaining trees were analyzed to find the posterior probability of resulting nodes. A consensus tree was generated with the 50 % majority-rule algorithm in PAUP 4.0b10 (Swofford 2002). The percentage of samples recovered in a particular clade was assumed to be the posterior probability of that clade in PAUP 4.0b10 using a heuristic search with 1,000 replicates and swapping with the TBR algorithm.

Maximum-likelihood (ML) analyses were performed in PAUP ver. 4.0b10 (Swofford 2002) algorithm (Felsenstein 1981) using a heuristic search with 1,000 replicates and swapping with the TBR algorithm. Reliability was assessed using each of the three codon positions individually while applying equal weights and nodal support using nonparametric bootstrapping. Members of each genus were used because although some phylogenetic analyses were done using allozymes (Yates and Greenbaum 1982; Moore 1986) the phylogenetic relationships among moles of North America were not previously examined using gene sequencing. Trees were rooted with Scalopini (*Scalopus aquaticus*), Urotrichini (*Neurotrichus gibbsii*), and Condylurini (*Condylura cristata*; Motokawa 2004).

Results

Phylogenetic analyses. AIC tests revealed that the best evolution model was a GTR model: *Cytb* (GTR + I + G), *Co1* (GTR + I), *Co3* (GTR + G), and the concatenated genes (GTR + I + G). BI and ML trees for *Cytb*, *Co1*, and *Co3*, and the concatenated data with four partitions converged on an essentially identical topology (Figure 2).

Analyses of the three genes within *Scapanus* resolved five haplogroups with strong bootstrap support (>95 %), as follows. Haplogroup 1: only specimens from San Pedro Mártir, Group C of *S. latimanus*; Haplogroup 2: specimens from southern California and northern Baja California peninsula, Group B of *S. latimanus*; Haplogroup 3: all *S. latimanus* specimens from Group A of central and northern California; Haplogroup 4: specimens of the two subspecies of *S. townsendi* with a very low percentage of differences between them; and Haplogroup 5: containing two groups, each with specimens of different subspecies of *S. orarius* (Figure 2).

Scapanus latimanus Group C (Haplogroup 1) is separated from the other *S. latimanus* Groups A and B by two different species, *S. townsendi* (Haplogroup 4) and *S. orarius* (Haplogroup 5). The percentage of pairwise genetic differences (p-distance) for the three genes between Group C (San Pedro Mártir) and each of Group A (northern California) and Group B (southern California and northern Baja California) ranged from 7.22 to 10.50 %. The genetic differences between Group A and Group B ranged from 2.49 to 5.75 % (Table 2).

Discussion

Genetic data revealed that *S. townsendi* and *S. orarius* are monophyletic and sibling taxa, as reported by Shinohara *et al.* (2003), and are substantially different from *S. latimanus*, as previously reported by Moore (1986). However, the geographic groups of *S. latimanus* do not exhibit a north-south phylogenetic relationship. The *S. latimanus* Group C from San Pedro Mártir formed an inconsistent relationship with the other two *S. latimanus* haplogroups from California and the northern Baja California peninsula (Haplogroups 2 and 3). Genes *Co3* (boot = 56) and *Cytb* (boot = 68) show *S. latimanus* Group C (San Pedro Mártir) as a sister group to specimens from southern California and Baja California. However, analyses of *Co1* (boot = 95) and the concatenated group (boot = 95) show *S. latimanus* Group C basal to all *Scapanus* clades (boot = 95), including *S. townsendi* and *S. orarius* (Figure 2). Each of the topologies show that *S. latimanus* Group C differs from Groups A and B. Although Hutchinson (1987) reported that *S. anthonyi* shared characteristics with *S. orarius*, this was not supported by the sequence data.

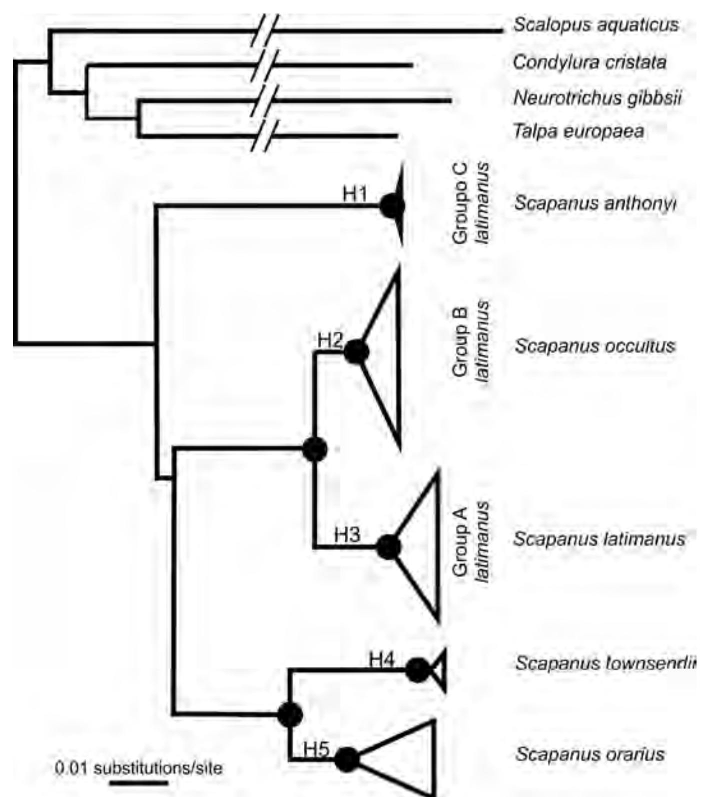

Figure 2. Bayesian tree constructed from three mitochondrial DNA genes (cytochrome *b*, cytochrome oxidase subunit I, and cytochrome oxidase subunit III) from members of the subfamily Scalopinae in North America and one of *Talpa* from Europe. Haplogroup 1 contains a single specimen from San Pedro Mártir, Group C of *S. latimanus* (*S. anthonyi*). Haplogroup 2 contains specimens from southern California and northern the Baja California peninsula, Group B of *S. latimanus* (*S. occultus*). Haplogroup 3 contains specimens from central and northern California, Group A of *S. latimanus* (*S. latimanus*). Haplogroup 4 is represented by two subspecies of *S. townsendi*. Haplogroup 5 is represented by two subspecies of *S. orarius*.

We did not perform a morphometric analysis of the craniodental measurements because this previously was reported by Yates and Salazar-Bravo (2005). Yates and Salazar-Bravo (2005) reported statistical differentiation in morphological characters between *S. l. occultus* and *S. anthonyi* separated by a distance > 50 km. In addition, they found significant differences between *S. l. occultus* and *S. l. latimanus sensus* (Yates and Salazar-Bravo 2005), with *S. l. occultus* being smaller overall. No specimens of *S. l. occultus* and *S. l. latimanus* have been collected in sympatry and in the two areas where *S. l. occultus* and *S. l. latimanus* occur, namely the southern part of Sierra Nevada and northern portion of Santa Barbara, it appears that *S. l. occultus* occurs in lower altitudes, and *S. l. latimanus* at higher altitudes.

Based on the genetic distance values between Groups A, B, and C, coupled with the morphological differences between them (Yates and Salazar-Bravo 2005), these groups can be considered as different species. The main morphological variations in the specimens of these groups are a smaller size in relation to the northern populations of *S. latimanus* and the variation in the number of upper premolars (Palmer 1937; Yates and Salazar-Bravo 2005).

Table 2. Genetic distances (pairwise distance, *p*) among samples of the three geographical groups of *Scapanus latimanus, S. orarius,* and *S. townsendi* for the mitochondrial cytochrome *b* (*Cytb*), cytochrome oxidase subunit I (*Co1*), and cytochrome oxidase subunit III (*Co3*).

	Group A	Group B	Group C
S. latimanus Group A (central and northern California)			
Cytb	0.13-1.96	4.13-5.75	8.00-9.67
Co1	0.00-1.22	2.59-3.65	9.59-10.50
Co3	0.15-0.61	2.49-3.60	7.89-8.58
Concatenated	0.24-1.23	3.27-4.17	8.53-8.95
S. latimanus Group B (southern California and northern Baja California)			
Cytb	4.13-5.75	0.0-1.50	7.88-9.52
Co1	2.59-3.65	0.00-0.90	8.68-9.98
Co3	2.49-3.60	0.0-0.92	7.22-7.55
Concatenated	3.27-4.17	0.00-0.99	7.82-8.01
S. latimanus Group C (San Pedro Mártir)			
Cytb	8.00-9.67	7.88-9.52	0.00-1.75
Co1	9.59-10.50	8.68-8.98	0.00-0.15
Co3	7.89-8.58	7.22-7.55	0.00-0.15
Concatenated	8.53-8.95	7.82-8.01	0.05-0.09
S. orarius			
Cytb	6.75-8.66	7.50-9.50	8.00-9.89
Co1	8.98-10.05	9.52-9.89	9.44-10.20
Co3	8.06-10.14	6.88-9.09	8.40-9.61
Concatenated	8.05-9.00	7.82-8.43	8.72-9.00
S. townsendi			
Cytb	8.00-9.39	8.00-9.50	9.75-11.14
Co1	8.98-8.98	7.91-8.98	9.59-10.20
Co3	8.92-9.79	7.72-8.75	9.09-9.61
Concatenated	8.67-9.19	8.40-8.72	9.43-9.62

Further, based on the sequence data, *Scapanus latimanus* from northern and southern California form two haplogroups. Haplogroup 3 includes all the specimens assigned to *S. latimanus* Group A (northern California) and Haplogroup 2 includes Group B (southern California and north Baja California peninsula). The population from San Pedro Mártir previously was considered as a distinct species, *S. anthonyi* (Allen 1893; Jackson 1915; Huey 1936; Yates and Salazar-Bravo 2005) and later subsumed into *S. latimanus* based on morphological characters (although a large series of specimens was never reviewed, which may have biased the interpretation), based primarily on the smaller size and number of upper premolars (Palmer 1937; Hutterer 2005). The morphological analyses (Yates and Salazar-Bravo 2005) and genetic analyses performed in this study support the consideration of *S. anthonyi* as a distinct species and indicates that *S. anthonyi* is restricted in distribution to the San Pedro Mártir mountain range.

Based on our phylogenetic analysis and its morphological characteristics (Allen 1893; Jackson 1915; Huey 1936; Yates and Salazar-Bravo 2005), we support that *S. anthonyi* is a different species from *S. latimanus.* Additionally, we propose that specimens known as *S. latimanus occultus* (including *S. l. grinnelli*) from southern California and northern Baja California peninsula should be considered as a distinct species (*S. occultus*) different from *S. latimanus* from central and north California and from *S. anthonyi* inhabiting San Pedro Mártir. Therefore, we consider that the genus *Scapanus* contains five species that should be recognized as *S. anthonyi*, *S. latimanus, S. occultus*, *S. orarius,* and *S. townsendi.*

Scapanus anthonyi Allen 1893

1893. *Scapanus anthonyi* Allen, Bull Amer. Mus. Nat. Hist., 5:200, August. Type locality: "Sierra San Pedro Martir, 7000 ft, Baja California [México]". Adult male, skin and skull, American Museum of Natural History number 6313, collected by A. W. Anthony.

1937. *Scapanus latimanus anthonyi* Palmer, J. Mamm. 18:312, August. Name combination.

Geographic range. Restricted to the highlands of Sierra San Pedro Mártir, Baja California, México.

Diagnosis and comparison. Scapanus anthonyi can be differentiated from the other species of *Scapanus* in having fewer than seven unicuspid teeth behind the incisors in the mandible and maxilla and total skull length <32.5 mm. Projection present in the braincase between the interparietal and the mastoid (Jackson 1915). Manus more square and smaller, with broader and heavier phalanges, tips of the pterygoids bones of the upper palate paral-

lel (Huey 1936). Smaller in all craniodental and somatic measurements relative to all other subspecies of *S. latimanus*, and teeth larger and crowded (Yates and Salazar-Bravo 2005:494 in table 3). Differing from *S. orarius* and *S. townsendii* in a smaller in size; dorsal coloration darker, almost black; no spaces between all unicuspid teeth, usually crowded; and rostrum short and broad.

Comments. Scapanus anthonyi has a distribution restricted to the upper portions of the Sierra San Pedro Mártir, within the pine and oak-pine forest. Collecting moles in the region is complex for several reasons. First, the gopher *Thomomys nigricans* is very abundant in the same area, so it is common to find gophers galleries that impinge upon and destroy mole galleries. Second, both species share a sympatric distribution throughout the mountain range. Third, although this region is a protected area, large numbers of cattle graze in the area and destroy the mole galleries. Fourth, specimens of *S. anthonyi* are very small in size, so their galleries also are small and the soil relief (molehill) that results from gallery construction is very difficult to determine. Fifth, galleries have a simple structure, and raise just 3 cm above the ground, and any leaf litter makes these molehills invisible (Cortés-Calva pers. obs.).

In the area, both *Scapanus* and *Thomomys* are named "*topos"* (moles) with no distinction between them, and only old ranchers give different names to them. *Scapanus* are called "*topos de manoplas*" (baseball-gloved moles) in reference to its forefoot size. *Thomomys* are known only as "*topos".*

Scapanus occultus Grinnell and Swarth 1912

1912. *Scapanus latimanus occultus* Grinnel and Swarth, Univ. California, Publ. Zool., 10:131, April. Type locality: "Santa Ana canyon, 400 ft (12 mi NE Santa Ana), Orange County California". Subadult female, skin and skull, Museum of Vertebrate Zoology, University of California, Berkeley, number 2369, collected by H. S. Swarth.

1914. *Scapanus latimanus grinnelli* Jackson, Proc. Biol. Soc. Washington, 27:56. Considered as junior synonym.

Geographic range. From Laguna Hanson (Sierra de Juárez) Baja California, México northwestward to Santa Barbara and northward to Yosemite Valley in Mariposa County, California.

Diagnosis and comparison. Scapanus occultus can be differentiated from *S. latimanus* in its smaller size and longer and wider skull (Yates and Salazar-Bravo 2005:494 in table 3). Some specimens have fewer than seven unicuspid teeth, but only on a single side of the mandible or maxilla. It differs from *S. orarius* and *S. townsendii* by the same characteristics mentioned in *S. anthonyi.*

Scapanus latimanus (Bachman 1842)

1842. *Scapanus latimanus* Bachman, Boston Jour. Nat. Hist., 4:34. Type locality "probably from Santa Clara, Santa Clara, California" Mounted specimen with imperfect skull, Berlin Museum, collected during October 1834.

1912. *Scapanus latimanus latimanus* Grinnell and Swarth, Univ. California, Publ. Zool., 10:131, April. First use of current name combination.

Geographic range. From Santa Barbara and Yosemite Valley, California, northward to southcentral Oregon.

Diagnosis and comparison. Scapanus latimanus can be differentiated from *S. orarius* and *S. townsendii* by the same characteristics mentioned in *S. anthonyi.*

Keys for the species of *Scapanus*

1. Dorsal coloration usually brown to gray. All unicuspid teeth with variable spacing between them and usually crowded; rostrum short and broad 2

1a. Dorsal coloration almost black. All unicuspid teeth with regular spacing between them, and not crowded; rostrum long and narrow 4

2. Fewer than seven unicuspid teeth behind the incisors in the mandible and maxilla; total skull length less than 32.5 mm .... *Scapanus anthonyi*

2a. Seven unicuspid teeth behind the incisors in the mandible and maxilla; total skull length >32.5 mm 3

3. Total length >161.0 mm. Skull length >34.0 mm in males and 33.4 mm in females. Ratio of mastoidal breadth to greatest skull length <49%, including the population of Alameda Island, California .... *Scapanus latimanus*

3a. Total length <161.0 mm. Skull length <34.0 mm in males and 33.4 mm in females. Ratio of mastoidal breadth to greatest skull length >49%, not including the population of Alameda Island, California .... *Scapanus occultus*

4. Total length >200.0 mm on average. Sublacrimal–maxillary ridge well developed; skull > 40.0 mm ....
.... *Scapanus townsendii*

4a. Total length <200.0 mm on average. Sublacrimal–maxillary ridge little developed; skull <40.0 mm ....
.... *Scapanus orarius*

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On the utility of taxonomy to reflect biodiversity: the example of Lasiurini (Chiroptera: Vespertilionidae)

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The taxonomic history of bats of the tribe Lasiurini (Chiroptera: Vespertilionidae) has undergone significant changes over time. Authors at different times have recognized various numbers of genera and subgenera within the tribe. The most recent proposed change to generic level taxonomy (that there should be three genera recognized instead of a single genus) has been debated in the literature. We reviewed papers that commented on the recent changes to lasiurine generic taxonomy, as well as those that have adopted the new taxonomy and the ones that have not. We also reviewed the relevant taxonomic literature from 1942 to the present that shows the fluid taxonomic history of these bats. The literature review shows that the recently proposed taxonomic change recognizing the three groups of lasiurine bats as distinct genera is the only taxonomy that differentiates the tribe from the genera. Examination of times to most recent common ancestor (TMRCA) of 24 vespertilionid genera shows *Lasiurus*, if it comprises all Lasiurini, to be an outlier. Here, we support the recognition of three genera and explain how this arrangement best reflects the evolutionary history and biodiversity of the tribe by bringing the three distinct lineages in line with other vespertilionid genera with respect to divergence times and genetic distances. Considering the Lasiurini to comprise a single genus, *Lasiurus*, that genus has the greatest TMRCA of all vespertilionid genera analyzed, comparable only to the genus *Kerivoula* of the monotypic subfamily Kerivoulinae. However, recognizing the three deeply diverged lasiurine lineages (red bats, yellow bats, and hoary bats) as genera brings their TMRCAs in line with other genera and approximates the mean TMRCA of the 24 genera analyzed. Opponents of Baird *et al.'s* taxonomy argued that these three lineages should be considered as subgenera to avoid changing scientific names for purpose of nomenclatural stability and ease of conducting a literature search and because the three deep lineages are all monophyletic. That argument ignores the biological reality that these lineages are morphologically distinct, and that they are genetically as distinct from one another as other genera of vespertilionid bats; there is ample precedent in the mammalian literature to use values of TMRCA as a metric to maintain consistency of higher taxonomic categories such as genus. We encourage other mammalogists to utilize taxonomy to its maximum descriptive potential, while taking into account phylogenetic relationships of the taxa of interest.

La historia taxonómica de murciélagos del tribu Lasiurini (Chiroptera: Vespertiliónidae) muestra grandes cambios significativos con el tiempo. Varios autores han reconocido diferentes números de géneros y subgéneros dentro del tribu. La taxonomía mas recién propone cambios a nivel de géneros (sugeriendo que existen tres géneros y no solamente uno). Este punto de vista ha sido debatiado en la literatura. Hemos revisado los trabajos que tratan de la taxonomía de estos murciélagos, notando que algunos autores aceptan la nueva taxonomía y otros autores no. Hemos revisado la bibliografía desde 1942, y por lo general, subraya la taxonomía fluida de estos murciélagos. La bibliografía muestra que solamente los estudios recientes que reconocen los 3 grupos de murciélagos como tres géneros distintos pueden diferenciar filogenéticamente el tribu del género o géneros. Considerando el tiempo del ancestro en común mas recién (TMRCA) de 24 géneros de vespertiliónidos muestra que *Lasiurus*, si incluye el total de Lasiurini, es un caso aparte. En este trabajo apoyamos el reconocimiento de tres géneros distintos y notamos como describe la evolución de los Lasiurini cuando se comparen géneros de Lasiurini con géneros de los vespertiliónidos con respeto a las divergencias evolucionarias y distancias genéticas. Si consideramos que los Lasiurini efectivamente está descrito solamente por el género *Lasiurus*, tal género va a tener el TMRCA mas grande, y comparable solamente con la subfamilia Kerivoulinae. Sin embargo, si reconocmos los tres géneros muy divergidos (murciélagos rojos, amarillos, canosos) como géneros hace sus TMRCA comparables a los otros géneros y a los otros 24 géneros analizados. Los que difieren con nosotros piden estabilidad de nomenclatura porque hace mas fácil la búsqueda de información bibliográfica y porque cada lineaje es monofilética. Ese argumento ignora la realidad biológica que estas lineas son tan distintas una del otra que de los otros géneros de vespertiiónidos. Recomendamos que mastozoólogos utilicen la taxonomía que tiene el máximo poder explicativa e incluye las relaciones filogenéticas del tribu, es decir los valores de TMRCA como una medida más para describir las categorías mas altas, como género, de la taxonomía.

Keywords: Genus; lasiurine bats; phylogeny; subgenus; taxonomy.

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Introduction

Recent studies on the evolutionary history of lasiurine bats (Chiroptera: Vespertilionidae: Lasiurini; Baird *et al.* 2015, 2017) have spurred discussion in the mammal literature regarding the broader implications of taxonomic revisions to long-standing nomenclature. Baird *et al.* (2015, 2017) recommended the separation of lasiurines into three genera: *Lasiurus* (red bats), *Aeorestes* (hoary bats), and *Dasypterus* (yellow bats). Until Baird *et al.* (2015, 2017), the vespertilionid tribe Lasiurini had been considered monotypic, comprised solely of the monophyletic genus, *Lasiurus*. At the time, some authors also recognized two subgenera: *Dasypterus* (yellow bats) and *Lasiurus* (red + hoary bats; Figure 1A). In the more distant past, other authors recognized each of these groups (red, hoary, and yellow bats) as separate genera. Throughout the taxonomic history of these bats, their status has been in flux (Figure 2). The purpose of this paper is to review the relevant literature regarding generic and subgeneric taxonomy within the Lasiurini and to address the concerns expressed by Ziegler *et al.* (2016), Novaes *et al.* (2018), and Teta (2019).

Methods

We reviewed the literature beginning with Tate (1942) who first recognized the Tribe Lasiurini and included the bats commonly referred to as red bats, hoary bats, and yellow bats. The literature of this group is extensive, but we restrict our assessment to 13 papers that we consider most influential for taxonomy (Figure 2), represent all the various taxonomic proposals, and are illustrative of the numerous changes, back and forth, between recognizing one or two genera over the course of nearly 80 years. We use this information to address the criticisms of our proposed arrangement of three genera of lasiurine bats (Baird *et al.* 2015, 2017) by Ziegler *et al.* (2016), Novaes *et al.* (2018), and Teta (2019).

Additionally, times to most recent common ancestor (TMRCA) for vespertilionid bat groups were estimated from the data provided in Amador *et al.* (2016). We recorded all estimated TMRCA for vespertilionid genera, tribes, and subfamilies for those taxa that were monophyletic. Two exceptions were *Hypsugo/Falistrellus* and *Eptesicus/Histiotus*. Genera represented by a single specimen in Amador *et al.* (2016) were not included in our analysis. Finally, dates for the TMRCA within Lasiurini were obtained from the estimates of Baird *et al.* (2017) because Amador *et al.* (2016) did not include multiple specimens of all lineages within Lasiurini. Those dates were not all specified in the Baird *et al.* (2017) paper, but they were extracted from the original analysis. The date for Lasiurini and the monotypic *Lasiurus* were included in the dates obtained from Amador *et al.* (2016). We sorted the TMRCA for each taxon by date and plotted them as a histogram using R.

Results

Phylogenetic relationships of the major groups of Lasiurini and the various generic and subgeneric taxonomic arrangements are shown in Figure 1. The taxonomic changes proposed by Baird *et al.* (2015, 2017, Figure 1B), who recognized three genera of lasiurine bats instead of the single genus *Lasiurus*, have been accepted by many authors (Alurralde *et al.* 2017; Amador *et al.* 2016; Best and Hunt 2020; Schmidly and Bradley 2016; Decker *et al.* 2020; Espinosa-Martínez *et al.* 2016; Geluso and Bogan 2018; Gimenez and Giannini 2017; Krejsa *et al.* 2020; Lew and Lim 2019; Tirira 2018); however, others have not followed our taxonomy including Upham *et al.* (2019) who report the most extensive phylogeny of mammals. Ziegler *et al.* (2016), Novaes *et al.* (2018), and Teta (2019) have argued that changes to the taxonomy of Lasiurini are not warranted because *Lasiurus* was a monophyletic genus. They all suggest recognizing the red, yellow, and hoary bats as different subgenera of *Lasiurus* (Figure 1C).

Table 1 shows the estimated Time to Most Recent Common Ancestor (TMRCA) for 24 genera, five tribes, and four subfamilies of vespertilionid bats. Figure 3 shows a histogram of these estimates and illustrates how the subdivision of Lasiurini into three genera changes the monotypic *Lasiurus* from an outlier among genera to three genera having approximately average vespertilionid TMRCAs.

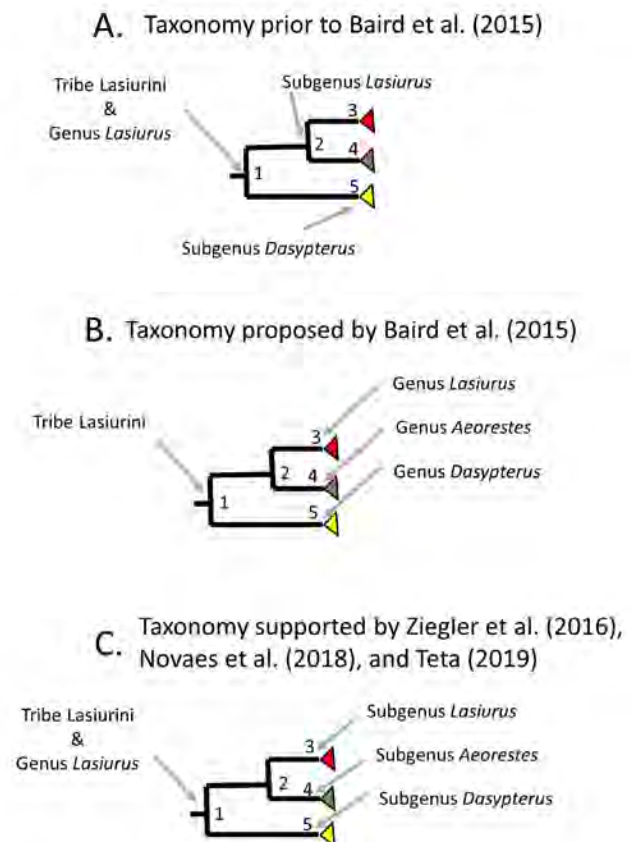

Figure 1. Phylogenetic relationships of the major groups of lasiurine bats according to Baird *et al.* (2015, 2017). The taxonomy proposed by Baird *et al.* (2015, 2017) in B differentiates the phylogenetic node that demarcates the tribe from the node, or nodes, that demarcates the genus or genera. In the taxonomies shown in A and C, node 1 demarcates both the tribe and genus.

Discussion

Teta (2019) asserted that a "main goal of the zoological nomenclature is to promote nomenclatural stability." Nomenclatural stability also is a key point raised by Ziegler *et al.* (2016) and Novaes *et al.* (2018). We agree that it is good to strive for stability; however, we reject the idea that stability is the main purpose of nomenclature. The history of nomenclature is one of frequent change and this is true for lasiurine bats (Figure 2). With the current rapid rate of change in technology, including increasing computing capacity and the increased speed and decreased cost of genetic sequencing, it is inevitable that change will be rapid in our understanding of biosystematics and consequently in taxonomy. Zoological nomenclature is a powerful tool that should be utilized to its maximum descriptive potential, not simply conserved because of the status quo and to make the lives of scientists, and online searches, easier. When used to its potential, nomenclature should convey evolutionary relationships, diversity, divergence, and the potential to clarify conservation priorities. Teta (2019) promoted the use of subgenera in cases such as Lasiurini where a monophyletic genus contains multiple distinct lineages. This was considered herein, but for reasons outlined below it is apparent that full generic recognition of the lineages is warranted due to their genetic and morphological distinction, and to keep them consistent with other vespertilionid genera. Having a distinct tribe comprised of a single genus containing three morphologically diverse lineages that are genetically as distinct and old as other vespertilionid genera does not adequately reflect the true biodiversity or history of the tribe.

Table 1. Time to most recent common ancestor (TMRCA) of vespertilionid taxa as estimated from the genetic data discussed herein. Dates are rounded to the nearest million years. Taxa with asterisks (*) follow the taxonomy of Lasiurini proposed by Baird *et al.* 2015.

Name	Taxonomic Level	TMRCA (Ma)
Harpiocephalus	Genus	2
Laephotis	Genus	3
Nyctophilus	Genus	6
Vespertilio	Genus	8
Otonycteris	Genus	8
*Lasiurus**	Genus	9
Nyctalus	Genus	11
*Aeorestes**	Genus	11
Chalinolobus	Genus	11
Corynorhinus	Genus	11
Vespadelus	Genus	12
Hypsugo + Falistrellus	Genus	12
*Dasypterus**	Genus	12
Scotophilus	Genus	14
Neoromicia	Genus	14
Scotophilini	Tribe	14
Barbastella	Genus	15
Rhogeessa	Genus	16
Glauconycteris	Genus	17
Murina	Genus	17
Eptesicus + Histiotus	Genus	17
Arielulus	Genus	19
Plecotus	Genus	19
Pipistrellus	Genus	19
Myotis	Genus	19
Lasiurus (monotypic)	Genus	20
Lasiurini	Tribe	20
Kerivoula	Genus	20
Kerivoulinae	Subfamily	20
Pipistrellini	Tribe	20
Murininae	Subfamily	21
Myotinae	Subfamily	22
Antrozoini	Tribe	23
Vespertilionini	Tribe	24
Plecotini	Tribe	27
Vespertilioninae	Subfamily	34

Novaes *et al.* (2018) also argued against the splitting of the vespertilionid genus *Lasiurus* into three distinct genera (*Aeorestes*, *Dasypterus*, and *Lasiurus*), as proposed by Baird *et al.* (2015, 2017). The first argument made by Novaes *et al.* (2018) is that genetic distance, and divergence times calculated from genetic distance, is not a useful character for defining genera because it is not comparable between different groups. They cite the examples of primate genera, *Tarsius* and *Homo*, which have vastly different divergence times from their respective sister taxa. Although this is certainly true for widely divergent taxa, Baird *et al.* (2015, 2017, and references therein) stated that divergence times and genetic distance among genera within Vespertilionidae are generally consistent, with the notable exception of *Lasiurus*.

Splitting the clades within *Lasiurus* into three distinct genera aligns their divergence times and genetic distances more closely to most other splits within Vespertilionidae. Examples of vastly different divergence times in other mammalian taxa, such as those in Primates cited by Novaes *et al.* (2018), are irrelevant to the discussion of lasiurine taxonomy because those discrepancies are not seen to the same degree in vespertilionids. Evidence of the relative consistency in age of vespertilionid taxa can be seen in Figure 3 and Table 1. The TMRCA of Lasiurini, and of *Lasiurus* prior to our subdivision of the genus, is 20 MYA making this the oldest genus in the family (tied with *Kerivoula* of the monotypic subfamily Kerivoulinae). The three genera recognized by Baird *et al.* (2015, 2017) have TMRCA estimates that range from 9 to 12 MYA and which are close to the mean TMRCA of 12.58 calculated for the 24 genera in Figure 3 and Table 1 (not including the monotypic Kerivoulinae, *Kerivoula*, and Lasiurini, *Lasiurus*, which are clear outliers).

The equivalence of taxonomic categories at the same rank and the decision as to what appropriate taxonomic level a group of species should be included have long been issues that have vexed taxonomists. Schaefer (1976, p. 2)

recommended a clear and logical solution: "Should a natural group of species, clearly distinct from other groups, be treated as a genus, tribe, or family? The answer of course appears to lie in comparing the group with other genera, tribes, and families in the higher category to which the group in question belongs." This is precisely what was done by Baird *et al.* (2015, 2017).

Secondly, Novaes *et al.* (2018) disputed that morphological differences distinguish *Aeorestes*, *Dasypterus*, and *Lasiurus* and thus stated that the taxonomic arrangement of Baird *et al.* "attributes unnecessary weight to clades whose phenotypic distinction is merely superficial". The most obvious morphological difference among the three genera is pelage color, which has given rise to the colloquial names of each group: the hoary, yellow, and red bats. *Aeorestes* (the hoary bats) are characterized by grayish pelage, *Dasypterus* (yellow bats) by yellowish pelage, and *Lasiurus* (red bats) by reddish pelage. Moreover, members of *Dasypterus* have only one premolar on each side of the upper jaw (Hall and Jones 1961) compared to two in *Lasiurus* and *Aeorestes*. *Aeorestes* species generally are larger in size than the other two genera. Other diagnostic characters of *Aeorestes* include multiple unique dental and skeletal features (Shump and Shump 1982). Although Handley (1960) considered the differences among the three "species groups" (as he referred to them) insufficient to warrant their distinction as different genera, he nonetheless provided a table of distinguishing characteristics for each (see Table 3 in Handley 1960).

The final point made by Novaes *et al.* (2018) was that vernacular names "cannot be used as an argument to take (sic) taxonomic decisions." There is a reason that the vernacular names red bats, hoary bats, and yellow bats exist: it is because the names reflect morphological distinction among the groups. We do not support changing taxonomy simply because vernacular names exist; it is the basis for their existence that supports the taxonomic change.

Characters in support of taxonomic revision. Novaes *et al.* (2018) admitted that separating a monophyletic group into different genera can be supported if "well-marked phenotypic discontinuities are detected among them" and "if supported by a suite of consistent characters, preferably from multiple datasets." But their bias towards the use of morphology in making taxonomic decisions is evident from the first part of the title of their paper. "Separation of monophyletic groups into distinct genera should consider phenotypic discontinuities." We argue that the split of Lasiurini into three distinct genera is supported by both morphology and, more importantly, genetics. Lasiurine bats are among the most easily distinguishable group of vespertilionids, even by those who are not experts in vespertilionid morphology. It is trivial to identify most species to genus from some distance away, without having to measure skull characters, etc. Moreover, given the new taxonomy proposed by Baird *et al.* (2015), it would be useful to have a morphological revision of the tribe that could provide morphological diagnoses of the genera and, importantly, include species that were unavailable to Baird *et al.* (2015) for genetic analysis. As for the "suite of consistent characters" required by Novaes *et al.* (2018) to define genera, Baird *et al.* (2015, 2017) certainly have defined a suite of characters that consistently group the three genera into reciprocally monophyletic groups and can be used to define them. Those characters are genetic data from multiple mtDNA and nuclear loci.

Despite the historical importance of morphological characters in taxonomy, we are now on the cusp of the genomics age in mammalogy (Baird *et al.* 2019), and molecular markers, not morphology, are the current gold standard for conducting phylogenetic analysis. Since it is generally agreed that taxonomy must reflect phylogeny, then it follows that genetic characters are the most useful in taxonomy as well. But morphology will continue to be used to diagnose taxa because those characters are useful for identification of specimens. In fact, morphological characters are useful to diagnose living and extinct taxonomic groups *because* they are characters with a genetic basis. If they were not, they could not be used. Nonetheless, they are not the best genetic-based characters available to us. DNA sequences are easily understood, discrete, and quantifiable.

2010s, 2000s, 1990s, 1980s, 1970s, 1960s, 1950s, 1940s

Reference	Tribe	Genera recognized	Subgenera recognized	Other
Roehrs et al. 2010	Lasiurini	*Lasiurus*	none	
Gardner and Handley 2007	Lasiurini	*Lasiurus*	none	
Wilson and Reeder 2005	Lasiurni	*Lasiurus*	*Lasiurus Dasypterus*	
Barquez, Mares, and Braun 1999	Not specified	*Lasiurus Dasypterus*	none	
Kurta and Lehr 1995	Lasiurini	*Lasiurus*	*Lasiurus Dasypterus*	
Baker et al. 1988	Not specified	*Lasiurus*	none	Results consistent with Hall and Jones 1961 – 3 kinds
Hill and Harrison 1987	Lasiurini	*Lasiurus Dasypterus*	none	
Bickham 1987	Lasiurini	*Lasiurus*	none	
Husson 1962	Not specified	*Lasiurus Dasypterus*	none	
Hall and Jones 1961	Not specified	*Lasiurus*	none	3 "named kinds" – red, yellow, hoary bats
Handley 1960	Not specified	*Lasiurus*	none	3 "species groups"
Simpson 1945	none	*Lasiurus*	none	
Tate 1942:	Lasiurini (defined the tribe)	*Lasiurus Dasypterus*	none	

Figure 2. Studies showing the various generic and subgeneric taxonomic relationships of the Tribe Lasiurini prior to Baird *et al.* (2015, 2017).

Novaes *et al.* (2018) "agree that clades may be separated into different genera if well-marked phenotypic discontinuities are detected among them." Their condemnation of our decision to recognize three well differentiated lasiurine genera is based in part on a vague and undefinable metric of morphological divergence. What exactly is a well-marked phenotypic discontinuity? No such scale exists. They went on to say that "the decision will always be arbitrary." Our decision, however, was *not* arbitrary. It was based on our use of genetics to estimate percent sequence divergences and divergence time estimates based on multiple genetic loci. These metrics were compared to other genera of vespertilionid bats and determined to be comparable. Consequently, we concluded that not only are *Aeorestes*, *Dasypterus*, and *Lasiurus* easy to distinguish based on morphology, there is also no doubt that they are distinct, highly divergent, and easily definable groups based on genetics.

Taxonomy and Phylogeny. Prior to Baird *et al.* (2015), the tribe Lasiurini was a monophyletic group consisting of a single, monophyletic genus. Therefore, the same node on the tree defined both a tribe and a genus (Node 1 in Figure 1A). The only taxonomic status given to the divergent clades within the tribe were the subgenus *Lasiurus* (node 2 in Figure 1A) and subgenus *Dasypterus* (node 5 in Figure 1A); however, the subgeneric taxonomy was not recognized by many authors (Figure 2). Even the authors who recognized subgenera would not normally use those names when referencing a particular group of lasiurines; they only used the genus name, which references node 1 in Figure 1A.

The taxonomic change proposed by Baird *et al.* (2015) maintains the monophyletic tribe defined by one node (node 1 in Figure 1B), but shifts the generic levels to their own nodes on the tree (nodes 3–5 in Figure 1B). This arrangement maximizes the use of taxonomy to describe the variation present in the lasiurine tree. The old arrangement (Figure 1A) did not assign taxonomic status to several important nodes in the lasiurine phylogeny. In using the Baird *et al.* (2015) taxonomy, researchers can now reference specific parts of the lasiurine tree by utilizing a genus name.

Critics of the Baird *et al.* (2015) taxonomy, including Ziegler *et al.* (2016), Novaes *et al.* (2018), and Teta (2019), all supported the following taxonomy: Tribe Lasiurini and Genus *Lasiurus*, with subgenera *Lasiurus*, *Aeorestes*, and *Dasypterus*. As shown in Figure 1C, this arrangement does not help resolve the issue of having the Tribe and Genus both defined by the same node on the phylogeny (*i.e.*, both the genus and the tribe still reference node 1 in Figure 1C). Although the subgenera would clarify the specific part of the phylogeny, researchers do not generally use subgenera, and therefore this proposed taxonomy does not meet our criteria of maximizing the potential of taxonomy to reflect phylogenetic divergence. Naming each of the major nodes within the lasiurine phylogeny is the most powerful way to utilize taxonomy.

The fact that the three critical papers all suggested that the recognition of three subgenera of *Lasiurus* would be appropriate, indicates that all three studies do in fact recognize these lineages as being distinct, and that we are only arguing about the taxonomic level at which they should be recognized. Patterson and Norris (2016) faced a similar dilemma in that all chipmunks were placed in a monophyletic genus *Tamias,* but it included three distinct lineages recognized as subgenera. Patterson and Norris (2016) elevated the subgenera to genera based on 1) the degree of genetic differentiation among the subgenera being comparable to other genera of ground squirrels, 2) the chipmunk lineages are older than the ground squirrel lineages as indicated in the fossil record, and 3) morphological distinction. Thus, we now have three genera of chipmunks despite the original genus *Tamias* being monophyletic. As with the lasiurine bats addressed here, the key metric to determine if the lineages represent genera or subgenera was the level of genetic differentiation and TMRCA of the lineages as compared to others in their taxonomic group.

Novaes *et al.* (2018), Teta (2019), and Ziegler *et al.* (2016) supported the status quo of recognizing a single, monophyletic genus, *Lasiurus*, within the tribe Lasiurini. They suggested that the names *Aeorestes* and *Dasypterus* should be used as subgenera (Figure 1C). Although we agree that splitting a monophyletic genus should not be done without strong evidence, we do not think that sub-generic taxonomy is the best way to handle the lasiurine situation. If, as Ziegler *et al.* (2016), Novaes *et al.* (2018), and Teta (2019) suggested, one were to recognize *Aeorestes* and *Dasypterus* as subgenera of *Lasiurus*, it would not solve the problem of having a single node on the tree defining both a genus and a tribe, thus still rendering the taxonomy ambiguous and lacking in resolution (Figure 1C). Additionally, and more importantly, it does not reflect the true degree of differentiation of these three highly distinctive lineages that are comparable in age and genetic distance to average vespertilionid generic lineages (Table 1, Figure 3).

Finally, Novaes *et al.* (2018) concluded their paper by wrongly suggesting that the taxonomic arrangement of Baird *et al.* (2015, 2017) has not been widely accepted. Multiple papers cited above have followed Baird *et al.* (2015), but it is especially worthwhile to note Amador *et al.* (2016). This is a comprehensive molecular systematic review of bats based on a study of 796 species using 9 nuclear and mitochondrial genetic markers. They report data for 270 species from 48 genera of vespertilionid bats. Notably, they report no subgenera.

We encourage other mammalogists to view taxonomy as we have outlined here. It should be a tool used to convey evolutionary relationships and biodiversity. A taxonomic arrangement is a hypothesis; therefore, it is subject to change when better data are available. The taxonomy of Baird *et al.* (2015, 2017) is a hypothesis that will be tested in future studies as better methods and more samples become available. Future studies may support or falsify the

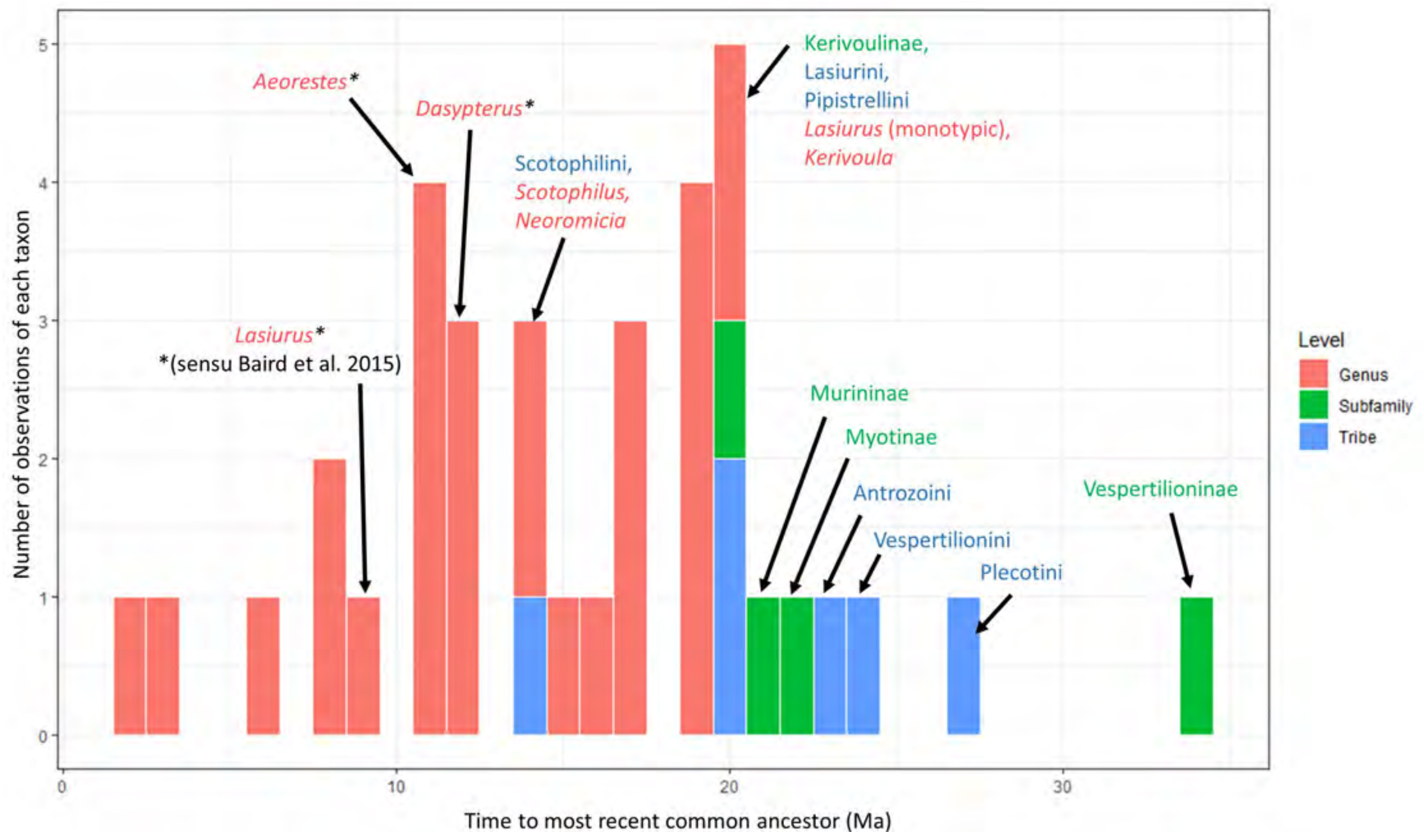

Figure 3. Observations of time to most recent common ancestors (TMRCA) of vespertilionid taxa. The data used to create this figure are detailed in Table 1.

hypothesis of three genera, but as it stands now, Baird *et al.* (2015, 2017) is the most complete and modern analysis of phylogeny and taxonomy of lasiurine bats ever conducted. Thus, their phylogenetic and taxonomic hypotheses should be accepted pending studies presenting data and analyses that falsify them.

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About the specific status of *Baiomys musculus* and *B. brunneus*

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The southern pygmy mouse, *Baiomys musculus*, is distributed in arid and semiarid lowlands, from southern Nayarit and central Veracruz in México to northwestern Nicaragua, excluding the Yucatán Peninsula and the Caribbean tropical lowlands. Previous reports suggest that *B. musculus* includes two clades that may be eligible for specific status, although this remains uncertain. We used mitochondrial DNA (cytochrome b) and morphometric data to test whether two lineages exist within the species. Molecular data support the existence of two monophyletic groups with genetic distances of 6.69 % between them: clade I, a western clade found in Colima, Jalisco, and Michoacán; and clade II, an eastern clade found in Guerrero, Morelos, Oaxaca, and Veracruz. Morphologically, clade I individuals are larger than clade II. Moreover, these clades seem to be allopatric, and their geographic boundaries are located in the complex topography of western México. Examination of previous reviews in addition to the data from this study suggest that it may be appropriate to recognize each clade as a species: clade I as *B. musculus* (Merriam, 1892) and clade II as *B. brunneus* (Allen and Chapman, 1897). Future studies with nuclear or genomic data, including Central American populations, would verify this taxonomic hypothesis.

El ratón pigmeo del sur, *Baiomys musculus*, se distribuye en las tierras bajas áridas y semiáridas, desde el sur de Nayarit y el centro de Veracruz en México, hasta el noroeste de Nicaragua, excluyendo la península de Yucatán y las tierras bajas tropicales del Caribe. Previamente fue reportado que existen dos clados genéticos que podrían representar especies diferentes, aunque esto sigue siendo incierto. Utilizamos DNA mitocondrial (citocromo b) y datos morfométricos para analizar la existencia de dos linajes al interior de la especie. Los datos moleculares confirman la existencia de dos grupos monofiléticos con distancias genéticas entre ellos de 6.69 %: el clado I, o el clado del oeste que se distribuye en Colima, Jalisco y Michoacán; y el clado II, o clado del este que habita en Guerrero, Morelos, Oaxaca y Veracruz. Los análisis morfométricos mostraron que los individuos del clado I son más grandes que los del clado II. Además, estos clados parecen ser alopátricos, y sus límites geográficos se localizan en la compleja topografía del oeste de México. Después de integrar previas investigaciones con nuestros datos, sugerimos apropiado renombrar al clado I como *B. musculus* (Merriam, 1892), y al clado II como *B. brunneus* (Allen and Chapman, 1897). Es necesario realizar estudios con datos nucleares o genómicos, incluyendo a las poblaciones de Centro América, para poder validar esta hipótesis taxonómica.

Keywords: Mitochondrial DNA; morphometric data; southern pygmy mouse; taxonomic change; western México.

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Introduction

The mice in the genus *Baiomys* belong to the cricetid tribe Baiomyini (Pardiñas *et al.* 2017) and includes some of the smallest North American rodents in the subfamily Neotominae, commonly known as pygmy mice (Packard 1960). *Baiomys* comprises two extant species: the northern pygmy mouse (*B. taylori*) and the southern pygmy mouse (*B. musculus*; Packard 1960; Pardiñas *et al.* 2017). The southern pygmy mouse primarily inhabits arid and semiarid lowlands (Packard and Montgomery 1978), from southern Nayarit and central Veracruz in México to northwestern Nicaragua, excluding the Yucatán Peninsula and the Caribbean tropical lowlands (Packard and Montgomery 1978; Pardiñas *et al.* 2017). This distribution overlaps with *B. taylori* in the west-central Mexican states of Colima, Jalisco, Michoacán, and Nayarit (Pardiñas *et al.* 2017). In this sympatric area, *B. musculus* and *B. taylori* are more strongly morphologically differentiated from each other than are other allopatric populations of each species (Packard 1960).

Baiomys musculus originally was described as *Sitomys musculus* (Merriam, 1892), and later the subgenus *Baiomys* was defined based on morphological differences (True 1894). *Baiomys* was recognized as an independent genus by Mearns (1907), who also was the first to use the binomial *Baiomys musculus*. Two years later, *Baiomys* was considered a subgenus of *Peromyscus* (Osgood 1909), but Miller (1912) subsequently re-recognized it as an independent genus. Currently, eight subspecies are recognized within *B. musculus*: *B. m. musculus* (Merriam, 1892; type specimen from Colima, México); *B. m. brunneus* (Allen and Chapman, 1897; type specimen from Veracruz, México); *B. m. nigrescens* (Osgood, 1904; type specimen from Chiapas, México); *B. m. grisescens* Goldman, 1932 (type specimen from Tegucigalpa, Honduras); *B. m. infernatis* Hooper, 1952 (type specimen from Oaxaca, México); *B. m. pallidus* Russell, 1952 (type specimen from Morelos, México); *B. m. handleyi* Packard, 1958 (type specimen from El Quiche, Guatemala); and *B. m. pullus* Packard, 1958 (type specimen from Esteli, Nicaragua; Figure 1).

Baiomys musculus mice have been studied from different perspectives, including assessment of morphometry (Osgood 1909; Hooper 1952; Packard 1960), karyotypes (Lee and Elder 1977), allozymes (Calhoun *et al.* 1989), demographic features and habitat preferences (García-Estrada *et al.* 2002; Schnell *et al.* 2008), geometric morphometrics (Abuzeineh 2006), singing behavior (Miller and Engstrom 2007), intra-specific niche modeling (Martínez-Gordillo *et al.* 2010), landscape genetics (Vargas *et al.* 2012), and ecotoxicology (Galván-Ramírez 2020). With respect to taxonomic relationships, analysis of the mitochondrial gene cytochrome b (*CytB*), detected two clades within *B. musculus*, one located in the Mexican states of Jalisco and Michoacán, and the other in Chiapas, Guerrero, Oaxaca, and Puebla (Amman and Bradley 2004). The genetic divergence between these two clades (genetic p-distance = 6.46 %) suggests that both units may be eligible for species status (Amman and Bradley 2004), however, the eight subspecies are still recognized (Pardiñas *et al.* 2017).

Our objective was to revisit the taxonomic status of these two monophyletic groups within *B. musculus* using additional genetic and morphological data. New *CytB* sequences were obtained from GenBank and others were generated herein, including individuals from the previously unanalyzed states of Colima, Morelos, and Veracruz, and the previously unsampled subspecies *B. m. brunneus*. The mitochondrial *CytB* gene was chosen because of its availability and its proven utility to clarify phylogenetic relationships in other Neotominae rodents (Edwards and Bradley 2002; Arellano *et al.* 2005; Bradley *et al.* 2007; Rogers *et al.* 2007; Vallejo and González-Cózatl 2012). Although more than 1,700 specimens of *B. musculus* have been analyzed in previous morphological studies (Osgood 1909; Hooper 1952; Packard 1960), and it was validated that differences in size and coloration among the eight subspecies exist, none of these morphological studies specifically attempted to detect morphometric evidence to confirm or reject the hypothesis of two genetic clades (Amman and Bradley 2004).

Materials and Methods

DNA sequence data. Mitochondrial sequences of the complete *CytB* gene (1,143 base pairs) were obtained from five specimens (four *B. musculus* and one *B. taylori*) housed in the mammal collection of the Museo de Zoología, Facultad de Ciencias, Universidad Nacional Autónoma de México, Ciudad de México, México (MZFC). A Qiagen DNEasy Blood & Tissue kit (Qiagen, Germantown, Maryland) was used to extract whole genomic DNA following the manufacturer's recommended protocols. Polymerase chain reaction (PCR) was used to amplify this gene using the primers MVZ05 (Smith and Patton 1993) and H15915 (Irwin *et al.* 1991). Each PCR had a final reaction volume of 13 µL and contained 6.25 µL of GoTaq Green Master Mix (Promega, Madison, WI, U.S.A.), 4.75 µL of H_20, 0.5 µL of each primer (10µM), and 1 µL of DNA stock. The PCR thermal profile included 2 minutes of initial denaturation at 95 °C, followed by 38 cycles of 30 seconds of denaturation at 95 °C, 30 seconds of annealing at 50 °C, and 68 seconds for extension at 72 °C. We included a 5-minute final extension step at 72 °C. PCR products (3 µL) were visualized using electrophoresis in 1 % agarose gels stained with SYBR Safe DNA Gel Stain (Life Technologies, Carlsbad, CA, U.S.A.). Each PCR product was purified with 1 µL of a 20% dilution of ExoSAP-IT (GE Healthcare Bio-Sciences Corp. Piscataway, NJ, U.S.A.), then incubated for 30 minutes at 37 °C followed by 15 minutes at 80 °C. Samples were cycle-sequenced using 6.1 µL of H_20, 1.5 µL of 5x buffer, 1 µL of 10µM primer, 0.4 µL of ABI PRISM Big Dye v. 3.1 (Applied Biosystems, Foster City, CA, U.S.A.), and 1 µL of the purified template. The cycle-sequencing profile included 1 minute of initial denaturation at 96 °C, followed by 25 cycles of 10 seconds for denaturation at 96 °C, 5 seconds for annealing at 50 °C, and 4 minutes for extension at 60 °C. Cycle sequencing products were purified using an EtOH-EDTA precipitation protocol and were read with an ABI 3130xl genetic analyzer (Applied Biosystems, Foster City, CA, U.S.A.). DNA sequences were edited, aligned, and visually inspected using Mega X (Kumar *et al.* 2018) and FinchTV 1.4 (Patterson *et al.* 2004). Nineteen additional *CytB* sequences of *B. musculus* were recovered from GenBank (Amman and Bradley 2004; Miller and Engstrom 2008; Light *et al.* 2016), so in total we analyzed 23 individuals of *B. musculus* (representating five subspecies: *B. m. brunneus*, *B. m. infernatis*, *B. m. musculus*, *B. m. nigriscens*, and *B. m. pallidus*; Figure 1) and one *B. taylori* individual was used as the outgroup (Appendix I). With this new sampling, we almost doubled the genetic samples analyzed by Amman and Bradley (2004), including samples from new localities, states, and subspecies previously not analyzed (Figure 1).

Maximum likelihood (ML) and Bayesian inference (BI) were used to estimate phylogenetic relationships of *B. musculus*. Prior to phylogenetic analyses, the best model and partition scheme (maximally divided by codon position) among all available models in MrBayes 3.2 (Ronquist *et al.* 2012) was selected based on the Bayesian Information Criterion (BIC) in PartitionFinder 2 (Lanfear *et al.* 2016). The IQ-TREE 1.6.12 (Nguyen *et al.* 2015) was used to estimate the ML gene tree, with branch support estimated by 1,000 replicates of nonparametric bootstrap. In MrBayes 3.2, three hot chains and one cold chain were used in two independent runs of 10 million generations, sampling data every 1,000 iterations. Convergence of MCMC results was determined by examining trace plots and sample sizes in Tracer 1.7 (Rambaut *et al.* 2018). The final topology was obtained using a majority rule consensus tree and considering a burn-in of 25 % (with effective sample sizes > 200). To evaluate levels of genetic differentiation, p-distances were estimated in Mega X using the pairwise deletion option and the Kimura 2-parameter model (Kimura 1980). These setting were chosen to facilitate comparison with previous works (Bradley and Baker 2001; Baker and Bradley 2006).

Morphological analyses. Following the removal of subadult and damaged specimens, morphometric variation was analyzed in 47 specimens of *B. musculus* from three subspecies (*B. m. brunneus*, *B. m. musculus*, *B. m. pallidus*; Figure 1; Appendix II). Twelve cranial measurements as defined by Ávila-Valle *et al.* (2012) and Hurtado and Pacheco (2017) were obtained using a digital caliper (0.01 mm resolution) as follows: condyle-incisive length (CIL), braincase depth (BCD), braincase breadth (BCB), zygomatic breadth (ZB), interorbital constriction (IOC), rostral breadth (BR), maxillary toothrow length (MTL), breadth across M3-M3 (BMM), breadth of M1 (BM1), length of auditory bulla (LAB), dentary greatest length (DGL), and dentary height (DH). Age classes were assigned to the specimens following tooth eruption and wear patterns, and we only analyzed adult specimens. To determine if the molecular results were congruent with the morphological data, we specifically tested for morphological differences between the clades detected in the molecular analyses. Because we were interested in recognizing measurements useful to detect groups within *B. musuculus*, and sexual size dimorphism has not been supported in this species (Packard 1960; Abuzeineh 2006), females and males were analyzed together.

All univariate analyses and summary statistics were performed in R 3.6.2 (R Core Team 2014). QQ-plots and the Shapiro-Wilk test were used to analyze the normality of the data in each group (clades I and II) and the Levene test from the CAR 3.0-6 package (Fox and Weisberg 2019) to test for homogeneity of variances between groups. All variables fulfilled the normality assumption, and all variables except for IOC and MTL fulfilled the assumption of homogeneity of variance. A Student's t-test was used to determine whether the two clades differed in each of the morphological measurements, using the Welch approximation to degrees of freedom to account for the heteroscedasticity for IOC and MTL (using var.equal = FALSE in the t.test function of R). A significance threshold (α) of 0.05 was implemented. Boxplots were plotted in ggplot2 (Wickham 2011) to better visualize the results.

Results

DNA sequence data. The final alignment included 207 variable characters, 92 singleton sites, and 115 parsimony informative characters. The best evolutionary model schemes were K80+I, F81+I, and GTR+G applied to the first, second,

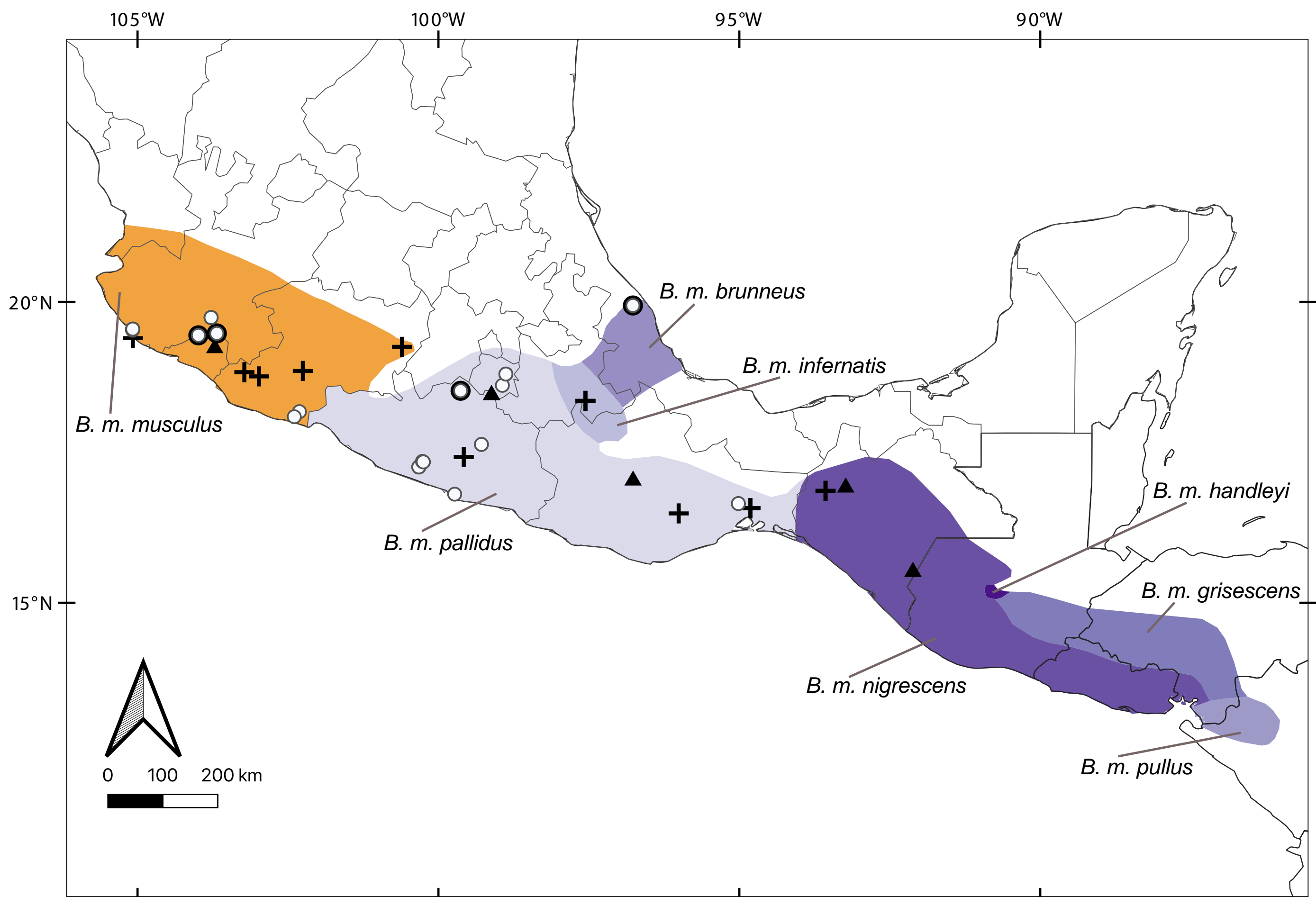

Figure 1. Geographic ranges of the eight recognized subspecies of *Baiomys musculus*, and specimens analyzed in this study. Individuals with cytochrome b sequences are represented by black crosses (sequences from Amman and Bradley 2004), black triangles (sequences from Miller and Engstrom 2008; Light *et al.* 2016), and black dots (this study). White dots represent individuals with morphological data (this study).

and third codon positions, respectively. Topologies from ML and BI trees were almost identical, but nodal support values were higher in BI (Figure 2). As previously reported, two well-supported clades were recovered within *B. musculus* (Amman and Bradley 2004). These clades were geographically structured, with clade I including samples from western México (Colima, Jalisco and Michoacán), and clade II including the rest of the samples. Clade II contained two sub-clades, one from central México (Guerrero, Morelos, and Puebla; clade II.a), and the other from eastern México (Chiapas, Oaxaca, and Veracruz; clade II.b). The K2P genetic distances between *B. taylori* and the two clades within *B. musculus* were > 11 %, the distance between clades I and II was 6.69 %, and between clades II.a and II.b was 3.98 %.

Morphological analyses. Based on molecular results, individuals were assigned to two *a priori* groups: clade I (Colima, Jalisco, and Michoacán; n = 26, 1 of them sequenced) and clade II (Guerrero, Morelos, Oaxaca, and Veracruz; n = 21, 2 of them sequenced; Appendix 2). Although some measurements overlapped between clades, individuals from clade I had significantly larger measurements than clade II in nine morphometric variables (Figure 3 and Table 1; all of the morphometric variables measured, except for BR, BMM, and LAB).

Discussion

The two mitochondrial lineages within *B. musculus* previously detected by Amman and Bradley (2004; Figure 2) were recovered, because their clade C is equivalent to our clade I. In addition, the K2P genetic distance between clades I and II was similar to those observed in other recognized sister species in Neotominae, such as in the genus *Megadontomys* (Vallejo and González-Cózatl 2012), *Neotoma* (Hernández-Canchola *et al.* 2021), *Peromyscus* (Bradley *et al.* 2007), among others (Bradley and Baker 2001; Baker and Bradley 2006). These two clades seem to be allopatric, but more thorough sampling is needed to determine if there is a distinct boundary between them. The complex topography between the Sierra Madre del Sur and the Transmexican Volcanic Belt, in addition to the mouth of the Balsas River, could be acting as geographic barriers to gene flow between clades in the southern pygmy mouse (Amman and Bradley 2004; Figure 4), and this limit aligns well with boundaries between other cryptic sister mammal taxa such as the mouse opossums (Arcangeli *et al.* 2018) and the Osgood's deermice (Ruiz-Vega *et al.* 2018). Additionally, multiple diversification events in western México generated many Mexican endemic mammal species, including

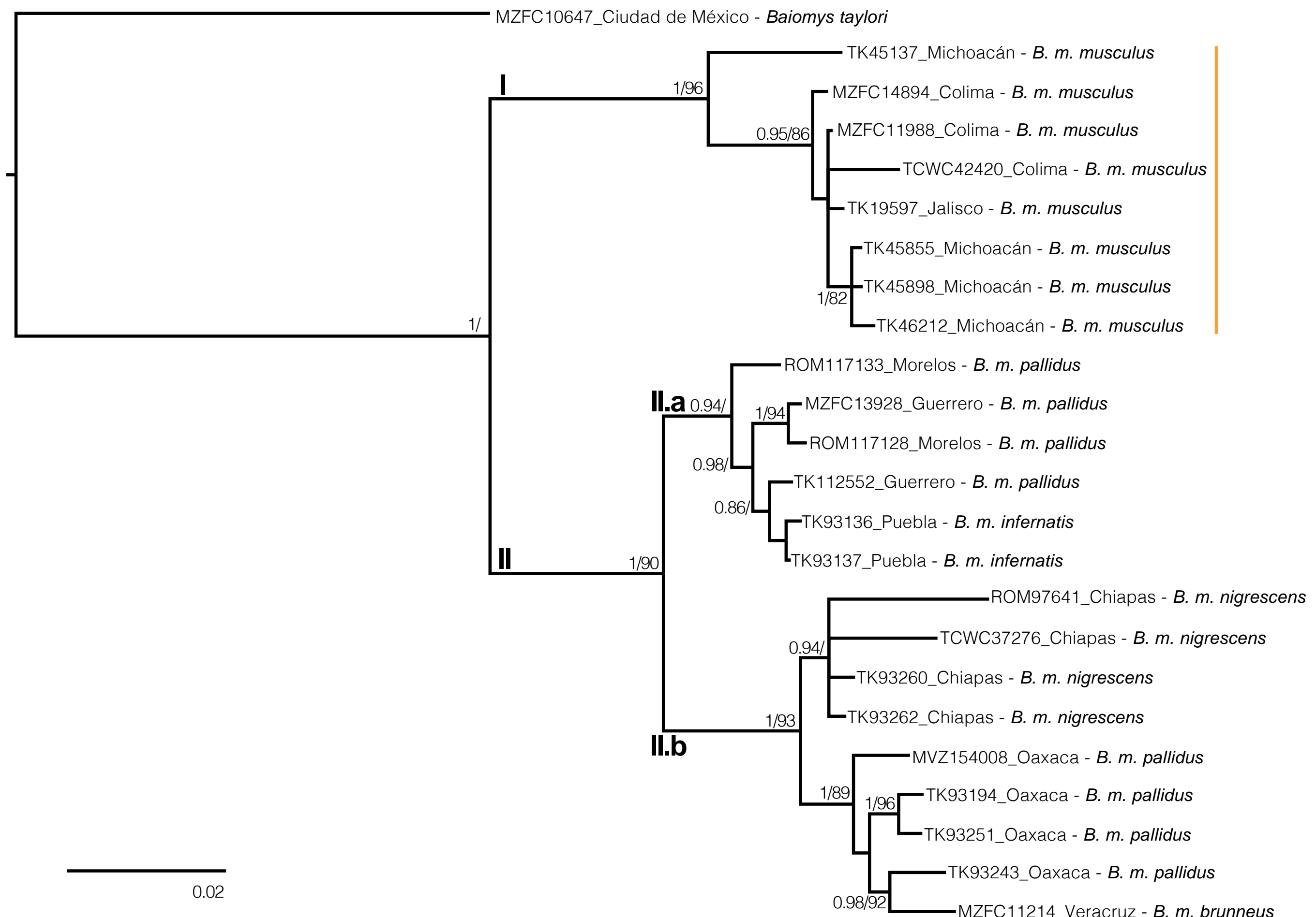

Figure 2. Majority rule consensus tree for *Baiomys musculus*, obtained from the Bayesian analysis of the mitochondrial cytochrome b sequences. Support values are shown as posterior probabilities followed by bootstrap values from a maximum likelihood analysis. Support values < 0.8/80 are not shown. Tip labels show the catalog number, the Mexican state of each sample and the taxa of each tip. Clade I is in orange and clade II in purple.

other neotomine rodents (*Osgoodomys banderanus*, *Xenomys nelson*i, *Hodomys alleni*, and *Peromyscus perfulvus*), in addition to the pygmy spotted skunk (*Spilogale pygmaea*), two species of mouse opossums (*Tlacuatzin*), the Mexican shrew (*Megasorex gigas*), and the banana bat (*Musonycteris harrisoni*), among others (Ceballos 2014; Arcangeli *et al.* 2018). This evidence suggests that the evolutionary history of clades I and II within *B. musculus* could have been influenced by the complex topography of western México, as in many other mammal taxa.

Baiomys musculus from clade I are distributed in Colima, Jalisco, and Michoacán, and specimens from these Mexican states were the largest individuals examined. Although the sample size was relatively small, our results are consistent with previous morphological evaluations that analyzed larger numbers of specimens from Guatemala and México (*n* = 299, Osgood 1909), México (*n* = 351, Hooper 1952), and from El Salvador, Guatemala, Honduras, México, and Nicaragua (*n* = 1,748; Packard 1960). In these previous morphological reviews, individuals from western México (Nayarit, Jalisco, Colima, and Michoacán) were larger in external and cranial dimensions than other Mexican and Central American specimens. This morphological differentiation of the populations from western México has been recognized for many years, as evidenced by their recognition as the subspecies *B. m. musculus* (Osgood 1909; Hooper 1952; Packard 1960; Figure 1).

Packard (1960) also noted increasing size in *B. musculus* from south to north. This general trend follows Bergmann's rule. However, *B. m. musculus* is the most distinct subspecies of the southern pygmy mouse, and it was proposed that its difference could be related to character displacement (Packard 1960) that could magnify the Bergmann's rule, because in western México *B. musculus* and *B. taylori*

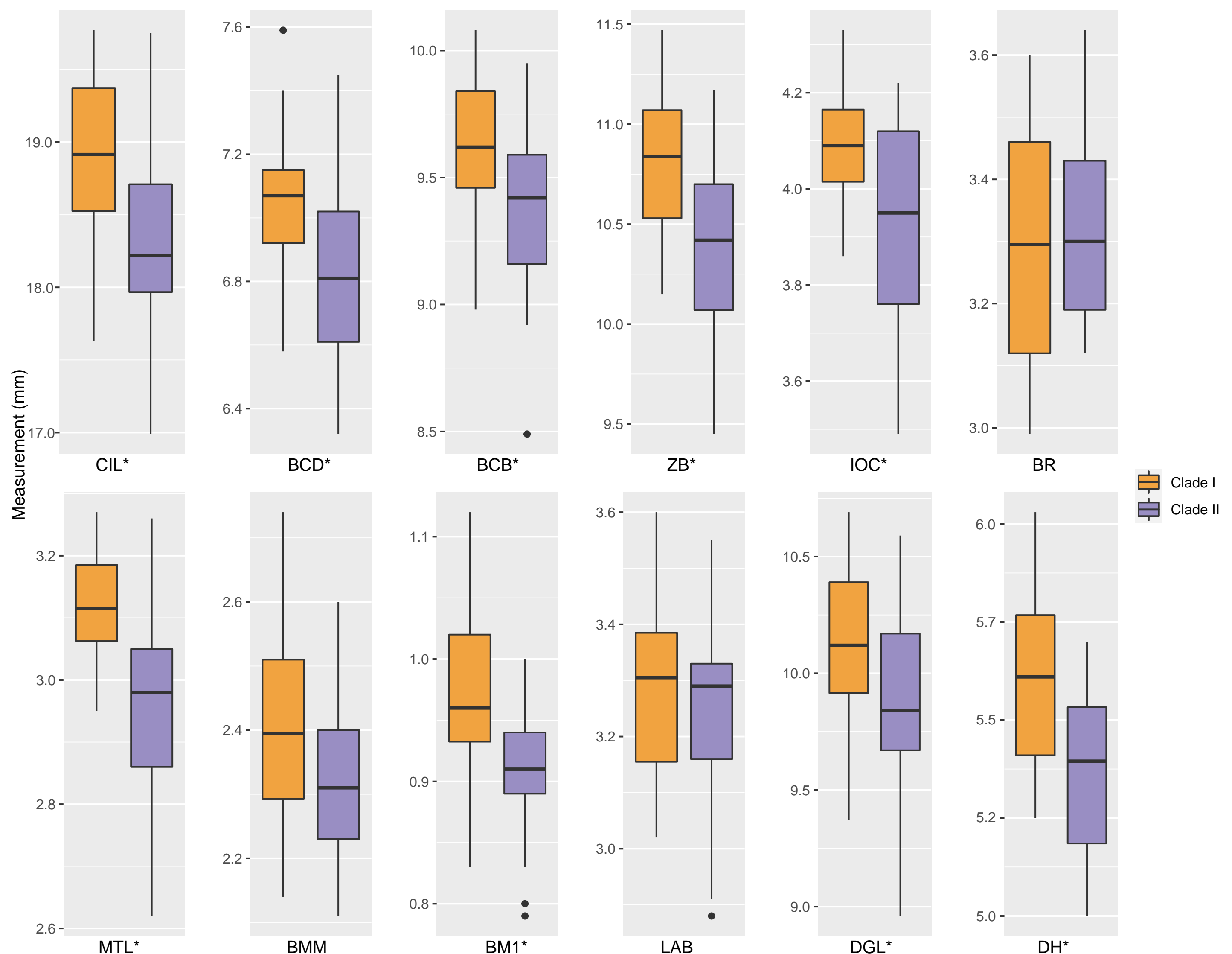

Figure 3. Boxplots summarizing the cranial and mandibular measurements of *Baiomys musculus*. Each plot represents a morphometric variable: condyle-incisive length (CIL), braincase depth (BCD), braincase breadth (BCB), zygomatic breadth (ZB), interorbital constriction (IOC), rostral breadth (BR), maxillary toothrow length (MTL), breadth across M3-M3 (BMM), breadth of M1 (BM1), length of auditory bulla (LAB), dentary greatest length (DGL), and dentary height (DH). *P*-values that were significant different (< 0.05) between clades are shown with an asterisk. Horizontal lines represent medians, boxes span the interval between the 25th and 75th percentiles, and the range of the vertical lines show the minimum and maximum values for each variable. Black dots show measurements that are farther from the mean than 1.5 times the interquartile range.

Table 1. Summary statistics and test statistics of morphological variables measured in *B. musculus* (condyle-incisive length, CIL; braincase depth, BCD; braincase breadth, BCB; zygomatic breadth, ZB; interorbital constriction, IOC; rostral breadth, BR; maxillary toothrow length, MTL; breadth across M3-M3, BMM; breadth of M1, BM1; length of auditory bulla, LAB; dentary greatest length, DGL; and dentary height, DH). Mean and standard deviation (sd) are shown for each clade, followed by the Student's t statistic (*t*), degrees of freedom (df; estimated using the Welch approximation for IOC and MTL to account for heteroscedasticity), and the *P*-value (*P*). *P*-values that were significant (< 0.05) are shown in bold type.

	Clade I		Clade II				
	Mean	**sd**	**Mean**	**sd**	***t***	**df**	***P***
CIL	18.90	0.576	18.30	0.687	3.20	44.0	0.003
BCD	7.05	0.229	6.83	0.293	2.84	45.0	**0.007**
BCB	9.65	0.265	9.36	0.344	3.20	44.0	**0.003**
ZB	10.80	0.361	10.40	0.480	3.32	44.0	**0.002**
IOC	4.09	0.116	3.95	0.219	2.76	28.9	**0.010**
BR	3.30	0.185	3.32	0.163	-0.35	45.0	0.729
MTL	3.12	0.088	2.97	0.155	3.92	30.2	**< 0.001**
BMM	2.40	0.152	2.32	0.128	1.86	45.0	0.070
BM1	0.98	0.072	0.91	0.056	3.84	45.0	**< 0.001**
LAB	3.28	0.169	3.23	0.175	0.92	45.0	0.365
DGL	10.10	0.358	9.85	0.445	2.25	42.0	0.030
DH	5.60	0.226	5.37	0.217	3.33	40.0	**0.002**

are sympatric and they are more morphologically distinct than where they are allopatric (Hooper 1952; Packard 1960). Analyses of cranial geometric morphometrics rejected the character displacement hypothesis between *B. musculus* and *B. taylori* (Abuzeineh 2006), but this proposal has not been tested in postcranial structures, or with other methodologies, and deserves attention to understand the processes that originated sympatric populations of *B. musculus* clade I / *B. taylori*, and the allopatric populations of *B. musculus* clade II / *B. taylori*. Martínez-Gordillo *et al.* (2010) tested whether clades I and II had different ecological niches, and although clade I inhabits warmer and drier environments than clade II, the ecological models showed nesting of the niches of the two clades (ecological niche modeling of clade I recovered most of the distribution of clade II, and vice-versa). Interestingly, *B. taylori*, which inhabits from the United States of America to central México, also has two detected clades with a lower genetic divergence than *B. musculus* (2.82 %; Amman and Bradley 2004), but the ecological niches were different between the two (Martínez-Gordillo *et al.* 2010). These contrasting genetic and environmental results between the northern and southern pygmy mice could suggest that different evolutionary processes are acting in each taxon within *Baiomys*.

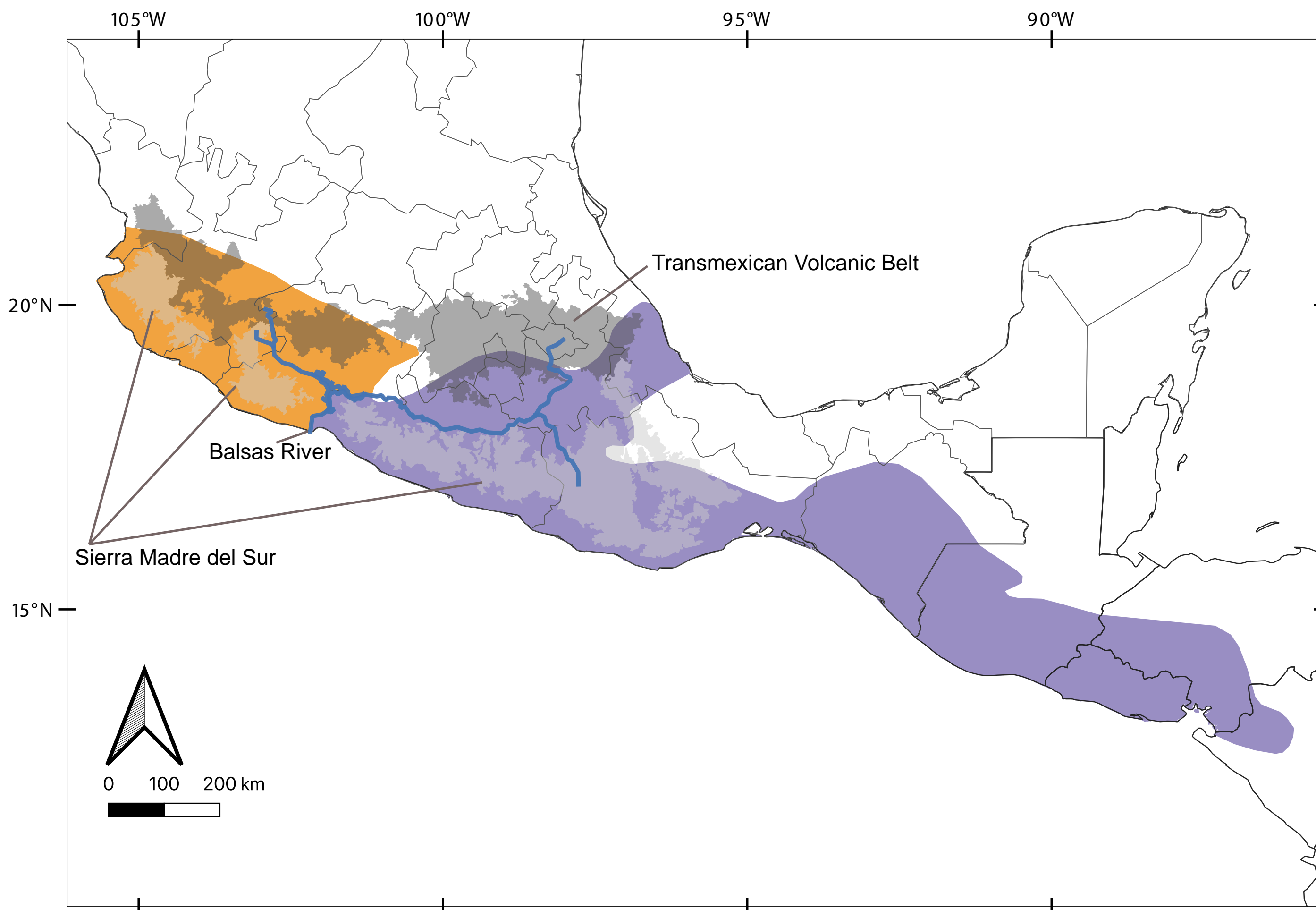

Figure 4. Revisited geographic ranges in the southern pigmy mice. Map colors show the preliminary suggested geographic ranges within *Baiomys musculus* of clade I (orange) and *B. brunneus* clade II (purple). The main biogeographic barriers discussed in this work also are shown.

In addition to the high levels of mitochondrial divergence (Amman and Bradley 2004) and morphological differences (external size, coat color, and certain cranial measurements; see Packard 1960 for more details) between *B. m. musculus* and the remaining subspecies, a substantial subdivision between *B. musculus* samples from Jalisco and Colima ($n = 9$) versus Chiapas, Oaxaca, and Veracruz ($n = 11$) was reported using allozymes (Calhoun *et al.* 1989). In the present work, more than using our limited sampling to draw conclusions about the taxonomic status of *B. m. musculus* (Figures 2, 3, Table 1), we aim to integrate the multiple and impressive reviews that have analyzed the variation within *B. musculus* (Osgood 1909; Hooper 1952; Packard 1960; Calhoun *et al.* 1989; Amman and Bradley 2004).

Considering all of these data, collected independently over more than a century, it appears that clade I, the Mexican endemic pygmy mice that inhabits in Colima, Jalisco, Michoacán, and Nayarit, could be named as *B. musculus* (Merriam 1892), and that all other populations of the southern pygmy mouse (including the brunneus, grisescens, handleyi, infernatis, nigrescens, pallidus, and pullus populations) seem to merit specific status and, following taxonomic priority, could be referred to as *B. brunneus* (Allen and Chapman, 1897). However, studies of additional data sets, such as nuclear / genomic data or Central American populations, will be required to definitively confirm the taxonomic status of these specimens. The substructure detected within clade II (Figure 2) also deserves additional attention: clade II.a appears to be restricted to the Balsas Basin and clade II.b to southeastern México. This phylogeographic pattern also has been reported in other mammal species, such as the Mesoamerican yellow-shouldered bat (Hernández-Canchola and León-Paniagua 2017) and the nine-banded armadillo (Arteaga *et al.* 2012) and this genetic differentiation does not agree with the geographic boundaries between subspecies of *B. musculus* (Figures 1, 2), so future studies will be needed to verify their validity. Basic aspects of the neotomine rodents, such as the number of species that inhabit North and Central America, are still not clear (Miller and Engstrom 2008; Platt *et al.* 2015; Sullivan *et al.* 2017). This knowledge gap will likely continue to exist in taxa that are rare and/or have restricted distributions (Gardner and Carleton 2009; Fernández 2014), unless the use of Next Generation Sequencing methods allow DNA data to be obtained from ancient specimens hosted in mammal collections (Castañeda-Rico *et al.* 2020). However, in more common and abundant species, as *B. musculus*, it will be easier to obtain and analyze data to solve these taxonomic uncertainties, which will allow us to understand the processes that generate and maintain biodiversity (Upham *et al.* 2019).

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Appendix 1

Specimens with the mitochondrial cytochrome b analyzed in this work.

Taxa	Mexican state	Lat	Long	Catalog #	Tissue #	Genbank
B. taylori	Ciudad de México	19.325	-98.986	MZFC 10647	5043	MZ056876
B. musculus	Colima	19.478	-103.683	MZFC 11988	4439	MZ056877
B. musculus	Colima	19.445	-103.989	MZFC 14894	6295	MZ056878
B. musculus	Colima	19.233	-103.717	TCWC 42420		MZ056878
B. musculus	Jalisco	19.398	-104.962	TTU 37737	TK19597	AF548478
B. musculus	Michoacán	19.254	-100.491		TK45137	AF548484
B. musculus	Michoacán	18.763	-102.868		TK45855	AF548483
B. musculus	Michoacán	18.833	-103.109		TK45898	AF548485
B. musculus	Michoacán	18.854	-102.137		TK46212	AF548482
B. b. brunneus	Veracruz	19.941	-96.769	MZFC 11214	3281	MZ056880
B. b. infernatis	Puebla	18.356	-97.442	TTU 82658	TK93136	AF548488
B. b. infernatis	Puebla	18.356	-97.442	TTU 82659	TK93137	AF548489
B. b. nigrescens	Chiapas	15.517	-92.117	ROM 97641		EF989933
B. b. nigrescens	Chiapas	16.917	-93.233	TCWC 37276		KU298967
B. b. nigrescens	Chiapas	16.861	-93.453		TK93260	AF548486
B. b. nigrescens	Chiapas	16.861	-93.453		TK93262	AF548487
B. b. pallidus	Guerrero	18.525	-99.632	MZFC 13928	3614	MZ056879
B. b. pallidus	Guerrero	17.424	-99.464	TTU 90341	TK112552	AF548490
B. b. pallidus	Morelos	18.454	-99.119	ROM 117128		EF989934
B. b. pallidus	Morelos	18.454	-99.119	ROM 117133		EF989935
B. b. pallidus	Oaxaca	17.033	-96.767	MVZ 154008		KU298966
B. b. pallidus	Oaxaca	16.486	-95.893		TK93194	AF548481
B. b. pallidus	Oaxaca	16.575	-94.701		TK93251	AF548479
B. b. pallidus	Oaxaca	16.575	-94.701		TK93253	AF548480

Appendix 2

Specimens with morphological data analyzed in this work. Specimens that have cytochrome b sequences are showed with an asterisk.

Taxa	state	Lat	Long	Sex	Catalog #	Field #
B. musculus	Colima	19.446	-103.989	Female	MZFC 14896	MLR160
*B. musculus**	Colima	19.478	-103.683	Male	MZFC 11988	MYACH430
B. musculus	Jalisco	19.531	-105.083	Female	MZFC 9818	MCHA001
B. musculus	Jalisco	19.742	-103.778	Male	MZFC 10458	FCR074
B. musculus	Jalisco	19.742	-103.778	Female	MZFC 10459	FCR075
B. musculus	Jalisco	19.742	-103.778	Female	MZFC 10460	FCR076
B. musculus	Jalisco	19.548	-105.082	Male	MZFC 10685	CHAM036
B. musculus	Jalisco	19.548	-105.082	Male	MZFC 10686	CHAM060
B. musculus	Jalisco	19.546	-105.082	Female	MZFC 12368	MCHAM119
B. musculus	Jalisco	19.546	-105.082	Female	MZFC 12369	MCHAM123
B. musculus	Jalisco	19.546	-105.082	Female	MZFC 12370	MCHAM126
B. musculus	Jalisco	19.546	-105.082	Female	MZFC 12371	MCHAM127
B. musculus	Jalisco	19.546	-105.082	Male	MZFC 12373	MCHAM132
B. musculus	Jalisco	19.546	-105.082	Male	MZFC 12374	MCHAM133
B. musculus	Jalisco	19.550	-105.080	Male	MZFC 12775	MCHAM111
B. musculus	Jalisco	19.550	-105.080	Male	MZFC 12777	MCHAM110
B. musculus	Jalisco	19.550	-105.080	Female	MZFC 12778	MCHAM108
B. musculus	Jalisco	19.550	-105.080	Female	MZFC 12779	MCHAM107
B. musculus	Jalisco	19.547	-105.081	Male	MZFC 12781	MCHAM098
B. musculus	Jalisco	19.550	-105.080	Male	MZFC 12783	MCHAM103
B. musculus	Jalisco	19.742	-103.778	Female	MZFC 12795	FCR085
B. musculus	Jalisco	19.742	-103.778	Male	MZFC 12798	FCR092
B. musculus	Jalisco	19.742	-103.778	Female	MZFC 12799	FCR097
B. musculus	Jalisco	19.742	-103.778	Male	MZFC 13616	FCR073
B. musculus	Michoacán	18.178	-102.310	Female	MZFC 10196	MBB108
B. musculus	Michoacán	18.093	-102.396	Female	MZFC 10197	MBB113
B. b. brunneus	Veracruz	19.941	-96.769	Female	MZFC 11094	MRM031
*B. b. brunneus**	Veracruz	19.941	-96.769	Male	MZFC 11214	MRM038
B. b. brunneus	Veracruz	19.941	-96.769	Female	MZFC 11219	MRM032
B. b. pallidus	Guerrero	16.806	-99.731	Male	MZFC 727	2205-132MTB
B. b. pallidus	Guerrero	17.342	-100.252	Female	MZFC 2356	953JJG
B. b. pallidus	Guerrero	17.258	-100.327	Male	MZFC 2357	1150JJG
B. b. pallidus	Guerrero	17.342	-100.252	Female	MZFC 2358	949JJG
B. b. pallidus	Guerrero	17.342	-100.252	Male	MZFC 2359	957JJG
B. b. pallidus	Guerrero	17.258	-100.327	Male	MZFC 2360	1105JJG
B. b. pallidus	Guerrero	17.258	-100.327	Female	MZFC 2361	1104JJG
B. b. pallidus	Guerrero	17.258	-100.327	Male	MZFC 2362	1103JJG
B. b. pallidus	Guerrero	17.352	-100.266	Male	MZFC 2363	916JJG
B. b. pallidus	Guerrero	17.342	-100.252	Male	MZFC 2364	958JJG
*B. b. pallidus**	Guerrero	18.525	-99.632	Female	MZFC 13928	GHC035
B. b. pallidus	Guerrero	17.633	-99.286	Male	MZFC 14875	MLR113
B. b. pallidus	Guerrero	17.633	-99.286	Male	MZFC 14879	MLR109
B. b. pallidus	Morelos	18.614	-98.938	Female	MZFC 9591	RAG375
B. b. pallidus	Morelos	18.802	-98.880	Female	MZFC 13918	GHC076
B. b. pallidus	Morelos	18.802	-98.880	Female	MZFC 13919	GHC086
B. b. pallidus	Oaxaca	16.650	-95.017	Male	MZFC 10055	NIZA043
B. b. pallidus	Oaxaca	16.650	-95.017	Male	MZFC 10056	NIZA044

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Evidence of differential genetic introgression at multiple localities between *Neotoma floridana* and *N. micropus*

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To determine the extent of genetic introgression along the parapatric border between *Neotoma floridana* and *N. micropus*, 140 woodrats were sampled from 21 localities in Kansas, Oklahoma, and Texas, at varying distances from the proposed species boundaries. All individuals were examined at the mitochondrial cytochrome-*b* gene (*Cyt*b) and two nuclear introns: intron seven of the Beta fibrinogen gene (*Fgb*-I7) and intron 2 of the vertebrate alcohol dehydrogenase gene (*Adh*1-I2). Additionally, individuals from a putative contact zone were genotyped using six microsatellite loci to better analyze population structure. Evidence of mixed ancestry was detected in 55 of 140 (39 %) individuals, at 10 of 21 (48 %) localities up to ~150 km from the proposed parapatric boundary. A pattern of differential admixture detected between the two nuclear markers suggested variation in selection pressures at the *Adh*1-I2 and *Fgb*-I7 markers is dependent upon the genomic makeup of the individual. Together, the mitochondrial and nuclear markers indicate evidence of historical hybridization and suggest that hybrid zones within this system are transient in nature.

Para determinar la extensión de la introgresión genética a lo largo del borde parapátrico entre *Neotoma floridana* y *N. micropus*, se tomaron muestras de 140 ratas de campo de 21 localidades en Kansas, Oklahoma y Texas, a diferentes distancias de los límites de las especies propuestas. Todos los individuos fueron examinados en el gen del citocromo-b mitocondrial (Cytb) y dos intrones nucleares: el intrón siete del gen del fibrinógeno Beta (Fgb-I7) y el intrón 2 del gen del alcohol deshidrogenasa de los vertebrados (Adh1-I2). Además, de los individuos de una zona de contacto putativa se obtuvo su genotipo utilizando seis loci de microsatélites para analizar mejor la estructura de la población. Se detectó evidencia de ascendencia mixta en 55 de 140 (39 %) individuos, en 10 de 21 (48 %) localidades hasta ~ 150 km del límite parapátrico propuesto. Un patrón de mezcla diferencial detectado entre los dos marcadores nucleares sugirió una variación en las presiones de selección en los marcadores Adh1-I2 y Fgb-I7 depende de la composición genómica del individuo. Juntos, los marcadores mitocondriales y nucleares indican evidencia de hibridación histórica y sugieren que las zonas híbridas dentro de este sistema son de naturaleza transitoria.

Keywords: Differential introgression; hybridization; microsatellites; parapatry.

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Introduction

Two non-sister species of woodrats (*Neotoma floridana* and *N. micropus*; Edwards and Bradley 2002; Longhofer and Bradley 2006) occur parapatrically from the Gulf of Mexico to southeastern Colorado (Figure 1; Hall 1981). Although both species can be found in a variety of habitats, *N. floridana* typically occupies more mesic riparian habitats (Wiley 1980), whereas *N. micropus* generally exploits more shrub-like, xeric habitats (Braun and Mares 1989). The distributions of these two species are separated by a few kilometers at several localities, and by less than one kilometer at others (Spencer 1968; Birney 1973, 1976; Stangl *et al.* 1992; Schmidly 2004; Mauldin *et al.* 2014, 2021). Based on the results of morphologic, allozymic, karyotypic, and genetic data, previous studies determined hybridization occurred along the North Canadian River in Major County, Oklahoma (Spencer 1968; Birney 1973, 1976; Mauldin *et al.* 2014).

Recently, Mauldin *et al.* (2021) examined genotypic variation in individuals collected from Major and Woodward counties and reported that hybridization was intermittent with potentially transient contact zones in this region, as evidence of genetic introgression was present at 11 of 12 sampled localities. Despite this apparent widespread evidence of genetic introgression, only two localities contained mitochondrial DNA (mtDNA) haplotypes of both species and individuals with highly admixed nuclear genomes (Mauldin *et al.* 2021). Additionally, two temporal sampling events (separated by 22 years) from the hybrid zone indicated ongoing and potentially ephemeral hybridization is occurring between the two species in western Oklahoma (Mauldin *et al.* 2021). Similarity of hybrid zone characteristics (*i. e.*, location of the zone, frequency of hybrids detected, directionality of hybridization, level of population substructure detected, etc.) in both datasets

indicated short term stability of the hybrid zone; however, expanded geographic sampling detected varying levels of nuclear admixture at 10 of 11 peripheral localities. Presence of individuals with *N. floridana* mtDNA haplotypes and *N. micropus* nuclear genomes at two localities west of the known area of sympatry suggested the location of the parapatric boundary between these taxa may: 1) be larger than the hybrid zone examined by Mauldin *et al.* (2021) or 2) there may be multiple sites of active hybridization (Mauldin *et al.* 2021).

Evidence of intermittent hybridization in Major County, Oklahoma (Mauldin *et al.* 2014, 2021), has lent support to the possibility that additional areas of hybridization may exist throughout the area of parapatry (Spencer 1968; Birney 1973). A second potential area of contact, along the south bank of the Red River (Locality 20, Figure 1) was sampled at intervals over several years (Stangl *et al.* 1992). Although no morphological evidence of hybridization was reported, Stangl *et al.* (1992) collected *N. floridana* and *N. micropus* within 100 m of each other, thereby establishing the possibility that the two species were in contact. Superficially, this region is similar to that of the known hybrid zone in Major County (Spencer 1968; Birney 1973; Mauldin *et al.* 2014, 2021), as the Red River bisects the parapatric border of these species, and riparian habitat typically exploited by *N. floridana* interdigitates with sage brush and sand dunes, more commonly inhabited by *N. micropus*. In addition to current areas of hybridization, detection of admixed individuals at localities peripheral to the current parapatric boundary could provide insight into the stability of the distributions of these species, and the effect dynamic distributions may have on hybridization in this system.

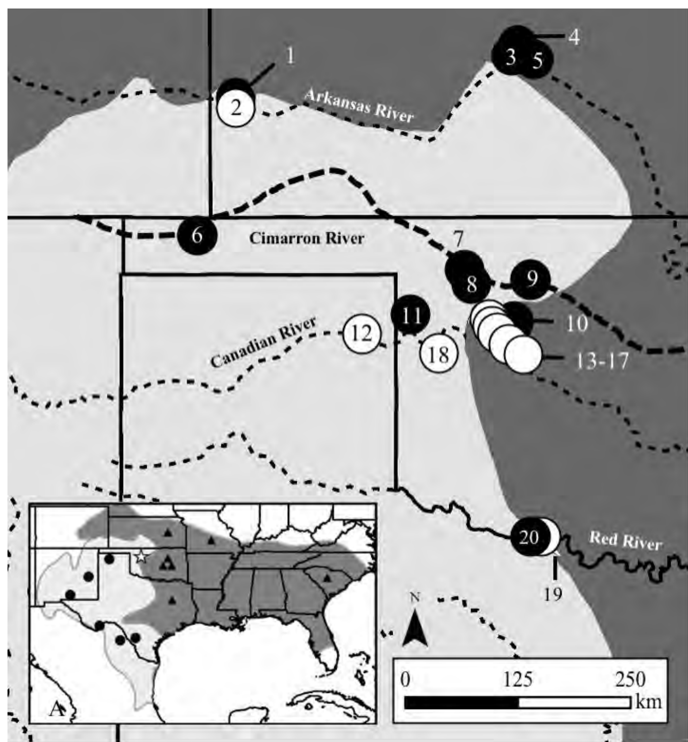

Figure 1. The delineation of the parapatric border that separates the distributions of *N. floridana* and *N. micropus* (shown in dark and light gray, respectively). Collection localities examined in this study are indicated by circles with corresponding locality numbers (Table 1). Localities from which hybrids were detected are shown in black. The white star represents the reported area of hybridization near Seiling, Major County, Oklahoma (Mauldin *et al.* 2014, 2021), of which inset B gives a closeup view. Inset A displays the entire geographic range of both species. For inset A, triangles and circles indicate the collection localities for reference specimens of *N. floridana* and *N. micropus,* respectively.

Given the potential ephemeral nature of the previously studied hybrid zone, along with the long parapatric border shared by these species, Mauldin *et al.* (2021) advocated for further taxonomic sampling along the border of parapatry. They suggested further study was need to determine if 1) additional areas of hybridization exist and 2) evidence of dynamic species distributions could be substantiated. Therefore, the goal of this study was to examine potential areas of sympatry for evidence of hybridization, and to inspect areas peripheral to the parapatric border for evidence of genetic introgression. To this end, multiple objectives were addressed: 1) collect and genotype individuals from localities along and at varying distances from the proposed parapatric border, 2) examine localities for presence or absence of evidence of genetic introgression, 3) determine the maximum recorded distance of hybrid individuals from the current estimated border of parapatry, and 4) utilize microsatellite data to examine population substructure and level of genetic introgression in areas of sympatry.

Materials and methods

Samples. State collecting permits, as well as permission of property owners or appropriate state agencies (*e. g.*, Kansas Department of Wildlife and Parks, Oklahoma Department of Wildlife Conservation, Texas Parks and Wildlife) were received prior to any collection efforts. One hundred and forty woodrats were collected from 21 localities throughout Kansas, Oklahoma, and Texas (Figure 1) between July 2009 and May 2012. Spatial distribution of individual capture sites (middens) were identified with UTM coordinates. Most woodrats were collected with Sherman live-traps (Sherman live-trap Co. Tallahassee, Florida), others were collected with Havahart ® live-traps (Woodstream Corporation, Lititz, Pennsylvania, USA), and some were captured by hand after excavation of middens (nests) to ensure all occupants were collected. Individuals and embryos of sufficient size to ensure extraction of embryonic DNA were given a unique identification number (TK number), sexed, measured, and sacrificed. Individual woodrats were assigned putative species identifications based on morphologic characteristics (Hall 1981; Schmidly 2004), however, given previous results and the inability to distinguish hybrids based solely on morphology, a formal morphological identification based on pelage color was not considered in hybrid identification. Animal care and use guidelines conformed to those proposed by the American Society of Mammalogists (Animal Care and Use Committee 1998) and were approved by the Texas Tech University Institutional Animal Care and Use Committee (IACUC protocol 11009-03). In cases where females and their offspring were captured in the same mid-

den, mother and offspring were cross-referenced; similarly, pregnant females were cross-referenced to embryos. Blood and tissue samples (heart, kidney, liver, lung, muscle, and spleen) were obtained and tissues were immediately frozen in liquid nitrogen, and subsequently archived at the Natural Science Research Laboratory (NSRL) at the Museum of Texas Tech University. Voucher specimens (skulls, postcranial skeletons, and skins) were prepared and deposited in the NSRL (Appendix 1). Additionally, liver samples of four woodrats (indicated by prefix TJM in Tables A1 and A2) were obtained from the lab of Ivan Castro-Arellano at Texas State University.

DNA Isolation. Total genomic DNA (nuclear and mitochondrial) was isolated from each individual using approximately 0.1 g of liver and the Qiagen DNeasy kit (Qiagen Inc.; Valencia, California, USA). In some cases, entire embryos were required to isolate sufficient DNA. DNA samples were stored at -20° C for subsequent analyses.

Genotype Analyses. All genotype analyses followed the protocol outlined in detail by Mauldin *et al.* (2014, 2021). Eight *N. floridana* and seven *N. micropus* collected a minimum distance of 125 km from the parapatric border, and previously utilized by Mauldin *et al.* (2014, 2021) were included as reference samples (Figure 1; Appendix 1). Three loci were examined, two autosomal loci (intron two of the vertebrate alcohol dehydrogenase gene (*Adh*1-12) and intron seven of the beta-fibrinogen gene (*Fgb*-I7)) and one mitochondrial DNA locus (Cytochrome-*b, Cyt*b). Additionally, individuals from Locality 20 were genotyped for six microsatellite loci (*Nma*01, *Nma*04, *Nma*05, *Nma*06, *Nma*10, and *Nma*11) developed by Castleberry *et al.* (2000) to detect genetic structure within the population.

Adh1-12 Assay. A banding pattern unique to *N. floridana* was produced using the restriction enzyme *Nsi*I (ATGCA/T) with a fragment of the *Adh*1-12 region of either 566 bp or 390 bp that had been amplified using PCR methods modified from Amman *et al.* (2006) and Longhofer and Bradley (2006) using one of the following primer pairs: ExonII-F and 2340-II (566 bp product) or 350F and 2340-II (390 bp product; Amman *et al.* 2006). Restriction digests were conducted following manufacturer's methods and are outlined in Mauldin *et al.* (2014).

Fgb-I7 *Assay*. Mauldin *et al.* (2014) reported that although no restriction enzyme was diagnostic, three diagnostic nucleotide substitutions were identified (positions 428, 497, and 493). Therefore, sequence data was collected on a 609-610 bp fragment amplified using PCR primers *Fgb*-I7L-*Rattus* and *Fgb*-I7U-*Rattus* from Wickliffe *et al.* (2003) and following PCR methods modified from Prychitko and Moore (2000) as outlined in Carroll and Bradley (2005). Sequence data has been deposited in GenBank (Appendix 1). Though previous studies have utilized this as a diagnostic marker (Mauldin *et al.* 2014, 2021), it is possible unsorted polymorphisms not detected in reference samples may exist.

*Cyt*b *Assay*. The entire *Cyt*b gene was amplified using two PCR primers (LGL765 forward—Bickham *et al.* 1995 and LGL 766 reverse—Bickham *et al.* 2004) and conditions outlined in Edwards and Bradley (2002). The restriction enzyme [*Bsa*I (GGTCTC(N)$_1$/)] produced a cut that was unique to *N. floridana* following methods outlined by the manufacturer and reported by Mauldin *et al.* (2014, 2021).

Microsatellite Assay. The six microsatellite loci developed by Castleberry *et al.* (2000) and utilized by Mauldin *et al.* (2014, 2021) were amplified and analyzed for all individuals collected at Locality 20 (Figure 1) as described by Haynie *et al.* (2007). Alleles were scored using GeneMapper software (version 4.0; Applied Biosystems Inc.).

Data Analysis. Based on the results of molecular assays outlined above, each individual was scored as either *N. micropus* or *N. floridana* for the mitochondrial genome, and as homozygous *N. micropus*, heterozygous, or homozygous *N. floridana* for the *Adh*1-I2 and *Fgb*-I7 markers. GenAlEx (version 6.5; Peakall and Smouse 2012) was utilized to identify presence of duplicate genotypes, test microsatellite loci for deviation from Hardy-Weinberg equilibrium (HWE) expectations, and format data for use in Structure (v2.3.4; Pritchard *et al.* 2000) as codominant nuclear markers with only adults and subadults being included in the analyses. Mitochondrial data were not analyzed in Structure or NewHybrids (*v.1.1Beta3*; Anderson and Thompson 2002) but were included in result plots to aid in identification of hybrid individuals and examine any potential bias present in directionality of introgression.

Based on preliminary results (nuclear introgression and geographic proximity of both mtDNA haplotypes), complete nuclear genotypes (*Adh*1-I2, *Fgb*-I7, and six microsatellite loci) for all individuals collected at Locality 20 were analyzed in Structure to examine population structure and quantify potential admixture between the two species. Structure runs utilized the admixture model with independent allele frequency option, a burnin of 500,000, run length of 1,000,000 iterations, and examined values of K (clusters) from 1-5. Two separate parameter sets were run, one assigned reference individuals to a priori populations using the popflag designation (parameter set A), whereas the other did not (parameter set B). Neither dataset used prior population assignment information for study samples. Structure result files were uploaded to Structure Harvester (Earl and vonHoldt 2012) to determine the value of K which best fit the data using the Evanno method (Evanno *et al.* 2005).

Results of the Structure run with the smallest variance value from parameter set A (K = 2) were used to generate a plot for examination of admixture between the two species. Furthermore, individuals from Locality 20 were analyzed in NewHybrids to determine the posterior probability values (PPVs) of individuals belonging to one of six classifications (pure parental *N. floridana*, pure parental *N. micropus*, F_1, F_2, backcross to *N. floridana*, backcross to *N. micropus*) based on admixture of nuclear genomes with no prior allele frequency data, Uniform priors, a burnin of 100,000, and 1,000,000 sweeps after burnin. Structure and NewHybrids output files were visualized using Excel

2010 (Microsoft Corporation, Redmond, Washington, USA). Assignment to hybrid classifications followed the protocol outlined by Mauldin *et al.* (2014, 2021).

Electronic species distributions of *N. floridana* and *N. micropus* (Patterson *et al.* 2007) generated by digitizing previously published range maps (*i. e.*, Hall 1981) were used to approximate the location of the parapatric border. Distance of each sampling locality to the closest point along the approximated parapatric boundary was then measured with the use of ArcGIS Software (ESRI, Redlands, California, USA), based on UTM coordinates of localities. Distances were measured to each distributional boundary (*N. floridana* and *N. micropus*) along the same vector, and the two distances were averaged for the final estimate. Additionally, samples from Locality 20 were collected from two nonadjacent parcels of private property; however, given the proximity of localities (all samples collected within ~2.5 km), samples from both properties were consolidated into a single locality for simplicity. However, these localities are examined both jointly (Locality 20) and independently (Localities 20a and 20b) to better examine patterns of interspecific genetic introgression at multiple scales.

Randomization tests of goodness-of-fit utilized 20,000 iterations and were conducted with Excel 2010 (Microsoft Corporation, Redmond, Washington, USA) following methods described by McDonald (2009) to determine if the following proportions deviated significantly from an equal contribution: 1) proportion mtDNA haplotypes of each species at Localities 20, 20a, and 20b, 2) proportion of *Adh*1-I2 and *Fgb*-I7 alleles detected at localities within each presumed species distribution, and 3) proportion of *Adh*1-I2 and *Fgb*-I7 alleles detected east and west of the proposed center of the hybrid zone in Major County, Oklahoma (data from Mauldin *et al.* 2014). The proportion test within the statistical package R (Team 2008) was utilized to compare the following proportions: *N. floridana* mtDNA haplotypes detected at Localities 20a and 20b, hybrid individuals with introgression detected at the *Adh*1-I2 locus within the distributions of *N. micropus* and *N. floridana*, respectively, and hybrid individuals with introgression detected at the *Fgb*-I7 locus within the distributions of *N. micropus* and *N. floridana*, respectively.

Results

Results of molecular assays are available in Appendix 2. Evidence of mixed ancestry was detected in 55 of 140 (39 %) sampled individuals, at 10 of 21 (48 %) localities (Figure 1). A high percentage of individuals with mixed ancestry (>50 %) was recorded at three localities (4, 9, and 20). Genetic introgression was detected at both nuclear loci at only two localities (9 and 20), whereas only Locality 20 contained mitochondrial DNA (mtDNA) haplotypes of both species. Given that Locality 20 is the only site at which both mtDNA haplotypes were detected, and the possibility that it represents an area of current or recent contact (Stangl *et al.* 1992), individuals from Locality 20 were not included in examination of differential detection of admixture between loci. Of the 22 woodrats with mixed ancestry collected within the distributional limits of *N. micropus*, evidence of nuclear admixture was detected at the *Adh*1-I2 locus in one animal, and at the *Fgb*-I7 locus in 21 individuals ($P < 0.0001$). Admixture was detected only at the *Adh*1-I2 locus for all 21 admixed individuals identified within the distribution of *N. floridana* ($P < 0.0001$). No individuals were heterozygous at both loci. A similar bias was identified through use of randomization test of goodness-of-fit that examined data from the Major County hybrid zone, as detection of admixture in individuals collected west of the proposed center of the zone was significantly biased towards *Fgb*-I7 locus ($P = 0.012$), and detection of admixture east of the zone was biased, although not significantly, to the *Adh*1-I2 locus ($P = 0.073$). The furthest distance from the parapatric border at which nuclear admixture was detected was approximately 150 km within the species distribution of *N. micropus* (Locality 6; Figure 1). Additional distance data for localities and individuals is available in Table 1.

Examination of microsatellite data with GenAlEx identified no duplicate genotypes. The following markers was determined to deviate significantly from HWE expectations within the sampled population, *Nma*05 in Locality 20b ($P = 0.030$). Results of Structure and Structure Harvester analyses determined K = 2 as the most appropriate number of clusters for both parameter sets. Results of Structure analyses detected no genetic introgression or population substructure at Locality 20 (Figure 2). Results of NewHybrids analyses of samples from Locality 20 identified only one sample as less than 90 % probability of belonging to the classification of 'pure' *N. micropus* (Figure 3; TK179266 = 87.76 %; mean *N. micropus* PPV = 96.56 %, median *N. micropus* PPV = 98.77 %). Spatial distribution of mtDNA haplotypes within Locality 20 is depicted in Figure 4. Results of randomization test of goodness-of-fit determined the proportion of mtDNA haplotypes of each species present at Locality 20 did not vary significantly from the null model of equal contribution ($P = 0.118$), nor did Locality 20a ($P = 0.690$); however, Locality 20b was significantly biased towards *N. floridana* mtDNA, with no *N. micropus* mtDNA haplotypes detected ($P = 0.004$). Results of analyses of the proportion of *N. floridana* mtDNA haplotypes at Locality

Table 1. The mean, median, minimum, and maximum distances (in kilometers) of each category from the closest point of the estimated parapatric border.

Category	Mean	Median	Minimum	Maximum
All localities	42	27	4	152
hybrid localities	45	29	12	152
'pure' localities	40	27	4	152
all individuals	34	26	4	152
hybrid individuals	27	26	12	152
'pure' individuals	38	26	4	152

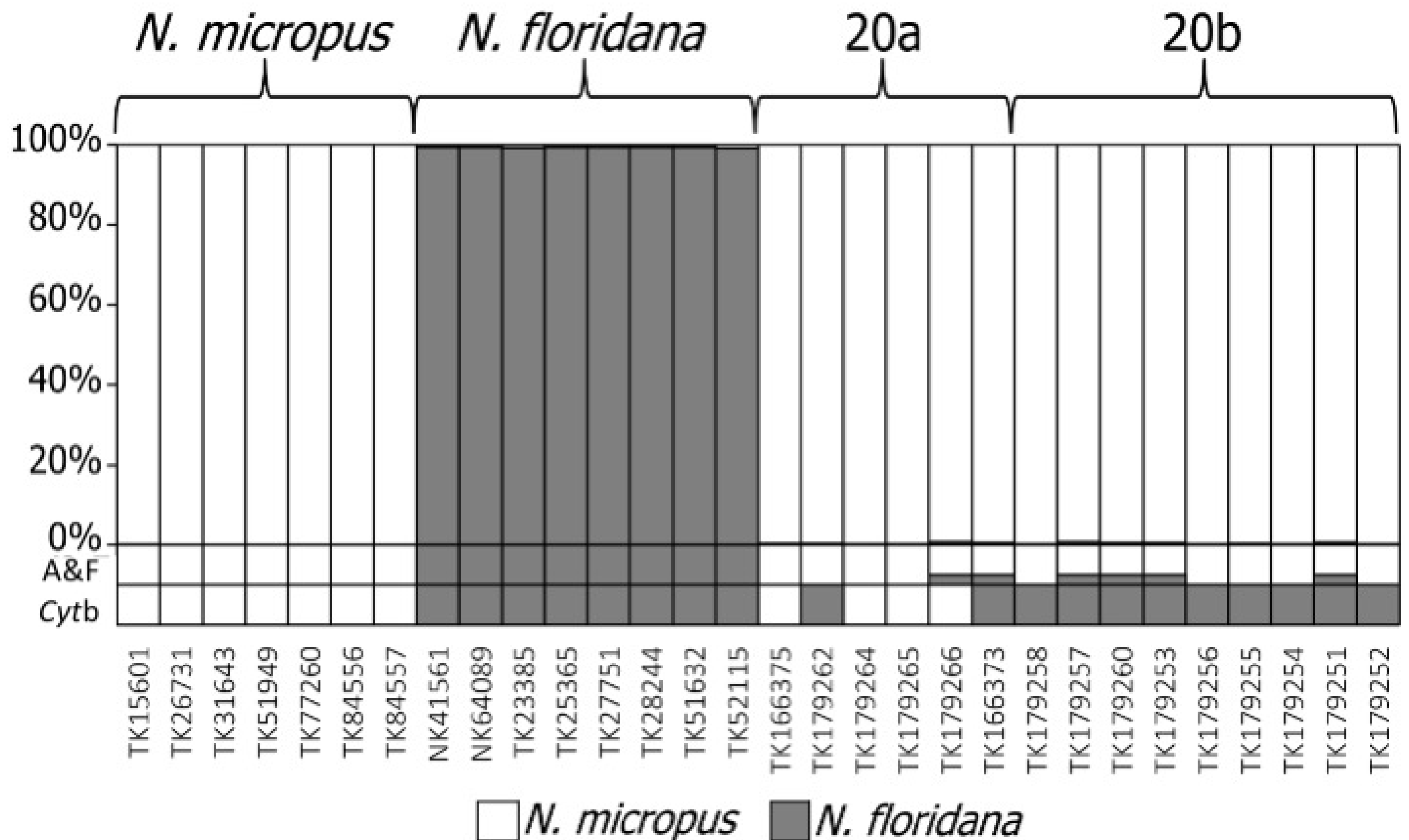

Figure 2. Results of Structure analyses: genotype information for all individuals collected from Locality 20. Specimen identification numbers are shown below the respective bar. Shading signifies the proportion of alleles contributed by each species (White: *N. micropus*, Gray: *N. floridana*). The top tier denotes the estimated proportion of the specimen's microsatellites as determined by Structure analyses, the second tier (*A&F*) indicates the proportion of *Adh*1-I2 and *Fgb*-I7 alleles, the third tier (*Cyt*b) identifies the mtDNA haplotype of the individual. Brackets and labels indicate reference samples of each species, and collection localities for all study individuals.

20b were significantly higher than that of Locality 20a (P = 0.024). Results of analyses of the proportion of hybrids with introgression detected at the *Adh*1-I2 (P < 0.0001) and *Fgb*-I7 (P < 0.0001) loci varied significantly depending upon the distribution from which they were collected.

Discussion

A high proportion of sampled woodrats (39 %) were determined to be of mixed ancestry, including individuals from 10 of 21 (48 %) sampled localities throughout Texas, Oklahoma, and Kansas (Appendix 2). Given the small number of molecular markers examined, these values likely underestimate the true number of genetically admixed individuals and localities at which they are found. These results suggest some degree of hybridization has occurred, or currently occurs, at multiple localities along the parapatric border. Additionally, at three localities (4, 9, and 20) greater than 50 % of examined individuals exhibited some level of genetic introgression; although no genetic admixture was detected at three localities (13, 14, and 19) of similar or lesser distances to the border. Furthermore, the geographic distance between some sampled hybrids and the putative location of the parapatric boundary is substantial (*e. g.*, Locality 6: ~150 km).

Finally, the locus at which admixture was detected was dependent upon the species distribution from which the samples were collected. For individuals collected within the distribution of *N. micropus*, genetic introgression was detected most frequently at the *Fgb*-I7 locus; however, for individuals collected within the distribution of *N. floridana*, introgression was detected only at the *Adh*1-I2 marker. The statistically significant difference in the locus at which exotic alleles were detected within each species distribution suggests that selection favors inclusion of foreign DNA sequences at different loci based upon the genomic background of the organism (*i. e.*, predominantly *N. floridana* or *N. micropus* nuclear genomes). Examination of the Major County hybrid zone data generated by Mauldin *et al.* (2014) identified a similar bias at a smaller geographic scale.

Results of Structure analyses failed to detect nuclear admixture at Locality 20 and estimated that nuclear genomes of sampled woodrats were predominantly (>99 %) composed of *N. micropus* alleles, although the majority contained *N. floridana* mtDNA haplotypes. Examination of results of NewHybrids analyses in combination with *Adh*1-I2, *Fgb*-I7, and *Cyt*b data determined all individuals from Locality 20 were either backcrosses to *N. micropus* (12) or putatively pure *N. micropus* (3), with no *N. floridana* parental types

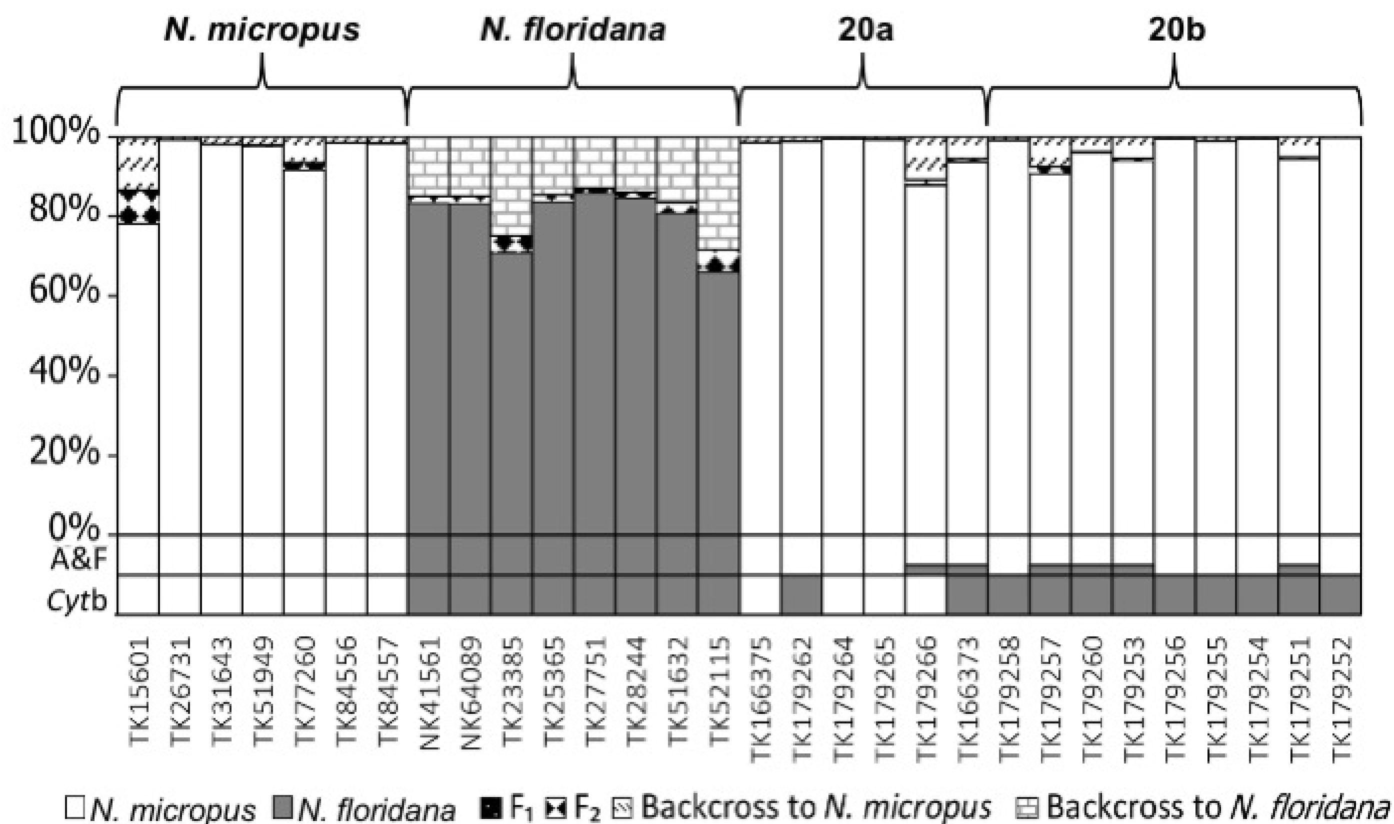

Figure 3. Results of NewHybrids analyses: genotype information for all individuals collected from Locality 20. The posterior probability that the individual belongs to a specified hybrid classification (*i. e.*, pure parental, F_1, F_2, etc.) based on microsatellite data is indicated by the proportion of each color or pattern within the top tier. The second tier (*A&F*) indicates the proportion of *Adh*1-I2 and *Fgb*-I7 alleles, and the third tier (*Cyt*b) identifies the mtDNA haplotype of the individual. Brackets and labels indicate reference samples of each species or collection localities of specimens for this study.

detected. The statistically significant change in proportion of mtDNA haplotypes present between Localities 20a and 20b, combined with paucity of *N. floridana* parental types, and nuclear genomes of all individuals composed primarily of *N. micropus* alleles suggests the location of the hybrid zone has shifted from the approximate location of Locality 20a to some location east of the sampled area. The easternmost sample (TK 179251) was collected ~100 m east of the I-44 Bridge reported to be an area of contact (Stangl *et al.* 1992) and appeared to be *N. micropus* (pelage and nuclear genome). Therefore, it is possible that the shift in area of sympatry began prior to the study by Stangl *et al.* (1992), at which time it had reached the I-44 Bridge, and has subsequently continued east along the Red River, with the mtDNA haplotype of *N. floridana* occurring throughout its now displaced range.

Similar cytonuclear discordance, although smaller and directionally reversed, was reported between the positions of the mitochondrial and nuclear boundaries between these species in Major County, Oklahoma, suggesting that the areas of hybridization are somewhat transient as distributional borders of these species shift over generations (Mauldin *et al.* 2021). Given the large variation in degree of genetic introgression detected over relatively small distances and the small proportion of individuals detected with highly admixed nuclear genomes (F_1 and F_2-like individuals) reported along the North Canadian River (Mauldin *et al.* 2021), distance to the putative area of sympatry cannot be estimated with any certainty. However, it is worth noting that an individual identified as a putatively pure *N. floridana* was collected from Locality 19 (~10 km east of Locality 20) in Oklahoma.

Various methodologies, including morphologic, karyotypic, allozymic, and genotypic data have been used to examine hybridization at various geographic scales within this system (Spencer 1968; Birney 1973, 1976; Mauldin *et al.* 2014, 2021). Examination of these studies and the research presented herein has determined the following characteristics are demonstrative of hybridization between these species: a high percentage of genetically admixed individuals, no evidence of reduced fertility in hybrid individuals, a paucity of F_1 and F_2-like genotypes, significant linkage disequilibrium, limited population structure, differential genetic introgression of nuclear loci, and varying levels of hybrid zone ephemerality. Examination of these characteristics in the framework of mechanistic models of hybrid zone maintenance and criteria set forth by Endler (1977) and Moore (1977), as summarized by Van Den Bussche *et al.* (1993), indicate that either the hybrid equilibrium model (wherein hybrids and parental types are equally fit) or the

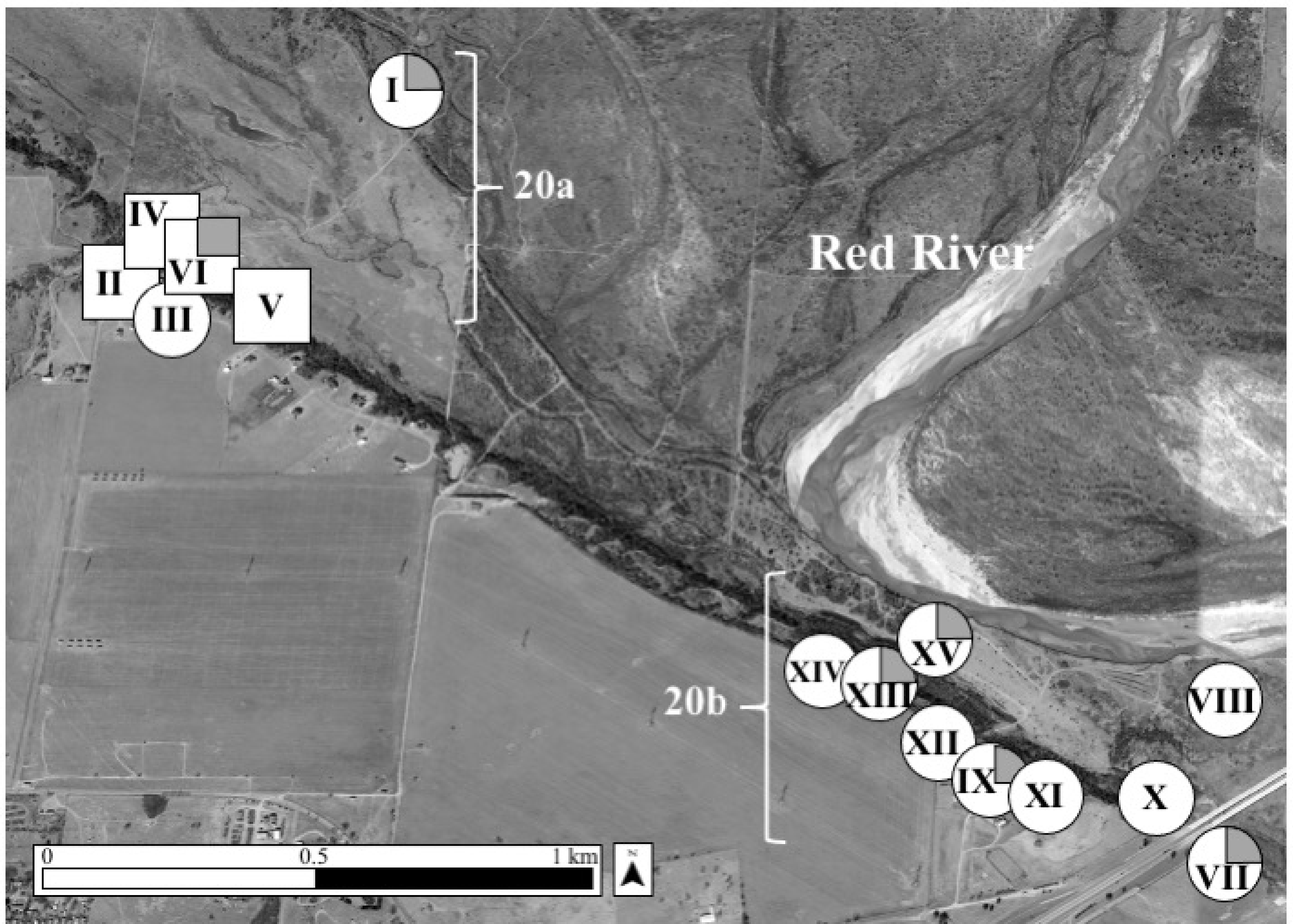

Figure 4. Map depicting Locality 20 near the Red River in Wichita County, Texas. Numbers signify midden locality and shape (Circles: *Neotoma floridana* and Squares: *N. micropus*) indicates the mtDNA haplotype (*Cyt*b) detected at each midden site. Shading represents the proportion of *Adh*1-I2 and *Fgb*-I7 alleles contributed by each species (Gray: *Neotoma floridana* and White: *Neotoma micropus*). No evidence of introgression was identified based on the microsatellite data (see Figure 2) at this locality; however, genotypes based on the combination of the *Cyt*b, *Adh*, and *Fgb* markers indicated that introgression occurred at all middens except 2, 4, and 5. For reference, the highway/bridge in the lower right corner is Interstate-44.

hybrid superiority model (in which hybrids have higher fitness within an ecotone or certain set of environmental conditions) might be responsible for maintaining hybridization between these species. Additional data concerning hybrid fitness, selection pressures, and possible correlations to environmental conditions are needed to distinguish between these models relative to the dynamics responsible for maintaining hybrid zones between these species.

Historical distribution changes of *N. floridana* have been documented by Quaternary fossil records (Richards 2013), 'recent fossil' remains dating to the late Holocene (~1,450 years before present – Eshelman 1971; Richards 2013), and temporal sampling in the 19th century (Cope 1872; Blatchley 1897). Additionally, a study examining distributions of *N. micropus* and *N. albigula* in southern New Mexico, determined interspecific competition led to displacement of *N. micropus* over a portion of the study area (Wright 1973). Given the evidence presented herein, the ephemeral nature of distributional boundaries, and the documented occurrence of interspecific displacement within the genus *Neotoma*, it is possible that the evidence of introgression detected at peripheral localities is a result of some combination of distributional shifts and dispersal of alleles over generations. Subsequently, the differential detection of alleles at distinct nuclear loci might be the result of disparity in persistence of certain alleles within populations, the rate at which those same alleles disperse over generations, or some combination thereof. Additionally, the possibility of unsorted polymorphisms may exist, potentially impacting the nuclear introgression calculations; however as all but one of the molecularly identified hybrids exhibited cytonuclear discordance, this would not change the overall results or the classification for most animals examined.

In conclusion, nuclear introgression was detected at multiple localities throughout a large portion of the parapatric border including sites near Burkburnett, Texas, Seiling, Oklahoma, as well as Great Bend and Syracuse, Kansas, among others (see black dots in Figure 1). Additionally, this introgression appears to be variable with regard to prevalence of admixture detected at separate nuclear markers

dependent upon the genomic background of the organism, as the *N. micropus* genome appears to tolerate *N. floridana* alleles at the *Fgb*-I7 locus better than at the *Adh*1-I2 locus, and *N. floridana* genome is more commonly infiltrated with *N. micropus* alleles at the *Adh*1-I2 locus than the *Fgb*-I7 locus. The presence of cytonuclear discordance at Locality 20, and similar evidence reported in Major and Woodward counties (Mauldin *et al.* 2021) provide evidence of nuclear genome displacement, likely caused by distributional shifts. Although introgression appears common throughout the parapatric border, the differential introgression of alleles and paucity of individuals determined to have highly admixed nuclear genomes, suggest hybridization does not pose a major threat to the gene pools of either species.

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Dedication. This manuscript is dedicated to Dr. David J. Schmidly for his devotion to mammalogy and the training of graduate students. I (RDB) first met Dr. Schmidly in the fall of 1982, while enrolled in his mammalogy course. His mammalogy class was one of the most challenging courses I had as an undergraduate, and I loved it! Dr. Schmidly asked me to work on a M.S. degree, under his tutelage, and it was the best professional decision I could have made. In the summer of 1983, I accompanied Dr. Schmidly and his NSF research team to Mexico, where we spent 6 weeks collecting specimens of *Peromyscus* for his research project. That summer changed my life and I was hooked on mammalian systematics. Later, Dr. Schmidly insisted that I go to Texas Tech University and obtain a PhD with the late Dr. Robert J. Baker; I would not have been confident enough to do so without Dave's encouragement. Joining me on the authorline are two of my former PhD students (MLH and MRM), as well as a current PhD student (SCV) who received her MS from MLH, so the tradition of training graduate students in mammalogy continues! Dave, given that this paper pertains to woodrats and hybridization, two topics on which you have several publications, we hope we have well-represented your teachings. On a personal note, I thank you for all you have done for me and my students over the years. You set the bar high and we continue to try and keep up with you!!!

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Appendix 1

Specimens examined– A comprehensive list of all specimens examined for this study. Specimen identification numbers (TK – Museum of Texas Tech University; NK – Museum of Southwestern Biology, University of New Mexico; TJM = collection number of specimens from Texas State University) followed by *Cyt*b, *Adh*1-I2, and *Fgb*-I7 GenBank accession numbers (*e. g.*, Museum ID # – *Cyt*b GenBank #, *Adh*1-I2 GenBank #, *Fgb*-I7 GenBank #). All specimens are from the United States unless noted otherwise.

Reference samples:

Neotoma floridana– United States; Kansas; Lyon County, Ross Natural History Preserve, 4 mi W, 1 mi S Americus (TK28244 – AF186818, AY817640, DQ180021); Missouri; Pulaski County, Fort Leonard Wood (NK41561 – AF294333, KF860899, KF861009); Oklahoma; Creek County, Heyburn State Recreation Area (TK27751 – AF294341, AY817639, DQ180020); Oklahoma; McIntosh County, 3.1 mi E Dustin (TK23385 – AF294339, EU284810, KF861010); Pottawatomie County, 2.5 mi N, 5.9 mi E Tecumseh (TK25365 – AF294340, KF860901, KF861011); South Carolina; Richland County, Congaree Swamp NM (NK64089– AF294335, AY817637, DQ180054); Texas; Anderson County, Gus Engeling Wildlife Management Area (TK52115 – AF294344, KF860902, KF861012); Brazoria County, Peach Point Wildlife Management Area (TK51632– AF294343, KF860903, KF861013).

Neotoma micropus– Mexico; Coahuila, 20 mi S Morelos (TK16501 – AF186824, KF860904, KF861014); United States; New Mexico; Otero County, Ft. Bliss Military Base (TK77270 – AF376474, AY817653, DQ180049); Roosevelt County, 16.5 mi S, 3 mi E Taiban (TK31643 – AF186822, AY817652, DQ180048); Texas; Brewster County, Black gap Wildlife Management Area (TK51949 – AF298845, KF860905, KF861015); Dimmit County, Chapparral Wildlife Management Area (TK84556 – AF186826, AY817654, DQ180050; TK84557– AF186827, AY817655, DQ180040); Moore County, 4 mi N, 1 mi E Dumas (TK26731 – EU286808, EU284813, KF861016).

Specimens from study sites: (Museum ID number, *Fgb*-I7 GenBank Accession number)

Locality 1.--- Kansas; Hamilton County, 1.5 mi N, 2.0 mi W Syracuse, Hamilton Co. Wildlife Management Area, (TK175812 – KJ611149; TK175813 – KJ611150; TK175814 – KJ611151; TK175815 – KJ611152; TK175816 – KJ611153; TK175818 – KJ611154; TK175819 – KJ611155)

Locality 2.— Kansas; Hamilton County, 0.5 mi S, 3.6 mi W Syracuse (Girlscout Camp: TK175806 – KJ611146; TK175807 – KJ611147; TK175808 – KJ611148)

Locality 3.— Kansas; Barton County; Cheyenne Bottoms Wildlife Area (TK165470 – KJ611062; TK165471 – KJ611063; TK165472 – KJ611064; TK165473 – KJ611065; TK165474 – KJ611066; TK165475 – KJ611067; TK165476 – KJ611068; TK165477 – KJ611069; TK165479 – KJ611070; TK165480 – KJ611071; TK165481 – KJ611072; TK165483 – KJ611073; TK169501 – KJ611128; TK169503 – KJ611129; TK169504 – KJ611130; TK169505 – KJ611131; TK169506 – KJ611132; TK169690 – KJ611137; TK169691 – KJ611138; TK169694 – KJ611139)

Locality 4.— Kansas; Barton County; 3.5 mi N Great Bend (TK169598 – KJ611133; TK169599 – KJ611134; TK169600 – KJ611135; TK169601 – KJ611136)

Locality 5.— Kansas; Barton County; 1.0 mi S, 0.2 mi W Ellinwood (TK175771 – KJ611140; TK175772 – KJ611141; TK175773 – KJ611142; TK175774 – KJ611143; TK175775 – KJ611144; TK175776 – KJ611145)

Locality 6.— Oklahoma, Cimarron County; Black Mesa State Park (TK160982 – KJ611043; TK163031 – KJ611044)

Locality 7.— Oklahoma; Woodward County, Boiling Springs State Park (TK167362 – KJ611104; TK167363 – KJ611105; TK167369 – KJ611106; TK167434 – KJ611121)

Locality 8.— Oklahoma; Woodward County, 2 mi S, 6 mi E Woodward (TK167500 – KJ611124; TK168001 – KJ611125; TK168007 – KJ611126; TK168009 – KJ611127)

Locality 9.— Oklahoma; Major County, 5 mi W Cleo Springs (TK167392 – KJ611107; TK167393 – KJ611108; TK167395 – KJ611109; TK167396 – KJ611110; TK167405 – KJ611111; TK167406 – KJ611112; TK167413 – KJ611113; TK167414 – KJ611114; TK167415 – KJ611115; TK167416 – KJ611116; TK167417 – KJ611117; TK167418 – KJ611118; TK167419 – KJ611119; TK167420 – KJ611120; TK167451 – KJ611122; TK167452 – KJ611123)

Locality 10.— Oklahoma; Dewey County, 1 mi N, 9 mi E Seiling (Canton WMA: TK167337 – KJ611089; TK167339 – KJ611090; TK167346 – KJ611091; TK167347 – KJ611092; TK167348 – KJ611093; TK167349 – KJ611094; TK167350 – KJ611095; TK167351 – KJ611096; TK167353 – KJ611097; TK167354 – KJ611098; TK167355 – KJ611099; TK167356 – KJ611100; TK167357 – KJ611101; TK167360 – KJ611102; TK167361 – KJ61110): Oklahoma; Blaine County, 2.9 mi S Canton Lake Recreational Area - Big Bend Campground (TK160840 – KJ611033; TK160841 – KJ611034; TK160843 – KJ611035; TK160845 – KJ611036; TK160846 – KJ611037; TK160847 – KJ611038; TK160849 – KJ611039; TK160850 – KJ611040; TK160851 – KJ611041; TK160865 – KJ611042)

Locality 11.— Oklahoma; Ellis County, Ellis Co. Wildlife Management Area (TK165342 – KJ611047; TK165382 – KJ611049; TK165383 – KJ611050; TK165384 – KJ611051; TK165385 – KJ611052; TK165386 – KJ611053; TK165387 – KJ611054; TK165388 – KJ611055; TK165389 – KJ611056; TK165390 – KJ611057)

Locality 12.— (Texas, Hemphill County, Gene Howe Wildlife Management Area, (TK165429 – KJ611058; TK165430 – KJ611059; TK165437 – KJ611060; TK165455 – KJ611061)

Locality 13.— Oklahoma; Dewey County, 6 mi N, 4 mi W Oakwood (TK166466 – KJ611083; TK166467 – KJ611084; TK166491 – KJ611086)

Locality 14.— Oklahoma; Dewey County, 1 mi S, 2.5 mi E Taloga (TK166493 – KJ611087; TK166494 – KJ611088)

Locality 15.— Oklahoma; Dewey County, 3 mi N, 6 mi W Oakwood (TK166441 – KJ611081)

Locality 16.— Oklahoma; Dewey County, 2 mi N, 7 mi W Oakwood (TK166402 – KJ611077; TK166403 – KJ611078; TK166404 – KJ611079; TK166405 – KJ611080)

Locality 17.— Oklahoma; Dewey County, 0.2 mi N, 0.5 mi W Fay (TK166462 – KJ611082; TK166474 – KJ611085)

Locality 18.— Oklahoma; Roger Mills County, 10.0 mi N, 2.5 mi W Cheyenne, Black Kettle National Grassland (TK165310 – KJ611045; TK165335 – KJ611046; TK165365 – KJ611048)

Locality 19.— Oklahoma; Cotton County, 5.5 mi S, 1mi E Randlett (TK166379 – KJ611076)

Locality 20a.— Texas; Wichita County, 1 mi N Burkburnett (TK166373 – KJ611074; TK166375 – KJ611075; TK179262 – KJ611165; TK179264 – KJ611166; TK179265 – KJ611167; TK179266 – KJ611168)

Locality 20b.— Texas; Wichita County, 0.5 mi N, 1 mi E Burkburnett(TK179251 – KJ611156; TK179252 – KJ611157; TK179253 – KJ611158; TK179254 – KJ611159; TK179255 – KJ611160; TK179256 – KJ611161; TK179257 – KJ611162; TK179258 – KJ611163; TK179260 – KJ611164;)

Locality 21.— Texas; Bastrop County, 10 mi S, 5 mi W Rosanky (TJM151 – KJ611169; TJM650 – KJ611170; TJM658 – KJ611171; TJM679 – KJ611172)

Appendix 2

Identification, demographic, locality, and genetic assay data for each individual woodrat examined in this study. Abbreviations are as follows: ID# = Unique identification number (TK = NSRL field identification number, TJM = collection number of specimens from Texas State University); sex: m = male, f = female, u = unknown sex; age: A = Adult, SA = Subadult, J = Juvenile, E = Embryo; genotype: M = homozygous for *N. micropus* alleles at the respective locus, F = homozygous for *N. floridana* alleles at the respective locus, H = heterozygous at the respective locus; Class = final classification of the individual: hyb = hybrid individual, mic = putatively pure *N. micropus* individual, flor = putatively pure *N. floridana* individual. Superscripts after TK numbers indicate the family unit (a-f) to which the individual belongs.

ID#	Sex	Age	Locality #	Adh1-I2	Cytb	Fgb-I7	Class
TK175812	f	A	1	M	M	H	hyb
TK175813	f	A	1	M	M	M	mic
TK175814	f	A	1	M	M	F	hyb
TK175815	m	A	1	M	M	M	mic
TK175816	m	A	1	M	M	H	hyb
TK175818	m	A	1	M	M	M	mic
TK175819	m	A	1	M	M	M	mic
TK175806	f	A	2	M	M	M	mic
TK175807	f	SA	2	M	M	M	mic
TK175808	f	A	2	M	M	M	mic
TK165470	f	A	3	H	F	F	hyb
TK165471	m	A	3	F	F	F	flor
TK165472	f	A	3	F	F	F	flor
TK165473	m	A	3	F	F	F	flor
TK165474	f	SA	3	F	F	F	flor
TK165475	f	A	3	F	F	F	flor
TK165476	f	A	3	F	F	F	flor
TK165477	f	A	3	H	F	F	hyb
TK165479	m	A	3	M	F	F	hyb
TK165480	f	SA	3	H	F	F	hyb
TK165481	m	A	3	H	F	F	hyb
TK165483	f	A	3	H	F	F	hyb
TK169501	f	A	3	F	F	F	flor
TK169503	f	A	3	F	F	F	flor
TK169504	f	A	3	F	F	F	flor
TK169505	f	A	3	F	F	F	flor
TK169506	f	A	3	F	F	F	flor
TK169690	f	SA	3	H	F	F	hyb
TK169691	f	A	3	H	F	F	hyb
TK169694	m	A	3	H	F	F	hyb
TK169598	f	A	4	H	F	F	hyb
TK169599	f	A	4	H	F	F	hyb
TK169600	m	A	4	H	F	F	hyb
TK169601	m	A	4	H	F	F	hyb
TK175771	f	A	5	F	F	F	flor

ID#	Sex	Age	Locality #	Adh1-I2	Cytb	Fgb-I7	Class
TK175772	f	A	5	F	F	F	flor
TK175773	m	A	5	F	F	F	flor
TK175774	f	A	5	F	F	F	flor
TK175775	m	A	5	F	F	F	flor
TK175776	m	A	5	F	F	F	flor
TK160982	f	SA	6	M	M	H	hyb
TK163031	f	SA	6	M	M	M	mic
TK167362	f	SA	7	M	M	M	mic
TK167363	m	A	7	M	M	M	mic
TK167369	f	A	7	M	M	H	hyb
TK167434	f	A	7	M	M	M	mic
TK167500	f	SA	8	M	M	M	mic
TK168001	f	A	8	M	M	M	mic
TK168007	f	A	8	M	M	M	mic
TK168009	f	A	8	M	M	H	hyb
TK167392	m	A	9	M	M	M	mic
TK167393	m	J	9	M	M	H	hyb
TK167395	m	J	9	M	M	H	hyb
TK167396[a]	f	A	9	M	M	F	hyb
TK167405[b]	f	A	9	M	M	H	hyb
TK167406	m	A	9	M	M	M	mic
TK167413	f	A	9	M	M	F	hyb
TK167414[a]	u	E	9	M	M	H	hyb
TK167415[a]	u	E	9	M	M	H	hyb
TK167416[a]	u	E	9	M	M	H	hyb
TK167417[a]	u	E	9	M	M	H	hyb
TK167418[a]	u	E	9	M	M	H	hyb
TK167419[b]	u	E	9	M	M	H	hyb
TK167420[b]	u	E	9	M	M	H	hyb
TK167451[c]	f	A	9	H	M	M	hyb
TK167452[c]	m	J	9	M	M	H	hyb
TK160840	m	A	10	F	F	F	flor
TK160841	f	A	10	H	F	F	hyb
TK160843	m	A	10	H	F	F	hyb
TK160845	m	A	10	M	F	F	hyb
TK160846	f	A	10	F	F	F	flor
TK160847	f	A	10	F	F	F	flor
TK160849	f	SA	10	F	F	F	flor
TK160850	f	A	10	H	F	F	hyb
TK160851	f	J	10	F	F	F	flor
TK160865	f	A	10	F	F	F	flor
TK167337	f	SA	10	F	F	F	flor
TK167339	m	SA	10	F	F	F	flor
TK167346	f	A	10	F	F	F	flor
TK167347	f	SA	10	F	F	F	flor
TK167348	f	A	10	F	F	F	flor
TK167349	f	A	10	M	F	F	hyb
TK167350	m	A	10	F	F	F	flor
TK167351	f	A	10	F	F	F	flor

Appendix 2

Continuation...

ID#	Sex	Age	Locality #	Adh1-I2	Cytb	Fgb-I7	Class
TK167353[d]	f	A	10	F	F	F	flor
TK167354	f	A	10	F	F	F	flor
TK167355	u	SA	10	M	F	F	hyb
TK167356	m	A	10	M	F	F	hyb
TK167357	f	SA	10	M	F	F	hyb
TK167360[d]	u	E	10	F	F	F	flor
TK167361[d]	u	E	10	F	F	F	flor
TK165342	m	SA	11	M	M	H	hyb
TK165382	f	A	11	M	M	M	mic
TK165383	f	A	11	M	M	M	mic
TK165384	f	A	11	M	M	M	mic
TK165385	m	A	11	M	M	M	mic
TK165386	f	A	11	M	M	M	mic
TK165387	f	A	11	M	M	H	hyb
TK165388	f	A	11	M	M	M	mic
TK165389	f	A	11	M	M	M	mic
TK165390	f	A	11	M	M	M	mic
TK165429	f	A	12	M	M	M	mic
TK165430	f	A	12	M	M	M	mic
TK165437	f	A	12	M	M	M	mic
TK165455	m	A	12	M	M	M	mic
TK166466	m	A	13	F	F	F	flor
TK166467	m	A	13	F	F	F	flor
TK166491	f	A	13	F	F	F	flor
TK166493	f	A	14	F	F	F	flor
TK166494	f	A	14	F	F	F	flor
TK166441	f	A	15	F	F	F	flor
TK166402	f	A	16	F	F	F	flor
TK166403	m	A	16	F	F	F	flor
TK166404	f	A	16	F	F	F	flor
TK166405	f	A	16	F	F	F	flor
TK166462[e]	f	A	17	F	F	F	flor
TK166474[e]	u	E	17	F	F	F	flor
TK165310	m	A	18	M	M	M	mic
TK165335	f	SA	18	M	M	M	mic
TK165365	f	A	18	M	M	M	mic
TK166379	m	A	19	F	F	F	flor
TK166373	m	A	$20a_{I}$	H	F	M	hyb
TK166375	m	A	$20a_{II}$	M	M	M	mic
TK179262	m	A	$20a_{III}$	M	F	M	hyb
TK179264	m	SA	$20a_{IV}$	M	M	M	mic
TK179265[f]	f	A	$20a_{V}$	M	M	M	mic
TK179266[f]	u	E	$20a_{VI}$	M	M	H	hyb
TK179251	f	A	$20b_{VII}$	M	F	H	hyb
TK179252	f	J	$20b_{VIII}$	M	F	M	hyb
TK179253	m	J	$20b_{IX}$	H	F	M	hyb
TK179254	f	A	$20b_{X}$	M	F	M	hyb
TK179255	f	SA	$20b_{XI}$	M	F	M	hyb
TK179256	f	A	$20b_{XII}$	M	F	M	hyb
TK179257	f	A	$20b_{XIII}$	H	F	M	hyb
TK179258	f	A	$20b_{XIV}$	M	F	M	hyb
TK179260	f	A	$20b_{XV}$	H	F	M	hyb
TJM151	f	A	21	F	F	F	flor
TJM650	m	A	21	F	F	F	flor
TJM658	m	A	21	F	F	F	flor
TJM679	f	SA	21	F	F	F	flor

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Chromosomal relationships among the native rodents (Cricetidae: Oryzomyini) of the Galápagos Islands, Ecuador

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Although the Galápagos Islands are recognized for their contribution to our understanding of evolutionary theory and have received the attention of scientists for over 185 years, our understanding of the native rodents there has been minimal relative to many other groups of organisms. Much of what we knew through most of the 20th century was based solely on species descriptions. Chromosome data has been limited to only *Nesoryzomys narboroughi* (2n = 32, FN (number of autosomal arms) = 50) and *Aegialomys galapagoensis* (2n = 56; FN = 58). We present the karyotypes of the only remaining extant species in the genus, *N. swarthi* (2n = 56; FN = 54) and *N. fernandinae* (2n = 44; FN = 54). Chromosomal banding reveals that extensive rearrangement has occurred within *Nesoryzomys*, including Robertsonian fusion and tandem fusion events but these alone cannot account for the diverse diploid numbers found within the genus. We propose that 1) *N. swarthi* represents the ancestral karyotype for the genus, similar to *A. galapagoensis*, 2) *N. swarthi* and *N. fernandinae* share the same fundamental number, suggesting divergence by Robertsonian fusions, and 3) *N. narboroughi* has the most derived karyotype, based on banding morphology and low diploid number.

Aunque las Islas Galápagos son reconocidas por su contribución a nuestra comprensión de la teoría de la evolución y han recibido la atención de los científicos durante más de 185 años, nuestra comprensión de los roedores nativos de dichas islas, ha sido mínima en comparación con muchos otros grupos de organismos. Gran parte del conocimiento obtenido durante la mayor parte del siglo XX se basó únicamente en descripciones de especies. Los datos cromosómicos se han limitado solo a *Nesoryzomys narboroughi* (2n = 32, FN (número de brazos autosómicos) = 50) y *Aegialomys galapagoensis* (2n = 56; FN = 58). Presentamos los cariotipos de las únicas especies que quedan en el género, *N. swarthi* (2n = 56; FN = 54) y *N. fernandinae* (2n = 44; FN = 54). El método de bandeo cromosómico revela que se ha producido un reordenamiento extenso dentro de *Nesoryzomys*, incluida la fusión robertsoniana y los eventos de fusión en tándem, pero estos por sí solos no pueden explicar los diversos números diploides que se encuentran dentro del género. Proponemos que 1) *N. swarthi* representa el cariotipo ancestral del género, similar a *A. galapagoensis*, 2) *N. swarthi* y *N. fernandinae* comparten el mismo número fundamental, lo que sugiere una divergencia por fusiones robertsonianas y 3) *N. narboroughi* tiene el cariotipo más derivado, basado en la morfología de bandas y en el bajo número diploide.

Keywords: *Aegialomys*; chromosomal rearrangements; G-bands; karyotypes; *Nesoryzomys*.

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Introduction

The Galápagos Islands have played a critical role in our understanding of evolution and have been the focus of thousands of studies dealing with the unique flora and fauna of this archipelago (Snell *et al.* 1996). Despite this, the rodent fauna has been poorly represented in scientific research relative to many Galápagos vertebrates. Until the late 20th century, original species descriptions were almost the only research published. This taxonomy of the native rodents has had a circuitous history with name changes at both the genus and species levels. Only a single study (Gardner and Patton 1976) has described cytogenetic data.

Taxonomic history of Galápagos rodents. Charles Darwin collected the first native rodents in the Galápagos Islands in 1835 on the island of Chatham (now known as San Cristóbal; Figure 1). The species, later described by Waterhouse (1839) as *Mus galapagoensis* (=*Aegialomys galapagoensis*), was never collected again on San Cristóbal (Clark 1984). Allen (1892) described a second species, *Oryzomys bauri* from the island of Barrington (now Santa Fé), recognizing both *bauri* and *galapagoensis* as belonging to the genus *Oryzomys*. In 1899, Oldfield Thomas described the first rodent from the island of Indefatigable (now Santa Cruz) as *Oryzomys indefessus*, and Heller (1904) later elevated this species to a separate genus, *Nesoryzomys,* on the basis of skull morphology. A second species within the genus, *N. narboroughi*, also was described by Heller (1904) on the westernmost island, Narborough (now Fernandina). Osgood (1929) described a third, smaller species, *N. darwini*, from Santa Cruz and Orr (1938) described another large form, *N. swarthi*, from James Island (now Santiago). A fifth species in the genus, *N. fernandinae*, was described in 1979 based on owl pellet remains from the island of Fernandina (Hutterer and Hirsch 1979) and was small in body size.

In 1983, Patton and Hafner published the most comprehensive systematic treatise on Galápagos rodents to date, summarizing the systematic relationships based on cranial, stomach and male reproductive tract morphology, pelage color, allozymes, and karyotypes. Despite the number of data sets analyzed, their study was hampered because at the time the manuscript was written, only 2 native species of rodents, *Nesoryzomys narboroughi* and *Oryzomys bauri,* were known to be extant. *Nesoryzomys swarthi* was known from four specimens in the type series

collected in 1906 (Orr 1938) and a single skull collected in 1965 (Peterson 1966); however, the species was considered extinct by most (Patton and Hafner 1983; Clark 1984). Both *N. indefessus* on Santa Cruz and Baltra, and *N. darwini* on Santa Cruz had been considered extinct since the 1940's (Brosset 1963; Clark 1984; Key and Muñoz 1994). *Nesoryzomys fernandinae*, described solely on skulls (Hutterer and Hirsch 1979), could not be included in the study by Patton and Hafner (1983) as the species had not yet been described at the time the manuscript was submitted. The analysis of available data by Patton and Hafner (1983) suggested that the large-bodied *Nesoryzomys*, represented by *N. indefessus*, *N. narboroughi*, and *N. swarthi*, were variants of a single species that should be recognized as *N. indefessus* and that *Oryzomys bauri* and *O. galapagoensis* should be synonymized with *O. galapagoensis* having priority. Musser and Carleton (2005) concurred, placing *narboroughi* and *indefessus* in synonymy under *N. indefessus*, but recognizing *N. swarthi* as a valid taxon. Most recently, in revisions of oryzomyines (Weksler 2006; Weksler *et al.* 2006), *Nesoryzomys* was retained as a valid genus, but *Oryzomys galapagoensis* was placed with *O. xanthaeolus* in the genus *Aegialomys* (Prado and Percequillo 2018). Currently, the Galápagos native rodents are composed of *A. galapagoensis*, *N. darwini*, *N. fernandinae*, *N. indefessus* and *N. swarthi*, following Musser and Carleton (2005). *Nesoryzomys narboroughi* was recognized as a fifth species of the genus by Dowler (2015). Herein, we treat the genus *Nesoryzomys* as including five named species. Three additional undescribed species based on fossil remains are known from the islands of Rábida and Isabela (Steadman *et al.* 1991) but Moreira *et al.* (2020) reported only one from both of these islands. In addition to these Galápagos species, fossil remains of giant rats, genus *Megaoryzomys*, are known from Santa Cruz and Isabela (Steadman and Ray 1982; Steadman *et al.* 1991).

Chromosomes of Galápagos rodents. The only chromosomal data for native Galápagos rodents were published by Gardner and Patton (1976) for *Nesoryzomys narboroughi* and *Aegialomys galapagoensis.* The karyotype of *N. narboroughi* had a diploid number (2n) of 32 and a fundamental number (FN - number of autosomal arms) of 50 with mostly metacentric chromosomes. Its karyotype was strikingly different from that of *A. galapagoensis* (2n = 56, FN = 58) with mostly acrocentric chromosomes. The karyotype of *A. galapagoensis* was reported to be essentially identical to that of *A. xanthaeolus*, a mainland from Peru and Ecuador. Not only was the karyotype of *N. narboroughi* considerably different from *A. galapagoensis* and *A. xanthaeolus*, but it was unlike any known oryzomyines at the time. On this basis, Gardner and Patton (1976) confirmed the generic status of *Nesoryzomys* first proposed by Heller (1904). Additional data from Patton and Hafner (1983) further supported the generic status of *Nesoryzomys* separate from *Oryzomys*. Other researchers (Ellerman 1941) have recognized *Nesoryzomys* as a subgenus of *Oryzomys*.

Field studies by Angelo State University researchers since 1995 have located living populations (Figure 1) of *Nesoryzomys fernandinae* on Fernandina (Dowler and Carroll 1996) previously known only from owl pellet material, and *N. swarthi*, previously considered extinct, on Santiago (Dowler *et al.* 2000). These discoveries have allowed an analysis of diploid and fundamental numbers of these previously unkaryotyped species. In addition, recent collections of all extant Galápagos species of rodents now permit the first comparison of chromosomal banding patterns to help elucidate the systematic relationships of these species.

Material and Methods

We surveyed the Galápagos rodent species on the islands of Fernandina, Santiago, and Santa Fé (Figure 1). In addition, we conducted survey trips to the islands of Baltra, Isabela, Rábida, San Cristóbal, and Santa Cruz that have had native rodent species historically or as recent fossils, but were unsuccessful in finding extant populations. Specimens were collected using Sherman live traps or small cage traps. All specimens were prepared as study skins or fluid-preserved specimens and deposited in the Angelo State Natural History Collections (ASNHC) of Angelo State University. Specific localities of capture and voucher specimen numbers are given in Appendix 1.

Up to four individuals were karyotyped from each of the three species of *Nesoryzomys* and *A. xanthaeolus*. Metaphase chromosomes were obtained in vivo from bone marrow following Lee and Elder (1980). Standard karyotypes were prepared and stained with conventional Giemsa and 8-10 spreads were examined for each species. Additional slides were prepared and counterstained with 4'6-Diamidine-2'-phenylindole dihydrochloride (DAPI) with antifade mounting reagent for visualization of banded chromosomes. DAPI positive bands are indicative of A-T rich regions of heterochromatin. These banding patterns correspond to G-bands produced by trypsin digestion of chromosomes, and subsequently will be referred to as G-bands (Heng and Tsui 1993).

All chromosomes were examined on an Olympus Vanox epifluorescent microscope (Olympus, Melville, NY, U.S.A.). G-bands were examined using a DAPI filter (excitation 350 to 460 nm; emission, longpass, 520 nm). Images were obtained using the SPOTÔ, CCD digital camera and Image Pro7 software package (Leeds Instruments, Irving, TX, USA). DAPI bands were obtained by inversion of the fluorescent image, creating banding patterns along the chromosomes. We examined karyotypes to determine phylogenetic relationships among species within the genus *Nesoryzomys*. For the purpose of establishing polarity of karyotypic characters, we used *Aegialomys* as an outgroup as recent molecular analyses have placed *Aegialomys* sister to *Nesoryzomys* (Parada *et al.* 2015; Castañeda-Rico *et al.* 2019; Brito *et al.* 2020).

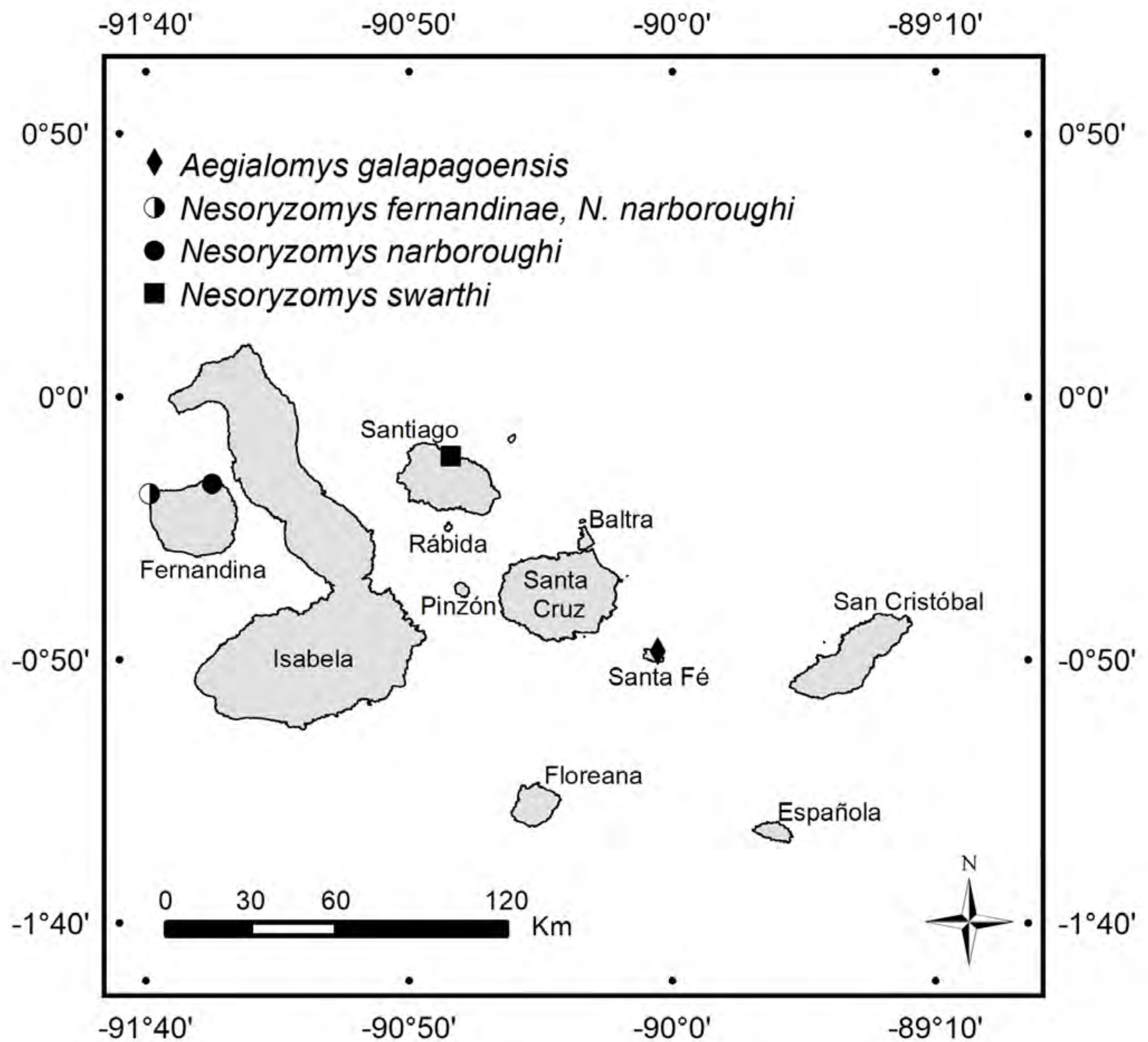

Figure 1. Map of Galápagos Islands, Ecuador with sampling localities of *A. galapagoensis* (diamond), *N. narboroughi* (closed circle, half circle), *N. swarthi* (square), and *N. fernandinae* (half circle) in the Galápagos Islands.

Results

Standard Karyotypes. The karyotype for *Aegialomys galapagoensis* is as previously reported by Gardner and Patton (1976). *Aegialomys galapagoensis* (2n = 56, FN = 58) is characterized by one distinctly large acrocentric pair and 24 pairs of acrocentric chromosomes ranging from large- to medium-sized, and two small metacentric pairs. The sex chromosomes, a medium-sized X and a small Y, are both acrocentric (Figure 2a).

The karyotype of *Nesoryzomys narboroughi* (2n = 32, FN = 50) presented herein is as described by Gardner and Patton (1976). It comprises eight metacentric pairs ranging from large- to medium-sized chromosomes, two subtelocentric pairs of large- and medium-sized chromosomes, five acrocentric pairs with one large pair and the others small (Figure 2d). The X and Y chromosomes are the same as previously described for the genus.

Karyotypic analysis for the previously undocumented extant species of *Nesoryzomys* revealed strikingly different karyotypes from that of *N. narboroughi*. Unlike the low diploid number found in *N. narboroughi*, *N. swarthi* (2n = 56, FN = 54) has a karyotype composed completely of 27 pairs of acrocentric chromosomes, with one large pair and 26 pairs ranging from medium to small (Figure 2b). The X chromosome is medium-sized and acrocentric, whereas the Y chromosome is small and acrocentric. *Nesoryzomys fernandinae* (2n = 44, FN = 54) is characterized by six pairs of metacentric chromosomes ranging from large to medium-sized, and one large pair and 14 small pairs of acrocentric chromosomes (Figure 2c). The X chromosome is large and acrocentric and the Y is a small acrocentric chromosome.

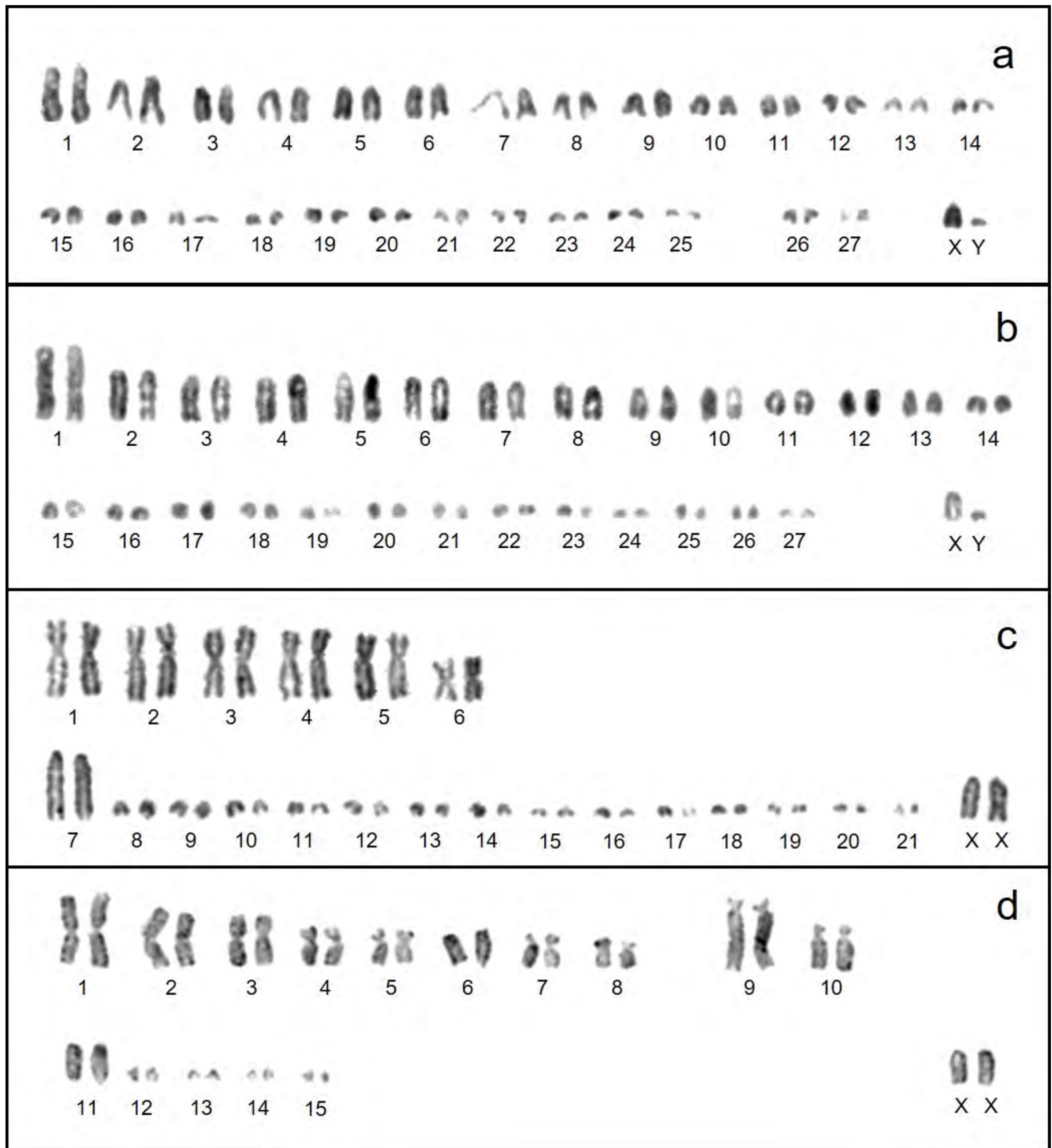

Figure 2. Representative standard karyotypes of the extant native Galápagos rodents. a) *Aegialaomys galapagoensis*, 2n = 56, FN = 58, male. b) *Nesoryzomys swarthi*, 2n = 56, FN = 54, male. c) *Nesoryzomys fernandinae*, 2n = 44, FN = 54, female. d) *Nesoryzomys narboroughi* Heller, 2n = 32, FN = 50, female. Chromosomes are numbered from longest to shortest, beginning with metacentrics and submetacentrics where present.

Banded Karyotypes. G-banded karyotypes (Figure 3) varied in quality but were sufficient to draw some conclusions regarding karyotypic rearrangements responsible for the observed changes in diploid and fundamental numbers. The karyotypes of *N. swarthi* and *N. fernandinae* have identical fundamental numbers, suggesting Robertsonian rearrangement events leading to the reduction in chromosome number and the appearance of biarmed chromosomes. Both *N. fernandinae* and *N. narboroughi* are similar in having biarmed chromosomes, but *N. fernandinae* has 15 pairs of acrocentric chromosomes while *N. narboroughi* has only five. The differences in fundamental numbers suggest tan-

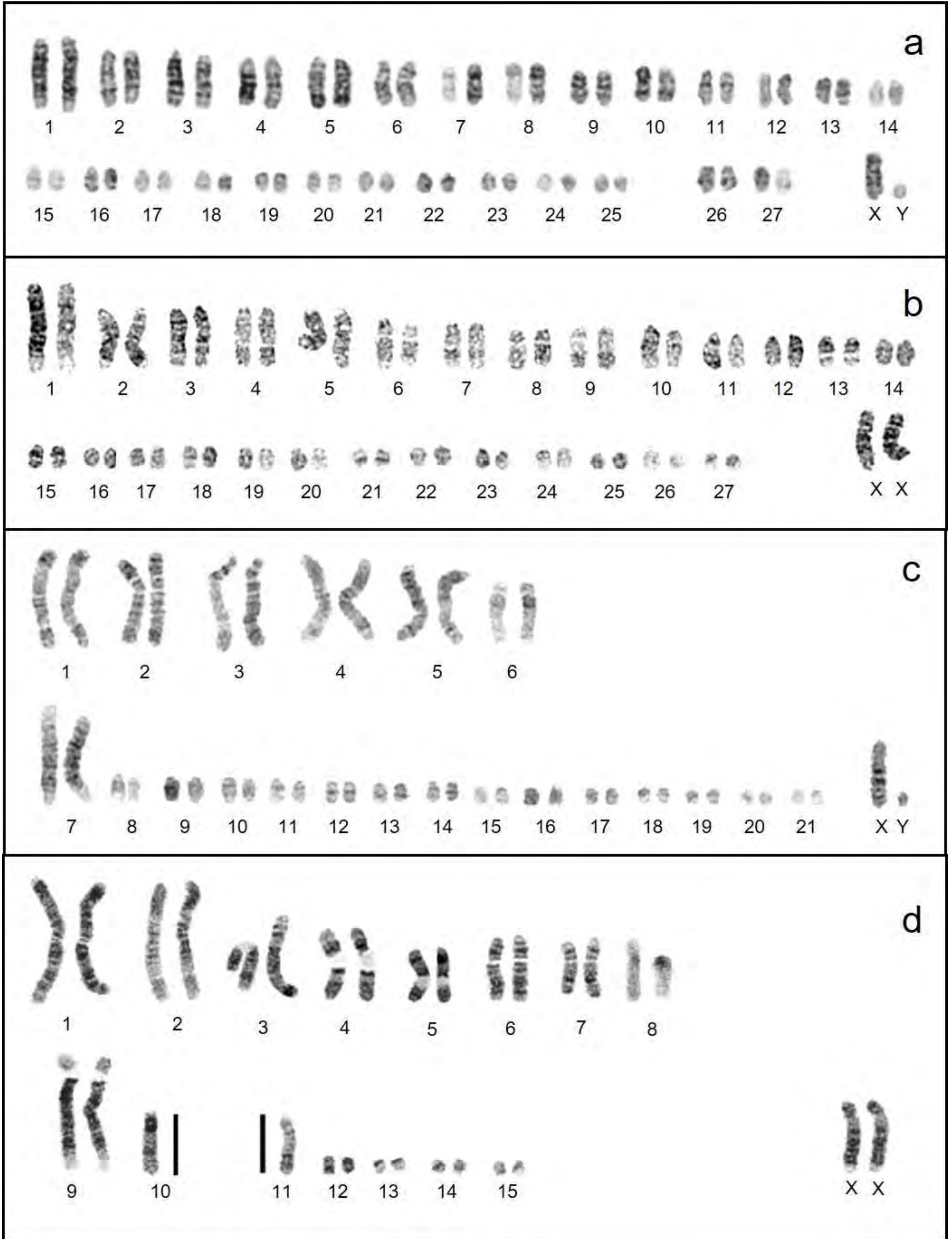

Figure 3. Representative DAPI karyotypes of the extant native Galápagos rodents. a) *Aegialaomys galapagoensis,* male. b) *Nesoryzomys swarthi,* female. c) *Nesoryzomys fernandinae*, male. d) *Nesoryzomys narboroughi,* female. Chromosomes are numbered from longest to shortest, beginning with metacentrics and submetacentrics where present.

dem fusions/fissions, inversions, translocations, or whole-arm heterochromatin additions or deletions. Homologous chromosomes and portions of chromosomes are evident and a composite karyotype of the G-banded chromosomes for the four species was constructed using some of the larger chromosomes (Figure 4).

Robertsonian translocations are evident in some cases across all four extant species of Galápagos rodents. Chromosome 9 in *N. narboroughi* (Figure 3d) is a large, subtelocentric chromosome explained by the fusion of a small acrocentric chromosome to the largest acrocentric chromosome that is found to be homologous in all other species (Figure 4g). Chromosome 7 in *N. narboroughi* (Figure 3d) is a medium-sized metacentric chromosome that corresponds to smaller, acrocentric chromosomes in *A. galapagoensis*, *N. swarthi*, and *N. fernandinae* (Figure 4f). Homologous metacentric chromosomes were identified between *N. fernandinae* and *N. narboroughi* for Chromosomes 6 and 8 respectively, determined by the fusion of acrocentric chromosomes in *A. galapagoensis* and *N. swarthi* (Figure 4e). Chromosome 10 in *N. narboroughi* is a medium-sized subtelocentric chromosome (Figure 3d), its longer arm detected in both *A. galapagoensis* and *N. swarthi* but not *N. fernandinae* (Figure 4i). Chromosome 11 is the largest acrocentric chromosome in *N. narboroughi* (Figure 3d) and was identified in *N. swarthi* but could not be detected in other species (Figure 4j).

Chromosome 1 in both *N. fernandinae* and *N. narboroughi* is large and metacentric and nearly identical between the species, with the exception of a small addition on the end of *N. narboroughi* (Figure 4a), indicated by an asterisk (*). Although some homologous portions of these chromosomes could be identified from both *N. swarthi* and *A. galapagoensis*, there are regions (*) that could not, either because of tandem fusions of smaller acrocentric chromosomes or insufficient staining quality. Similar observations can be made for Chromosome 3 in both *N. fernandinae* and *N. narboroughi* (Figure 4c).

Many of the chromosomes in *N. narboroughi* (Figure 3d) were found to be unique with variations that could not be found in the other species. It is possible that heterochromatic additions may play a role in these differences. Chromosome 4 in *N. narboroughi* is a metacentric chromosome with a homologous portion found in *A. galapagoensis* and *N. swarthi* but could not be identified in *N. fernandinae*. The lighter portion indicated by an asterisk (*) in Figure 4h contains an area considered to be a heterochromatic addition. Chromosome 5 in *N. narboroughi* could not be resolved with other species, but likely contains a heterochromatic addition as seen in Chromosome 4, based on banding pattern. All species within *Nesoryzomys* possess an X chromosome that is mostly identical to each other when compared to *Aegialomys*, but with *N. narboroughi* differing slightly by a possible heterochromatic addition (Figure 2d).

Discussion

This is the first study to include karyotypes for all extant endemic rodent species of the Galápagos Islands. Our

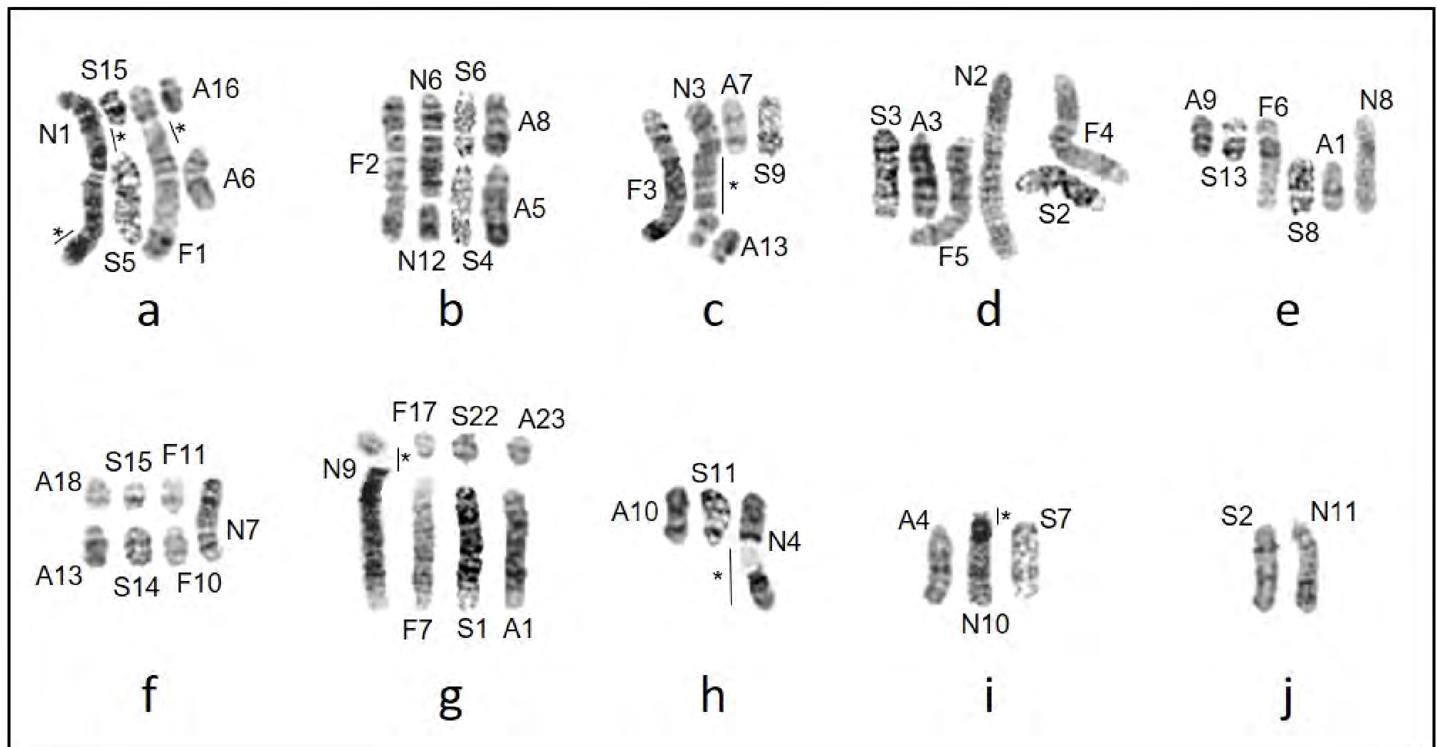

Figure 4. Comparison of banded haploid complements of the endemic Galápagos rodents for *Aegialaomys galapagoensis, Nesoryzomys swarthi, N. fernandinae*, and *N. narboroughi*. Figures a-j are composites where chromosome numbers correspond to those assigned to karyotypes in Figure 2 and prefixes represent corresponding species: A = *A. galapagoensis*, S = *N. swarthi*, F = *N. fernandinae*, N = *N. narboroughi*. Areas indicated by an asterisk (*) represent unique areas of chromosomes with unresolved homologies.

karyotype for *A. galapagoensis* agrees with those reported by Gardner and Patton (1976) where they suggested that *A. galapagoensis* and *A. xanthaeolus* were identical in all aspects and perhaps were conspecific. Moreira *et al.* (2020), however, pointed out that Prado and Percequillo (2016) determined that the same specimens karyotyped as *A. xanthaeolus* by Gardner and Patton (1976) were from Peru and in fact belong to *A. baroni* and *A. ica*. Despite the fact that true *A. xanthaeolus* from Ecuador and northern Peru have yet to be karyotyped, the lack of apparent chromosomal variation among the three other species suggests that *Aegialomys* may be karyotypically monomorphic. Prado and Percequillo (2018) found that *A. galapagoensis* was sister to the two southern mainland forms *A. baroni* and *A. ica*, concluding that it is a unique species and lineage.

With respect to *Nesoryzomys*, surveys by our field teams and others over the last several decades suggest the two species from the island of Santa Cruz, *N. darwini* and *N. indefessus* (also from Baltra) likely have been extinct since the 1930s (Patton and Hafner 1983; Clark 1984; Dowler *et al.* 2000). In addition, three undescribed species occurred on the island of Rábida and Isabela but are extinct (Steadman *et al.* 1991). Thus, our karyotypic knowledge of the known *Nesoryzomys* fauna of eight species is restricted to those reported here. In contrast to the chromosomes of *Aegialomys*, our study reveals striking intrageneric variation in the karyotype of *Nesoryzomys*. Previously known only from *N. narboroughi*, its aberrant arrangement of mostly biarmed chromosomes was used to establish generic status (Gardner and Patton 1976; Patton and Hafner 1983; Suárez-Villota *et al.* 2013; Moreira *et al.* 2020). We report two new additional, distinct karyotypes of the other *Nesoryzomys* that provide insight into the origin of such a unique arrangement and demonstrate a closer relationship with other oryzomyine sister taxa.

Of the four major clades (Clades A-D) described in the monophyletic lineage of oryzomyines (Weksler 2006), *Nesoryzomys* falls within Clade D. Within that group, *Nesoryzomys* is placed in the *Aegialomys-Megalomys-Melanomys-Nesoryzomys-Oryzomys-Sigmodontomys-Tanyuromys* clade (Pine *et al.* 2012; Salazar-Bravo *et al.* 2016; Timm *et al.* 2018) and most phylogenies agree that *Nesoryzomys* is sister to *Aegialomys* (Weksler 2003; Hanson and Bradley 2008; Pine *et al.* 2012; Machado *et al.* 2014; Parada *et al.* 2015; Steppan and Schenk 2017; Timm *et al.* 2018; Castañeda-Rico *et al.* 2019; Brito *et al.* 2020). *Aegialomys galapagoensis* shares the same 2n = 56 karyotype as the mainland forms *A. ica* and *A. baroni* (Gardner and Patton 1976; Prado and Persequillo 2018), and *N. swarthi* but differs in fundamental numbers, FN = 58 in *Aegialomys* and FN = 54 in *N. swarthi*. The karyotype of *A. galapagoensis* comprises mostly acrocentric autosomes but has two small metacentric chromosomes that are absent in the entirely acrocentric karyotype of *N. swarthi*. No small metacentric chromosomes were found in any of the three species of *Nesoryzomys* that we examined, suggesting that these form a chromosomal group distinct from that of *Aegialomys*. Homologies in *N. swarthi* are not apparent for the two small metacentric chromosomes in *Aegialomys*. Some molecular phylogenies have found *Nesoryzomys* to be sister with *Sigmodontomys* and *Melanomys* and these sister to *A. xanthaeolus* (Weksler 2003; Machado *et al.* 2014; Salazar-Bravo *et al.* 2016). Based on chromosomal morphology, *Melanomys* (2n = 56, FN = 58) differs from *N. swarthi* by its two small metacentric autosomes and subtelocentric sex chromosomes (Gardner and Patton 1976). All sex chromosomes found within *Nesoryzomys* are acrocentric. The karyotype of *N. swarthi* is identical with *Sigmodontomys alfari* (2n = 56, FN = 54) as described by Gardner and Patton (1976). *Sigmodontomys aphrastus* was elevated to generic status as *Tanyuromys aphrastus* by Pine *et al.* (2012), and *S. alfari* remained sister to *Melanomys*. No known karyotype for *Tanyuromys* is available for comparison. The relationship between *Melanomys* and *Sigmodontomys* has been found to be paraphyletic and new species have been described (Timm *et al.* 2018; Pine *et al.* 2012). The identical karyotype shared between *S. alfari* and *N. swarthi* could be convergent; however, future comparison of chromosomal rearrangements between these two could provide valuable insight on the chromosomal evolution of *Nesoryzomys*.

In considering *Aegialomys* as sister to *Nesoryzomys*, small metacentric chromosomes are absent in all karyotypes of *Nesoryzomys* and some small acrocentric chromosomes would likely be the result of a fission event that occurred sometime in the past. Given that up to five other endemic species are now extinct, it is probable that one of these may have retained a chromosomal arrangement identical to *Aegialomys*. These gaps will remain problematic in completely understanding the chromosomal evolution of the Galápagos endemic rodent fauna. With the available evidence, we propose that the 2n = 56 karyotype is basal for the *Nesoryzomys-Aegialomys* clade (Figure 5).

Patton and Hafner (1983) concluded that the three large forms of *Nesoryzomys (N. indefessus*, *N. narboroughi*, and *N. swarthi*) all allopatrically distributed on different islands were conspecific based on similarity of specimen morphology. Diploid and fundamental numbers for both *N. swarthi* and *N. narboroughi* differ significantly, leaving no question that they are distinct species, and this also has been supported in all molecular studies that included both of these species (Weksler 2003; Pine *et al.* 2012; Leite *et al.* 2014; Parada *et al.* 2015; Steppan and Schenk 2017; Castañeda-Rico *et al.* 2019; Brito *et al.* 2020). The question of whether *N. indefessus* is conspecific with *N. narboroughi* as proposed by Patton and Hafner (1983) and adopted by Musser and Carleton (2005) is yet to be answered. Given that each species in the genus thus far has had such uniquely distinct karyotypes, we believe that there is a strong likelihood that *N. indefessus*, endemic to Santa Cruz and Baltra, would differ from the other large *Nesoryzomys* and should be recognized as such (Dowler 2015). Future molecular analysis that includes *N. indefessus* may be able to settle this issue.

Both *N. fernandinae* and *N. narboroughi* on Isla Fernandina also differ markedly in diploid and fundamental number. The karyotype for *N. fernandinae* represents the only chromosomal data for the small body-size form in *Nesoryzomys*, the other being the presumed extinct *N. darwini.* This is the only case of sympatry among extant forms and both the karyotypic and morphological differences rule out any likelihood of hybridization between the two.

In addition to the obvious geographic barriers between island populations of organisms, chromosomal rearrangements are known to serve as reproductive barriers and can become established over short periods of time (Searle 1993; Moreira *et al.* 2020). Gardner and Patton (1976) established the foundation for understanding chromosomal evolution among the Sigmodontinae and suggested that the general trend for chromosomal evolution was one of decrease in both diploid and fundamental numbers. Moreira *et al.* (2020) concluded that chromosomal evolution of oryzomyines differ by a large variety of rearrangements and that diploid numbers both decrease and increase without any distinguishable pattern. In the case of the Galápagos endemic rodent fauna, we propose that speciation on the archipelago has resulted in a decrease in both diploid and fundamental numbers resulting from potentially rapid divergence as colonists arrived on unoccupied islands. Some of these speciation events may have been facilitated by chromosomal rearrangements (King 1993; Britton-Davidian *et al.* 2000; Wang and Lan 2000).

Of the 141 oryzomyine species for which karyotypic data exist, Moreira *et al.* (2020) reported that 55 included some chromosomal banding, but banded karyotypes only exist for members of *Oryzomys* outside the Galápagos rodent fauna (Haiduk *et al*. 1979) in the *Aegialomys-Megalomys-Melanomys-Nesoryzomys-Oryzomys-Sigmodontomys-Tanyuromys* clade (Pine *et al.* 2012; Salazar-Bravo *et al.* 2016; Timm *et al.* 2018). This is the first study to include banded karyotypes for all extant endemic rodent species of the Galápagos Islands. Banding revealed extensive chromosomal rearrangements in the Galápagos rodents, a pattern that is clear in many other oryzomyines (Suárez-Villota *et al.* 2013; Suárez *et al.* 2015; Pereira *et al.* 2016). Despite the utility of chromosome banding as a tool in identifying and examining interspecies homologies (Damas *et al.* 2021), lack of banding data from those considered close relatives to the Galápagos rodents makes comparisons impossible at this time.

We identified numbers and types of chromosomal rearrangements using the largest chromosomes found in *N. fernandinae* and *N. narboroughi* (Figure 4) and demonstrate Robertsonian fusions, tandem fusions, other translocations, and some that could not be identified from banding sequences. The banding patterns between *N. fernandinae* and *N. narboroughi* revealed at least three whole chromosome homologies, at least one whole arm translocation, and evidence of tandem fusions when compared to *N. swarthi* and *A. galapagoensis* (Figure 4). Banding found in *N. narboroughi* revealed unique regions not found in any of the other Galápagos rodents with large heterochromatic additions. The difference in 2n but not FN between *N. swarthi* and *N. fernandinae* also suggests Robertsonian fusions have occurred. These rearrangements could be supported further with C-banding, but we were unable to obtain

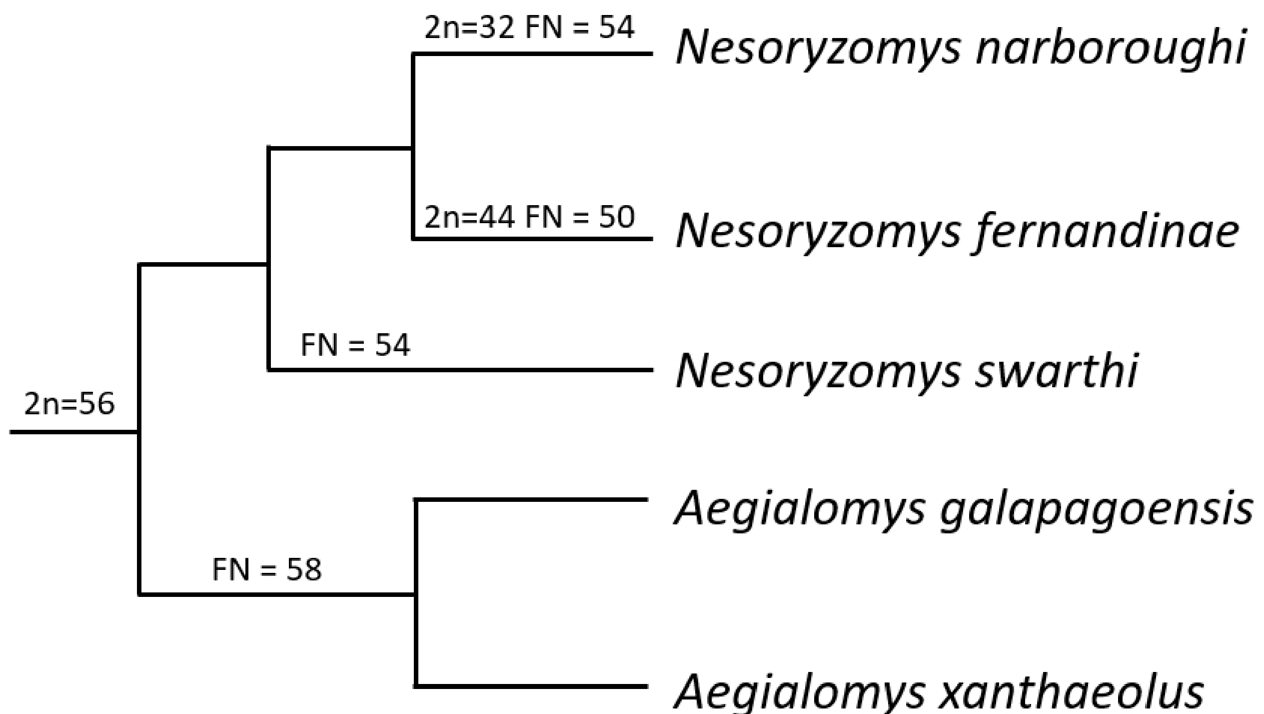

Figure 5. Cladogram depicting phylogeny of the native Galápagos rodents and *Aegialomys xanthaeolus* with chromosomal diploid (2n) and fundamental numbers (FN) included.

these data. Castañeda-Rico *et al.* (2019) determined these two species to be sister to *N. narboroughi* based on mtDNA D-loop sequence data, as did Steppan and Schenk (2017) based on concatenated sequences of multiple genes. In contrast, *N. narboroughi* has been placed with *N. swarthi* in other molecular phylogenies (Parada *et al.* 2015; Brito *et al.* 2020), but with lower statistical support. Other molecular studies only have included two of the three species in their molecular analyses.

Moreira *et al.* (2020) examined the karyotypic variation among all Oryzomyini rodents and found the majority of species are composed of acrocentric chromosomes with a diploid number between 48 and 64 with fundamental numbers ranging from 56 to 74. We use this generality to examine the evolutionary history of the native rodent fauna of the Galápagos Islands. Given that the chromosomal arrangements among the four extant species suggest a progressive reduction in the diploid number, the completely acrocentric karyotype of *N. swarthi* (2N = 56, FN = 54) should be regarded as the most representative of the ancestral form of the genus (Figure 5). The karyotypes of *N. fernandinae* (2n = 44, FN = 54) and *N. narboroughi* (2n = 32, FN = 50) represent derived species with different chromosomal rearrangements resulting in both metacentric and subtelocentric chromosomes. Based on banding patterns, it is plausible to infer that *N. fernandinae* represents an intermediate stage of the genus, with rearrangements that carried into *N. narboroughi*. Garagna *et al.* (2014), when examining the Robertsonian phenomenon in the house mouse (*Mus*), stated that a high occurrence rate of Robertsonian fusions and whole arm translocations suggest that there are inherent genomic traits in the centromeric region that promote these rearrangements. The differences in sex chromosomes between *Nesoryzomys* and *Aegialomys* further support that the ancestral form for *Nesoryzomys* is unique relative to present day *Aegialomys* occurring on the islands. Searle (1993) suggested that mutations in chromosomal races that lead to metacentric chromosomes contribute to increasing reproductive isolation and, in time, to speciation.

Colonization of oryzomyine rodents in the Galápagos Islands represents the greatest overwater dispersal distance for terrestrial mammals (Pine *et al.* 2012) and these rodents are the only terrestrial mammals that have naturally colonized and diversified within the archipelago. *Nesoryzomys* is known to occur on at least 6 of the 13 major islands (Harris and Macdonald 2007), which date from up to 3.5 to 4 mya to 60,000 ya (Geist *et al.* 2014). Garagna *et al.* (2014) stated that the best place to search for extremes in chromosomal variation is in geographically isolated populations. Piálek *et al.* (2005), in examining chromosomal variation in European *Mus*, identified 'islands' of *Mus* occurring in the Swiss Alps. The standard karyotype of *Mus* is an all telocentric karyotype (2n = 40), but metacentric rearrangements occurred in these 'island' populations and these polymorphisms have the potential to become fixed. In laboratory stocks of *Mus domesticus*, it was observed that once a Robertsonian fusion occurred, it acted as an 'infectious agent' and other fusions quickly followed, something that could occur in wild populations (Nachman and Searle 1995). King (1993), in discussing the role of chromosome change and species evolution, stated that it is evident that the formation of Robertsonian fusion events leading to metacentric chromosomes arise and spread in populations, constituting one of the main sources for karyotype evolution in mammals. Centromeres and telomeres play a role in maintaining genome stability and changes in chromosome number can result in centromere repositioning over time (Damas *et al.* 2021). These can become fixed by selection when fusion events are associated with changes in gene expression or meiotic drive and act as a reproductive barrier and promote speciation. A Robertsonian event may lead to a significant reduction in the DNA sequence that organizes the centromere making it difficult to regenerate functional telocentric chromosomes (Garagna *et al.* 1995). This suggests a tendency of ancestral all-telocentric karyotypes moving toward accumulation of metacentric chromosomes but without reverse tendencies towards fission events that result in telocentric chromosomes, once a largely metacentric karyotype has been established (Garagna *et al.* 2014). The chromosomes of the endemic Galápagos rodent fauna appear to follow these patterns.

The colonization history of native rodents in the Galápagos Islands and its timing continue to be uncertain. Most have supported the idea of three separate colonization events from mainland South America or Central America for each of the rodent genera, with *Megaoryzomys* the oldest, *Nesoryzomys* next and more recently, *Aegialomys* (Patton and Hafner 1983; Parent *et al.* 2008; Pine *et al.* 2012). For the extant genera, *Aegialomys* and *Nesoryzomys*, molecular phylogenies suggest they diverged in the Pliocene about 3.84 mya based on mtDNA D-loop sequences (Castañeda-Rico *et al.* 2019), although others have estimated their divergence at about 2.8 mya (Parada *et al.* 2015) and in the Pleistocene from 1.49 mya (Machado *et al.* 2014) and 2.4 mya (Parada *et al.* 2013). Some of this discrepancy is a result of Castañeda-Rico *et al.* (2019) using an origin of the Galápagos in their calculations of 5 mya based on Geist *et al.* (2014) whereas Machado *et al.* (2014) used 4 mya based on Geist (1984). Species divergences within *Nesoryzomys* occurred in the early Pleistocene about 2.23 mya between the clade represented by *N. swarthi*/*N. fernandinae* and *N. narboroughi*. This was followed by the divergence of *N. swarthi* and *N. fernandinae* at 1.58 mya (Castañeda-Rico *et al.* 2019). Most other studies that have attempted to date the divergence of oryzomyines also have placed the divergence of *Nesoryzomys* species in the Pleistocene (Parada *et al.* 2013; Leite *et al.* 2014; Parada *et al.* 2015).

An explanation of the sequence of colonization events that resulted in the seven known taxa of *Nesoryzomys* is likely impossible based on the geologic history of the Galápagos Islands. Recent studies by Ali and Aitchison (2014) and Geist *et al.* (2014) proposed that this archipelago's pat-

tern of subsidence and sea level changes have resulted in multiple small and large previous islands that were available for further isolation of evolving taxa. This phenomenon has alternated with sea level declines that allowed movement of populations across previous oceanic barriers between currently recognized islands. Geist *et al.* (2014) proposed that for lava lizards (*Microlophus*), both dispersal and vicariant allopatric speciation occurred based on the subsidence and sea level changes that shifted the amount of land area in the Galápagos Islands. For *Nesoryzomys*, a similar phenomenon may well have occurred. Dispersal and allopatric speciation allowed the oryzomyines that first colonized the islands, potentially *Sigmodontomys* or related forms, to diverge from these previous mainland ancestors. Following that event, a series of speciation events occurred, some by further dispersal to newly arising islands and others by vicariance. As sea level first declined during glacial events to unite land masses, such as the uniting of the central 'core' islands that included Santa Fé, Santiago, Isabela, and Fernandina, overland dispersal could occur for once isolated forms. As sea level then rose during interglacial periods, these larger islands were once again divided, isolating their flora and fauna. Ali and Aitchison (2014) compare patterns of distribution for most of the nonmammalian vertebrates (reptiles and Darwin's finches) and *Scalasia* land plants of the Galápagos. The known endemic rodents in the genus *Nesoryzomys* all follow a distribution in the 'core' area of the archipelago. These patterns of recurring isolation followed by widespread dispersal could well have provided a sufficient mechanism for the origin of the extensive chromosomal shuffling that is apparent in the three extant *Nesoryzomys* species and that likely occurred among all the existing forms of the genus.

Many oryzomyine rodents possess species-specific karyotypes (Gardner and Patton 1976; Suárez-Villota *et al.* 2013; Di-Nizo *et al.* 2017; Moreira *et al.* 2020) and provide ample evidence that chromosomal rearrangements contribute to the process of speciation (Damas *et al.* 2021). Thus, identification of chromosomal rearrangements contributes to our understanding of chromosomal evolution within *Nesoryzomys*. The role of chromosomal rearrangements has been a point of discussion for over half a century and chromosomes remain a valuable tool in systematics as they combine both morphological and genetic character traits, and represent the elements of variation and heredity (Bakloushinskaya 2016). Damas *et al.* (2021) suggested that chromosomal rearrangements are both a critical mechanism of reproductive isolation and a source of genetic variation that contributes to novel and adaptive traits during and after speciation has occurred. Adaptability applies not only to the organism as a whole but also to the genome, the structure of which changes under selection (Bakloushinskaya 2016). Charles Darwin, in formulating his concept of natural selection that originated from observations made on the Galápagos Islands, knew that natural selection occurs because of variation in a population (Darwin 1859). Yet, the mechanisms for which chromosomal variation give way to speciation is still not clearly understood. With few exceptions, both the 2n and FN are relatively constant in the known karyotypes found within the *Aegialomys-Megalomys-Melanomys-Nesoryzomys-Oryzomys-Sigmodontomys-Tanyuromys* clade, suggesting a stable karyotype that is not drastically changed by speciation events. The lone exception from those taxa that have karyotypic data is the genus *Nesoryzomys*. Based on what we understand of the chromosomal variation in these endemic rodents of the Galápagos Islands, chromosomal rearrangements either result from or play a key role in island speciation and adaptability of a population over time.

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Appendix 1

Specimens examined. Acronym for Angelo State Natural History Collections is ASNHC.

Aegialomys galapagoensis (3). ECUADOR: Galápagos Islands, Santa Fé, -0° 48.21'S, -90° 2.45' W (ASNHC 10613, ASNHC 10614, ASNHC 10615).

Nesoryzomys swarthi (4). ECUADOR: Galápagos Islands, Santiago, La Bomba, -0° 11.21' S, -90° 42.04' W (ASNHC 10597, ASNHC 10598, ASNHC 10599, ASNHC 10601).

Nesoryzomys fernandinae (4). ECUADOR: Galápagos Islands, Fernandina, Cabo Douglas, -0° 18.24' S, -91° 39.14' W (ASNHC 10578, ASNHC 10579, ASNHC 10580, ASNHC 10581).

Nesoryzomys narboroughi (4). ECUADOR: Galápagos Islands, Fernandina, Punta Espinoza, -0° 15.96' S, -91° 26.79' W (ASNHC 10591, ASNHC 10594, ASNHC 10595); Galápagos Islands, Fernandina, Cabo Douglas, -0° 18.24' S, -91° 39.14' W (ASNHC 10587).

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A re-examination of the molecular systematics and phylogeography of taxa of the *Peromyscus aztecus* species group, with comments on the distribution of *P. winkelmanni*

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The objectives of this study are to examine the available molecular data from the mitochondrial cytochrome-*b* gene (*Cytb*) and a concatenated dataset with this gene and two nuclear introns (*Adh*-1-I2 and *Fgb*-I7) to reexamine the systematic and phylogeographic conclusions reached by Sullivan *et al.* (1997) concerning the *Peromyscus aztecus* species group. The divergence of samples of *P. aztecus oaxacensis* across the Isthmus of Tehuantepec are further examined and taxonomic revisions are suggested. In addition, this study reviews the sources of data that lead to the conclusion that *P. winkelmanni* occurred in the Sierra Madre del Sur in Guerrero including a morphometric examination of a reported voucher. Bayesian and maximum likelihood analyses were conducted on a dataset of 31 *Cytb* sequences of all taxa in the *P. aztecus* group except for *P. a. cordillerae* and a concatenated dataset including five individuals of this group. Representative taxa of the *P. boylii*, *P. mexicanus*, and *P. truei* groups were included in both analyses. Body and cranial measurements of the voucher of the *P. winkelmanni* from Guerrero from which a *Cytb* sequence is reported to have been obtained was compared with measurements from specimens taken from the vicinity of Dos Aguas, Michoacán, including the type locality. We identified seven instances involving problematic identifications in GenBank. Once these issues were addressed, well-supported monophyletic sister clades of the *P. aztecus* and *P. boylii* species groups were recovered from phylogenetic analyses of *Cytb* sequences (Fig 1). Phylogenetic analyses of the *Cytb* and the concatenated datasets recover similar topologies that support the relationships of taxa of the *aztecus* group proposed by an earlier molecular study. Populations of *P. a. oaxacensis* southeast of the Isthmus of Tehuantepec represent a distinct species. Measurements of the voucher from Guerrero identified as the source of a *P. winkelmanni Cytb* sequence are smaller than *P. winkelmanni* for several characters. The divergent populations of *P. a. oaxacensis* from southeast of the Isthmus of Tehuantepec are recognized as two subspecies of *P. cordillerae*, *P. c. cordillerae* and *P. c. hondurensis*, whereas those northwest of the Isthmus are retained as *P. a. oaxacensis*. The lack of genetic divergence observed between *P. a. evides* and *P. a. oaxacensis* questions whether these two taxa should continue to be recognized as separate subspecies. Northern and southern populations of *P. spicilegus* demonstrate moderate divergence and additional examination of morphological and molecular differentiation within this taxon is warranted. The distribution of *P. winkelmanni* should be restricted to the vicinity of Dos Aguas, Michoacán, due to the lack of a voucher specimen that would confirm its reported occurrence in Guerrero.

Los objetivos de este estudio son examinar los datos moleculares disponibles del gen del mitocondrial citocromo-b (*Cytb*) y un conjunto de datos concatenados con este gen y dos intrones nucleares (*Adh*-1-I2 y *Fgb*-I7) para reexaminar las conclusiones sistemáticas y filogeográficas alcanzadas por Sullivan *et al.* (1997) sobre el grupo de especies de *Peromyscus aztecus*. Se examina más a fondo la divergencia de muestras de *P. aztecus oaxacensis* a lo largo del Istmo de Tehuantepec y se sugieren revisiones taxonómicas. Este estudio revisa las fuentes de datos que llevan a la conclusión de que *P. winkelmanni* se distribuye en la Sierra Madre del Sur de Guerrero, incluyendo un examen morfométrico de los ejemplares "voucher". Se realizaron análisis bayesianos y de máxima verosimilitud de 31 secuencias de *Cytb* de todos los taxa en el grupo de *P. aztecus,* excepto *P. a. cordillerae,* y un conjunto de datos concatenados que incluye cinco individuos de este grupo. En ambos análisis se incluyeron ejemplares representativos de los grupos *P. boylii, P. mexicanus* y *P. truei*. Las medidas somáticas y craneales de los ejemplares "voucher" de *P. winkelmanni* de Guerrero, de los que se obtuvo una secuencia de *Cytb,* se compararon con medidas de especímenes tomados en las cercanías de Dos Aguas, Michoacán, incluyendo la localidad tipo. Se detectaron siete casos que involucran identificaciones problemáticas en GenBank. Una vez que se abordaron estos problemas, se recuperaron los clados monofiléticos hermanos con buen soporte de los grupos de especies de *P. aztecus* y *P. boylii* a partir de análisis filogenéticos de secuencias de *Cytb*. Los análisis filogenéticos de *Cytb* y los conjuntos de datos concatenados recuperan topologías similares que apoyan las relaciones entre taxa del grupo *aztecus* propuesto por un estudio molecular anterior. La población de *P. a. oaxacensis* al sureste del Istmo de Tehuantepec representan una especie distinta. Las medidas de los "vouchers" de Guerrero identificado con secuencia *Cytb* como *P. winkelmanni* son más pequeñas que las de *P. winkelmanni* para varios caracteres. Las poblaciones divergentes de *P. a. oaxacensis* del sureste del Istmo de Tehuantepec se reconocen como dos subespecies de *P. cordillerae*, *P. c. cordillerae* y *P. c. hondurensis*, mientras que los del noroeste del istmo se conservan como *P. a. oaxacensis*. La falta de divergencia genética observada entre *P. a. evides* y *P. a. oaxacensis* cuestiona si estos dos taxones deberían seguir siendo reconocidos como subespecies independientes. Las poblaciones del norte y del sur de *P. spicilegus* demuestran una divergencia moderada y se justifica un examen adicional de la diferenciación morfológica y molecular dentro de este taxón. La distribución de *P. winkelmanni* debería estar restringida a las cercanías de Dos Aguas, Michoacán, debido a la falta de "vouches" que confirmara su distribución reportada en Guerrero.

Keywords: Isthmus of Tehuantepec*; Peromyscus cordillerae; P. aztecus oaxacensis; P. winkelmanni.*

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Introduction

The *Peromyscus aztecus* group was first recognized by Carleton (1989) with a content of three distinct species: *P. aztecus* (Saussure, 1860); *P. spicilegus* Allen, 1897; and *P. winkelmanni* Carleton, 1977. Five montane subspecies have been recognized within *P. aztecus* by Carleton (1979, 1989): *P. a. aztecus* occurring in the Sierra Madre Oriental; *P. a. cordillerae* Dickey, 1928, occurring in the highlands of Mt Cacahuatique of El Salvador; *P. a. evides* Osgood, 1904 (including the synonym *yautepecus* Goodwin, 1955); occurring in the Sierra Madre del Sur; *P. a. hylocetes* Merriam, 1898, occurring in the Transmexican Volcanic Belt; and *P. a. oaxacensis* Merriam, 1898, occurring in the highlands of central Oaxaca in the Sierra Madre del Sur, across the Isthmus of Tehuantepec in the Tierras Altas de Chiapas, and south to Guatemala, Honduras, and El Salvador. The divergence and phylogenetic relationships among and within the taxa of the *P. aztecus* species group have been characterized and estimated by examination of cranial morphology (Carleton 1977, 1979; Bradley *et al.* 1996), glans and bacular morphology (Bradley and Schmidly 1987; Bradley *et al.* 1989, 1990), karyotypes (Carleton *et al.* 1982; Smith *et al.* 1989; Smith 1990), allozymes (Sullivan and Kilpatrick 1991) and cytochrome-*b* (*Cytb*) sequences (Sullivan *et al.* 1997).

Carleton (1977) reported that *P. winkelmanni* occurs in the oak-pine forest at elevations between 6,900 and 8,000 feet from three localities SE and WSW of Dos Aguas in Michoacán. In a karyotypic study, Smith *et al.* (1989) reported a specimen from the vicinity of Filo de Caballos in Guerrero which expanded the range of *P. winkelmanni* from the mountains of the Sierra Madre del Sur in southwestern Michoacán to the main portion of the Sierra Madre del Sur in Guerrero. The occurrence of *P. winkelmanni* in the Sierra de Coalcomán in Michoacán and the Sierra Madre del Sur in Guerrero areas separated by a deep canyon of the Rio Balsas was viewed as biogeographically implausible by Musser and Carleton (2005). They concluded that the identification of the vouchers of *P. winkelmanni* from Guerrero needed to be reconfirmed.

The gleaning mouse, *P. spicilegus*, occurs in western México along the flanks of Sierra Madre Occidental from Sinaloa and Durango to Jalisco and northern Michoacán in the western Transmexican Volcanic Belt (Carleton 1977, 1989; Bradley *et al.* 1996). Fixed differences were observed in allozyme data reported by Sullivan and Kilpatrick (1991) from *P. spicilegus* suggesting that samples from Michoacán and samples from Nayarit possibly represent different species. Although considerable morphological (Bradley *et al.* 1996) and chromosomal (Carleton *et al.* 1982; Smith *et al.* 1989; Smith 1990) variation has been reported for this taxon, no geographic pattern of that variation has been detected. Sequence analysis by Sullivan *et al.* (1997) only examined samples from the southern portion of the range of this species, leaving the question of the sequence differentiation between northern and southern populations unaddressed.

A cladistic analysis by Sullivan and Kilpatrick (1991) including allozyme data, chromosomal characters reported by Smith *et al.* (1989) and morphological characters from Bradley *et al.* (1990) demonstrated considerable differentiation between *hylocetes* and other subspecies (*aztecus*, *evides*, and *oaxacensis*) of *P. aztecus*. The level of genetic identity and the degree of allozymic, chromosomal and morphological divergence exhibited by *hylocetes* led Sullivan and Kilpatrick (1991) to conclude that this taxon should be reinstated as a species, *P. hylocetes*. Analyses of *Cytb* sequence data supported this conclusion and found substantial levels of genetic divergence between *P. hylocetes* and *P. aztecus* (Sullivan *et al.* 1997).

The molecular analysis of Sullivan *et al.* (1997) recovered *P. a. oaxacensis* as polyphyletic and they suggested that populations south and east of the Isthmus of Tehuantepec represented a distinct species that was strongly divergent from populations of *P. a. oaxacensis* in Oaxaca. Musser and Carleton (2005) noted that populations of *P. aztecus* occupying the highlands south of the Isthmus of Tehuantepec warrant further scrutiny but continued to recognize them as subspecies of *P. aztecus, P. a. oaxacensis*. Duplechin and Bradley (2014) recognized *oaxacensis* populations in México as a distinct species citing the genetic data and inferences of Sullivan *et al.* (1997) but stated that it was unclear whether populations in Oaxaca were referable to *P. a. aztecus*, *P. a. evides*, or *P. oaxacensis*. Bradley *et al.* (2017) recognized populations northwest and southeast of the Isthmus of Tehuantepec as *P. oaxacensis* without comment on the genetic differentiation of populations separated by this geographic feature.

Although considerable morphological variation has been observed among allopatric populations of *P. aztecus* that has warranted the recognition of subspecies (Carleton 1977, 1979, 1989), little genetic differentiation has been observed (Sullivan and Kilpatrick 1991; Sullivan *et al.* 1997) other than between populations northwest and southeast of the Isthmus of Tehuantepec. Qualitative data of glans and bacular morphology (Bradley and Schmidly 1987; Bradley *et al.* 1990) showed a close relationship of *aztecus* to *oaxacensis* and *evides* to *hylocetes*, whereas quantitative data depicted *aztecus* as being distinct from the other subspecies (Bradley *et al.* 1990). Phenetic analysis of allozymic data (Sullivan and Kilpatrick 1991) found that *evides* clustered with *oaxacensis* to the exclusion of *aztecus*. However, analyses of allozymic data (Sullivan and Kilpatrick 1991) and *Cytb* sequence data (Sullivan *et al.* 1997) recovered *evides* and *oaxacensis* in the same cluster or clade with *aztecus* in a separate cluster or a sister clade. The lack of congruence among datasets makes it difficult to resolve the relationships among the subspecies of *P. aztecus*.

Since the initial molecular systematic analyses of the *P. aztecus* group by Sullivan *et al.* (1997), mitochondrial and nuclear sequence data have been obtained from 15 additional specimens from this group. The objectives of this study are to analyze an expanded dataset of *Cytb* sequences

and a concatenated dataset of mitochondrial (*Cytb*) and nuclear (*Adh*-1-I2 and *Fgb*-I7) markers to further resolve the phylogeography and phylogenetic relationships among the taxa of this group. Specifically, these analyses will address: 1) the differentiation between northern and southern populations of *P. spicilegus*; 2) The differentiation between *P. hylocetes* and subspecies of *P. aztecus*; 3) The differentiation and relationships of populations recognized as *P. a. oaxacensis* separated by the Isthmus of Tehuantepec; and 4) the relationships among subspecies of *P. aztecus*. Additionally, this paper examines the voucher of a specimen from which tissue and a karyotype were reported to have been obtained that led to the conclusion of Smith *et al.* (1989) and that was supported by Sullivan *et al.* (1997) that *P. winkelmanni* occurs in the vicinity of Filo de Caballos in Guerrero.

Materials and Methods

Collection and analyses of molecular data. All available cytochrome *b* (*Cytb*) sequences in GenBank (https://www.ncbi.nlm.nih.gov/genbank/) as of November 15, 2020, for taxa of the *P. aztecus* species group (*n* = 31); representative *Cytb* sequences from 9 taxa of its sister group, the *P. boylii* species group (*n* = 21); and two outgroup taxa from the *P. truei* species group (Table 1) were downloaded and aligned using ClustalW (Thompson *et al.* 1994) in MEGA7 (Kumar *et al.* 2016). After an initial Neighbor-Joining analysis (Saitou and Nei 1987) in MEGA7 and a Nucleotide Blast search of an outlying sequence, sequences from three taxa of the *P. mexicanus* species group were added to the *Cytb* dataset (Table 1). PartitionFinder 2.1.1 (Lanfear *et al.* 2016) was used to find the best partitioning scheme and the best model for each partition based on the AIC criterion under a likelihood framework using PhyML (Guindon *et al.* 2010) and the greedy algorithm (Lanfear *et al.* 2012). Maximum likelihood (ML) analysis with 1,000 bootstrap replicates and GTR+I+G model on all partitions was conducted with RAxML ver. 8.0 (Stamatakis 2014). The majority-rule consensus tree (MRC) with bootstrap support values was constructed in Mesquite ver. 3.5 (Maddison and Maddison 2011). A Bayesian analysis was conducted on the CIPRES portal (https://www.phylo.org; Miller *et al.* 2010) using the partitions and models identified with PartitionFinder for the *Cytb* dataset in MrBayes 3.2.3 (Ronquist and Huelsenbeck 2003). Default settings with two simultaneous MCMC runs of 10,000,000 generations with sampling every 1,000 generations were used and the log files for both runs were examined in Tracer 1.6 (Rambaut *et al.* 2014). A burn-in of 1,000,000 generations was set for each run and the runs were combined after discarding the burn-in to produce a MRC tree with posterior probabilities in Mesquite.

Kimura 2-parameter distances (=K2P; Kimura 1980) were estimated using MEGA7 based on *Cytb* alignments of individuals of the *P. aztecus* group in Table 1. Individuals were generally grouped by clades recovered in the phylogenetic analyses to determine mean K2P distances. However, subspecies of *P. aztecus* samples from México were grouped by biogeographic areas (mountain ranges). These mean K2P values were used to examine species boundaries based on levels of interspecific divergence observed among rodents and other mammals (Bradley and Baker 2001).

Sequences for two nuclear introns, alcohol dehydrogenase intron 2 (*Adh*-1-I2) and beta-fibrinogen intron 7 (*Fgb*-I7) were downloaded from GenBank for all available samples from the *P. aztecus* species group (*n* = 13), six taxa of the *P. boylii* species group, two taxa of the *P. mexicanus* species group and two taxa of the *P. truei* species group (Table 1). Sequences for these two nuclear introns were concatenated with a *Cytb* sequence obtained either from the same specimen or from a specimen from the proximity of the collecting locality of the nuclear sequence source (Table 1). A concatenated dataset of a *Cytb* and one or two nuclear genes was constructed for five samples reported to be from taxa of the *P. aztecus* group, nine samples from six taxa of the *P. boylii* group, and two samples from both the *P. mexicanus* and *P. truei* groups. Six additional samples represented by only *Cytb* sequences from *P. a. oaxacensis* from either side of the Isthmus of Tehuantepec were included in the analyses with concatenated sequences.

PartitionFinder 2.1.1 was used to find the best partitioning scheme and the best model for the concatenated dataset. A bootstrapped ML analysis was carried out for the concatenated dataset as with the *Cytb* dataset and the MRC tree with bootstrap support values was constructed in Mesquite. A Bayesian analysis was conducted on the CIPRES portal using the partitions and models identified with PartitionFinder for the concatenated dataset in MrBayes 3.2.3. The same parameters were used in this Bayesian analysis as used in the analysis of the *Cytb* dataset to produce a MRC tree with posterior probabilities in Mesquite.

Pairwise uncorrected p-values were estimated with MEGA7 for sequences of the two nuclear introns. These values were used to identify the species of the source of nuclear sequences from specimens determined as likely misidentified in the *Cytb* analysis, from different non-identical sequences reported from the same specimen, and sequences reported from species well outside of their known range.

Morphometrics. External measurements (in mm) including total length (TL), length of tail (LT), length of hind foot (HF), and ear length (EL) were taken from the collectors' field tags. Head and body length (HB) was calculated by subtracting the tail length from the total length to provide comparable data for specimens with broken tails. Measurements were taken from specimens of *P. winkelmanni* collected from the following locations: Michoacán: 7.4 mi WSW Dos Aguas (*n* = 10); 6.3 mi WSW Dos Aguas (*n* = 3); 6.9 mi WSW Dos Aguas (*n* = 3) housed in the Texas Cooperative Wildlife Collection (TCWC). Seven dimensions of the skull, following those reported by Carleton (1977) were measured (±0.01 mm) with dial calipers including: skull greatest length (SGL), rostral length (RL), brain-case width (BCW), zygomatic breadth (ZB), interorbital width (IOW), molar row length

Table 1. Sequence data examined from specimens of the *Peromyscus aztecus*, *P. boylii* groups and out-group taxa (*P. attwateri*, *P. gratus*, and *P. mexicanus*). References: 1) Miller and Engstrom 2008; 2) Sullivan *et al*. 1997; 3) Bradley *et al*. 2014; 4) Platt *et al*. 2015; 5) Sullivan *et al*. 2017; 6) Saasa *et al*. 2012; 7) Amman *et al*. 2006; 8) Amman 2005; 9) Bradley *et al*. 2007; 10) Tiemann-Boege *et al*. 2000; 11) Bradley *et al*. 2000; 12) Bradley *et al*. 2017; 13) Bradley *et al*. 2004; 14) Cabrera *et al*. 2007; 15) López-González *et al*. 2014; 16) Reeder and Bradley 2007; 17) Durish *et al.* 2004; and 18) Bradley *et al.* 2016.

Taxon[1]	State	Location	GenBank Accession Number			Field or Catalog Number	Ref
			Cytb	*Adh*-1-I2	*Fgb*-I7		
***P. aztecus* Species Group**							
P. aztecus	Michoacán	5 km E Dos Aguas	FJ214683[2]			FN 22401	GenBank
	Michoacán	5 km E Dos Aguas		FJ214669[3]	FJ214695[3]	TK 45255[4]	GenBank
	El Salvador	Santa Ana, Parque Nacional Montecristo, Los Pines	EF989968			ROM 101489	1
	El Salvador	Santa Ana, Parque Nacional Montecristo, Los Pines	EF989969			ROM 101490	1
P. a. aztecus	Veracruz	Teocelo	U89966			2204	2
	Veracruz	Teocelo	U89967			235	2
	Veracruz	8.8 km N Huatusco	U89968			GK 4053	2
P. a. evides	Oaxaca	5.6 km S. Suchixtepec	U89970			GK 3439	2
	Oaxaca	9.7 km E Juquila	U89969			GK 3407	2
	Guerrero	6.4 km SSW Filo de Caballos	FJ214685*	FJ214670*	FJ214700*	TK 93391	3, 4, 4
	Guerrero	4 mi SSW Filo de Caballos	KY707306			TK 93385	5
	Guerrero	17.22 N x 99.28 W	AB703007			1950/171	6
P. a. oaxacensis	Honduras	Francisco, Morazan, La Tigra Parquae Nacional	FJ214688*[3]	FJ214675*[3]	FJ214714*[3]	TK 101037	GenBank
	Guatemala	Zacapa, 2 km N San Lorenzo	U89971			34194	2
	Guatemala	Alta Verapaz Yalijux Mountain, Chelemha Reserve	KF201657			TK 151047	3
	Oaxaca	1.4 km N Llano de las Flores	U89972			GK 3516	2
	Oaxaca	2.1 km S Llano de las Flores	U89973			CWK 2117	2
P. hylocetes	Michoacán	Estacion Cerro Burro, Microodas, 3,270 m	DQ000481*	AY994235*	FJ214705*	TK 45309	3, 7, GenBank
	Michoacán	Puerto Garnica	U89974			CWK 2040	2
	Morelos	2.43 km W Huitzilac	U89975			GK 2781	2
	Michoacán	Puerto Garnica	U89976			CWK 2035	2
	Michoacán	3.6 km W Mil Cumbres	U89977			GK 4229	2
	Michoacán	4.9 km S Los Azufres	U89978			GK 2853	2
P. spicilegus	Michoacán	Dos Aguas		AY994233	FJ214719	TK 45262	8, GenBank
	Michoacán	5 km E Dos Aguas		AY994234		TK 45255[4]	4
	Michoacán	Km 81 carr. Ario de Rosales and La Huacana	DQ000480*	AY994232*		TK 47888	3, 7
	Durango	San Juan de Camarones	AY322512			TK 70912	3
	Durango	San Juan de Camarones	DQ973107			TK 70919	9
	Michoacán	10.7 km E Uruapan	U89979			GK 4217	2
	Nayarit	8.1 km W Villa Carranza	U89980			GK 3253	2
P. winkelmanni	Michoacán	6.9 mi WSW Dos Aguas	AF131930*	FJ214678*	FJ214721*	GK 3311	3, GenBank
	Michoacán	19.3 km WSW Dos Aguas	U89981			GK 3287	2
	Michoacán	19.3 km WSW Dos Aguas	U89982			GK 3286	2
	Guerrero	Filo de Caballo	U89983			GK 3388	2
***P. boylii* Species Group**							
P. b. boylii	California	Monterey Co., Hastings Natural History Reservation	AF155386*			MVZ: K, Nutt 120	9
	California	San Diego Co., Heise County Park		AY994225*		TK 90233	7
P. b. rowleyi	Jalisco	30 km W Huejuquilla del Alto	AF155388*		AY274208*	TK 48636	10, 4
	Jalisco	2 km NW Mesconcitos		AY994227*		TK 93089	4
P. b. utahensis	Utah	Garfield Co., Henry Mts., Mt. Pennell, Sidehill Springs	AF155392*			MSB-NK 39457	9
	Utah	Washington Co., Beaver Dam Wash		AY994226*		TK 24389	8
P. beatae	Chiapas	Yalentay		AY994223		TK 93279	7

Table 1. Continuation...

Taxon[1]	State	Location	GenBank Accession Number			Field or Catalog Number	Ref
			Cytb	*Adh*-1-I2	*Fgb*-I7		
	Veracruz	Xometla	AF131921*	AY994222*		GK 3954	3, 8
	Veracruz	6.7 km NE, 81.6 km SE Perote			FJ214696*	TK 150106	GenBank
	Oaxaca	3 mi S Suchixtepec	AF131923			GK 3450	11
	Chiapas	12 km SE Ixtapa	AF131917			FN 33058	11
P. carletoni	Nayarit	Ocota de la Sierra	KF201663			TK 148445	3
	Nayarit	Ocota de la Sierra	KF201664			TK148432	3
	Nayarit	Ocota de la Sierra	KF201671			TK148428	3
P. kilpatricki	Michoacán	Km 81 between Ario de Rosales and La Huacana	KX523179			TK 47887	12
	Michoacán	Km 81 between Ario de Rosales and La Huacana	KX523180			TK 47890	12
	Michoacán	13.5 km SW Zitacuro	KX523183			Tk 150627	12
P. levipes	Michoacán	Las Minas, 3 km SW Tuxpan	DQ000477*	AY994224*		TK 47819	7, 7
	México	12 km S Acambay	AY322509*	KT361507*		TK 93400	13, GenBank
	México	14.1 km NW Villa del Carbon	KX523178*		FJ214707*	TK 112532	
						TK 113532*	12, 4
P. l. ambiguus	Nuevo Léon	Cola de Caballo	AF131928			GK 3840	3
P. l. levipes	Tlaxcala	2 km W Teacalco	AF131929			GK 4031	3
P. schmidlyi	Durango	6.2 km W Coyotes, Hacienda Coyotes	AY370610*	AY994228*	FJ214718*	TK 72443	9, 7, GenBank
	Durango	30 km SW Ojitos	AY322524*	AY994229*		TK 70812	13, 8
	Sonora	0.8 km N, 1.4 km E Yecora	EU234540			10889 CIB	14
	Chihuahua	3.2 km S, 0.8 km E Hueleyvo	KC403898			CRD 4001	15
Out-Group Taxa							
P. attwateri	Oklahoma	McIntosh Co., 4.9 km E Dustin	AY155384*	AY817626*	AY274207*	TK 23396	9, 7, 16
P. gratus	Michoacán	Aquililla, 4 km E Cuitzeo	AY376421*	AY994218*	FJ214703*	TK 46354	9, 7, 4
P. mexicanus	Chiapas	9 mi N Ocozocozulta	AY376425*		AY274210*	TK 93314	17, 16
P. nudipes	Nicaragua	Madriz, San Lucas, Los Mangos	FJ214687*	AY994238*	FJ214713*	TK 93600	4, 4, 4
P. nicaraguae	Nicaragua	Matagalpa, Selva Negra	KX998947			TK 93678	18

1. As designated in GenBank Accessions
2. Determined not from this location
3. Determined not from this taxon
4. Sequences from TK 45255 attributed to both *P. aztecus* (FJ214669 and FJ214695) and *P. spicilegus* (AY994234)
* Sequence concatenated

(MRL), and palatal breadth (PB) from specimens from 7.4 mi WSW Dos Aguas (*n* = 7). Measurements were also taken from a specimen from Guerrero that has the same field number (GK 3388) as the specimen from which a *Cytb* sequence was reported of *P. winkelmanni* by Sullivan *et al.* (1997).

Estimation of descriptive statistics (mean, range, and standard deviation) of all measurements was calculated for specimens of *P. winkelmanni*. Measurements from the Guerrero specimen (GK 3388, TCWC 045175) were compared to the descriptive statistics obtained from this sample of *P. winkelmanni* and those provided by Carleton (1977) including those of the holotype.

Results

The initial Neighbor-Joining analysis of the *Cytb* dataset recovered sequence FJ214688, reported from a *P. aztecus oaxacensis*, as an outlier to both the *P. aztecus* and *P. boylii* clusters. A Nucleotide Blast of this sequence recovered 99 to 98 % identities with sequences of *P. nicaraguae* and *P. nudipes* of the *P. mexicanus* species group.

The expanded *Cytb* dataset, including representative taxa of the *P. mexicanus* species group, was partitioned by codon position. The Bayesian analysis using a GTR+I+G model for codons 1 and 3 and a GTR+I model for codon 2, recovered a well-supported phylogenetic tree (Fig. 1). The *Cytb* sequence (FJ214688) reported to be from a *P. a. oaxacensis* from Honduras (TK 101037) was recovered in a well-supported clade with sequences from taxa of the *P. mexicanus* species group sister to a sequence from a *P. nudipes*. The K2P distance between this sequence and sequences from the *P. aztecus* species group ranged between 13.4 to 17.5 % (Table 2) whereas the differentiation from a *P. nudipes*

sequence was only 1.9 %. The Bayesian analysis of concatenated sequences using a GTR+I+G model for *Cytb* codons 1 and 3, HKY+I for *Cytb* codon 2, and HKY+G for *Adh*-1-I2 and *Fgb*-I7 recovered specimen TK 101037 from Honduras in the *mexicanus* species group clade as the sister taxon to *P. nudipes* (Figure 2). A p-distance of 0.3 % of the *Fgb*-I7 sequence FJ214714 was found with a *P. nudipes* sequence, but the *Adh*-1-I2 sequence FJ214675 from this specimen had a p-distance of 5.7 % from the *P. nudipes* sequence and values > 3.2 % from all taxa of the *P. aztecus* group for which *Adh*-1-I2 sequences were available.

The remaining 30 *Cytb* sequences reported to be from taxa of the *P. aztecus* species group were recovered in a well-supported clade (ML = 87; PP = 1.00) that was sister to a well-supported clade (ML = 99; PP = 1.00) of taxa of the *P. boylii* species group (Figure 1). Five subclades were recovered in the *aztecus* group clade representing *P. winkelmanni*, *P. spicilegus*, *P. a. oaxacensis* southeast of the Isthmus of Tehuantepec, *P. hylocetes*, and a clade containing *aztecus*, *evides*, and *oaxacensis* from northwest of the Isthmus of Tehuantepec. Although *P. winkelmanni* showed the greatest divergence from other taxa of the *P. aztecus* group (mean K2P = 8.79 %), its affinities were clearly with this group and not with the *boylii* group.

Sequences of *Cytb* from the gleaning mouse, *P. spicilegus*, were recovered in a well-supported (ML = 99; PP = 1.00) clade (Figure 1) that was divergent from other clades in the *aztecus* group with a mean K2P distance of 8.71 % (Table 2). Two subclades were recovered in the *spicilegus* clade, one containing northern samples from Durango and the other more southern samples from Michoacán and Jalisco. The mean K2P differentiation between these southern and northern groups was 3.33 %.

Although a *Fgb*-I7 sequence and three *Adh*-1-I2 sequences (Table1) are available from specimens of *P. spicilegus*, only one of the *Adh* sequences is from a specimen (TK 47888) for which a *Cytb* sequence is available. The analyses of the concatenated sequences from TK 47888 recover

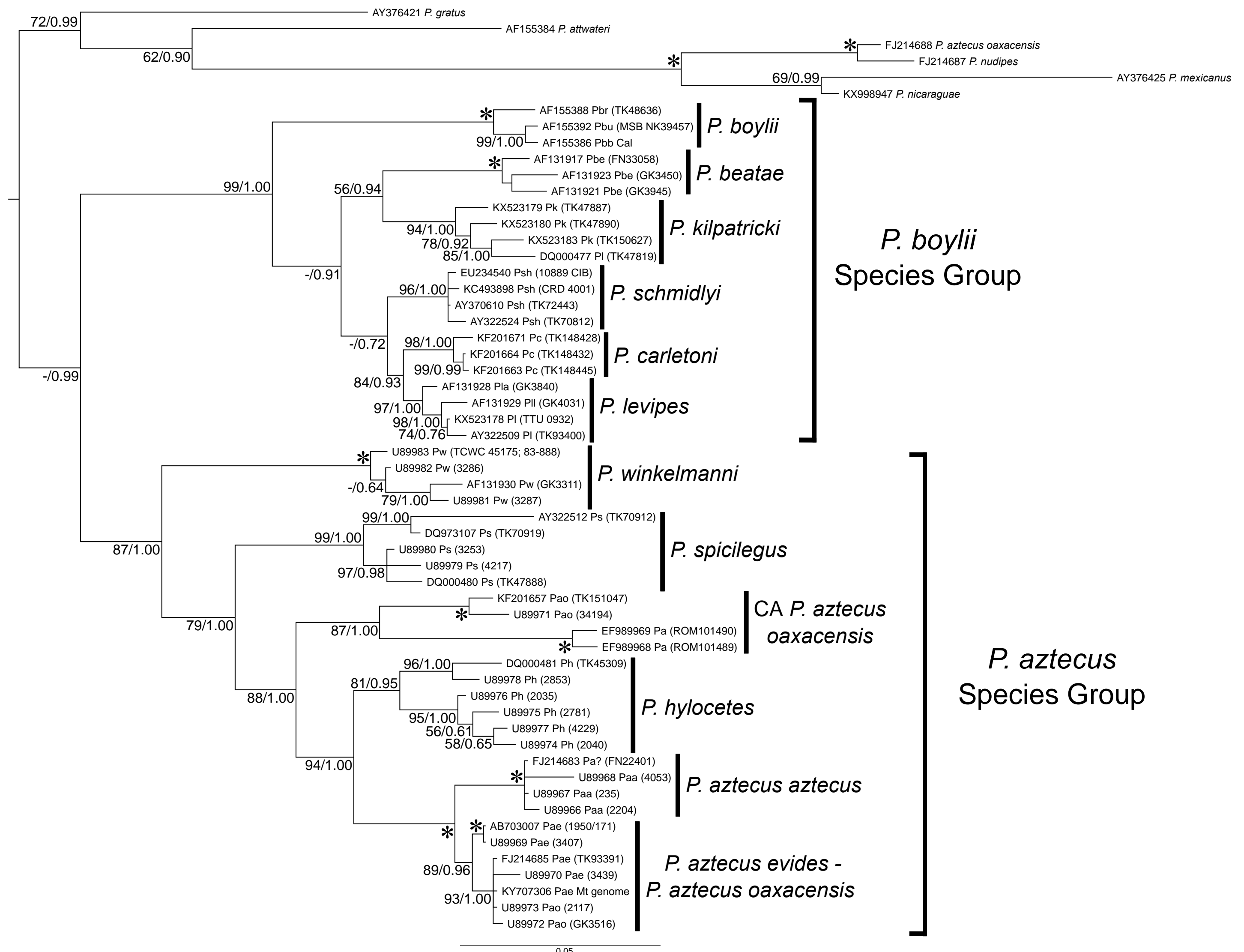

Figure 1. Bayesian tree of 31 *Cytb* sequences from samples reported to be from taxa of the *P. aztecus* species group and *Cytb* sequences from representative taxa of the *P. boylii*, *P. truei*, and *P. mexicanus* species groups. Nodal support is provided as Maximum Likelihood bootstraps and Bayesian posterior probability values (ML/PP: only if > 50 %). An asterisk (*) identifies nodes with fully realized support (ML = 100 and PP = 1.00).

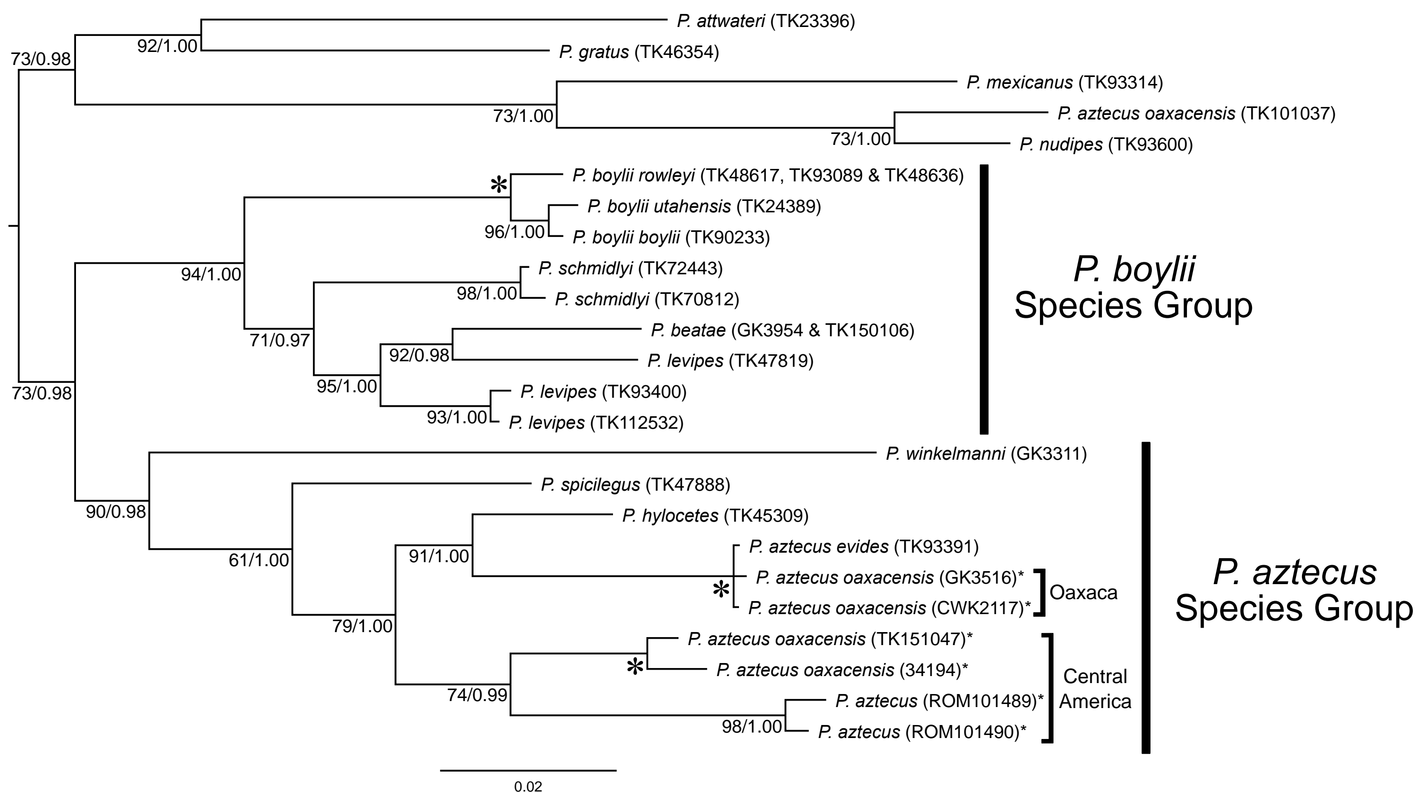

Figure 2. Bayesian tree of concatenated sequences (*Cytb*, *Adh*-1-I2, and *Fgb*-I7) from samples reported from five taxa of the *P. aztecus* species groups and representative samples from the *P. boylii, P. truei*, and *P. mexicanus* groups. Individuals represented only by a *Cytb* sequence are indicated with an asterisk (*) following the sample number. Nodal support is provided as Maximum Likelihood bootstraps and Bayesian posterior probability values (ML/PP: only if > 50 %). An asterisk (*) identifies nodes with fully realized support (ML = 100 and PP = 1.00).

this specimen of *P. spicilegus* as sister to a *hylocetes-aztecus*-Central American *oaxacensis* clade (Figure 2).

Populations of *P. a. oaxacensis* from Central America (southeast of the Isthmus of Tehuantepec) were recovered in a well-supported (ML = 87; PP = 1.00) clade (Figure 1) containing two well-supported subclades, one with samples from Guatemala and the other samples from El Salvador. The mean genetic differentiation (K2P) between these two subclades was 7.5 %. Samples of *P. a. oaxacensis* from northwest and southeast of the Isthmus of Tehuantepec had a mean K2P divergence of 7.9 % (Table 2). A similar topology for these samples was recovered in the analyses of the concatenated dataset (Figure 2), though only *Cytb* sequence data were available for samples of *P. a. oaxacensis*.

The remaining samples representing populations of *aztecus*, *evides*, *hylocetes*, and *oaxacensis* from north of the Isthmus of Tehuantepec were recovered in a well-supported (ML = 94; PP = 1.00) clade (Figure 1) sister to the *oaxacensis* clade from south of the Isthmus. Within the former clade, samples of *hylocetes* form a moderately well-supported (ML = 81; PP = 0.95) clade with a mean genetic differentiation (K2P) of 5.5 % from the *aztecus-evides*-northern *oaxacensis* clade (Fig. 1). Analyses of the concatenated dataset recovered *hylocetes* within a well-supported clade (ML = 91; PP = 1.00) as the sister taxon of *evides* and northern *oaxacensis* (Figure 2).

Two well-supported subclades were recovered in the *P. aztecus* clade from northwest of the Isthmus of Tehuantepec, one including samples of *P. a. evides* and northern samples of *P. a. oaxacensis* (Figure 1). Little genetic differentiation (mean K2P = 0.005) was observed between samples of *P. a. evides* from the Sierra Madre del Sur and the *P. a. oaxacensis* from the highlands of central Oaxaca. The other subclade contained samples of *P. a. aztecus* and a sequence (FJ214683) of a *P. aztecus* reported to be from 5 km E Dos Aguas in Michoacán (Figure1), a location from which *P. aztecus* has not previously been reported.

If the sequences for *P. a. aztecus* from two specimens (FN 22401 and TK 45255) both reported from 5 km E Dos Aguas, Michoacán, a locality well outside of the known range of this subspecies (Carleton 1989) are included in the concatenated dataset, this concatenated sequence of *P. aztecus* is recovered as sister to a *hylocetes*-*evides*-northern *oaxacensis* clade. The *Cytb* sequence from FN 22401 (FJ214683) was recovered in the *P. a. aztecus* clade with strong support (ML = 100; PP = 1.00) in the phylogenetic analyses of the *Cytb* dataset (Figure 1). The *Fgb*-I7 sequence from TK 45255 (FJ214695) was found to have p-distances of 1.0 % from *P. spicilegus*, 2.5 % from *P. nudipes*, and 3.2 % from *P. hylocetes* and *P. a. evides* sequences. The *Adh* sequence from TK 45255 (FJ214669) had a p-value > 3.2 % from all comparisons with *P. aztecus* species group taxa. A concatenation of these sequences was not included in our analyses.

Body and skull measurements from the voucher from Guerrero (GK 3388, TCWC 45175) for *Cytb* sequence U89983 from a *P. winkelmanni* was smaller for head and body length,

Table 2. Mean pairwise Kimura-2-parameter distances between clades recovered in the phylogenetic analysis of *Cytb* sequences below the diagonal and within the clades on the diagonal.

Taxa	*P. a. aztecus*	*P. a. evides*	*P. a. oaxacensis*	*P. hylocetes*	*P. spicilegus*	*P. winkelmanni*	*P. aztecus* El Salvador	*P. aztecus* Guatemala	*P. aztecus* Honduras
P. a. aztecus	0.0076								
P. a. evides	0.0295	0.0077							
P. a. oaxacensis	0.0302	0.0050	0.0015						
P. hylocetes	0.0642	0.0503	0.0494	0.0262					
P. spicilegus	0.0901	0.0876	0.0826	0.0805	0.0273				
P. winkelmanni	0.0881	0.0836	0.0824	0.0836	0.0833	0.0135			
P. aztecus El Salvador	0.0881	0.0912	0.0781	0.0770	0.1019	0.1049	0.0125		
P. aztecus Guatemala	0.0838	0.0708	0.0798	0.0777	0.0835	0.0892	0.0747	0.0175	
P. aztecus Honduras[1]	0.1597	0.1655	0.1589	0.1530	0.1534	0.1454	0.1749	0.1338	NA

1. Sequence from a *P. nudipes*

the greatest length of skull and brain-case width, and at the minimal range for hind foot and molar row length compared with measurements from *P. winkelmanni* (Table 3). This specimen is cataloged as TCWC 45175 and identified as a *P. a. evides* collected on 30 July 1983 from Filo de Caballos, 7,900 ft, Guerrero, México. The sequence U89983 does, however, belong to the *P. winkelmanni* clade (Figure 1).

Discussion

Sequences associated with incorrect data. Several misplaced or problematic sequences were detected while downloading sequences or in our phylogenic analyses, including sequences FJ214688 (*Cytb*), FJ214675 (*Adh*), and FJ214714 (*Fgb*) from specimen TK 101037 (TTU 83698) from Francisco, Morazán, La Tigra Parque Nacional in Honduras identified in GenBank as from *P. a. oaxacensis* (Table 4). The specimen, TTU 83698 (TK 101037) is catalogued in Vertnet as a *P. mexicanus* and the results of the analysis of the *Cytb* sequence (FJ214688) and an analysis including a concatenation of these three sequences recovered this specimen within the *P. mexicanus* species group, sister to a specimen of *P. nudipes*. Based on our molecular analyses, sequences from TK 101037 from Honduras appear to be from a *P. nudipes* and not a *P. aztecus*, however, the *Adh* sequence FJ214675 from this specimen demonstrates a p-distance greater than 5.0 % from *P. nudipes*, suggesting contamination or concatenation of this sequence with some other taxon (Table 4).

Other problematic sequences include the collecting locality for FJ214683 (*Cytb*) and the taxon from which sequences FJ214669 (*Adh*), FJ214695 (*Fgb*) and AY994234 (*Adh*) were obtained (Table 4). These issues were resolved by examining the identification of the voucher specimen in Vertnet and/or calculating K2P (*Cytb*) or the p-distance (*Adh* and *Fgb*) to sequences of reference taxa. Cytochrome *b* sequence FJ214683 appears to be from a *P. a. aztecus* from Veracruz whereas AY994234 (*Adh*) and FJ214695 (*Fgb*) appear to be from a *P. spicilegus* from Michoacán (Table 4). Although the *Adh* sequence FJ214669 is reported to by from the same specimen (TK 45255) as *Adh* sequences AY994234, these two sequences have a p-distance of 3.9 %.

Once taxon source misidentifications or incorrect localities are recognized for sequences, the database (GenBank) needs to be corrected (see https://ncbi.nlm.nih.gov/genbank/update/ for instructions). Without correction of taxon misidentification and incorrectly reported source localities in the database the use of these sequences and their reported collecting localities will continue and may cause confusion in the literature. Sequence FJ214669 should be excluded from future analyses until its source can be verified.

Distribution of P. winkelmanni. *Peromyscus winkelmanni* was described by Carleton (1977) from a series of 12 specimens collected by John R. Winkelmann and Floyd Downs from 6.3 mi (by road) WSW Dos Aguas, Michoacán, México at an elevation of 8,000 feet. Additional specimens of this

Table 3. Quantitative morphometric data (mean, standard deviation and range) for selected characters from specimens of *P. winkelmanni* from the vicinity of Dos Aguas, Michoacán, and a voucher (TCWC 045175) associated with the development of a hypothesis of a population in the vicinity of Filo de Caballo, Guerrero.

		Body Measurements				Skull Measurements				
Source	n	TL	HB	LT	HF	GLS	BCW	ZB	IOW	MRL
Holotype		263	123	140	29	33.3	14.3	-----	5.3	5.2
Carleton (1977)	32	249.2±11.2	-----	129.1±7.9	27.6±0.67	32.5±0.88	-----	16.2±0.56	-----	5.3±0.14
Range		235–265	-----	120–140	27–29	31.2–33.9	-----	15.4–17.1	-----	5.1–5.6
Michoacán	16, 7	254.9±13.3	122.9±4.66	132±10.4	27.4±0.96	32.4±0.88	14.1±0.32	16.0±0.70	5.5±0.16	5.3±0.24
Range		230–273	113–133	117–149	26–28	30.7–33.6	13.4–14.3	14.8–16.8	5.3–5.7	5.1–5.5
Guerrero	1	190+	112	78+	26	29.8	13.3	15.5	5.4	5.1

taxon from the vicinity of Dos Aguas have been reported by Carleton (1977), Álvarez *et al.* (1987) and this study (Appendix 1). Although Carleton (1977) speculated that this taxon inhabited other areas of the coastal sierra in Michoacán, no additional populations have been discovered in Michoacán.

Smith *et al.* (1989) reported a specimen of *P. winkelmanni* based on the detection of a karyotype with a FN = 70 and only three large pairs of biarmed chromosomes from the vicinity of Filo de Caballos in Guerrero. Based on this karyotype, which was identical to karyotypes of *P*. *winkelmanni* from Dos Aguas, Smith *et al.* (1989) concluded that the geographic range of *P. winkelmanni* extended at least from southwestern Michoacán through the Sierra Madre del Sur in Guerrero. Although no voucher number was provided by Smith *et al.* (1989) for the Guerrero specimen, the field catalog (examined by CWK) records only one specimen (GK 3388) collected from "Guerrero, Filo de Caballo vicinity". This male specimen was initially identified as a "*P. evides*" and later noted to have a FN = 70 with comments later written in the margin including "*P. evides*? or in *P. mexicanus* group" and "*P*. *winkelmanni*".

Sullivan and Kilpatrick (1991) reported on the allozymes of *P. winkelmanni* from three locations WSW of Dos Aguas and two specimens from 4 mi S Filo de Caballos. Phenetic and cladistic analyses of these biochemical data supported the contention of Smith *et al.* (1989) as they placed these samples from Filo de Caballo in a *P. winkelmanni* cluster or clade. However, vouchers were not identified for the sources of tissues used by Sullivan and Kilpatrick (1991).

The molecular analysis of the *P. aztecus* species group by Sullivan *et al.* (1997) identifies the vouchers associated with the *P. winkelmanni* sequences in the appendix (page 439). Three sequences were obtained from two specimens (3286 and 3287) from 19.3 km WSW Dos Aguas, Michoacán, and a specimen (3388) from Filo de Caballo, Guerrero.

The specimen with the field number GK 3388 was cataloged as TCWC 045175 and is smaller in several measurements than a series of *P. winkelmanni*, including GK 3286 (TCWC 045614) and GK 3287 (TCWC 045615). The voucher for GK 3388 is cataloged as a *P. aztecus evides* and is not a *P. winkelmanni* based on our comparison of measurements. Thus, there are no vouchers available for specimens of *P. winkelmanni* from Guerrero. We suspect that the sequence obtained by Sullivan *et al.* (1997) was likely obtained from specimen GK 3288, a *P*. *winkelmanni* from 19.3 km WSW of Dos Aguas and cataloged as TCWC 045616. Mislabeling of Nunc tubes, slides, and other material associated with specimen GK 3288 led to reports of *P. winkelmanni* occurring in Guerrero. Given the absence of a voucher of a specimen of *P. winkelmanni* from Guerrero, the distribution of the forest mouse should be restricted to the vicinity of Dos Aguas, Michoacán.

Taxonomy of the P. aztecus *species group.* With the utilization of molecular data and the expansion of species concepts, the content of the genus *Peromyscus* has been expanded from 53 species recognized by Carleton (1989) and Musser and Carleton (1993), to 56 species recognized by Musser and Carleton (2005), to 66 species recognized by Pardiñas *et al.* (2017) and to 78 species currently recognized in the Mammal Diversity Database (Mammal Diversity Database 2020). Over 20 new species of *Peromyscus* have been recognized just in the past 10 years (Ávila-Valle *et al.* 2012; Bradley *et al.* 2014, 2015, 2017, 2019; Pérez-Consuegra and Vazquez-Domínguez 2015; Greenbaum *et al.* 2019; Lorenzo *et al.* 2016; Álvarez-Castañeda *et al.* 2019; López-González *et al.* 2019; Léon-Tapia *et al.* 2020).

Molecular data have been used to examine the phylogeography and phylogenetic relationships of several species groups including the *P. aztecus* (Sullivan *et al.* 1997), *P boylii* (Bradley *et al.* 2000; Tiemann-Boege *et al.* 2000), *P. maniculatus* (Bradley *et al.* 2019; Greenbaum *et al.* 2019), *P. mexicanus* (Pérez Consuegra and Vázquez-Domínguez 2015; Bradley *et al.* 2016) and *P. truei* (Durish *et al.* 2004) species groups. The molecular study of the *P. aztecus* species group by Sullivan *et al.* (1997) was conducted, however, before the development of several modern molecular phylogenetic approaches and their associated software and was based on short (<750 bp) fragments of the *Cytb* gene.

The content of the *P. aztecus* species group has increased from three species, *P. winkelmanni*, *P. spicilegus*, and *P. aztecus* proposed by Carleton (1979, 1989), to four with the reinstatement of *P. hylocetes* as a distinct species (Sullivan and Kilpatrick 1991; Sullivan *et al.* 1997; Musser and Carleton 2005), to five species with the reinstatement of *P. oaxacensis* as a distinct species (Duplechin and Bradley 2014; Bradley 2017). Although our molecular analyses are congruent with the recognition of five distinct species in the *P. aztecus* species group, we do not support recognition of *P. oaxacensis* as a distinct species.

Peromyscus oaxacensis was described as a distinct species by Merriam (1898) based on specimens from Cerro San Felipe, Oaxaca, México, 10,000 ft. This taxon was recognized as a species (Osgood 1909; Hall and Kelson 1959; Hooper and Musser 1964; Hooper 1968; Goodwin 1969; Hall 1981) with a distribution in the highlands of Oaxaca and Chiapas, México. Musser (1969) pointed out that the range of *P*. *oaxacensis* extended southward into Guatemala, El Salvador, and western Honduras. Hooper (1968) questioned whether *P*. *oaxacensis* and *P*. *hylocetes* might represent disjunct populations of a single species. Carleton (1977) concurred with Hooper's (1968) hypothesis and later formally placed *oaxacensis* and *hylocetes* together with *evides* as subspecies of *P. aztecus* (Carleton 1979).

The Isthmus of Tehuantepec has been hypothesized to be an effective barrier to gene flow acting as a vicariant event contributing to the isolation, diversification, and speciation of rodent populations. Isolation by the Isthmus resulting in speciation has been reported for *Habromys* (León-Paniaqua *et al.* 2007), *Microtus* (Conroy *et al.* 2001), and *Neotoma* (Ordóñez-Garza *et al.* 2014). Ordóñez-Garza and Bradley (2018) examined DNA sequence variation

within populations of 11 species of cricetid rodents distributed across the Isthmus of Tehuantepec and found that the Isthmus only appeared to be an effective barrier to gene flow in the montane species *Reithrodontomys sumichrasti*. Sullivan *et al.* (2000) compared the phylogeography of this highland forest dwelling harvest mouse, *R. sumichrasti*, to the previously published (Sullivan *et al.* 1997) phylogeography of the co-distributed *P. aztecus*/*P. hylocetes* complex and concluded that these two species share a more common biogeographic history than can be accounted for by the independent response hypothesis.

Our analyses support the conclusion of Sullivan *et al.* (1997) that forms of *P. a. oaxacensis* southeast of the Isthmus of Tehuantepec represents a distinct species. Specimens from northwest of the Isthmus, including samples from near the type locality of *P. oaxacensis* in the highlands of Oaxaca, show little genetic differentiation from *P. a. evides* in the Sierra Madre del Sur. Our analyses do not support the inclusion of populations of *oaxacensis* from the Oaxacan highlands as a distinct species from *P. aztecus* as suggested by Bradley *et al.* (2017). Duplechin and Bradley (2014) questioned the taxonomic affinities of these Oaxacan highland populations, but we conclude they should be recognized as conspecific with *P. aztecus* (Figure 3) following Carleton (1979).

Samples of *oaxacensis* from southeast of the Isthmus form a well-supported clade, sister to a P. *hylocetes*-*P. aztecus* clade (Figures 1 and 2) but demonstrate considerable genetic differentiation from taxa of that sister clade. Two names appear to be available for this taxon. *Peromyscus hondurensis* Goodwin, 1941, was described from specimens from western Honduras (Muya, 5 mi N Chinacla, department La Paz, Honduras, 3,000 to 4,000 ft.), but was considered a southern representative of *P. oaxacensis* by Musser (1969). This taxon is represented in our sampling by two specimens from Guatemala (TK 151047 and 34194). Another potentially available name for this taxon is *cordillerae* described from specimens from northeastern El Salvador (Mt. Cacahuatique, Dept. San Miguel, 3,500 feet) as a subspecies of *P. boylii* by Dickey (1928) but considered a subspecies of *P. aztecus* by Carleton (1979). The samples from Parque Nacional Montecristo, El Salvador (ROM 101489 and ROM 101490), may or may not correspond to this taxon. Regardless, the available data advocate for the recognition of all populations of the *P. aztecus* species group located south of the Isthmus of Tehuantepec as a distinct species, and *Peromyscus cordillerae* Dickey, 1928, has priority (International Commission on Zoological Nomenclature 1999).

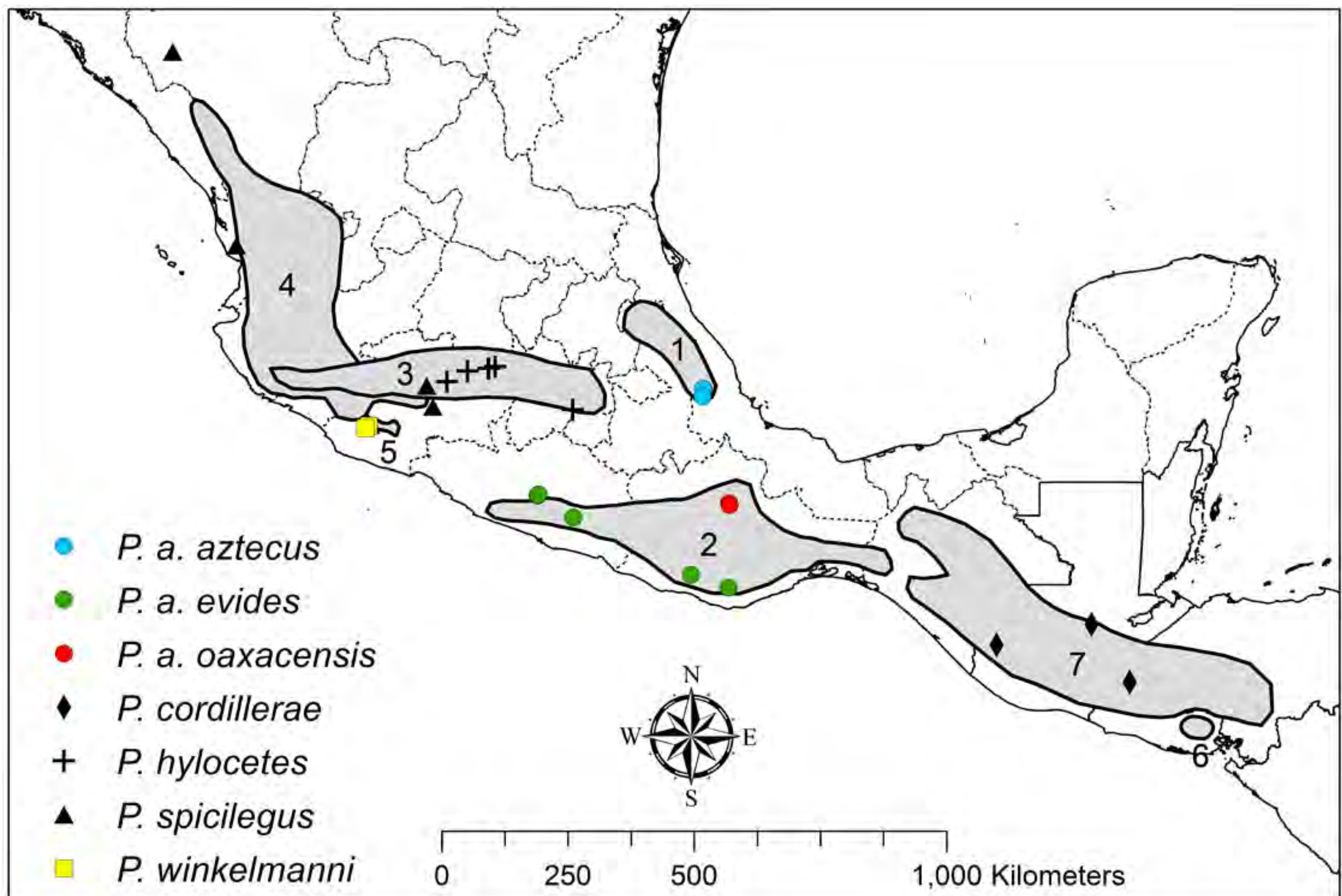

Figure 3. Distribution of taxa of the *P. aztecus* species group (map modified from Carleton 1989): 1) *P. aztecus aztecus*. 2) *P. a. evides* – *P. a. oaxacensis*. 3) *P. hylocetes*. 4) *P. spicilegus*. 5) *P. winkelmanni*. 6) *P. cordillerae cordillerae*, and 7) *P. cordillerae hondurensis*.

We suggest that *P. cordillerae* occurs in Guatemala, El Salvador, western Honduras, and the southern highlands of Chiapas, México (Figure 3). Further field and laboratory work are needed to resolve the geographic separation of *P. a. oaxacensis* and *P. cordillerae* in Chiapas. Two well-supported and highly differentiated subclades were recovered in the *P. cordillerae* clade, thus more than a single species may be present. Despite the high level of differentiation observed we refrain from further expansion of the number of Central American taxa of the *P. aztecus* group until samples are available from near the type localities on Mt. Cacahuatique (*cordillerae*) and in western Honduras (*hondurensis*). Instead, we tentatively consider *cordillerae* and *hondurensis* as distinct at only a subspecies level.

Levels of divergence and cladistic analyses of sequence data in this study support the earlier conclusion that *P. hylocetes* should be recognized as a distinct species (Sullivan and Kilpatrick 1991; Sullivan *et al.* 1997). Although morphological similarities are present between *hylocetes* and *P. a. oaxacensis*, Carleton (1979) pointed out that these morphological features appear to be subject to elevational effects and these two taxa both occur at higher elevations. In addition to biochemical and genetic divergence, Smith *et al.* (1989) reported differences in karyotypes with *P. hylocetes* having a FN = 72–74 and *P. aztecus* a FN = 68–70.

The data concerning the relationships of the allopatric subspecies of *P. aztecus* are difficult to interpret due to the inclusion of *hylocetes* as a subspecies but are generally incongruent (Bradley *et al.* 1990). However, phenetic analysis of both quantitative data for the glans and bacula (Bradley *et al.* 1990) and allozymic data (Sullivan and Kilpatrick 1991) identified *P. a. aztecus* as being distinct from the other subspecies. Phylogenetic analyses (Sullivan *et al.* 1997; this study) of sequence data recovered *P. a. evides* and *P. a. oaxacensis* in the same clade. In this study, less mean divergence was observed between *Cytb* sequences of *P. a. evides* and sequences of *P. a. oaxacensis* (K2P = 0.5 %) than among sequences of *P. evides* (K2P = 0.77 %). This lack of differentiation between these populations in the highlands of central Oaxaca and the Sierra Madre del Sur questions whether these two subspecies are allopat-

Table 4. Determination of collecting locality and likely source taxon for problematic GenBank data. Most likely source taxon is designated with an asterisk in the remarks.

Sequence Accession Number	Gene or Intron	GenBank ID	Specimen Catalog Number	Collecting Locality	Vertnet ID	Bayesian Analyses A) *Cytb*; B) concatenated	Remarks
FJ214688	Cytb	*P. a. oaxacensis*	TK 101037 TTU 83698	Francisco, Morazán La Tigra Parque Nacional, Honduras	*P. mexicanus*	A: Sister to *P. nudipes*	K2P distance of 1.9% from *P. nudipes*[1]; >13 % from taxa of *P. aztecus* group *P. nudipes**
FJ214675	*Adh*-I2	*P. a. oaxacensis*	TK 101037 TTU 83698	Francisco, Morazán La Tigra Parque Nacional, Honduras	*P. mexicanus*	B: Sister to *P. nudipes*	p-distance of 5.7 % from *P. nudipes*[2]; *contamination or concatenation with sequence of some other taxon*
FJ214714	*Fgb*-I7	*P. a. oaxacensis*	TK 101037 TTU 83698	Francisco, Morazán La Tigra Parque Nacional, Honduras	*P. mexicanus*	B: Sister to *P. nudipes*	p-distance of 0.3 % from *P. nudipes*[3] *P. nudipes**
FJ214683	Cytb	*P. aztecus*	FN 2401 ROM 100795	5 km E Dos Aguas, Michoacán, México	*P. aztecus*	A. within *P. a. aztecus* clade	Vertnet locality given as Veracruz. *P. a. aztecus**
FJ214669	*Adh*-I2	*P. aztecus*	TK 45255	5 km E Dos Aguas, Michoacán, México	Not found		p-distance of 6.0 % from *P. a. evides*[4] and 3.2 % from *P. spicilegus*[5] Unknown taxon*
FJ214695	*Fgb*-I7	*P. aztecus*	TK 45255	5 km E Dos Aguas, Michoacán, México	Not found		p-distance of 5.1 % from *P. a. evides*[6] and 1.2 % from *P. spicilegus*[7] *P. spicilegus**
AY994234	*Adh*-I2	*P. spicilegus*	TK 45255	5 km E Dos Aguas, Michoacán, México	Not found		p-distance of 0.3 % from *P. spicilegus*[5] *P. spicilegus**

Reference sequences used for comparison 1) *P. nudipes* FJ214687. 2) *P. nudipes* AY994238. 3) *P. nudipes* FJ214713. 4) *P. a. evides* FJ214670. 5) *P. spicilegus* AY994232. 6) *P. a. evides* FJ214700. 7) *P. spicilegus* FJ214719

ric. More thorough sampling in the rugged mountains of Oaxaca is needed to understand the level of differentiation and distribution of these two subspecies. Unlike species, however, subspecies need not exhibit reciprocal monophyly (Patton and Conroy 2017).

Considerable local and individual variation was observed among samples of *P. a. evides* from Oaxaca (Goodwin 1969). In general *P. a. evides* is smaller in size, has less inflated bullae, sparsely haired tails, and exhibits subtle differences in pelage coloration when compared to *P. a. oaxacensis* (Carleton, 1989). Whether such differences are diagnostic and geographically discrete enough to warrant subspecies status remains to be seen. Goodwin (1969, map 67) found samples of these two taxa to overlap broadly. However, these reported morphological differences might function on a gradient (see Carleton 1979) from the lower elevation (*evides*) to the higher elevation (*oaxacensis*). Both a morphological and molecular evaluation of these taxa in the context of broader geographic sampling that includes type material is required. Until subject to more detailed study, we do not yet recommend that *evides* be synonymized with *P. a. oaxacensis*.

Considerable morphological (Bradley *et al.* 1996) and chromosomal variation (Carleton *et al.* 1982; Smith *et al.* 1989; Smith 1990) has been reported among populations of *P. spicilegus* but no apparent congruence was found (Bradley *et al.* 1996). A possible association of the morphological data with the allozymic data of Sullivan and Kilpatrick (1991) was discussed by Bradley *et al.* (1996). The fixed allelic difference reported by Sullivan and Kilpatrick (1991) between samples of *P. spicilegus* from Michoacán and Nayarit, occurred in populations that were quite distinct morphologically (Bradley *et al.* 1996). Although sequence data are not available for specimens of *P. spicilegus* from Nayarit, data are available from southern Durango. Considerable differentiation (K2P = 0.033) was detected between northern (Durango) and southern samples (Jalisco and Michoacán) in this study, like what was found in the morphometric study (Bradley *et al.* 1996). In addition, morphological divergence was reported along an elevational gradient in Jalisco, with individuals at higher elevations being larger (Sánchez-Cordero and Villa-Ramírez 1988). Further examination of molecular and morphological data is needed before subspecific recognition can be proposed.

Although additional research is needed to clarify the correct taxonomic position of several forms, we believe the following represents a concise summary of the most appropriate taxonomic designations in the *Peromyscus aztecus* species group based on available data. Nine taxa have been named in this species group and we here recognize five of these at the rank of species, three as additional subspecies, and one as a junior synonym (Figure 3). *Peromyscus winkelmanni* Carleton, 1977, is found in the vicinity of Dos Aguas, Michoacán, and is sister to all other members of the species group. *Peromyscus spicilegus* J. A. Allen, 1897, is found on the flanks of the Sierra Madre Occidental. *Peromyscus cordillerae* represents all members of the species group southeast of the Isthmus of Tehuantepec and is comprised of two subspecies, *P. c. cordillerae* Dickey, 1928, and *P. c. hondurensis* Goodwin, 1941, the boundaries of which remain poorly understood. *Peromyscus hylocetes* Merriam, 1898, is found at mid to high elevations in the Transmexican Volcanic Belt. The range of *P. aztecus* appears to be restricted to northwest of the Isthmus of Tehuantepec and the species contains three subspecies. *Peromyscus a. aztecus* (Saussure, 1860) is found in the Sierra Madre Oriental. The geographic delineation of the remaining two subspecies, *P. a. oaxacensis* Merriam, 1898, and *P. a. evides* Osgood, 1904 (including *yautepecus* Goodwin, 1955), remains poorly defined. Future research on the *P. aztecus* species group should focus on clarifying the status of *P. a. oaxacensis* versus *P. a. evides* and on *P. c. hondurensis* versus *P. c. cordillerae*. Such studies should also investigate the potential for unrecognized species or subspecies diversity in *P. cordillerae*, *P. spicilegus*, and *P. hylocetes*.

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This paper is dedicated to my friend and colleague David J. Schmidly. My (CWK) collaborations with Dave stem from when we were both graduate students in the early seventies and both examining the relationships among populations of the *Peromyscus boylii* species group. Our collaborations on this complex group of rodents led to a series of collecting trips in México between 1982 and 1985 supported by NSF. While those collecting trips provided specimens and other materials that have contributed to numerous publications, their greatest contributions were in the training of future mammalogists. On each of those trips, Dave invited students from Universities in México to join us in conducting this field work and many of those students have gone on to become very prominent mammalogists. Dave, we are indebted to you not only for your scholarly contributions, but also for the academic offspring that that you have fostered and nurtured that continue to contribute greatly to our knowledge of mammals of the southwestern United States, México, and Central America. Congratulation on having this issue of Therya published in your honor with contributions from your students, colleagues and friends.

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Appendix 1

Measurements from specimens of *P. winkelmanni* housed in the TCWC at Texas A&M University. Body measurements include total length (TL), length of tail (LT), hind foot (HF), ear height (EH) and cranial measurement of skull greatest length (SGL), rostral length (RL), brain case width (BCW), zygomatic breadth (ZB), interorbital width (IOW), molar row length (MRL), and palatal breadth (PB).

Field #	Sex	TL	LT	HF	EH	Catalog #
7.4 mi WSW Dos Aguas, 7900 ft., Michoacán, México						
GK 3281	M	263	134	28	22	TCWC 045610
GK 3283	M	272	149	28	24	TCWC 045611
GK 3284	F	258+	129+	28	23	TCWC 045612
GK 3285	M	242	122	27	23	TCWC 045613
GK 3286	F	250	128	26	22	TCWC 045614
GK 3287	F	261	140	28	21	TCWC 045615
GK 3288	M	230	117	27	23	TCWC 045616
GK 3568	F	240	119	26	22	TCWC 045618
GK 3570	F	261	139	27	25	TCWC 047665
GK 3565	F	256	133	27	24	TCWC 45617
6.3 mi WSW Dos Aguas, 7900 ft., Michoacán, México						
GK 3302	M	265	140	29	23	TCWC 045622
GK 3303	F	260	138	29	23	TCWC 045623
GK 3304	?	241	117	26	21	TCWC 045624
6.9 mi WSW Dos Aguas, 7900 ft., Michoacán, México						
GK 3309	F	230+	110+	27	23	TCWC 045619
GK 3310	M	273	140	28	24	TCWC 045620
GK 3311	M	233+	115+	27	23	TCWC 045621

Cranial Measurements

Field #	SGL	RL	BCW	ZB	IOW	MRL	PB
GK 3281	32.7	12.6	14.27	16.83	5.59	5.28	3.96
GK 3283	32.86	12.0	14.3	16.59	5.46	5.45	3.19
GK 3284	33.62	13.12	13.35	16.64	5.32	5.15	3.52
GK 3285	32.42	12.35	14.1	15.97	5.33	5.11	3.48
GK 3286	30.68	10.09	14.28	14.82	5.73	5.5	3.82
GK 3287	32.75	13.2	14.19	15.3	5.43	5.4	3.68
GK 3288	31.65	11.86	13.86	15.71	5.71	5.31	3.48

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Rejection of the monotypic status of *Peromyscus furvus* (Rodentia: Cricetidae), with consequences for its species group

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Previous studies using Cytochrome-*b* or ND3-ND4 mitochondrial gene have yielded intriguing evidence about the phylogenetic relationships among populations of *Peromyscus furvus*; however, those studies each based on phylogenies for a single type of genes, yielded conflicting topologies. In addition, analyses with traditional morphometrics have revealed differences in skull size among certain populations of the species. Therefore, in order to reassess the systematic and taxonomic status of *P. furvus*, we incorporated a suite of genetic and morphometric characters and employed cladistic analyses. Herein, we present results mostly derived from our genetic analyses (results from the phylogenetic examination of skull size and shape will appear later). Phylogenetic analyses were conducted using four mitochondrial genes (*Cytb* and ND3-ND4L-ND4) with the respective data analyzed separately or combined, followed by an analysis with genetic and morphometric data (size and shape characters). Most phylogenetic constructions were made with parsimonious methods, but probabilistic methods also were used in the analyses with the genes separated by type. Similar topologies were recovered from all analyses of the *Cytb* gene and from all parsimony analyses of the NADH genes; however, conflicting topologies were obtained with the probabilistic methods for the NADH genes. Additionally, to better understand the genetic variation in each type of gene, analyses for genetic divergence were conducted within and among genetic groups and haplotype networks were constructed. All the topologies obtained using genetic data questioned the monotypic status of *P. furvus*, as two additional clades were identified that seemingly correspond to unrecognized entities. The first of these, *P. latirostris*, occurs in the northern region and could be considered as either a species or subspecies. An unknown *Peromyscus species nova* that occurs to the south is considered as a valid species. Further, *P. furvus s. s.* becomes a polytypic species by recognizing at least two subspecies (*P. f. angustirostris* and *P. f. furvus*). Phylogenetic analyses also rejected membership of *P. melanocarpus* and *P. ochraventer* within the *furvus* species group. Instead, *P. melanocarpus* showed a greater affinity to *P. mexicanus totontepecus*, whereas, *P. ochraventer* either joined to the clade containing *P. melanocarpus* and *P. m. tototepecus* or to *Megadontomys cryophilus* in a sister clade. Finally, *Osgoodomys banderanus* (subgenus *Haplomylomys*) always remained basally positioned and segregated from all members of the subgenus *Peromyscus*.

Estudios previos con los genes mitocondriales citocromo-*b* y ND3-ND4, han arrojado evidencia intrigante acerca del estado monotípico de *Peromyscus furvus*; sin embargo cada uno de esos estudios basados en filogenias para un solo tipo de genes, produjeron topologías conflictivas entre ellos. Además, análisis con morfometría tradicional han revelado diferencias en el tamaño del cráneo entre ciertas poblaciones de la especie. Por ende, con el fin de reevaluar el estado sistemático y taxonómico de *P. furvus*, incorporamos un juego de caracteres genéticos y morfométricos, empleando análisis cladísticos. Aquí presentamos resultados derivados en su mayoría de nuestros análisis genéticos (los resultados del examen filogenético del tamaño y la forma del cráneo aparecerán después). Se utilizaron análisis filogenéticos en cuatro genes mitocondriales (*Cytb* y ND3-ND4L-ND4) con las respectivas bases de datos por separado o combinadas, seguidos de un análisis con datos genéticos y morfométricos (caracteres de tamaño y forma). La mayoría de las construcciones filogenéticas se realizaron con métodos parsimoniosos, pero también se utilizaron métodos probabilísticos en los análisis con los genes separados por tipo. Se recuperaron topologías similares en todos los análisis del gen *Cytb* y en todos los análisis de parsimonia con los genes NADH; sin embargo, se obtuvieron topologías en conflicto con los métodos probabilísticos para los genes de la NADH. Además, para entender mejor la variación genética en cada tipo de genes, se condujeron análisis de divergencia genética dentro y entre grupos genéticos y se construyeron redes de haplotipos. Todas las topologías obtenidas utilizando los datos genéticos, cuestionaron el estado monotípico de *Peromyscus furvus*, ya que se identificaron dos clados adicionales que al parecer, corresponden a entidades sin reconocer. La primera de estas, *P. latirostris*, ocurre en la región norteña y podría ser considerada ya sea como una especie o como una subespecie. Una *species nova* de *Peromyscus* que ocurre hacia el sur se considera como especie válida. Además, *P. furvus s. s.* se convierte en una especie politípica al reconocerse al menos dos subespecies (*P. f. angustirostris* y *P. f. furvus*). Los análisis filogenéticos también rechazaron la pertenecia de *P. melanocarpus* y *P. ochraventer* dentro del grupo de especies *furvus*. En cambio, *P. melanocarpus* mostró una mayor afinidad hacia *P. mexicanus totontepecus*, mientras que *P. ochraventer* se unió, ya sea al clado que contenía a *P. melanocarpus* y a *P. m. tototepecus*, o a *Megadontomys cryophilus* en un clado hermano. Finalmente, *Osgoodomys banderanus* (subgénero *Haplomylomys*) siempre permaneció posicionada basalmente y segregada de todos los miembros del subgénero *Peromyscus*.

Keywords: Cytochrome-*b* gene; *furvus* species-group; multiple-character-phylogenies; NADH genes; *Peromyscus*.

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Introduction

Peromyscus furvus is an endemic Cricetid species from southeastern México that inhabits the temperate cloud and mixed forests from montane highlands and currently is considered as a monotypic species (Rogers and Skoy 2011; but see Ramírez-Pulido *et al.* 2014). Its current distribution ranges from southeastern San Luis Potosí, along the Sierra Madre Oriental, through the Faja Transvolcánica Mexicana and southward to the Sierra Norte de Oaxaca (Rogers and Skoy 2011). The species includes in synonymy (Musser 1964; Hall 1968; Huckaby 1980) *Peromyscus latirostris* (Dalquest 1950 – type locality Xilitla, San Luis Potosí) and *P. angustirostris* (Hall and Álvarez 1961 – type locality Zacualpan, Veracruz), as well as unassigned specimens from Puerto de la Soledad, Oaxaca.

Although *P. furvus* currently is considered a monotypic species (Rogers and Skoy 2011), several previous studies, under different epistemological and methodological approaches, have noted differences among its synonyms and assigned populations. Such differences include skull size (Musser 1964; Hall 1968; Martínez-Coronel *et al.* 1997; Ávila-Valle *et al.* 2012), allozymes (Harris and Rogers 1999), and mitochondrial genetic sequences (cytochrome-*b* gene, Harris *et al.* 2000, and ND3-ND4 genes, Ávila-Valle *et al.* 2012). In particular, genetic studies have added two distinct scenarios to the status of populations within *P. furvus*. One scenario (Harris *et al.* 2000) based on data from the *Cytb* gene is: a) that the southernmost populations from Puerto de la Soledad, Oaxaca genetically are distinct from others; and b) that the northernmost populations in Xilitla, San Luis Potosí are distinct from *P. furvus* proper. In the geographically intermediate zone, populations from Hidalgo, northern Veracruz and Puebla, historically attributed to *angustirostris*, appear separated from populations from central Veracruz (Xalapa, type locality of *furvus*) but are contained in the same basal clade

The second scenario, based on data from NAD3-ND4 genes (Ávila-Valle *et al.* 2012), suggests: a) that the most genetically distinctive populations are the northernmost ones from Xilitla, San Luis Potosí and Santa Inés, Querétaro (*latirostris*) and b) that populations from Hidalgo, Puebla, Veracruz, and Oaxaca are grouped together in a central-southern clade. This southern clade appears to contain three subclades: one from Mesa de la Yerba and Xico, Veracruz (*furvus*), a second from El Salto, Puebla (*angustirostris*), and a third containing populations from Puerto de la Soledad, Oaxaca and the remaining populations from Hidalgo, Puebla, and Veracruz, including the type locality of *angustirostris.* These scenarios lead to the following questions: how many different entities or distinctive genetic groups are included within the "monotypic" *P. furvus*? What is the level of genetic variation associated with the recognizable taxonomic units, *e. g.*, species and subspecies? Do the specimens from the northern and southern ends of the distribution deserve specific recognition? What is the systematic and taxonomic status of the intermediate populations in Hidalgo, Puebla, and Veracruz?

Herein, the degree and level of divergence in populations from throughout the geographic range of *P. furvus* was assessed in order to understand if *P. furvus* consisted of one or more evolutionary and taxonomic entities. Our analyses included genetic (mitochondrial genes), morphogeometric (ventral shape of skull), and magnitude characters (centroid size of the latter and linear measurements for skull). Results from the genetic analyses are reported herein, whereas additional morphometric analyses will be presented in a separate manuscript. Additional genetic analyses were conducted to shed light into the complex relationships among populations of the intermediate geographic zone (Harris *et al.* 2000; Ávila-Valle *et al.* 2012).

Finally, Bradley *et al*. (2007) reported some uncertainty in the composition of the *furvus* species group within the genus *Peromyscus*. For example, *P. furvus* historically has been included in different species groups (*e. g*., *mexicanus* species group; Hooper 1968; Huckaby 1980) until it was assigned to its own group (Carleton 1989). However, the species composition within the *furvus* species group also has varied among authors (Carleton 1989; Wade *et al.* 1999; Musser and Carleton 2005). Herein, results from our analyses contribute to refine the composition of the *furvus* species group, in the light of the latest phylogenies provided for the genus (Bradley *et al.* 2007; Platt *et al.* 2015).

Materials and methods

Definition of groups. Populations from the geographic range of *Peromyscus furvus* were arranged into Operational Taxonomic Units (OTUs) according to their historical or current taxonomic designation and to their geographic provenance (Appendix 1, Figure 1). Hereafter, we refer to the OTUs of the Ingroup (IG) as Genetic Groups (GG1–5). GG1–3 with their taxonomic designations and their States of origin are: GG1 (*latirostris, Pl*) from San Luis Potosí and Querétaro; GG2 (*angustirostris, Pa*) from Hidalgo, Puebla, and Veracruz; and GG3 (*furvus*, *Pf*) from central Veracruz. The specimens with no formal designation include samples from two localities in Oaxaca, Puerto de la Soledad, referred to as GG4 (*Oax18*), and La Esperanza, GG5 (*Oax19*); however, GG5 samples lacked genetic data and were included only in morphometric analyses. We also selected five OTUs as Outgroups (OGs) based on evidence from recent phylogenies for peromyscines (Bradley *et al.* 2007; Platt *et al.* 2015). The three congeneric species include *P. ochraventer* (*Poc*) and *P. melanocarpus* (*Pml*), which have been associated with the *furvus* species group by Carleton (1989) and Musser and Carleton (2005), respectively, and *P. mexicanus totontepecus* (*Pmt*) from the closely related *mexicanus* group. Also, based on the aforementioned studies (Bradley *et al.* 2007; Platt *et al.* 2015), we included two additional species from different genera; *Megadontomys cryophilus* (*Mcr*), given its relative closeness to *P. furvus*, and *Osgoodomys banderanus* (*Oba*) as the most distant and possibly more conserved species (*i. e*., tentative root).

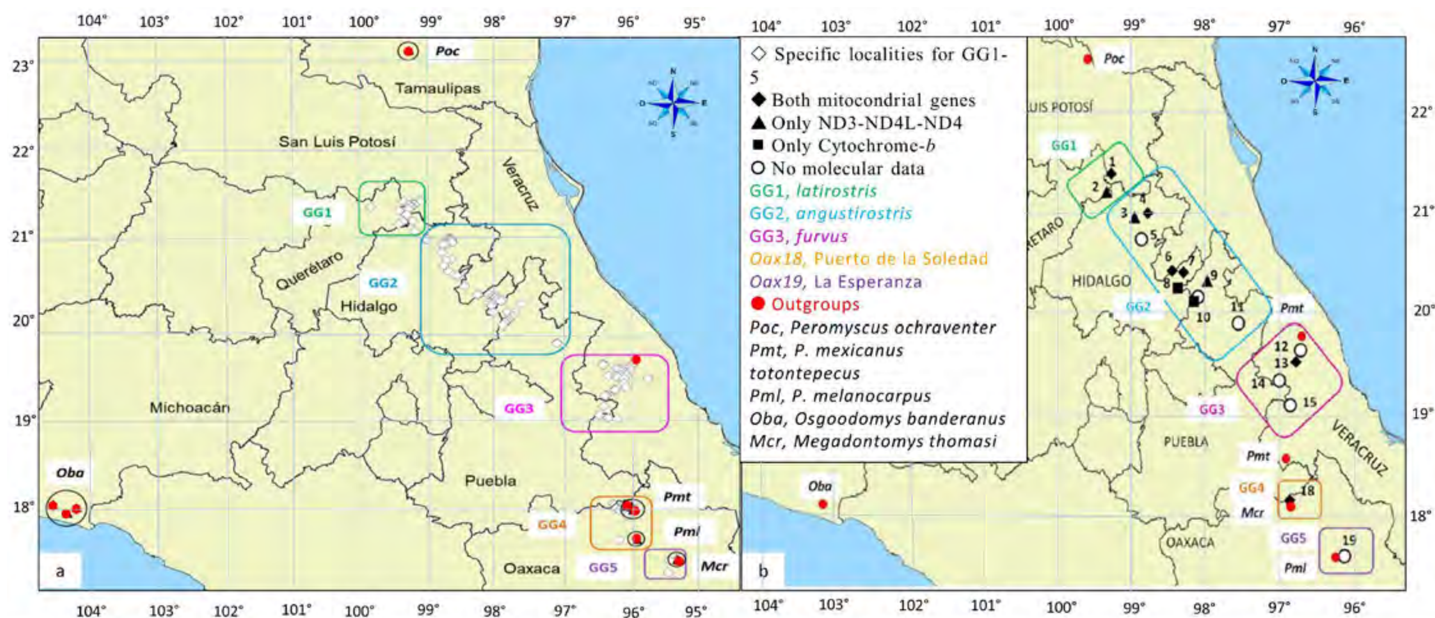


Figure 1. Geographical delimitation of the Genetic Groups (GG1–5) in the distribution of *Peromyscus furvus* used in this study as Ingroups (IGs), together with five outgroups (see legend); GG5 was included only in morphometric analyses. The map in (a) shows the location of specific localities that were pooled into group localities (numbers 1–19) in further analyses (b). See Appendix for details.

Molecular characters. Genetic sequences were downloaded, as complete as possible, from GenBank® (Genetic Sequence Database) for Cytochrome *b* gene (*Cyt-b;* IG $n = 53$, OG $n = 13$) and for three genes of the Nicotinamide Adenine Dinucelotide Hydride (NADH: ND3-ND4L-ND4; IG $n = 57$, OG $n = 6$). The sole exception was a sequence of NADH genes for *Poc* obtained from Wade *et al.* (1999). The sequences were aligned beginning at the start codon with the MEGA7 ClustalW option (Molecular Evolutionary Genetics Analysis, Ver. 7.0–7.2, Kumar *et al.* 2016) and truncated to similar lengths in all taxa. We analyzed 719 bases from the *Cytb* gene and 971 bases from the NADH genes (ND3, 306; ND4L, 293; ND4, 373; the tRNA-Arg interegion was eliminated, as it was unknown for *Pml*). Information about the geographic origin of sequences, GenBank accession numbers, and references appear in the Appendix.

Morphometric characters (continuous linear measurements). In our analyses of overall skull size, we included all OTUs (GG1–5 + five OGs). Herein, 233 specimens from 129 specific localities (SL) represented the IG, and 116 specimens from 11 SL represented the OG. We georeferenced all SLs using QGIS Wien (ver. 2.080–2.93) to project them onto a map (Figure 1A) and to pool the IG samples into 19 group localities (GLs), which in turn were assigned to GG1–5. We also used geographic coordinates of GLs to estimate the geographic distances (km) between every pair of GLs. Morphometric characters for analysis of overall skull size comprised 18 cranial measurements from adult specimens following Ávila-Valle *et al.* (2012; see figure 1:167 therein). Additional specimens were added to increase sample sizes in all OTUs ($n \geq 15$, except for *Mcr*, $n = 14$). Appendix provides the geographical data, sample sizes, and museum designations.

Analyses of the ventral shape and size of the skull were based on only complete and undamaged skulls (listed in bold in the Appendix). We selected five structures from the ventral view of skull as morphogeometric characters. The details for the anatomical origin of the structures (and substructures contained in them), as well as the quantity and position of the landmarks and semilandmarks outlining each morphogeometric configuration, will be described elsewhere. We measured each skull with type 1 and 2 landmarks *sensu* Bookstein (1991), taken from the literature (Myers *et al.* 1996; Cordeiro-Estreia *et al.* 2008; Grieco and Rizk 2010; Cordero and Epps 2012; Holmes *et al.* 2016), using utilities in the TPS series (Thin Plate Spline, Ver 2.31, TPS, available at https://life.bio.sunysb.edu/morph/ ...html; Rohlf 2015). In the examination of cranial contours, we followed Sheets *et al.* (2004) to adjust the semilandmarks. In the data matrix for morphogeometric characters, the columns had the consecutive coordinates of the landmarks and semilandmarks for the respective configuration (Sheets 2002). We also calculated the centroid size (CSs) of each configuration in each specimen as the square root of the sum of the squared distances, using each landmark toward the centroid (Zelditch *et al.* 2004). We then computed the average centroid size for the respective configuration, according to OTUs. All geometric morphometrics programs of the IMP8 series (Integrated Morphometric Package, Ver. 8.0) were downloaded from Canisius College (Buffalo, NY, https://www3.canisius.edu/~sheets /morphsoft.html; Sheets 2002). Prior statistical analyses (descriptive statistics and normality tests), as well as other univariate and multivariate statistics of these data, were conducted with PAST (PAleontological STatistics, Ver. 3.15; Hammer *et al.* 2001) at a significance level of $P \leq 0.05$ (unpublished results).

Molecular phylogenies based on Cytb and NADH genes. We constructed single gene phylogenies with all available sequences of the mitochondrial genes, using maximum parsimony (MP), Bayesian inference (BI), and maximum likelihood (ML) methods. In the MP analysis, the evolutionary model used Farris 'optimization as the standard option in TNT program (Tree analysis using New Technology, ver. 1.1; Goloboff *et al.* 2008) with the algorithm new technologies (NTS) in the search of the most parsimonious trees. In the probabilistic methods, we performed searches for the most suitable evolutionary model by type of mitochondrial genes (Jmodeltest, ver. 2.0, https://github.com/ddarriba/jmodeltest; Guindon and Gascuel 2003; Felsenstein 2005; Posada 2008) under the Bayesian Information Criteria (BIC) and Akaike Information Criteria (AIC). To construct the Bayesian phylogenies, we used four Markov chains and the Metropolis routine of MrBayes (ver. 3.2.6, https://mrbayes.sourceforge.net; Ronquist *et al.* 2012). We searched for the most likely tree with two runs of 1,000,000 generations for the *Cytb* gene and twice that for the NADH genes. In each case, temperature was 0.01, 0.25 % of the data was discarded, the most plausible trees were separated in the runs, and the most probable tree was selected. We pooled the two trees from each run based on each gene in a strict consensus cladogram. The MV analyses were performed on the CIPRES web page (Cyber - Infrastructure for Phylogenetic Research, https://www.phylo.org), loading the respective molecular data matrices to search for the most probable tree, obtain the branch lengths, and estimate bootstrap support (1,000 replications).

Phylogeny with genetic and morphometric characters. In this multicharacter phylogenetic analysis, we used only the same or the closest SLs represented by sequences from both genes and that had data for both the entire cranial size and for the ventral shape and size of skull. The Appendix shows in italics the IG OTUs arranged by GLs, according to GG1–4. The character matrix combined data from molecular together with morphometric characters of the size and shape of the cranium, resulting in 29 IGs, 5 OGs as OTUs, and 1,718 characters (34r x 1,718c).

Regardless of character type, all integrated analyses used MP in the TNT program because it allowed us to examine different types and large number of characters (Goloboff *et al.* 2008). In all cases, we used bootstrap resampling with 1,000 repetitions to assess branch support of the resulting topologies. A bootstrap support ≥ 90 was considered as strong. In the molecular analyses, 29 IG sequences from 13 SLs (*Cyt-b*, *n* =6; ND3-ND4L-ND4, *n* = 7) were selected for each gene, considering either that all genes were represented or that the SLs were ≤ 10 km distant from each other. We included five more sequences in each gene to represent the respective OTUs of the OGs. The geographic data of the examined genetic sequences, arranged by GG1–4, appear as italics in the Appendix. We used the sequences, saved in FASTA and ASCII formats by type of mitochondrial genes, to construct the matrix for molecular characters with the number of bases in the columns and the OTUs (GG1–4) in the rows. The search for the most parsimonious trees was sectorial with 20 changes per sector, Wagner trees (ratchet) with 100 substitutions, drift with 100 substitutions, and merging five trees per replication. If the analysis resulted in more than one tree, a strict consensus cladogram was constructed. Likewise, to determine *a priori* the number of steps in Wagner trees, we used Farris (1970) optimization and an evolutionary model where the position of the four bases (A, T, C, G) was allowed to change in any direction without penalizing reversals. Each change was counted as a single step.

Analyses of genetic variation and divergence in the IG. To assist in the interpretation of the topologies in the molecular phylogenetic analyses, as well as to facilitate recognition of the taxonomic level of the GGs, we examined the genetic variation within and among GG1–4 in each mitochondrial gene (Appendix). Following Ávila-Valle *et al.* (2012), we used the Tamura and Nei (1993) genetic distances for GG1–4 with ARLEQUIN (ver. 3; Excoffier *et al.* 2005) to construct the respective genetic data matrices. Genetic distances between pairs of OTUs were estimated, and the probability for statistically significant differences ($P \leq 0.05$) by each type of gene was analyzed using AMOVA (Excoffier *et al.* 1992, 2005). These analyses also provided the *Phi* genetic diversity index and the *Theta Phi* statistics, with their respective standard deviations for summarizing the degree of differentiation between the divisions of a population (variance components; Excoffier *et al.* 1992) in GG1–4. In addition, these analyses computed base frequency, or number of mutations, according to their type (transitions, ts; transversions, tv; substitutions, sb; indels, in), and the respective genetic distance averages and SD *per* GG. The AMOVA analyses were repeated each time significant differences were identified within the same GG, in order to test the significance of subgroups.

In order to visualize the relative position of sequences, GLs, and GG1–4, we used an analysis of haplotype networks. This analysis allowed identification of related groups and determine degrees of genetic divergence between GGs. Haplotypes were obtained from each population (Appendix) and DNAsp (Ver. 5.10.01; Librado and Rozas 2009) was used to build the corresponding network using the software Network (Ver. 5.0.01, https//:www.fluxus-engeneering.com). We labeled each network with a color code to identify allotted haplotypes in different GGs (GG1, green; GG2, blue; GG3 pink; and GG4, orange); when a GG contained more than one GL, we used additional tones of its assigned color. In these analyses, we interpreted the number of mutations between the haplotypes assigned to GLs and GGs as indicative of genetic divergence (Excoffier *et al.* 1992). We conducted additional analyses to interpret the respective distance matrices in terms of the criteria outlined by Bradley and Baker (2001:963) with 2-parameter model of nucleotide substitution (Kimura 1980) assuming minimum evolution in the *Cytb* gene. First, we estimated

the genetic distances under the Tamura and Nei (1993) evolutionary model with a gamma distribution using the Distance menu and the Compute Between Group Mean Distance option in MEGA7 (ver. 7.0; Kumar *et al*. 2016) and then transformed them into percentages. We first performed this analysis with the IG and their respective GLs used in the integrated phylogenies and then we averaged the data of the GLs for the respective GG and included the five OGs. Second, in order to determine phylogenetic and taxonomic distinctions between GG1–4, we used the criteria of Baker and Bradley (2006) for applying genetic distances to the genetic species concept.

Results

Phylogenies based on mitochondrial genes. The most suitable evolutionary model for the *Cytb* data set was GTR I+G (BIC, -*ln*L = 2729.48; AIC = -*ln*L 2725.67). The parameters for this evolutionary model used a gamma distribution and included base frequencies of A = 0.3302, C = 0.2874, G = 0.1347, T = 0.2478. A fixed shape = 1.2190 indicated a low rate of variation with nucleotides evolving slowly; 0 invariable sites; fixed rate of transversions and transitions: AC = 5.1938; AG = 18.6338; AT = 8.3552; CG = 1.2041; CT= 92.4518; and GT= 1.0. Number of substitution types (6) indicated that all transitions and transversions were treated differently.

For the NADH dataset, the best evolutionary model was TPM 2 uf + G (BIC, -*ln*L = 5860.0478; AIC, -*ln*L = 5860.0478). This evolutionary model was used with a gamma distribution and included base frequencies of A = 0.3492, C = 0.2685, G = 0.0788, and T = 0.3035. Fixed shape = 0.3350, indicating there were different rates of change, and 0 invariable sites. Fixed rate of transversions and transitions: AC = 0.6516; AG = 5.3993; AT = 0.6516; CG = 1.0000; CT = 5.3993; and GT = 1.0, and the number of substitution types was 6.

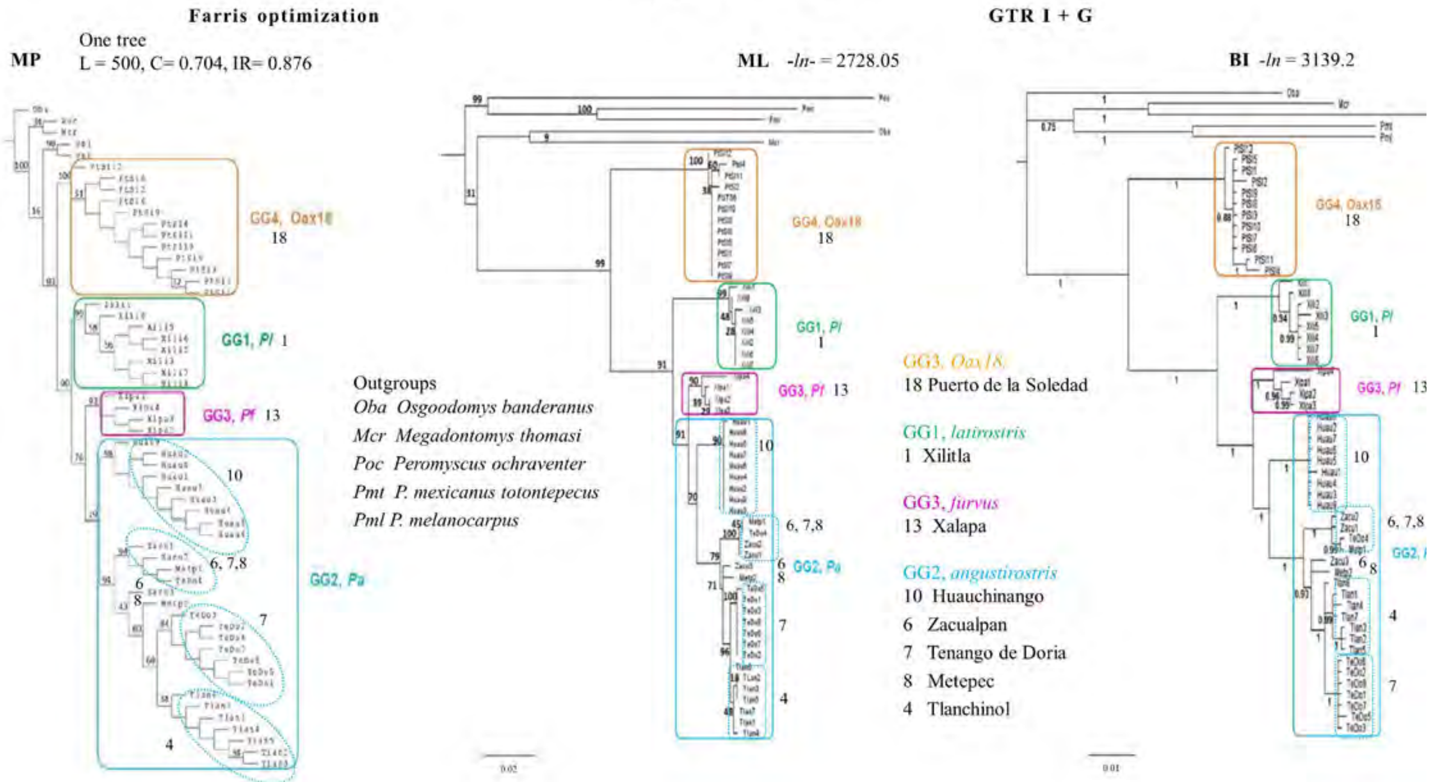
The MP phylogeny for *Cytb* yielded one tree (L = 500, IC= 0.704, IR= 0.876). The MP analysis of the NADH genes (L = 963, C= 0.565, IR= 0.876746) yielded 41 trees; a strict consensus tree was used to represent the single topology. These two MP topologies (Figure 2), together with both ML and BI for *Cyt-b*, provided the same topology as the integrated phylogenies based on molecular characters (Figure 4). Most differences were due to the location of the OGs in the two probabilistic topologies for the *Cytb* gene (Figure 2a). In the ML tree, *P. ochraventer* (*Poc*), was basal to a strongly supported clade containing *P. melanocarpus* (*Pml*) and *P. mexicanus totontepecus* (*Pmt*). *O. banderanus* (*Oba*) and *M. cryophilus* (*Mcr*) were the sister group of the IG in a poorly supported clade (9 %). In the Bayesian analysis, *Oba* formed a separate clade basal (1.0) to all the other OGs (0.75). *Mcr* and *Poc* were sister (1.0) to the other *Peromyscus* species (1.0), and all IG members formed a separate clade (1.0).

The two probabilistic topologies for the NADH genes (Figure 2b) rendered a strikingly different topology in both the location for the OGs and for the IG. *Mcr* and the three OG *Peromyscus* were located in a basal clade (50 %) with internal branches well supported (87 to 99 %). In both ML and BI topologies, *Oba* was the sister group to the IG in a moderately supported clade (ML 50 %, BI 0.63). For the IG, GG1 (*latirostris Pl*, 89 %, 0.99) was separated from the other GGs (100 %, 1.0). The largest clade (93 %, 0.66) was comprised of a polytomy containing three subclades. The first of these subclades possessed all samples of GG3 (*furvus*, *Pf* 55 %, 0.85). The second clade contained individuals with half the sequences from Xicotepec de Juárez, Puebla (GL9) of GG2 (*angustirostris Pa*), located between GG3 (*Pl*) and GG4 (Oax) in the ML (34 %). The third clade contained GG4 (*Oax18*, 98 %, 1.0) and the GLs from Hidalgo and northern Veracruz (69 %, 0.94) of GG2 (*Pa*). Within the third clade, three sequences from Zacualpan (GL6 74 %, 0.99) and all sequences from Otongo (GL3, 84 %, 0.64) did not group with other subpopulations (11%, 0.95).

Interpopulation genetic variation in the IG: genetic distances and tests. The respective AMOVAs for *Cytb* gene and NADH genes revealed significant differences both among the GGs and within their group localities (GLs, Table 1) as reflected in the single and combined genetic topologies (Figures 2, 4). In the *Cytb* gene, all the genetic fixation indices (FI) scored >70 %, whereas in the three NADH genes, only the variation within populations (FST) was greater. FST values were higher in NADH genes than in *Cytb*, indicating a higher level of genetic differentiation within the GLs of the same GG. Similarly, in all genes, the second highest index (FSC) indicated divergence among GGs, although it was lower for the NADH genes. Low FSC values were detected for NADH among populations among GLs within a GG. The variance component that explained most percentage of variance was variation among GG1–4 (FCT) in both mitochondrial genes, followed by variation among the GLs of a GG (FSC), and then variation among the sequences in the same GL (FST).

Table 1. AMOVA results for mitochondrial genes showing degrees of freedom (df), sum of squares (SS), variance components (VC), percentage of variance explained (% V), and fixation indexes (FI) indicating average variation: among GG1–4 (FCT); among all populations (GLs) among GGs (FSC); and within a GLs of the same GG (FST). All *p*-values were significant (*).

	Cytochrome b					ND3-ND4L-ND3				
Variation source	**df**	**SS**	**VC**	**% V**	**FI***	***df***	**SS**	**VC**	**% V**	**FI***
Among GGs (FCT)	3	603.7	16.8	78.3	0.78	3	803.4	17.7	63.24	0.63
Among populations within GGs (FSC)	7	130.0	3.6	16.9	0.77	7	158.1	3.08	10.63	0.28
Within populations in GG (FST)	42	44.1	1.0	4.9	0.95	50	366.4	7.3	26.13	0.73
Total	52	777.7	21.5			60	1328.0	28.0		



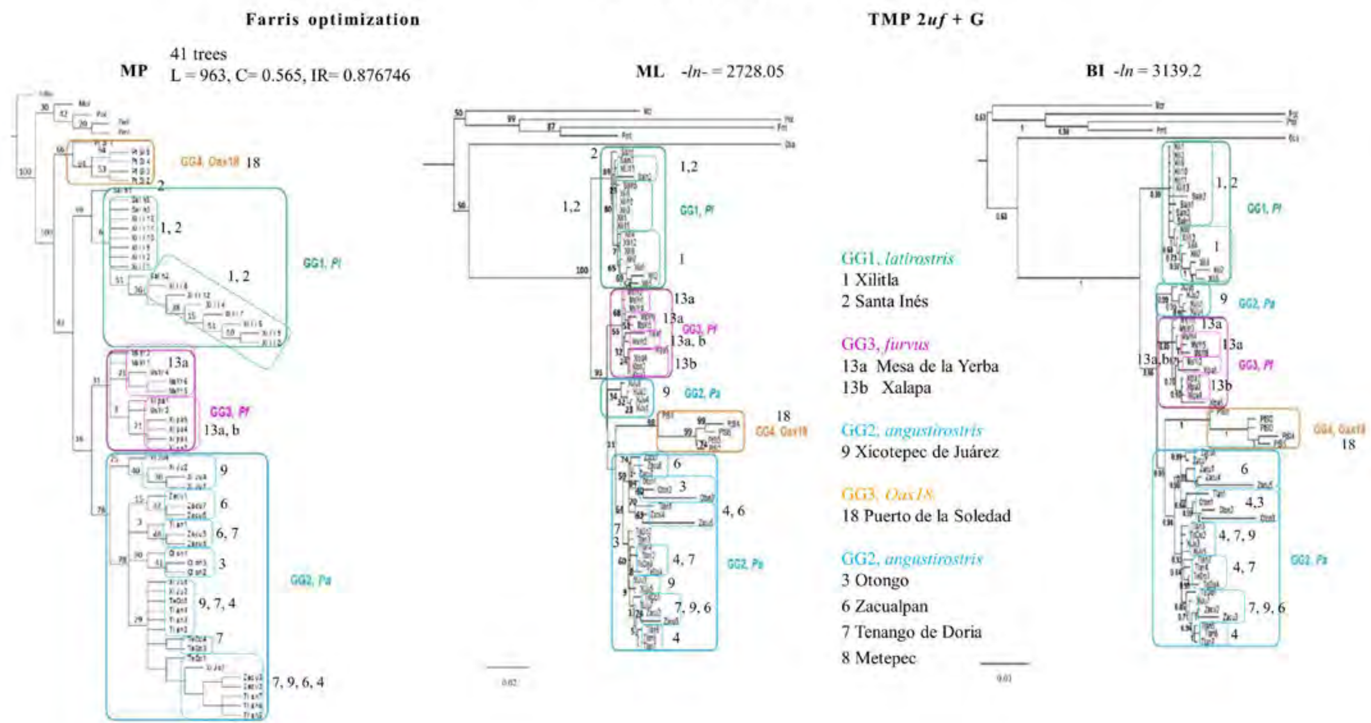


Figure 2. Topologies resulting from phylogenetic analyses using Maximum Parsimony (MP), Maximum Likelyhood (ML), and Bayesian Inference (BI) with sequences for the Cytochrome-b gene with 719 bases (a) and sequences for the NADH genes with 971 bases (b). The numbers on the nodes indicate percentage of support for the branches per 1000 bootstrap replicas. See text for description of support values. See Appendix for details.

Overall, most paired comparisons between GLs among GG1–4 were statistically significant for the *Cytb* and NADH genes (Table 2). GGs at the ends of the distribution range (GG1 *Pl*; GG4 *Oax18*) diverged from the intermediate GGs (GG, *Pa*; GG3 *Pf*) and from each other with greater and significant genetic distances in both genes (Table 2). For NADH genes, the northernmost GG1 (*Pl*), included all the sequences from San Luis Potosí (GL1 Xilitla) and was not

Table 2. Genetic divergence in mitochondrial genes Cytochrome-*b* (A) and ND3-ND4L-ND4 (B), depicted by distances of Tamura and Nei's (N-T, below diagonal) between pairs of Group Localities (GL#) in the Genetic Groups (G1–4), arranged in a NW-SE direction. Geographic distances (*e. g.*, straight lines for km between GLs) are also shown as reference (above diagonal). Number of asterisks depict significant N-T genetic distances among and within GLs (and GGs), according to level of *p*-values: * $p < 0.05$ and ** $p < 0.01$; bold distances had non-significant *p*-values (> 0.05). Colors correspond to color-code in Figures.

A. Cytochrome-*b*

GL#	GG	Code	Xili	Tlan	Zacu	TeDo	Metp	Huau	Xalp	PtSl
1	**GG1**	**Xili**	0	65.17	137.18	147.53	158.52	182.87	322.11	447.53
4	GG2	Tlan	0.96*	0	124.61	136.07	95.83	166.99	256.61	380.72
6	GG2	Zacu	0.92*	0.73*	0	14.65	**24.41**	45.24	190.24	306.96
7	GG2	TeDo	0.93*	0.53**	0.55*	0	19.93	34.38	177.45	298.48
8	GG2	Metp	0.91**	0.69*	**0.28**	0.48**	0	27.99	**173.64**	292.28
10	GG2	Huau	0.97*	0.97**	0.93**	0.92**	0.92*	0	146.03	271.31
13	GG3	Xlpa	0.89*	0.86**	0.73*	0.82**	**0.65**	0.90**	0	161.77
18	GG4	PtSl	0.97*	0.98**	0.96**	0.96*	0.96*	0.98**	0.96**	0

B. ND3-ND4l-ND4

GL#	GG	Code	Xili	Saln	Oton	Tlan	Zacu	TeDo	XiJu	MsYb	Xlpa	PtSl
1	**GG1**	**Xili**	0	**21.86**	58.62	65.17	137.18	147.53	175.43	342.11	322.11	447.53
2	GG1	Saln	0.1	0	46.76	58.29	124.61	136.07	165.7	332.87	312.87	429.32
3	GG2	Oton	0.81**	0.71*	0	17.51	78.83	82.88	118.76	286.55	266.55	386.75
4	GG2	Tlan	0.80**	0.75**	0.36*	0	137.18	**147.53**	117.07	276.61	256.61	380.72
6	GG2	Zacu	0.75**	0.66**	0.20**	0.16*	0	14.65	46.46	210.24	190.24	306.96
7	GG2	TeDo	0.83**	0.80*	0.39*	**0.04**	0.22*	0	**32.10**	197.45	177.45	298.48
9	GG2	XiJu	0.75**	0.66**	0.43**	0.28*	0.3**	**0.29**	0	167.10	147.10	276.65
13	GG3	MsYb	0.84**	0.83**	0.68**	0.65**	0.53**	0.76**	0.44**	0	20.00	161.67
13	GG3	Xlpa	0.79**	0.74**	0.64*	0.65**	0.56**	0.71**	0.46**	0.26**	0	181.67
18	GG4	PtSl	0.84**	0.76**	0.72**	0.78**	0.72**	0.78*	0.73**	0.83**	0.77**	0

genetically different from samples from Querétaro (GL2 Santa Inés). Also, all sequences in GG4 (*Oax18*) from Puerto de la Soledad, Oaxaca (GL18) were genetically segregated from other GLs based on greater geographic distance.

In addition to the lack of genetic divergence between Xilitla (GL1) and Santa Inés (GL2) in GG1 (*Pl*) in the NADH genes, there were two additional exceptions (Table 2) for significant divergence between the GLs from the intermediate GGs (GG2 *Pa*; GG3 *Pf*). In the *Cytb* gene, Metepec, Hidalgo (GL8, GG2, *Pa*) showed the least genetic divergence from the type localities of either GG2 (GL6, Zacualpan, Veracruz) or GG3 (*Pf*, GL13, Xalapa, Veracruz), regardless of the geographic distance (24.4 and 174 km, respectively). Likewise, for the NADH genes, the lack of genetic divergence involved only GLs from GG2 (*Pa*), including Tenango de Doria (GL7), located almost 150 km from Tlanchinol (GL4) in Hidalgo, and 32 km distant from Xicotepec de Juárez (GL9) in Puebla (Table 2). All remaining GLs at each gene possessed significant distances of genetic divergence (Table 2), regardless of their geographic proximity (*e. g.*, 20 km between Mesa de la Yerba and Xalapa, in Veracruz, NADH). These results suggest that there may be different genetic subgroups within the intermediate portion of the geographic range.

The genetic structure of the GLs in GG1–4 (Table 3) with the *Cytb* gene indicated that the two populations at the end of the geographic range (GL Xilitla, GG1 Pl; GL18 Puerto de la Soledad, GG4 *Oax18*) had the lowest genetic divergence. The northern populations of GG2 (*Pa*), Huauchinango in northern Puebla (GL10) and Tlanchinol in northern Hidalgo (GL4) were the most genetically conserved. Tenango de Doria, a southern location in Hidalgo (GL7) and to the southeast of the type locality of GG2 (*Pa*), Zacualpan, Veracruz (GL6), also was genetically conserved with a divergence slightly higher than GL1 and GL18. Zacualpan (GL6), which is geographically closer to populations at Tenango de Doria (GL7) and to the populations of Metepec (GL8), showed twice the amount of genetic variation than the former but 50 % less than the latter. Finally, Xalapa (GL13), in central Veracruz and the type locality for GG3 (*Pf*), showed four times more genetic divergence than Metepec (GL8) and ranked as the most variable GL for the *Cytb* gene. However, the number of indels in the Xalapan sequences (Table 3) were responsible for the higher genetic diversity. Excluding these indels, Xalapa had genetic diversity more similar to Tenango de Doria (GL7, GG2 *Pa*) for the *Cytb* data.

The three NADH genes showed a relative higher amount of genetic diversity among GG1–4 than did the *Cytb* gene

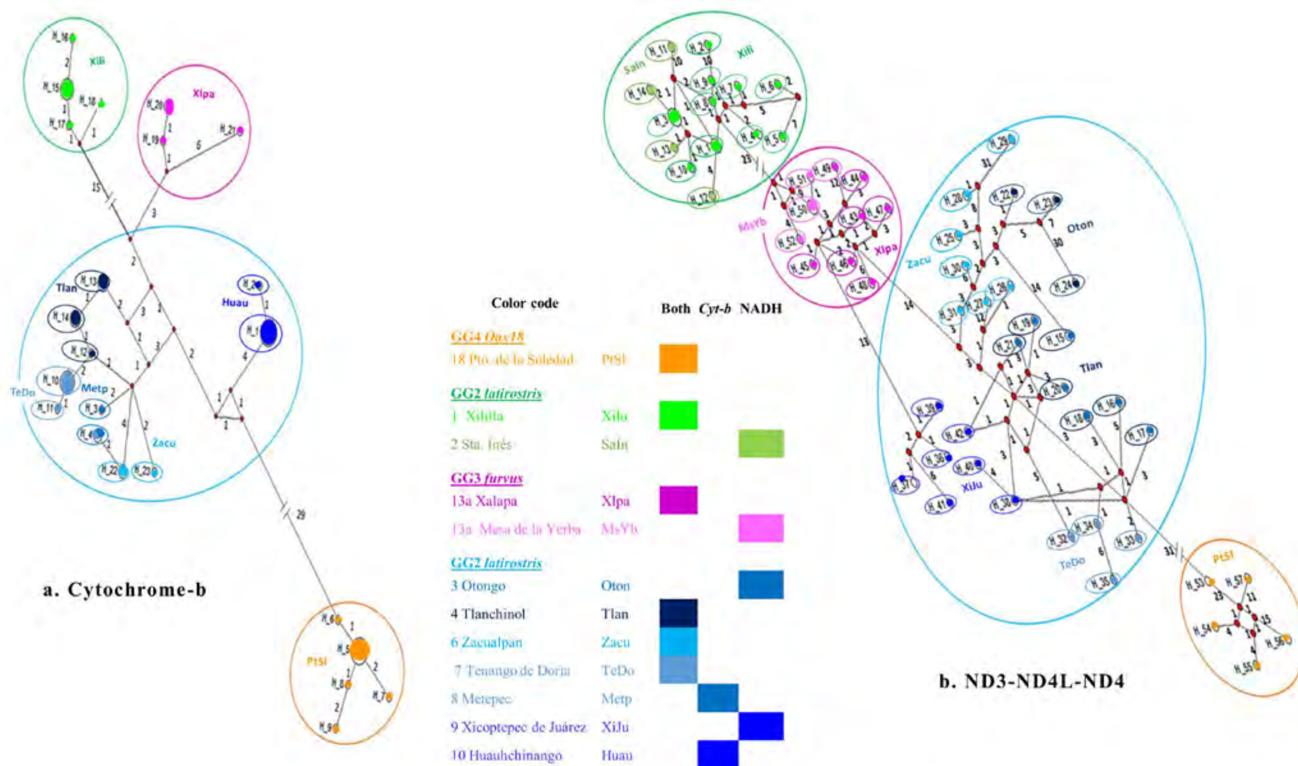


Figure 3. Haplotype networks for the Genetic Groups within the Ingroup in the Cytochrome-*b* gene (a) and in the NADH genes (b). Red diamonds depict unknown haplotypes; digits in the joining lines among haplotypes, indicate the number of mutations (genetic divergence); the underlined haplotype H4 is shared in two nearby group localities in Hidalgo. The tones of the colors aim to facilitate location of the group localities within the same GG. See text and Table 4.

(Table 3). The GLs with the lowest genetic divergence were Mesa de la Yerba (GL13b, GG3 *Pf*) in central Veracruz and the most northern and distant Xilitla (GL1, GG1 Pl). Tenango de Doria (GL7 *Pa*) and Santa Inés (GL1 *Pl*) were the next least divergent localities. Xalapa (GL13 *Pf*) and Tlanchinol (GL4 *Pa*) possessed intermediate levels of genetic diversity with Tlanchinol (GL4 *Pa*) being similar to the northernmost populations in Puebla, Xicotepec de Juárez (GL9 *Pa*). Finally, although Puerto de la Soledad in northern Oaxaca was located geographically near Zacualpan (GL6 *Pa*) and Tenango de Doria (GL7 *Pa*), it was genetically divergent based on the NADH genes.

Haplotype networks in the Internal Group. As in all phylogenetic topologies with molecular data (Figures 2, 4), each GG1–4 is readily recognizable in the haplotype networks (Figure 3) because all haplotypes from the GLs joined, regardless of geographic proximity between their respective GLs (Figure 1b, Table 2). Both haplotype networks reflected the overall geographical distribution of the G1–4 (Figures 1, 3), with sequences of GG1 (*latirostris*, *Pl*) and GG4 (*Oax18*) at the geographical extremes, and GG2 (*angustirostris*, *Pa*) and GG3 (*furvus*, *Pf*) join in the intermediate zone. However, in both haplotype networks, GG3 (*Pf*) appears to be closer to GG1 (*Pl*) than GG2 (*Pa*). The distinctiveness of GG1–4 is supported by the large amount of the variance as seen in the AMOVA (78.30 % in *Cytb* and 63.24 % in the NADH genes, Table 1). The haplotype networks are consistent in depicting geographic representation (Table 4, Figure 1, Appendix), but differ in the intrinsic amount of genetic variation when *Cytb* is compared to NADH (Tables 1, 2). Accordingly, each network displayed its own number of mutations (steps in each branch), including those between known haplotypes, between haplotypes and unknown haplotypes, and between unknown haplotypes. Unknown haplotypes appear as "UnkH" in Table 4 and as red diamonds on Figure 3.

The 53 sequences in the *Cytb* gene coalesced into 24 distinctive haplotypes inside the eight GLs representing GG1–4 with one exception (Table 4, Figure 3a). GG2 (*Pa*) from Hidalgo, Tenango de Doria (GL7), and Metepec (GL8), shared the same haplotype (H4). For *Cytb*, all GLs had 1–2 shared haplotypes except for Metepec (GL8). The number of mutations ranged from 1 to 29 with most found within the GGs, except for each of the type localities of GG3 (*Pf*, GL13, H21, 6) and GG3 (Pa, GL6, H22, 4). Among GGs represented by a single GL (Figure 3a), populations from Oaxaca (GG4 *Oax18*) had no unknown haplotypes, whereas San Luis Potosí (GG1 *Pl*) and (GG3 *Pf*) had one each. GG2 (*Pa*) had five GLs and included seven unknown haplotypes (Figure 3a), most of which joined the GLs from Hidalgo (GL4, GL7, and GL8) with Zacualpan, Veracruz (GL6), the type locality of *angustirostris*. GL4 (Tlanchinol) and GL7 (Tenango de Doria), located 82.93 km apart (Table 2), joined through two mutations; whereas an unknown shared haplotype joined four geographically closer GLs (Table 2) through either 1, 2, or 4 mutations. Zacualpan (GL6) joined through to Metepec (GL8, 21.41 Km) or Tenango de Doria (14.65 km) by a single mutation (H4). Tenango de Doria (GL7) and Metepec

Table 3. Indexes of genetic diversity (*Pi*) in the representative populations of the genetic groups (GG1–4) of the Ingroup, indicating the related statistics (*Theta Pi* with one standard deviation, SD), the number (No.) of mutations by type, and the respective mean with its standard deviation (SD) for cytochrome-*b* and ND3-ND4L-ND4. Colors correspond to those in the Figures. See Appendix for details.

Cytochrome-*b*										
Group Locality#	GL1	GL4	GL6	GL7	GLG8	GL10	GL13	GL18		
Genetic Group	GG1	GG2	GG2	GG2	GG2	GG2	GG3	GG4		
GL Code	Xili	Tlan	Zacu	TeD	Metp	Huau	Xlpa	PtSl		
									Mean	SD
n of sequences	8	7	3	8o	2	9	4	12		
Statistic					Genetic diversity					
Pi	1.43	0.86	5.33	3	10	0.22	61.5	1.44	10.47	19.5
Theta Pi	1.43	0.86	5.33	3	10	0.22	61.5	1.44	10.47	19.5
SD *Theta Pi*	1.1	0.78	4.4	1.99	10.49	0.33	40.56	1.06	7.59	12.8
					Mutations, number of					
transitions, ts	5	2	7	11	10	0	8	6	6.13	3.5
transversions, tv	0	0	1	1	0	1	1	1	0.63	0.5
substitutions, sb	5	2	8	12	10	1	9	7	6.75	3.6
indels, in	0	0	0	0	0	0	114	0	14.25	37.7
Sum	10	4	16	24	20	2	132	14		
No. of ts sites	5	2	7	11	10	0	8	6	6.13	3.52
No. of tv sites	5	2	7	11	10	0	8	6	0.63	0.48
No. of ss sites	5	2	8	12	10	1	9	7	6.75	3.6
No. of in sites	0	0	0	0	0	0	114	0	14.25	37.7
Sum	15	6	22	34	30	1	139	19		

ND3-ND4L-ND4												
Group Locality#	GL1	GL2	GL3	GL4	GL6	GL7	GL9	GL13	GL13	GL18		
Genetic Group	GG1	GG1	GG2	GG2	GG2	GG2	GG2	GG3	GG3	GG4		
Code	Xili	Saln	Oton	Tlan	Zacu	TeDo	XiJu	MsY	Xlpa	PtSl		
											Mean	SD
n of sequences	13	5	3	7	7	4	7	6	5	12		
Statistic						Genetic diversity						
Pi	7.9	9.83	28.7	13.05	23.29	9	18.19	6.33	12	22.8	15.11	7.26
Theta Pi	7.9	9.83	28.7	13.05	23.29	9	18.19	6.33	12	22.8	15.11	7.26
DE *Theta Pi*	4.42	6.83	21.8	7.68	13.4	6.28	10.55	4.04	7.67	14.21	9.69	5.19
						Mutations, number of:						
transitions, ts	9	11	30	27	39	11	28	10	25	34	22.4	10.6
transversions, tv	18	8	13	11	27	6	8	5	4	19	11.9	7.02
substitutions, sb	27	19	43	38	66	17	36	15	29	53	34.3	15.6
indels, in	1	0	0	0	0	0	0	0	0	0	0.1	0.3
Sum	30	38	86	76	132	34	70	30	58	106		
No. of ts sites	9	11	30	27	39	11	28	10	25	34	22.4	10.6
No. of tv sites	9	11	30	27	39	11	28	10	25	34	11.9	7.02
No. of ss sites	27	19	43	38	64	17	36	15	29	53	34.1	15.2
No. of in sites	1	0	0	0	0	0	0	0	0	0	0.1	0.3
Sum	46	41	103	92	142	39	92	35	79	121		

(GL8), which are separated by 19.93 km, also differed by one mutation. The most divergent subpopulation in GG2 (*Pa*) was Huauchinango, Puebla (GL10) which is 28 km from Metepec (GL8) but that was separated from it (and from all the other GLs) by 5 to 6 unknown haplotypes and at least 11 mutations.

Although Huauchinango (GL10) is the most proximate (271.31 km) subpopulation of GG2 (*Pa*) to Puerto de la Soledad, Oaxaca (GG4 *Oax18*), the number of mutations between these two GGs scored highest in the *Cytb* network, with 29 mutations and 1 to 2 unknown haplotypes (Figure 3a). Branches joining GG2 (*Pa*) to GG3 (*Pf*) included 3 to 7 unknown haplotypes. In the network analysis (Figure 3a), Xalapa (GL13, *Pf*) was genetically divergent from Tlanchinol (GL4, 256.6 km) and to the group formed by Zacualpan (GL6, 190. 24 km), Tenango de Doria (GL7, 177.45 km),

Table 4. Haplotypes (H#) analyzed in gene networks for Cytochrome-*b* and three NADH genes, according to OTU (genetic group, GG1–4, taxonomic entity and key). Geographic data include the group localities (GL#) in a NW-SE direction (see Figure 1b); the asterisk (*) indicates type localities or first known localities. The total number of genetic sequences (#Sequences) in each OTU appears in the next column with the GL code, whereas in the last column, the number inside parenthesis indicates how many were shared in a common haplotype. See Appendix 1. Colors for OTUs are as in Figure 3.

OTU	GL#. Name, State	GL Code # Sequences	Haplotypes H#	Common H#s (#Sequences)
		Cytochrome -*b gene*		
GG1 *latirostris Pa*	1. Xilitla, San Luís Potosí*	Xili 1–8	H15–H18	H15(5)
GG2 *angustirostris Pa*	4. Tlanchinol, Hidalgo	Tlan 1–7	H12–H14	H13 (3), H14(2)
	6. Zacualpan, Veracruz*	Zacu 1–3	H22, H23	H22(2)
	7. Tenango de Doria, Hidalgo	TeDo 1–9	H4, H10–H11	H10(7), H4(Metpc)
	8. Metepec, Hidalgo	Metp 1–2	H3, H4	H4(TeDo)
	10. Huauchinango, Puebla	Huau 1–9	H1–H2	H1(8)
GG3 *furvus Pf*	13. Xalapa, Veracruz*	Xlpa 1–4	H19–H21, H20	H20(2)
GG4 unknown *Oax18*	18. Puerto de la Soledad, Oaxaca*	PtSl 1–12	H5–H9	H5(8)
		ND3-ND4L-ND4 genes		
GG1 *latirostris Pa*	1. Xilitla San Luís Potosí*	Xili 1–13	H1–H10	H3(3) ,H1(2)
	2. Santa Inés, Qro (SaIn1–4)	SaIn 1–4	H11–H14	none
GG2 *angustirostris Pa*	3. Otongo, Hgo (Oton1–4)	Oton 1–4	H22–H24	none
	4. Tlanchinol, Hidalgo	Tlan 1–4	H15–H21	none
	6. Zacualpan, Veracruz*	Zacu 1–7	H25–H31	none
	7. Tenango de Doria, Hidalgo	TeDo 1–4	H32–H35	none
	9. Xicotepec de Juárez, Pue (XiJu1–7)	XiJu 1–7	H36–H42	none
GG3 *furvus Pf*	13a. Xalapa, Veracruz*	MsYb 1–6	H43–H48	H50(2)
	13b. Mesa de la Yerba, Veracruz*	Xlpa 1–6	H49–H52	none
GG4 unknown *Oax18*	18. Puerto de la Soledad, Oaxaca*	PtSl 1–5	H53–H57	none

and Metepec (GL8, 173.64) by 10 mutations. Xalapa (GL13 *Pf*) compared to that of Huauchinango (GL10), to which it is geographically closer (146.04 km), differed by 11 mutations. Finally, the branches between GG1 (*Pl*, Xilitla, San Luis Potosí, GL1) and GG3 (*Pa,* Xalapa, Veracruz, GL13) showed a genetic divergence of 19 mutations (Figure 3a).

In the NADH genes network (Figure 3b), the 58 sequences resulted in 57 distinctive haplotypes, the sole exception being H50 from Mesa de la Yerba in central Veracruz (GL13, GG3 *Pf*), which was presented by two sequences. With the exception of Puerto de la Soledad, Oaxaca (GL18, GG4 *Oax18*), all other GGs had more than one GLs representing them: two in either GG1 (*Pl*) and GG3 (*Pf*) and five in GG2 (*Pa*). Overall, the number of mutations in the network branches ranged from 1 to 31. In the NADH network, the total number of unknown haplotypes was slightly more than triple that found in *Cytb* (Figure 3b). Although most haplotypes from the same GL were spatially closer, there was more intermingling among haplotypes from different GLs, thus allocating haplotypes among some GLs. Furthermore, the higher and more fluctuating number of mutations among the GLs, the greater intermingling of haplotypes from different GLs in the same genetic group. The lower number of shared haplotypes rendered a lower percentage of variance explained by the variation among GG1–4 in the AMOVA (Table 1). For the NADH genes, variation within subpopulations (GLs) accounted for a higher amount of variance than in the *Cytb* gene.

In GG4 (*Oax18*, GL1), no sequence joined another except through unknown haplotypes (number of mutations was 4, 11, 15, and 23; Figure 3b). H53, the most divergent haplotype, diverged by 31 mutations from an unknown haplotype that join a GL of GG2 (*Pa*) from Puebla (GL9, Xicotepec de Juárez, 276 km) and two from Hidalgo (GL7, Tenango de Doria, 298 km; GL4 Tlanchinol, 380 km). The haplotypes of three GLs formed their own subpopulation and shared haplotypes from others through unknown haplotypes. These three GLs contained 1 to 6 mutations and only Tenango de Doria (GL7; H34, H35) and Xicotepec de Juárez (GL9; H38, H20) had haplotypes that joined to the same subpopulation. Xicotepec de Juárez, Puebla (GL9) is 32 km from Tenango de Doria (GL7) and 117 km from Tlanchinol (GL4) and their haplotypes diverge from unknown haplotypes by 1, 2, or 6 mutations. Only H15 from Tlanchinol (GL4) joins Otongo (GL3), 17 km distant, through a genetic divergence of at least 17 unknown haplotypes. The level of divergence

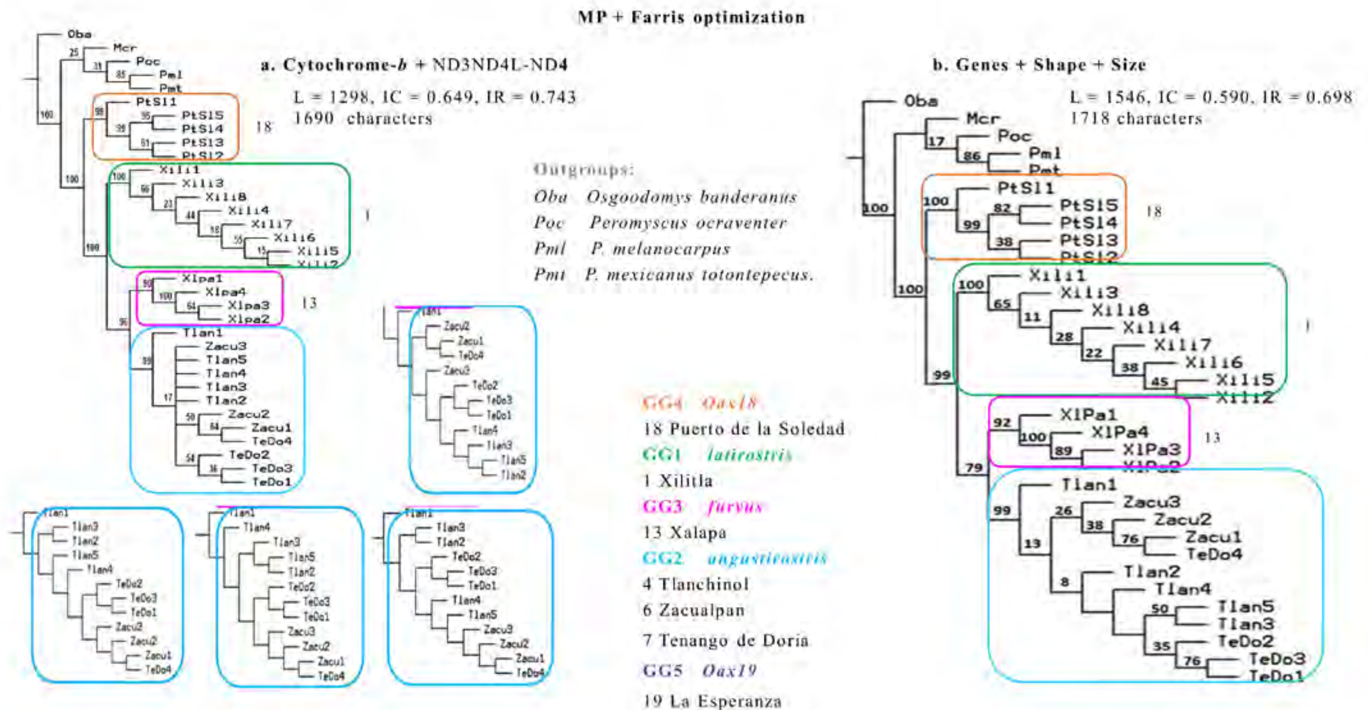


Figure 4. Topologies from selected populations of *Peromyscus furvus*, integrating two types of mitochondrial genes in a strict consensus cladogram (a; note the four outcomes for GG2), and the former with 28 morphometric characters for skull shape and size in a completely resolved single phylogenetic tree (b). The numbers on the nodes indicate percentage of support for the branches per 1000 bootstrap replicas. See text for description of support values.

in the sequences from Otongo (GL3) includes 1, 7, and 30 mutations from unknown haplotypes. The sequences from the type locality Zacualpan, Veracruz (GL6) also formed a separate group but joined by 7 or 11 mutations to Otongo (GL3), 78 km away. Most genetic divergence among subpopulations contained 1 or 3 mutations, however, haplotypes had 6, 8, 12, and 31 mutations to an unknown haplotype. Therefore, the overall number of mutations in GG2 (*Pa*) was high. Consistent with its intermingling pattern, GG2 (*Pa*) joins to GG3 (*Pf*) through clusters of two different subpopulations separated by 46 km (Zacualpan GL6, Xicotepec de Juárez GL9). The route from Zacualpan (GL6 *Pa*) involved 14 to 17 mutations between haplotypes connecting it to Xalapa (GL13 *Pf*). The alternative route from Xicotepec de Juárez (GL9 *Pa*) to Xalapa (GL13 *Pa*) encompasses 13 mutations between unknown haplotypes (GL9 is 147 km from GL13).

The two GLs in GG3 (*Pf*) are 20 km distant and joined by either 5 or 15 mutations (Table 2). In Xalapa (GL13a), the number of mutations from an unknown haplotype ranged from 1 to 3, and 6, but in Mesa de la Yerba (GL13b) they were higher with 1, 4, 9, or 12 mutations. The only joining of two haplotypes from the same subpopulation (Figure 3b) occurred in Mesa de la Yerba, which had a single mutation between haplotype H50 and H51. H50 was the only shared haplotype in the entire NDH network. Between GG3 (*Pf*) and GG1 (*Pl*), there are 23 mutations segregating the two unknown haplotypes to which 5 or 10 mutations join Mesa de la Yerba (GL13a) and another one joined Xilitla, San Luis Potosí (GL1). These two GLs are 322 km distant. In Xilitla (GL1), the number of mutations from a haplotype to an unknown haplotype was 1, but there also were 2, 5, 7, and 10 mutations. The number of mutations between GL1 and Santa Inés, Querétaro (GL2), was 2 or 4, and the latter joined to unknown haplotypes by 1, 2, or 10 mutations.

Combined datasets. In the MP analyses for combined datasets (Figure 4), all OGs were distinct from the members of the IG and each GG1–4 was readily recognizable with support values of > 90 to 100 %. *Oba* was always at the root, whereas the sister group to all IG was (*Mcr*(*Poc*(*Pml Pmt*))). In this sister group, only the clade of *Pml* and *Pmt* was well supported (85%, 86%). In the IG, the sequences from Puerto de la Soledad, Oaxaca (GG4, *Oax18*) were located basally and separated from other GGs. In the most derived clade, sequences from Xilitla, San Luis Potosí (GG1 *Pl*) showed full support and separated from a sister subclade with sequences from the two remaining GGs. Clade support was high in ML but lowered in the IB topology for the sequences from Xalapa, Veracruz (GG3 *Pf*) and in GG2 (*Pa*) with all the remaining sequences from Hidalgo and Veracruz (GG2 *Pa*). However, the clades of these two GGs were distinctive from each other and with strong support.

The MP strict consensus cladogram combining all molecular sequences (Figure 4a) resulted from four most parsimonious trees (L = 1298, IC = 0.649, IR = 0.743). The differences among these original trees (Figure 4a) were due to the relative position of the specimens of GG2 (*Pa*) from the two group localities in Hidalgo, Tlanchinol (GL4) and

Tenango de Doria (GL7) with respect to the specimens from Zacualpan, Veracruz (GL6). The addition of morphometric data in the multicharacter phylogeny (Fig 4b) resolved the aforementioned discrepancies in a single, though longer tree (L = 1546, IC = 0.590, IR = 0.698). In agreement to its geographic location, a sequence from Tlanchinol (GL4) preceded a group with all the sequences from Zacualpan (GL6) and one sequence from Tenango de Doria (GL7). This sister clade included sequences from GL4 and GL7 that are geographically more distant (Table 2) and poorly supported (8 %). However, some clades ranked from moderate (Tlanchinol, 50 %) to acceptable (Zacualpan and Tenago de Doria, or the latter alone, with 76 % each). In both combined datasets with genetic data (Figure 4), branches defining subclades associated with the separation of every GG were fully (100 %) or very highly supported (90 to 99 %).

Percentage genetic distances within the IG and with all OTUs for the Cytb gene. In the analysis of the IG (Table 5a), the larger genetic distances occurred between GG4 (*Oax18*) from Puerto de la Soledad, Oaxaca (GL18) and the other GGs (6.6 to 7.8 %), with the exception of Metepec, Hidalgo (GL8) of GG2 (*angustirostris*) which was <2 %. Likewise, although of smaller magnitude, the genetic distances associated with GG1 (*latirostris*) from Xilitla, San Luis Potosí (GL1) showed segregation from all the other GGs (3.8 to 7.8 %). GG3 (*furvus*) from Xalapa, Veracruz (GL13) differentiated from GG1 (*latirostris*, 3.8 %) and especially from GG4 (*Oax18*, 7.6 %). However, the genetic distances of GG3 (*furvus*) were lower (2.4 to 3.0 %) with respect to the remaining localities in Hidalgo, Puebla, and Veracruz (corresponding to GG2, *angustirostris*). The variation among the localities within GG2 (*angustirostris*) ranged 0.7 to 2.7 %. Noticeably, these analyses also showed minimal genetic distances even among individuals from the same locality (Table 5a) both in Metepec, Hidalgo (GG2 *angustirostris*) and in Xalapa, Veracruz (GG3 *furvus*).

In the genetic distances including all OTUs (Table 5b), all comparisons among the OGs and IGs, averaged >11 % with an average genetic divergence among the ten OTUs of 16.2 % (range values, 11.8 to 22.9 %); among the OGs from the IG, the values were 15.6 % (11.8 to 19.8 %). *Peromyscus ochraventer* (*Poc*, 18.4 %) had the greatest divergence from the IG, followed by *O. banderanus* (*Oba*, 16.4 %) and *P. melanocarpus* (*Pml*, 16.1 %). *P. mexicanus totontepecus* (*Pmt*, 14.2 %) and *M. cryophilus* (*Mcr*, 13.0 %) showed the lower genetic distances from the IG. The overall average genetic divergence of the IG from all the OGs was 15.6 %. GG3 (*Pf*, 16 %) was the most divergent to all OGs, followed by both GG2 (*Pa*, 15.7 %) and GG4 (*Oax18*, 15.7 %), whereas GG1 (*Pl*, 15.1 %) was slightly less divergent. When pooled together, GG3 (*Pl*) and GG2 (*Pa*) were slightly more divergent from the OGs (15.9 %) than GG4 (*Oax18*). When the IG was individually compared with all three *Peromyscus* in the OG (*Poc*, *Pml*, and *Pmt*), they diverged by an average genetic distance of 16.1 % if the GG3 (*furvus*) and GG2 (*angustirostris*) were pooled together, but with an average distance of 14.2 % if they were separated.

Table 5. Percentage genetic divergence (%) of the Tamura and Nei genetic distances, *sensu* Bradley and Baker (2001), for the Cytochrome-*b* gene in the genetic groups (GG1–4) of the ingroup (a) and in all the species examined herein (b). Genetic distances between pairs of entities appear below the diagonal the numbers on diagonal in the genetic groups are distances within populations (GLs).

a. Genetic Groups	**GL#. Name, State**	GL1	GL4	GL6	GL7	GL8	GL10	GL13	GL18
GG1 *latirostris Pl*	1. Xilitla, San Luis Potosí	0.0							
	4. Tlanchinol, Hidalgo	4.4	0.0						
	6. Zacualpan, Veracruz	4.5	1.1	0.0					
GG2 *angustirostris Pa*	7. Tenango de Doria, Hidalgo	5.0	0.7	1.2	0.0				
	8. Metepec, Hidalgo	4.1	1.2	0.7	1.2	**0.01**			
	10. Huauchinango, Puebla	4.9	2.4	2.5	2.7	2.6	0.0		
GG3 *furvus Pf*	13. Xalapa, Veracruz	3.8	2.4	2.8	3.0	2.7	2.5	**0.01**	
GG4 unknown *Oax18*	18. Puerto de la Soledad	7.8	7.2	7.3	6.7	1.2	6.6	7.6	0

b. All OTUs	***Oba***	***Mcr***	***Poc***	***Pml***	***Pmt***	***Pl***	***Pff***	***Pfa***	***Oax18***
Osgoodomys banderanus, Oba									
Megadontomys cryophilus, Mcr	16.7								
Peromyscus ochraventer, Poc	20.3	15.9							
P. melanocarpus, Pml	17.9	14.1	22.9						
P. mexicanus totontepecus, Pmt	18.9	13.5	21.9	12.5					
P. latirostris / P. f. latirostris, Pl	15.8	13.7	16.9	15.0	14.0				
P. f. furvus, Pff	16.9	13.3	19.1	16.8	13.8	5.0			
P. f. angustirostris Pfa	16.1	13.1	19.8	15.6	14.1	4.0	2.8		
Unknown *Peromyscus* from *Oax18*	16.8	11.8	17.8	17.1	14.9	8.4	7.3	8.1	

GG1–4 differed from each other with lower average genetic distances (5.9 %, 2.8 to 8.4 %; Table 5b). Puerto de la Soledad (GG4 *Oax18*) had an average genetic distance of 7.9 % from GG1–3. GG1 (*latirostris*) differed from GG2–4 by 5.8 %, and GG3 (*furvus*) and GG2 (*angustirostris*) diverged from the other three GGs by 5 %. Either pooled together or alone, the two latter GGs had an average divergence of 6.0 to 6.1 % from the two geographically extreme GG1 (*latirostris*) and GG4 (*Oax18*), but were 7.7 % from GG4 and 4.5 % from GG1. The least average genetic distance (2.8 %) was between GG2 (*furvus*) and GG3 (*angustirostris*).

Finally, the average genetic distance among the OGs was 17.5 % (12.5 to 22.9 %). *P. ochraventer* (*Poc,* 20.3 %) had the most divergent average genetic distance, followed by *O. banderanus* (*Oba*, 18.5 %). *P. melanocarpus* (*Pml*, 16.9 %) and *P. mexicanus totontepecus* (*Pmt*, 16.7 %) and *M. cryophilus* (*Mcr*, 15.1 %) showed lower genetic divergence. The least divergent OGs were *Pml* and *Pmt* (12.5 %), and the most divergent were *Poc* and *Pml* (22.9 %).

Discussion

The results presented herein were obtained from several analyses, including single-character phylogenies and all methods combined (MP, ML, and BI). Regardless of method, GG1 (*latirostris*, *Pl*), GG3 (*furvus*, *Pf*), and GG4 (*Oax18*) were always distinct because their sequences were reciprocally monophyletic. All analyses of the *Cytb* gene and the MP analyses for the NADH genes yielded a topology nearly identical to that reported by Harris *et al.* (2000:2131–2). However, the probabilistic topologies of the NADH genes concurred with the findings of Ávila-Valle *et al.* (2012:171). In Harris *et al.* (2000), the most distinctive entity was GG4 (*Oax18*), followed by GG1 (*Pl*), whereas in Ávila-Valle *et al.* (2012) the latter was the most distinctive entity and GG4 (*Oax18*) was associated with GG2 (*Pa*). Although these authors pointed out the distinctiveness of these two GGs, they did not propose their formal recognition. It is clear that the populations in the intermediate zone of the distribution range posed a challenge to interpreting the phylogenetic relationships among them, especially because their subpopulations (GLs) were not monophyletic in several of their topologies (Harris *et al.* 2000; Ávila-Valle *et al.* 2012). Aside from Huauchinango, Puebla (GL10), Otongo, some sequences of Tlanchinol, Tenango de Doria from Hidalgo, and some sequences of Mesa de la Yerba and Xalapa from Veracruz, combinations of sequences from different GLs (*i. e*., Zacualpan-Metepec-Tenango de Doria) had greater support than did all sequences representative of GG2 (*Pa*) in topologies of single types of genes. Similar results were obtained from the variance components in the AMOVA and in the haplotype networks herein.

Differences in the outcomes of these topologies were based on the method (non-parametric MP, parametric ML, BI; *e. g*., Steel and Penny 2001; Sober 2004), especially in the methods used to calculate branch length; probably due to different ontological and epistemological assumptions (De Luna 1995). Another possible source for disconcordance among topologies could have been from the evolutionary model used in the probabilistic methods and its relationship with the evolutionary assumptions in the MP analysis. The GTR I + G (GTR) used in the *Cytb* is more realistic, though more complex and thus with more probability of error because it considers six substitution parameters; whereas, the more simple model TMP 2 uf + G (TMP2) only has three parameters (Posada 2008). The GTR model, by assigning the same importance to the changes from one base to another and *vice versa*, is more like the Farris optimization model in MP analysis (Farris 1970; Lipscomb 1998). However, GTR considers different probabilities for the different types of mutations (Tavare 1986) and assigns a specific value to particular types of mutations. Conversely, the TPM2 model accepts and evaluates only three forms of base substitutions (Kimura 1981), thus ignoring possible informative mutations for phylogenetic analyses, unlike the Farris (1970) optimization. Use of GTR as an evolutionary model for the *Cytb*, with or without invariant sites, is quite frequent in studies on the systematics of *Peromyscus* (*e. g*., Harris *et al.* 2000; Bradley *et al.* 2007; Bradley *et al.* 2014; Platt *et al.* 2015; and other references therein and herein). However, exploration of TPM2 in *Peromyscus* (Walker and Greenbaum 2006; Ávila-Valle *et al.* 2012; Castañeda-Rico *et al.* 2014) and other species (León-Paniagua *et al.* 2007) is just beginning.

Ávila-Valle *et al.* (2012) also used the GTR model and obtained the same topology as presented herein for the NADH genes, even when they examined only whole ND3-ND4 sequences (1,043 base pairs). Therefore, the differences between the topologies may be due to a different evolutionary rate for nucleotide substitution (synonymous and non-synonymous) between the mitochondrial genes. *Cytb* evolves faster than do any of ND3, ND4L and ND4 genes in other mammalian orders (primates, carnivores, perissodactyls, and cetaceans; Pesole *et al.* 1999) and may have caused the disconcordance among topologies. For non-synonymous mutations, the rate of change for *Cytb* is at least two times faster than in any of the NDAH genes. For synonymous mutations, the rate of change in ND3 is 50 % less than that of the ND4 and *Cytb*, and the rate of change in ND4L is 25 % less than the *Cytb* and NADH genes. Therefore, GG4 may have grouped with GG2 and GG3 because of the influence of the ND3 and ND4L genes, whereas sequences from *Cytb* clearly separated it from the other GGs. The rate of change in *Cytb* has proven to be adequate to discern both intraspecific (intrapopulation, intrasubspecific, intraspecific) and intrageneric (sister species, interspecific) variation *sensu* Bradley and Baker (2001). It also is likely that our adjustment in the number of bases in the GI (*Pl*) and the OG in the ND3-ND4L-ND4 genes allowed us to avoid possible inconsistencies between the sequences recovered from the literature and GenBank due to indels.

In our integrated phylogeny, based on all characters, we used the same epistemological approach to perform our

analyses under the same evolutionary model (Farris optimization) and method (MP) in the TNT package (Goloboff *et al.* 2006). The decision to overcome technical restrictions for analyzing a big character matrix with different type characters allowed us to test the behavior of NADH genes under a more open evolutionary scenario. Moreover, it allowed us to include continuous morphometric characters for the shape and size of skull (Goloboff *et al.* 2006; Ramírez-Sánchez *et al.* 2016). As with the molecular data, we conducted single-character phylogenies for each size (linear measurements) and shape characters (morphogeometric configurations) with a much larger amount of individuals (in prep.), especially for size (*i. e*., those used in Ávila-Valle *et al.* 2012). Herein, morphometric characters solved the discrepancies among GLs in GG2 (*Pa*, Figure 2) in spite of reluctance of including them within combined molecular and morphometric phylogenies (Scotland *et al.* 2003). In other studies (*e. g*.,Lee and Camens 2009; Ledevin and Millien 2013), incorporation of morphometric characters into integrated phylogenies with molecular data has proven to be useful to understand and solve conflicting molecular outcomes.

After reduction of the number of genetic sequences in order to include only the GLs with both sequences, followed by an even more reduced number of GLs with all genetic and morphometric characters, GG1–4 appeared as well recognizable geographic and evolutionary entities with better supporting values compared to single-characters topologies. Herein, specimens from Puerto de la Soledad (GG4, *Oax18*), in the farther southern range, show the greatest genetic distances from the other GGs, and its geographic remoteness. Likewise, specimens (GG1, *latirostris*) from Xilitla, San Luis Potosí, with the second greatest geographic distances, showed twice the genetic divergence from specimens in the intermediate range. Finally, sequences from Xalapa, Veracruz and its surrounding locations (GG3, *furvus*), as well as those from Tlanchinol and Tenango de Doria, Hidalgo, and Zacualpan, Veracruz (GG2, *angustirostris*), were distinctly separated from each other as the subunits of a third genetic clade. Therefore, the monotypic status currently assigned to the populations in the overall distribution of *P. furvus* (Rogers and Skoy 2011) does not coincide with our genetic nor the morphometric data. Rather, the data presented herein confirms the morphometric (Musser 1964; Hall 1968; Martínez-Coronel *et al.* 1997; Ávila-Valle *et al.* 2012) and biochemical (Harris and Rogers 1999; Harris *et al.* 2000; Ávila-Valle *et al.* 2012) discrepancies reported previously.

Systematic and taxonomic remarks. Application of the genetic species concept (Wiley and Mayden 2000) *sensu* Baker and Bradley (2006), allows us to suggest the taxonomic level (clades and haplotypes clearly forming monophyletic groups) and the subspecific level (clades and shared haplotypes) of the distinctive genetic entities in the *Cytb* dataset. All GG1–4, with GG2–3 either separated (mean 14.2 %, 13.8 to 14.9 %) or pooled (16.1 %, 15.3 to 16.6 %), were similar in levels of genetic divergence as other congeneric species in the OG. When *M. cryophilus* and *O. banderanus* are included, the divergence level increased (15.6 %, 15.1 to 16 %) for the two genes. These levels of genetic distances among G1–4 indicate evidence supporting the recognition of genetic entities *sensu* Bradley and Baker (2001). The population of Puerto de la Soledad, Oaxaca (GG4) possessed the greatest genetic diversity of the four populations (GG1–4). GG4 had an average genetic distance of 7.5 % (or of 7.9 %, when the 10 OTUs were included), which is sufficient to distinguish it as a distinctive species from the other populations (GG1, *latirostris*; GG2, *angustirostris*, and GG3, *furvus*) *sensu* Bradley and Baker (2001) and Baker and Bradley (2006).

The northern population, (GG1, *latirostris*), possessed an average genetic distance of 4.2 % among GG1–3 (and of 4.9 % when the OGs are included in the analysis). Baker and Bradley (2006) mention a K2P distance interval of 2.8 to 10.8 % to validate species within *Peromyscus*, with several authors using a lesser distance than 7.0 %, although it is necessary to support genetic distance values with other kinds of evidence (Bradley and Baker 2001; Bradley *et al.* 2014). In the *boylii* species group, in addition to the genetic data of *Cytb*, the fundamental number of chromosomes and/or morphometric data have been used to describe new species in which the average lower limit of (*i. e*., the percentage genetic distance to its closest relative) is 4.3 % ± 1.1 SD (3.3 to 5.6 %). Following this logic, *P. schmidlyi* was described with a mean distance of 3.3 % from *P. levipes* (Bradley *et al*. 2004a), *P. carletoni* from *P. levipes* (3.4 %, Bradley *et al.* 2014), and *P. kilpatricki* from *P. levipes* (5.2 %, Bradley *et al.* 2016). Similarly, in the *mexicanus* species group, Lorenzo *et al.* (2016) described *P. gardneri* based on a K2P distance of 3.6 to 4.2 % from other species and reinforced the decision with morphometric data. In our analysis of linear (size) and morphogeometric (shape) characters, GG1 (*latirostris*) behaved as a separate entity, similar to that found in the morphometric data of Bradley *et al.* (2004a) and Lorenzo *et al.* (2016). In addition, GG1 (*latirostris*) has a larger skull size compared to GG2–4 (Martínez-Coronel *et al.* 1997; Ávila-Valle *et al.* 2012) and some distinctive qualitative cranial traits have been reported (Hall and Alvarez 1961; Musser 1964: Hall 1968).

GG2 (*angustirostris*) and GG3 (*furvus*) also possessed N-T genetic distances that averaged greater or similar to those K2P values reported for *Peromyscus* (Tiemann-Boege *et al.* 2000; Durish *et al.* 2004; Ordóñez-Garza *et al.* 2010). The average genetic distance between GG3 and GG2 was 2.7 % and 2.8 %, compared to other OTUs. Based on the logic presented in Bradley and Baker (2001), our results revealed that GG3 (*furvus* from the type locality Xalapa, Veracruz and its surroundings) and GG2 (*angustirostris* from Hidalgo, Puebla and locations further NW in Veracruz) would appear to be subspecies. The genetic distances between GG2 and GG3 are similar to that reported by Bradley *et al.* (2015) for two subspecies (ranging from 1.77 % in *P. p. laceianus* to 2.21 % in *P. p. pectoralis*). Similarly, Lorenzo *et al.* (2016) use a K2P genetic distance of 2.08–3.65% to define two subspe-

cies of *P. zarhynchus* in Chiapas (*P.* z. *zarhynchus* and *P. z. cristobalensis*). Although our analyses were based on N-T distances, our overall conclusion concurs with the framework of Bradley and Baker (2001).

Finally, we suggest the possibility of additional subspecies within *P. furvus*. For example, ambiguities in the phylogenetic topologies were found by Harris *et al.* (2000) and Avila-Valle *et al.* (2012), as well as in haplotype networks presented herein. This is especially true for populations assigned herein to *P. f. angustirostris* to the north of Puebla (Huauchinango) and to the SE of the type locality (Zacualpan, Veracruz) in Hidalgo (*i. e*., Tlanchinol, Metepec, and Tenango de Doria). Sequences from the intermediate group localities are needed to resolve this issue. Further, sequences are needed from geographic areas such as Molango, Hidalgo, and from vegetational transition zones between Puebla and Veracruz (*e. g*., Rogers and Skoy 2011; Peralta-Moctezuma 2011).

Remarks for the furvus species group. Peromyscus furvus as currently conceived (*sensu lato*), contains multiple species and subspecies. The population of Puerto de la Soledad Oaxaca (GG4, *Oax18*) is a *species nova*, distinctive from *P. furvus* by its evolutionary divergence. This interpretation agrees with the genetic distances reported by Harris and Rogers (1999) for the allozyme PGDH, as well as with the possible biogeographic scenario proposed by Harris *et al.* (2000). *Peromyscus latirostris* from Xilitla, San Luis Potosí, and Santa Inés, Querétaro, is either a sister species to *P. furvus*, as suggested initially by Dalquest (1950), or is valid subspecies, *P. f. latirostris*. Our morphometric analyses (in prep.) strongly support the recognition of a separate species. Finally, populations along the intermediate zone (Hidalgo, Puebla, and Veracruz) represent at least two different subspecies, *P. furvus angustirostris* (as described by Hall and Álvarez 1961) and *P. f. furvus* (as described by J. A. Allen and Chapman 1897). Based on these findings, the *furvus* species group now comprises three species (*P. latistrostis*, *P. species nova*, and *P. furvus*) and at least two subspecies (*P. f. angustirostris* and *P. f. furvus*).

The pairing of *P. m. totontepecus* and *P. melanocarpus* agrees with the observation by Bradley *et al.* (2007) that *P. melanocarpus* did not seem to belong to the *furvus* species group, as it was more closely associated with species of the *mexicanus* species group (see Hooper 1968, Huckaby 1980). *Peromyscus ochraventer*, which also was considered a member of the *furvus* species group (Carleton 1989), behaved as an even more distant taxon from the *furvus* species group, as defined here and by others such as Wade *et al*. (1999) and Musser and Carleton (2005). Herein, *P. ochraventer* was more basal in all phylogenetic analyses, suggesting that this species is closer to the more derived *difficilis* species group (Durish *et al.* 2004; Bradley *et al.* 2007). The grouping of *M. cryophilus* with *P. ochraventer* in the OG is consistent with recent *Cytb* gene phylogenies, where this species and *M. thomasi* were genetically closer to *P. furvus* and the Oaxacan *species nova* (Bradley *et al.* 2007:1115) and to other subgenera such as *Podomys* and *Neotomodon* (Platt *et al.* 2015:712). Finally, except for the single molecular phylogenies with probabilistic methods in the NADH genes, *O. banderanus* was always basal and remained segregated from the other taxa. Its position at the base of the topology suggests an affiliation to the subgenus *Haplomylomys*, the basal most subgenus of *Peromyscus* in recent phylogenies of the genus (Bradley *et al.* 2004b, 2007; Platt *et al.* 2015).

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Appendix 1

Sequences of four mitochondrial genes examined in the analyses. Mitochondrial genes included 719 bases of the Cytochrome-*b* gene (*Cytb* gene), and 971 bases comprising three genes (ND3-ND4L-ND3) of the coenzyme 1, Nicotinamide Adenine Dinucelotide Hydride (NADH). Data are arranged by operational taxonomic units (OTUs), comprise in parenthesis: Genetic Groups (GG1–4) and Ingroup (IG), assigned according to its geographic distribution (Figure 1), and former taxonomic designations with its respective key in italics. The other five OTUs are Outgroups (OGs). After each type of mitochondrial gene, with its total sample size inside parenthesis, follows the Mexican State, number and name of group locality (GL#; see map in Figure 1b), and numbered specific localities (SLs) *sensu* Cruz-Gómez *et al*. (2018). An asterisk (*) indicates close GLs to topotypic populations (GG1–3) or the first sampled Oaxacan populations (GG4). Following the SLs the list continues with the codes for the sequences inside parenthesis (abbreviations of GLs names + specimens consecutive numbers: 1-n, where n = total number of sequences) and their GenBank accession numbers (GnBnk#). In the list, note that:

a. All 130 sequences were used in phylogenies and genetic analyses of variation and divergence, according to type of mitochondrial genes.

b. Sequences and codes inside brackets were used in the integrated molecular phylogenies and in haplotype networks (*Cytb*, GG1–4 *n* = 31, OGs *n* = 6; NADH genes, GG1–4 *n* = 29, OGs *n* = 5, Cruz-Gómez *et al*. 2018).

c. Sequences and codes in italics, were used in the molecular integrated phylogeny with 29 sequences in each type of mitochondrial genes and one sequence for each one of the five OGs.

The total 53 *Cytb* sequences in GG1–4 previously were analyzed by Harris *et al.* (2000), while the 13 sequences in the OGs were analyzed elsewhere (a, Bradley *et al.* 2007; b, Light *et al*. 2016; c, Pérez-Consuegra and Vázquez-Domínguez 2015). Likewise, the total 57 sequences for the three NADH genes in the IG were analyzed by Ávila-Valle *et al.* (2012), along with seven more sequences for the OGs, examined there and in other studies (d, Ávila-Valle *et al.* 2012; e, Wade 1999; f, Ávila-Valle *et al*. 2005). Colors in GG1–4 correspond to the visual code used in figures.

OTUs currently assigned to *Peromyscus furvus*:

OTU1 (GG1, *latirostris*, *Pl*). *Cytb* gene (*n* = 8). San Luis Potosí: GL1 Xilitla*. 3) Ejido Aguayo, 6.2 km N Xilitla*, [*Xili1–8*]: [*AF270981, AF270987, AF271001, AF271004, AF271006, AF271015, AF271016, AF271020*]. NADH genes (*n* = 17, 11). San Luis Potosí: GL1 Xilitla*. 3) Ejido Aguayo, 6.2 km N Xilitla*, Xili1–13: [*Xili1–8, JN885476, JN885477, JN885478, JN885479, JN885480, JN885481, JN885482, JN885483*], JN885484, JN885485, JN885486, JN885487, JN885488. Querétaro: GL2 Santa Inés. 15) El Pemoche [SaIn1, JN885489]; 22) 2.5 Km NW Santa Inés [SaIn2–3, JN885490, JN885491]; 23) 2 Km W Santa Inés [SaIn4–5, JN88549, JN885493].

OTU2 (GG2, *angustirostris*, *Pa*). *Cytb* gene (*n* = 29 [*n* = 14]). Hidalgo: GL4 Tlanchinol. 35) 3 km E Tlanchinol, Tlan1–7: [*Tlan1–5*, *AF270989, AF270998, AF270999, AF2701002, AF2701008*], AF2701023, AF2701028. Veracruz: GL6. Zacualpan*. 65) La Colonia, 6.5 km W Zacualpan*, [*Zacu1–3*, *AF270985, AF270993, AF2701021*]. GL7 Tenango de Doria. 80) El Potrero, 10 km SW Tenango de Doria, TeDo1–8: [*TeDo 1–4, AF270991, AF271003, AF271005, AF271009*], AF271010, AF271019, AF271024, AF271028. GL8 Metepec. 74) 21.8 km NE Metepec, [Metp1–2, AF271014, AF271026]. Puebla: GL10 Huauchinango. 82) Rancho El Paraíso, 6 km SW Huauchinango, Huau1–9: [Huau1–5, AF270982, AF270983, AF270984, AF270986, AF270988], AF270990, AF2701018, AF2701022, AF2701025. NADH genes (*n* = 24 [*n* = 11]). Hidalgo: GL3 Otongo, [Oton1–3, JN885501, JN885502, JN885503]. GL4. Tlanchinol. 35) 3 km E Tlanchinol, Tlan1–7: [*Tlan1, JN885494; Tlan2–4, JN885495, JN885496, JN885497, JN885498*], JN885499, JN885500. Veracruz: GL6 Zacualpan*. 65) La Colonia, 6.5 km W Zacualpan*, Zacu1–7: [*Zacu1–3 JN885504, JN885505, JN885506*], JN885507, JN885508, JN885509, JN885510. Hidalgo: GL7. Tenango de Doria. 80) El Potrero, 10 km SW Tenango de Doria, [*TeDo1–4: JN885511, JN885512, JN885513, JN885514*]. Puebla: GL9. Xicotepec de Juárez. 83) El Salto, [XiJu1–7, JN885515, JN885516, JN885517, JN885518, JN885519, JN885510, JN885521].

OTU3 (GG3, *furvus*, *Pf*). *Cytb* gene (*n* = 4). Veracruz: GL13 Xalapa*. 115) Banderillas, 6 km NW Xalapa* [*Xalp1–4: AF270980, AF271030, AF271031, AF27103*]. NADH genes (*n* = 11 [*n* = 10]). Veracruz: GL13b Mesa de la Yerba*. 120) Mesa de la Yerba*, MsYb1–6: [MsYb1-4, JN885522, JN885523, JN885524, JN885525], JN885526, JN885527. GL13a Xalapa**.** 116) 1.5 Km SE Banderillas*, Xalp1–5: [*Xalp1–4*, *JN885528, JN885529, JN885530, JN885531*], JN885532.

OTU4 (GG4, unknown *Peromyscus* from Oaxaca, Oax18). *Cytb* gene (*n* = 12 [*n* = 4]). Oaxaca: GL18 Puerto de la Soledad*. 157) 1.5 km S Puerto de la Soledad*, PtSl1–12: [*PtSl1–5,_AF270992, AF270994, AF270995, AF270996, AF270997*], AF271000, AF271007, AF271011, AF271012, AF271013, AF271017, AF271027. NADH genes (*n* = 4). Oaxaca. GL18 Puerto de la Soledad*. 150) Puerto de la Soledad*, [*PtSl–5: JN885533, JN8855534, JN8855535, JN8855536, JN885537*].

Outgroups

Congeneric species formerly assigned to the *furvus* species group (sister Outgroups):

OTU6 (*Peromyscus melanocarpus, Pml*). *Cytb* gene (*n* = 2 [*n* = 1]). Oaxaca: La Esperanza, *Pml*1–2: [*Pml, EF028173* [a]], CMC29192[a]. NADH genes (*n* = 2 [*n*= 1]). No data, [*Pml*1–2: [*Pml*: JN885472[d]], JN885473[d].

OTU7 (*P. ochraventer, Poc*). *Cytb* gene (*n* = 2 [*n* = 1]). San Luis Potosí: Las Abritas, *Poc*1–2: [*Poc, JX910119* [b]], DQ973106 [a]. NADH genes (*n* = 1). San Luis Potosí: Santa Isabel, [*Poc*: ---[e]]

Congeneric species of a close species group (close Outgroup)

OTU8 (*P. mexicanus totontepecus, Pmt*). *Cytb* gene (*n* = 4 [*n* = 2]). Veracruz: Zongolica; Misantla, Pmt1-4: [*Pmt*1–2, *Pmt*: *AY376425* [a], EF028174[a]], TTU82759[c], CNMA34309[c]. NADH genes (*n* = 2 [*n* = 1]). No data, *Pmt*1–2: [*Pmt: U83862*[f]], JN885471[f].

Non-congeneric species (farther Outgroups)

OTU9 (*Megadontomys cryophilus, Mcr*). *Cyt-b* gene (*n* = 2 [*n* = 1]). Oaxaca: Puerto de la Soledad, *Mcr*1–2: [*Mcr: DQ861373*[a]], BYU16076[a]. NADH genes (*n* = 1). Oaxaca: Puerto de la Soledad, [*Mcr*, *DQ793119*[d]].

OTU10 (*Osgoodomys banderanus, Oba*) – root. *Cytb* gene (*n* =3 [*n* = 1]). Michoacán: Coalcomán, *Oba*1–2, *Oba*3: [*Oba: DQ000473*[a]], TK45952[a], EF98985[a]. NADH genes (*n* = 1). No data, *Oba*1: [*Oba, U83860*[d]].

Geographic representation and sample sizes, according to scientific collection, in the morphometric analyses. Due to the restrictions imposed by the COVID-19 pandemic, the catalog numbers of the specimens examined are not shown in this list and only the number of specimens in each locality is referred to. Authors can send catalog numbers later upon request, when the respective scientific collections can be accessed. This list follows the same overall arrangement as the previous list for sequences, beginning with OTUs (with the genetic group, GG1–5 in the Ingroup), taxonomic or geographic designation, and key inside parenthesis. The geographic data, organized in a NW-SE direction, begin with the GLs´ number and name (GL1–18; see Figure 1b), with sample sizes inside parenthesis, followed by the State and specific localities (SLs) with the number of examined specimens and collection acronyms inside parenthesis. Numbers for GLs and SLs are from Cruz Gómez *et al*. (2018). SLs with an asterisk (*) refer to closer records to the type localities of GG1–3, or to the first records in Oaxaca for GG4–5. The specimens are hosted at Universidad Autónoma Metropolitana, Iztapalapa Unit (UAMI), and at the Museum of Zoology "Alfonso L. Herrera", Facultad de Ciencias, Universidad Nacional Autónoma de México (MZFC-M). Total sample sizes, according to analyses, and character coding in the list (Brackets, italics) is as follows:

a) A total of 233 skull in SLs were examined in the traditional morphometric analyses for overall skull size, using 18 linear measurements; their sample sizes follow the geographic data inside parenthesis.

b) From the former, a total of 217 skulls in perfect condition (complete, not damaged, and clean), were also used in morphogeometric analyzes, using five morphogeometric configurations of ventral shape of skull; their sample sizes are mentioned inside brackets. If the same skulls were used in both morphometric analyses, the sample sizes appears only once after the SLs, inside brackets.

c) Finally, a selected set of 24 skulls were sequenced for all mitochondrial genes. The obtained sequences were used in the integrated phylogeny with all kind of characters; their sample sizes appear in italics inside brackets. These specimens are linked with the previous list by its GLs' code (abbreviation of GL name and number of sequences, *1-n*, where the *n* is total sample size).

OTUs currently assigned to *Peromyscus furvus* (Ingroup):

OTU1 (GG1, *latirostris, Pl, n* = 36 [n =; *Xili1–8*]). GL1 Xilitla*(*n* = 20 [*n* = 16]). San Luis Potosí: 8) Apetzco, 0.5 Km N, 2 Km W Xilitla, San Luis Potosí* (*n* = 9 [*n* = *3*] UAMI). 16) Km 241 carretera Xilitla, El Rizal (*n* = 7 [*n* = *3*] MZFC); 19) 11 Km S, 8 Km W Xilitla (*n* = 4 [*n* = *2*] UAMI). GL2 Santa Inés [*n* = 16]. Querétaro [*n* = 14]: 15) El Pemoche [n = 4 MZF]); 21) 2.8 Km 324 NW Santa Inés [*n* = 3 MZFC]; 22) 2.5 Km NW Santa Inés [*n* =5 MZF]; 23) 2 Km W Santa Inés [n = 2 MZFC]. Hidalgo [*n* = 2]: 20) 13.5 Km SE Pisaflores [*n* = 1 MZFC); 25) 3 km S Santa Ana de Allende [1 UAMI].

OTU2 (GG2, *angustirostris, Pa*, n = 100 [n = 96, *n* = *14*]). GL3 Otongo (*n* = 20 [n = 19]): Hidalgo: 29) 1.5 Km N Chilijapa [*n* = 5 UAMI]; 33) 4 km N Tepehuacán de Guerrero [*n* = 1 UAMI]; 34) 1 Km N Chilijapa, (*n* = 1 UAMI); 46) 1 Km S, 3.5 Km W Otongo [*n* = 2 UAMI]. 48) Km S, 6 Km W Otongo [*n* = 3 UAMI]. GL4 Tlanchinol (*n* = 18 [*n* = 17; *Tlan1–5*]). Hidalgo: 28) 5 Km N, 1.5 km E Tlachinol (*n* = 1 UAMI); 31) 4 Km N, 2 km E Tlachinol [*n* = 5 UAMI]; 32) 4 Km N, 1.5 Km E Tlanchinol [*n* = 1 UAMI]; 35) 3 Km N 1 Km E Tlachinol [*n* = 3 UAMI]; 36) 2.5 Km N, 1.5 O Tlachinol [*n* = 3 UAMI]; 43) 10 km N Carr. Tehuatlan-Huazalingo [*n* = 4 MZFC]; 44) 10 Km NW Tehuatlan, Car. Tehuatlan-Huazalingo [*n* = 1 MZFC]; 45) 1.5 Km S, 3.8 Km W Tlachinol [*n* = 4, *n* = *2* UAMI]; 47) 2 Km S, 3 Km W Tlanchinol [*n* = 6, *n* = *5* UAMI]. GL5 Molango [*n* = 1]. Hidalgo: 52) Tianguistengo [*n* = 1 UAMI]. GL6 Zacualpan* [*n* = 10, *Zacu1–3*]. Veracruz: 61) 9 Km W Zacualpan [*n* = 6 MZFC]; 62) 1 Km E Zacualpan* [*n* = 4, *n* = *3* MZFC]. GL7 Tenango de Doria (*n* = 34 [*n* = 33, *TeDo1–4*]). Hidalgo: 69) San Bartolo, Tutotepec [*n* = 1 MZFC]; 70) San Bartolo, Cueva El Cirio [*n* = 6 MZFC]; 76) El Texmé [*n* = 2 MZFC]; 78) Tenango de Doria (*n* = 20 [*n* = 19, *n* = *4* MZFC]; 80. Tenango de Doria, el Potrero [*n* = 5 MZFC]. GL8. Xicotepec de Juárez [*n* = 5, 5]. Puebla: 77) Xicotepec de Juárez, El Salto [*n* = 5 MZFC]. GL9. Huauchinango [*n* = 6]. Puebla: 82) 8 Km N Huauchinango [*n* = 1 UAMI]; 84) 0.2 Km N Honey [*n* = 2 UAMI]; 93) 5.6 KM SW Huachinango [*n* = 4 MZFC]. GL10 Zacapoaxtla [*n* = 6]. Puebla: 95) 5.5 Km N Zacapoaxtla [*n* = 4 UAMI]; 96) 5 Km N Zacapoaxtla [*n* = 2 UAMI].

OTU3 (GG3, *furvus, Pf*, *n* = 47 [*n* = 45]). GL11. Las Minas (*n* = 0). Veracruz (with no morphometric specimens in either UAMI or MZFC). GL12 Naolinco (*n* = 22 [*n* = 21]). Veracruz: 103) 4 Km N Naolinco [*n* = 2 UAMI]; 104) 1 Km W Tlacolulan [*n* = 6 UAMI];

106) 1 Km S Tlacolulan [*n* = 5 UAMI]; 112) 8 Km SW Naolinco [*n* = 9 UAMI]. GL13 Xalapa* (*n* = 4 [*n* = 3])*. Veracruz: 116. *1.5 Km SE Banderillas** [*n = 4* UAMI]. GL14 Ixhuacán de los Reyes [*n* = 12]. Veracruz: 129) 2 Km NO Ixhuacán de los Reyes (*n* = 5 UAMI); 130) 1 Km W Ixhuacán de los Reyes [*n* = 7 UAMI]. GL15 Coscomatepec [*n* = 9]. Veracruz: 132. 1.5 Km SE Quimixtlán [*n* = 5 UAMI]; 138) 5.5 km N, 6 km E Coscomatepec [*n* = 4 UAMI].

OTU4 (GG4, undescribed *Peromyscus*, *Oax18,* [*n* = 25, *PtSl1–5*]). GL18 Puerto de la Soledad [*n* = 25]. Oaxaca*: 148) 5 Km N, 1 Km W Huautla [*n* = 1 UAMI]; 152) 3 km N, 1 Km Huautla [*n* = 1 UAMI]; 151) Teotitlán, Puerto de la Soledad, Huehuetlán [*n* = 22, *n = 5* MZFC]; 166) San Juan Bautista, Cuicatlán. El Venado [*n* = 1 MZFC].

OTU5 (GG5, undescribed *Peromyscus, Oax19*, [*n* = 19]). GL19. La Esperanza, Oaxaca* [*n* = 19]: 169) 5 Km S, 3 Km W La Esperanza [*n* = 9 UAMI]; 170) 2.5 Km N, 1 Km E La Esperanza [*n* = 10 UAMI].

OTUs in the Outgroups:

a. Congeneric species formerly assigned to the *furvus* species group

Peromyscus ochraventer, Poc [*n* = 26]. Tamaulipas: 174) 8 KM NW Gómez Farías [*n* = 26 UAMI].

Peromyscus melanocarpus, Pml [*n* = 22]. Oaxaca: 182) 3.5 Km Santa María Pápalo, 2500 m, [*n* = 22 UAMI].

b. Congeneric species of a close species group:

Peromyscus mexicanus totontepecus, Pmt [*n* = 36]. Oaxaca: 177) 8 Km NW Huatla, 1150 m, [*n* = 6 UAMI]; 178) 3 km N, 1 Km W Huautla, 1140 m [*n* = 16 UAMI]; 180) Huatla, 1130 m [*n* = 6 UAMI]; 183) 5 Km N, 1 Km W Huautla, 1120 m [*n* = 8 UAMI].

c. Non-congeneric species

Megadontomys criophylus, Mcr [*n = 14*]**.** Oaxaca: 176) 5 Km S, 3 Km W La Esperanza, 1950 m [*n* = 4 UAMI]; 184) 2.5 Km N, 1 Km E La Esperanza, 1850 [*n* = 9 UAMI].

Osgoodomys banderanus, *Oba* [*n* = 18]. Michoacán: 175) Arteaga "Charco del Toro" [*n* = 8 MZFC]; 179) Lázaro Cárdenas «El Habillo" [*n* = 5 MZFC]; 181) Lázaro Cárdenas «La Bonetera» [*n* = 5 MZFC].

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Morphological differentiation of *Peromyscus leucopus* and *P. maniculatus* in East Texas

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The white-footed deer mouse (*Peromyscus leucopus*) and the North American deer mouse (*P. maniculatus*) are widely distributed throughout North America, often with overlapping distributions. These species are believed to be sympatric east of the Balcones fault zone in Texas, but records from natural history collections indicate that *P. maniculatus* is not common from this region. Given that these two species are notoriously difficult to differentiate morphologically, it is possible that specimens have been incorrectly identified and that *P. maniculatus* may be rare or not present in East Texas. This study aims to determine if *P. leucopus* and *P. maniculatus* can be differentiated morphologically east of the Balcones fault zone in Texas. Cranial and external characters from genetically identified specimens representing each species were analyzed using traditional and geometric morphometric methods. Morphological analyses revealed that genetically identified specimens of *P. leucopus* and *P. maniculatus* from east of the Balcones fault zone could be differentiated using a suite of morphological characters. Many of the specimens of *P. leucopus* used in this study were originally misidentified, suggesting that *P. maniculatus* is rare in East Texas.

El ratón ciervo de patas blancas (*Peromyscus leucopus*) y el ratón ciervo norteamericano (*P. maniculatus*) estan ampliamente distribuidos por toda Norteamérica, frecuentemente con distribuciones superpuestas. Se cree que en la región este de la falla de Balcones, Texas estas especies son simpátricas, pero los registros de su historia natural indican que *P. maniculatus* no es común en esta región. Debido a que estas dos especies son notoriamente difíciles de diferenciar morfológicamente, es posible que los especímenes hayan sido identificados incorrectamente y que *P. maniculatus* sea rara o pueda no estar presente en el este de Texas. Este estudio pretende determinar si *P. leucopus* y *P. maniculatus* pueden diferenciarse morfológicamente en la zona del este de la falla de Balcones, Texas. Los caracteres craneales y externos de especímenes identificados genéticamente que representan cada especie fueron analizados utilizando métodos morfométricos tradicionales y geométricos. Los análisis morfológicos revelaron que los especímenes genéticamente identifacados de *P. leucopus* y *P. maniculatus* del este de la zona de la falla de Balcones podrían diferenciarse utilizando un conjunto de caracteres morfológicos. Muchos de los especímenes de *P. leucopus* usados en este estudio fueron identificados erróneamente, lo que sugiere que *P. maniculatus* es raro en el este de Texas.

Keywords: Balcones fault zone; geometric morphometrics; species differentiation; traditional morphometrics.

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Introduction

Rodents belonging to the genus *Peromyscus* have been called the "*Drosophila* of North American mammalogy" (Musser and Carleton 1993), and as a model system, they have long been the focus of ecological, evolutionary, systematic, and biogeographic research (*e. g.*, King 1968; Harney and Dueser 1987; Kirkland and Layne 1989; Wolff 1996; Berl *et al.* 2017; Bedford and Hoekstra 2015; Lewarch and Hoekstra 2018). *Peromyscus* species also are of public-health interest due to their ability to serve as reservoirs for a variety of pathogens and viruses, such as hantaviruses and Lyme disease, that can be transmitted to humans (*e. g.*, Rand *et al.* 1993; Childs *et al.* 1994; Schmaljohn *et al.* 1995; Song *et al.* 1996; Drebot *et al.* 2001; Oliver *et al.* 2006; Larson *et al.* 2018). Given the importance of these species to a wide variety of scientific fields, it is essential to identify and differentiate *Peromyscus* species accurately. However, these ecologically and medically important species are morphologically variable across their geographic range (Dice 1940), thus making accurate identification difficult.

Rigorous analytical techniques may be necessary to differentiate morphologically similar taxa. Two techniques commonly used to separate organismal groups based on morphology are traditional (linear) morphometrics and two-dimensional (2D) geometric morphometrics. Traditional morphometrics focus on linear-distance measurements of traits (usually obtained using calipers) and often incorporate size components. In contrast, geometric morphometrics is a method that primarily captures variation in shape (Rohlf and Slice 1990; Slice 2007) and requires advanced imaging of specimens from various views (*e. g.*, ventral, dorsal, and lateral views) followed by careful placement of morphological landmarks on the image. The geometric relationships of these landmarks are then analyzed, allowing an independent analysis of shape after removing the influence of size, position, and orientation in landmark data (Rohlf and Marcus 1993; Adams *et al.* 2004). Geometric morphometrics are believed to have multiple benefits over traditional morphometric approaches, such as better visualization of among-group differences and provision of addi-

tional information for analysis (Breno *et al.* 2011). Traditional morphometrics, however, are generally more readily accessible in terms of data acquisition and have a demonstrated record of successfully differentiating taxa morphologically.

Two *Peromyscus* species that have been the subject of many studies involving morphological differentiation are the white-footed deer mouse (*P. leucopus*) and the North American deer mouse (*P. maniculatus sensu lato*; Bradley *et al.* 2019; Greenbaum *et al.* 2019). These species are distributed widely throughout North America, often with overlapping distributions (Kirkland and Layne 1989). Although not close phylogenetic relatives (Platt *et al.* 2015) and placed in different (but sister) species groups, *P. leucopus* and *P. maniculatus* are morphologically similar (Hall 1981), with tail length, extent of tail bicoloration, pelage color, hind-foot length, and ear size commonly used to differentiate these two species (*e. g.*, Palas *et al.* 1992; Bruseo *et al.* 1999). However, both external (*e. g.*, tail length) and cranial characters are geographically variable (*e. g.*, Osgood 1909; Choate 1973; Choate *et al.* 1979; Hall 1981; Dalquest and Stangl 1983; Myers *et al.* 1996; Pergams and Ashley 1999; Pergams and Lacy 2008; Grieco and Rizk 2010; Holmes *et al.* 2016; Millien *et al.* 2017). This geographic variation may result in higher likelihoods of species misidentification. Thus, genetic or molecular means of identification is often necessary to confidently identify morphologically similar species such as *P. leucopus* and *P. maniculatus* (*e. g.*, Aquadro and Patton 1980; Feldhamer *et al.* 1983; Rich *et al.* 1996; Sternburg and Feldhamer 1997; Bruseo *et al.* 1999; Reed *et al.* 2004; Tessier *et al.* 2004; Ridenhour and Cramer 2015; Seifert *et al.* 2016).

In Texas, the distributions of *P. leucopus* and *P. maniculatus* are thought to overlap throughout much of the state, often making species identification difficult (Schmidly and Bradley 2016). An examination of specimens on VertNet (9 November 2020) suggests that *P. leucopus* is far more common throughout the state than *P. maniculatus* (Figure 1). For example, there are 8,350 specimens of *P. leucopus* in collections, compared to 3,603 specimens of *P. maniculatus*, with *P. leucopus* recorded from 198 of Texas' 254 counties (78 %; Figure 1a) and *P. maniculatus* recorded from 159 counties (63 %; Figure 1b). The Balcones fault zone (Figure 1) divides the state into distinct western and eastern regions, which are further divided into four regions based on the ecological distribution of mammals: the Trans-Pecos and Plains Country west of the fault zone and East Texas and the Rio Grande Plains including and east of the fault zone (Schmidly 1983; Davis and Schmidly 1994; Schmidly and Bradley 2016; Figure 1c). These regions differ in climate, precipitation, flora, and fauna and many species meet their western or eastern limits at the Balcones fault zone (*e. g.*, Smith and Buechner 1947; Gehlbach 1991). According to Schmidly and Bradley (2016), approximately 18 terrestrial-mammal species occur primarily west of the Balcones fault zone, 13 species principally occur east of the Balcones fault zone, and 31 species (including *P. leucopus* and *P. maniculatus*) are distributed throughout the state. However, specimens from natural history collections, indicate that *P. maniculatus* is less common in East Texas (Figure 1) and is perhaps even rarer than is perceived given the difficulty in accurately identifying *Peromyscus* species.

A major objective of this study is to determine if *P. leucopus* and *P. maniculatus* of East Texas can be differentiated morphologically based on reference samples of genetically identified specimens of each species. This study also compares the utility of traditional (linear) and geometric morphometrics for differentiating *P. leucopus* and *P. maniculatus* and describes general morphological variation present in these species from East Texas. Lastly, the distribution of *P. maniculatus* east of the Balcones fault zone will be reassessed.

Materials and Methods

Specimens examined. Specimens, primarily from East Texas, were obtained from Angelo State University Natural History Collections, Texas A&M University Biodiversity Research and Teaching Collections, and The Museum of Texas Tech University (*n* = 61; Suplementary material 1). These specimens were identified to species by the collector or the natural history collection. To determine if traditional or geometric morphometric analyses could confidently differentiate *P. leucopus* and *P. maniculatus* from East Texas, only specimens from which genetic data were obtained (with four exceptions; see below) were included in the analyses.

Laboratory methods. Frozen tissues (stored at -20ºC) or destructive samples of toe clips, skin snips, or rib bones (stored at room temperature) were subjected to molecular assessment. DNA was extracted from frozen tissues using an Omega Bio-Tek E.Z.N.A. Tissue DNA Kit (Omega Bio-Tek, Norcross, Georgia) following manufacturer's instructions. For destructive samples of specimens from natural history collections, all DNA extractions were performed using a QIAmp DNA Micro Kit (QIAGEN Inc., Valencia, California) in a dedicated laboratory for historical samples; this laboratory was free of recent DNA and subjected to rigorous sterilization protocols to prevent contamination. DNA extractions of historical specimens were performed following manufacturer's instructions but also included a 24-h presoak in a 1x phosphate-buffered saline solution.

Because the mitochondrial cytochrome-*b* (*Cytb*) gene is one of the most frequently amplified and sequenced mammalian markers and is useful for differentiating *Peromyscus* species (*e. g.*, Zheng *et al.* 2003; Dragoo *et al.* 2006; Lucid and Cook 2007; Gering *et al.* 2009; Kalkvik *et al.* 2012; Greenbaum *et al.* 2017), fragments of this gene were targeted for genetic assessments of specimens included in this study. For DNA extractions of frozen tissues, a 414 base pair (bp) fragment of *Cytb* was amplified using the primers MVZ04 and MVZ05 (Smith and Patton 1991) following Benedict *et al.* (2019). Two fragments of *Cytb* from destructive samples were amplified using primers designed from alignments of *P. leucopus* and *P. maniculatus*. The first fragment amplified a *ca.* 163 bp fragment using prim-

ers Pero53F (5'-AATGAATCCTTCATTGATCTCCCCAC-3') and Pero216R (5'-GTAGTTKACGTCTCGGCAGAT-3') and the second fragment amplified a ca. 130 bp fragment using primers Pero268F (5'-GAGCCTCAATATTCTTYATCTGCTT-3') and Pero402R (5'-GATATTTGTCCTCATGGRAGTACAT-3'). In reference to the full *Cytb* gene, the 5' nucleotide of Pero53F, Pero216R, Pero268F, and Pero402R occur at base 43, 206, 257, and 392, respectively (determined via alignments of lab generated sequences to *P. leucopus* GenBank number KY064165 and *P. maniculatus* GenBank number EF666219). PCR cycling parameters for *Cytb* fragments from DNA from frozen tissues were initialized with a 5-minute denaturation step at 95°C, 35 cycles of 95°C (30 s), 52°C (60 s), and 72°C (90c), and a final extension of 72°C for 5 min. Cycling parameters were similar for DNA obtained from historical samples except for five additional cycles and an annealing temperature of 45°C (or 43°C if fragments failed to amplify). All amplified fragments (amplification success was determined via gel electrophoresis) were purified using ExoSAP-IT (USB Corporation, Cleveland, Ohio) and sequenced in forward and reverse directions using the primers listed above and ABI Prism BigDye Terminator cycle sequencing protocols (New Haven, Connecticut) at the DNA Analysis Facility on Science Hill at Yale University. Sequences were edited using Sequencher 4.10 (GeneCodes Corporation, Ann Arbor, Michigan) and compared to published sequences using the National Center for Biotechnology Information Basic Local Alignment Search Tool (BLAST). Top BLAST hits were used to determine species identifications. In our case, given the high prevalence of *P. leucopus* and *P. maniculatus Cytb* sequences on GenBank, there were > 20 sequences producing significant alignments with > 98 % identity and > 80 % query coverage when we performed our searches. All sequences were deposited to GenBank (Supplementary material 1). In total, 37 specimens of *P. leucopus* from 13 counties (from Denton Co. south to Willacy and Starr Cos.), and 24 specimens of *P. maniculatus* from seven counties (from Denton Co. south to Aransas Co.; Figure 1c; Appendix 1) were genetically identified to species and included in the morphological analyses. Four specimens of *P. maniculatus* failed to amplify for both *Cytb* fragments (three from Robertson Co. and one from Caldwell Co.); however, successful amplification and sequencing occurred for a minimum of five other specimens from the same or nearby collection locality (Supplementary material 1). We are therefore confident of their species identity and these specimens were retained for morphological analyses. Two of the 37 specimens of *P. leucopus* (TCWC 63781 and 63951) were identified to species by another mitochondrial gene (NADH dehydrogenase 2; from another study conducted by JEL). Laboratory methods and results of BLAST searches were similar to what was described above.

Morphological analysis. Morphological analyses were conducted using both traditional (linear) and 2D geometric morphometric techniques. All genetically identified specimens were adults, as determined by their complete dental eruptions and cheek tooth cusp patterns and wear (Koh and Peterson 1983; Rich *et al.* 1996). Sixteen standard morphological measurements were taken directly from the specimen tag (external measurements) or using digital calipers: total length (ToL), tail length (TL), hindfoot length (HL), ear length (EL), depth of braincase (DB), diastema length (DIA), length of incisive foramen (IFL), interorbital constriction (IOC), length of auditory bulla (LAB), mastoid breadth (MB), molar tooth row (MTR), nasal length (NL), occipital-incisor length (OIL), occipital-nasal length (ONL), post-palatal length (PPL), rostral width (RW), and zygomatic breadth (ZB; Figure 2). In some analyses described below, the ratio of tail length to head-body length (TL:TBL) was examined. We recognize that measurements recorded on specimen tags may not always be correct (especially if taken by an inexperienced collector). However, for the purposes of this study, we have accepted them as-is.

Prior to geometric morphometric analyses, specimens were photographed in ventral and lateral cranial views (Figure 2; Appendix 1). Landmark locations were selected, in part, based on previous analyses used to discriminate *P. leucopus* and *P. maniculatus* (*e. g.*, Myers *et al.* 1996; Grieco and Rizk 2010; Millien *et al.* 2017). Both traditional characters and landmark locations were selected to emphasize rostral length and width, tooth arrangement, and zygomatic breadth, regions of cranial morphology known to differ between *Peromyscus* species (Rich *et al.* 1996; Millien *et al.* 2017). All landmarks were placed using tpsDig2 (Rohlf 2001). The number of specimens used in each morphological analysis (i.e., dataset) described below varied as broken specimens or those missing landmark locations were removed from the analyses (traditional morphometrics cranial and external characters: $n = 29$ *P. leucopus* and 20 *P. maniculatus*; traditional morphometrics cranial characters only: $n = 34$ *P. leucopus* and 22 *P. maniculatus*; traditional morphometrics external characters only: $n = 32$ *P. leucopus* and 22 *P. maniculatus*; geometric morphometrics ventral view: $n = 35$ *P. leucopus* and 23 *P. maniculatus*; geometric morphometrics lateral view: $n = 35$ *P. leucopus* and 18 *P. maniculatus*; traditional morphometrics cranial and external characters combined with geometric morphometrics ventral and lateral views: $n = 27$ *P. leucopus* and 16 *P. maniculatus*; Supplementary material 1); analyses including external characters were run using either ToL and TL separately or the TL:TBL ratio.

All traditional morphometric characters were transformed logarithmically to decrease the effect of individual size variation (Gould 1966; dos Reis *et al.* 1990) and assessed for normality; no extreme outliers were identified and there were no significant departures from a normal distribution for any of the measured characters. Similar to the findings of previous studies (*e. g.*, Kamler *et al.* 1998; Pergams and Lacy 2008), secondary sexual dimorphism was not found to be associated with any of the cranial traits in either species ($P > 0.05$ in all Welch's unpaired *t*-tests of the log-transformed traditional characters); therefore, males and females were pooled in subsequent analyses of traditional morphomet-

ric characters. In the geometric morphometric analyses, a Procrustes superimposition was applied to remove non-shape related variation associated with location, rotation, and scale on all raw landmark data (Lawing and Polly 2010; Zelditch *et al.* 2012). Procrustes-corrected data were ordinated using a principal component analysis (PCA). Principal component (PC) scores were extracted from these analyses as independent components of shape variation. As with the traditional morphometric data, secondary sexual dimorphism was not detected in the geometric morphometric datasets as assessed using multivariate analyses of variance (MANOVA) in association with the PC scores of each species (ventral view: $F = 0.73$, $P > 0.05$, lateral view: $F = 0.99$, $P > 0.05$); therefore, all geometric morphometric analyses were conducted with pooled sexes. Significant size-related allometry was observed using a linear regression on the geometric morphometric datasets (ventral view: $F = 3.29$, $P < 0.01$; lateral view: $F = 3.50$, $P < 0.01$). This allometric relationship did not differ significantly between the species as assessed using a multiple linear regression (ventral view: $F = 0.58$, $P > 0.05$, lateral view: $F = 1.86$, $P > 0.05$; Appendix 2); geometric morphometric analyses were conducted using both allometry-minimized and non-allometry-minimized residuals. Both traditional and geometric morphometric analyses were conducted using R version 3.6.3, with the *MASS* and *geomorph* packages (Venables and Ripley 2002; Adams *et al.* 2020; R Core Team 2020).

Welch's unpaired *t*-tests were performed to assess differentiation between species for each individual traditional morphometric character; Bonferroni corrections were included to account for the number of individual tests. PCAs were performed on the log-transformed traditional morphometric variables using a covariance matrix (the scales of the variables are standardized after log-transformation; Croux and Haesbroek 2000). MANOVAs were conducted on both traditional and geometric morphometric datasets using PC scores to detect differentiation between species and analyses of variance (ANOVAs) were used to detect the specific PCs that differed between species. Discriminant function analyses (DFAs) were performed to determine if specimens could be separated based on the *a priori* hypothesis of group membership to genetically identified species. Leave-one-out cross-validation linear discriminant function analyses (DFA-CVs) also were performed to determine if *a priori* group membership could be appropriately predicted. In these analyses, individual specimens were assessed in an iterative process, removing each specimen from the training dataset to estimate the likelihood that it is included within either *P. leucopus* or *P. maniculatus* based on its morphology. Both DFAs and DFA-CVs were performed on all datasets (with allometry-minimized and non-allometry-minimized residuals for the ventral and lateral cranial views) as well as combined geometric and traditional morphometric datasets. For analyses including linear measurements from the traditional morphometric datasets, both the PCs of these linear measurements (as in geometric morphometrics) as well as the log transformed data were examined. For all DFAs and DFA-CVs, specimens were assigned posterior probabilities (pp) of membership to *P. leucopus* or *P. maniculatus* based on Mahalanobis distance.

Multiple logistic regressions were conducted to examine the relationship between specimen misidentification and morphology (for both traditional morphometric traits and PC scores extracted from geometric morphometric analyses). Specimen misidentification was tabulated for each specimen as a binary 'yes' or 'no' based on the specimen's genetic identification and the identification originally assigned by the collector or natural history collection.

To further examine the distribution of *P. maniculatus* in Texas, data were downloaded from VertNet (accessed 17 January 2021) for all specimens with the county of collection and external measurement information ($n = 386$). These were then further classified as either likely correct species identification (multiple localities of multiple individuals for

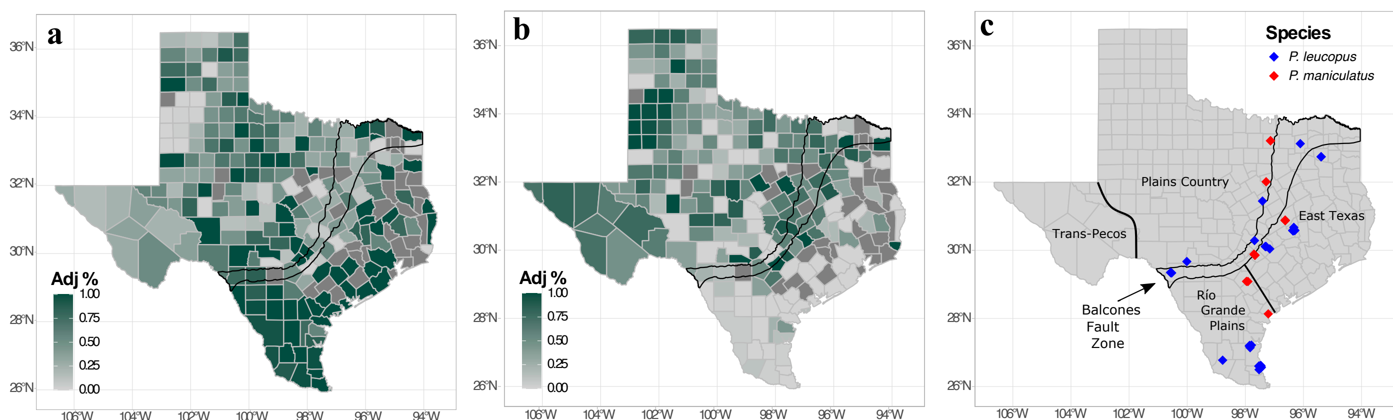

Figure 1. Heat map of specimens of *Peromyscus leucopus* and *P. maniculatus* across Texas and counties sampled per species. Percentage of specimens per county were calculated based on specimens in natural history collections (data obtained from VertNet on 9 November 2020; assumes all specimen identifications are correct). Percentages were calculated from the total number of specimens of each species across the entire state. Heat maps were generated as follows: a) percentage of *P. leucopus* divided by the sum of percentages of *P. leucopus* and *P. maniculatus* and b) percentage of *P. maniculatus* divided by the sum of percentages of *P. leucopus* and *P. maniculatus.* These adjusted percentages demonstrate the relative prevalence of specimens collected within Texas counties. c) Specimens of *P. leucopus* and *P. maniculatus* examined in this study indicated by blue and red diamonds, respectively. The Balcones fault zone is indicated with a black border.

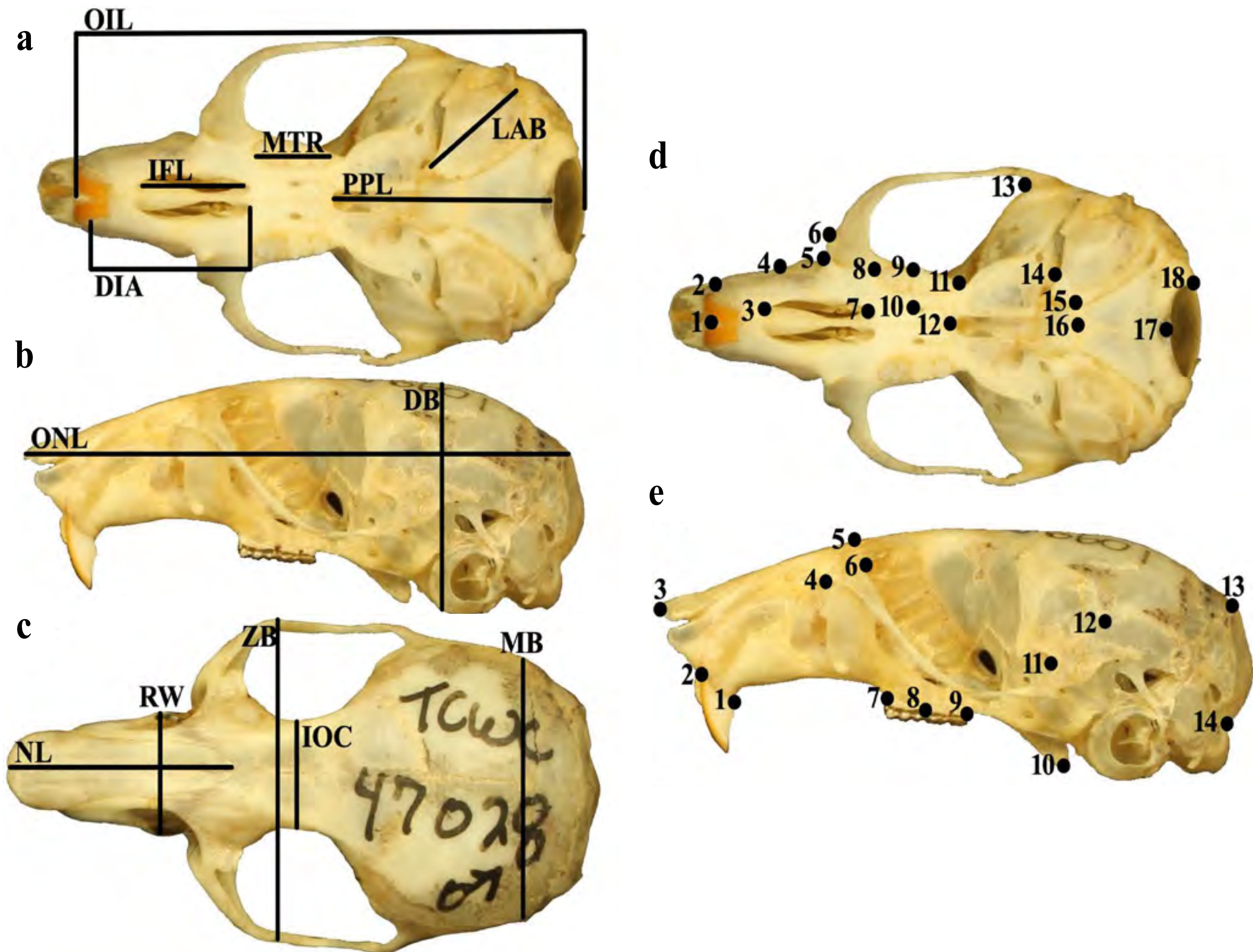

Figure 2. Traditional characters and landmark schemes used in morphometric analyses of *Peromyscus* specimens. Traditional morphological characters were obtained from a) ventral, b) lateral, and c) dorsal views of the skull (see text for definitions of abbreviations). Landmark schemes used in geometric morphometric analyses were obtained from d) ventral and e) lateral views of the skull (see text for anatomical definitions of landmarks).

that county) or "suspect" (only a single locality, often with only a single individual, for that county). This "suspect" classification based on county occurrence was considered reasonable due to the rather constant geographic area of counties in eastern Texas, forming a nearly uniform grid: the 126 counties of eastern Texas (as defined here; Figure 1) average 2,302 km^2, or about 50 km on a side, and a single county record could reasonably be considered of suspect identification. Suspect specimens were included as unassigned specimens in a DFA (SYSTAT 7.0, Wilkinson 1997) based on external measurements (ToL, TL, HL, and EL) with the genetically identified reference samples. Suspect specimens were assigned posterior probabilities (pp) of membership to the two reference groups based on Mahalanobis distance and grouped for comparison with those reference samples into geographic groups representing *P. leucopus* and the Texas subspecies of *P. maniculatus*: *P. m. pallescens* in East Texas, *P. m. blandus* in West and South Texas, and *P. m. luteus* in the Texas Panhandle. Specimens were assigned to subspecies of *P. maniculatus* based on geographic distribution, and differences among these taxa and the genetically identified reference groups were assessed using Tukey's honestly significant difference post hoc tests.

Results

Traditional morphometrics. Welch's Two-Sample *t*-tests indicated a significant difference between *P. leucopus* and *P. maniculatus* in many traditional linear characters. All cranial and external traits except length of auditory bulla (LAB) and length of incisive foramen (IFL) were significantly different between species ($P < 0.001$; Figure 3; Appendix 3). The first principal component (PC1) associated with the traditional characters accounted for 60.81 % of the total variation, all coefficients had the same sign, and occipital-nasal length (ONL) and occipital-incisor length (OIL) had the highest loadings (eigenvalue of PC1 = 9.73; Figure 4; Appendix 4). PC2 of the traditional morphometric dataset accounted for 9.75 % of the total variation and was primarily associated with length of auditory bulla (LAB) and length of incisive foramen (IFL; Figure 4; Appendix 4). MANOVA results indicated that PC scores of *P. leucopus* and *P. maniculatus* were significantly different ($P < 0.001$), including a significant difference between species associated with PC1 ($P < 0.001$) and PC2 ($P < 0.05$).

Geometric morphometrics.—Geometric morphometric analyses also detected a significant difference between *P.*

leucopus and *P. maniculatus* based on cranial size and certain axes of morphology. In the ventral view, PC1 accounted for 18.46 % of the overall variation in the allometry-minimized dataset and was primarily related to the relative skull length and cheek-tooth arrangement, whereas PC2 accounted for 13.34 % of the variation and was primarily associated with the relative rostral length and skull width (Figure 4). In the lateral view, PC1 accounted for 27.94 % of the variation in the allometry-minimized dataset and was associated with cranial width and depth and PC2 accounted for 17.69 % of the variation and was associated with skull length and post-dental cranial width (Figure 4). Results were similar for analyses of non-allometry-minimized datasets (data available upon request). MANOVAs associated with the allometry-minimized ventral- and lateral view analyses failed to detect a significant overall morphological difference between the species ($P > 0.05$), although this relationship was significant with the non-allometry-minimized cranial views ($P < 0.0001$; Appendix 5). ANOVAs of individual allometry-minimized PCs detected several axes of morphological differentiation between the species, including PC1 of the ventral view ($P < 0.01$) and PC2 and PC4 of the lateral view (both $P < 0.05$). Results were similar for ANOVAs of non-allometry-minimized datasets (data available upon request).

Specimen misidentification and distribution of P. maniculatus *in Texas*.—Genetic analyses revealed that 21 of 61 (34.43 %) specimens in our dataset, all *P. leucopus*, had previously been misidentified; 14 of these misidentified specimens are from east of the Balcones Escarpment (Appendix 1). Discriminant function analysis (DFA) on various suites of morphological characters correctly classified (posterior probability, or pp, = 1) the majority of specimens of *P. leucopus* and *P. maniculatus* to species, regardless of the dataset analyzed (*e. g.*, cranial characters only, cranial and external characters, and combined datasets; Table 1). For DFAs of traditional morphological characters, results were similar whether PCs or log-transformed data were used, or if total length (ToL) and tail length (TL) were analyzed separately or as part of the tail length to head-to-body length ratio (Table 1). ONL, OIL, and ToL tended to have the highest factor loadings. In DFAs of exclusively external characters, TL had the highest factor loadings and no specimens were misclassified. However, in analyses of log-transformed external data, there was low certainty in the classification of three specimens of *P. maniculatus* (pp = 0.54 for TCWC 46975, 0.61 for TCWC 46976, and 0.66 for 46994; Appendix 1). Similarly, DFA of the PCs of the external data resulted in four specimens of *P. maniculatus* misclassified (TCWC 46974, 46975, 46994, and 46998), and there was low certainty of classification for four specimens of *P. leucopus* (pp = 0.51 for TCWC 63240, 0.67 for TCWC 63355, 0.63 for TCWC 63781, and 0.65 for TCWC 64157) and one specimen of *P. maniculatus* (0.72 for TCWC 46976). DFA using the non-allometry-minimized residuals of the combined ventral and lateral cranial views resulted in high confidence of classification (pp > 0.80) for both species with TCWC 56617

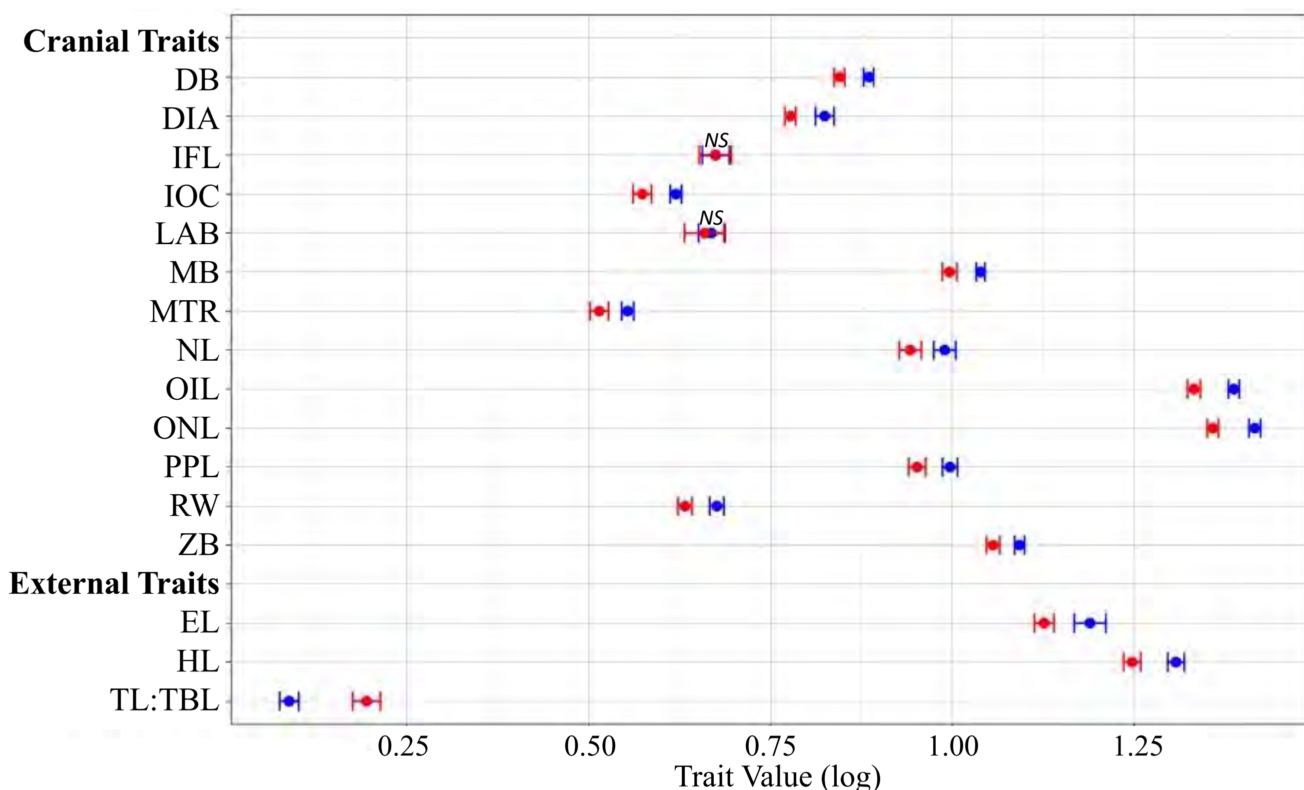

Figure 3. Traditional morphometric characters of *Peromyscus leucopus* and *P. maniculatus* (see text for definitions of abbreviations). Points represent the mean value of each trait and the associated 95 % confidence interval, where blue = *P. leucopus* and red = *P. maniculatus*. All traits, except those marked with "*NS*" (non-significant) were significantly different between the two species.

Table 1. Discriminate function analysis classification results conducted without (DFA) and with (DFA-CVs) leave-one-out cross-validation for all traditional and geometric morphometrics analyses and datasets. Type of data examined for each morphometric dataset is indicated.

Morphometric Analysis	Dataset	DFA % Correct		DFA-CVs % Correct	
		P. leucopus	*P. maniculatus*	*P. leucopus*	*P. maniculatus*
Log-Transformed Traditional Data					
Traditional	Cranial & External[1]	100	100	100	95
Traditional	External only[1]	100	86.36	96.88	77.27
Traditional	Cranial only	100	100	100	90.91
Principal Components					
Traditional	Cranial & External[1]	100	100	100	100
Traditional	External only	87.50	77.27	84.38	72.73
Traditional	Cranial only	100	100	97.06	95.45
Geometric[2]	Ventral & Lateral	94.12	83.33	94.12	83.33
Geometric[3]	Ventral & Lateral	73.53	27.78	73.53	27.78
Geometric[2] & Traditional	Ventral & Lateral, Cranial & External[1]	100	100	100	100
Geometric[3] & Traditional	Ventral & Lateral, Cranial & External[1]	100	100	100	100
Log-Transformed Traditional Data & Geometric Morphometric Principal Components					
Geometric[2] & Traditional	Ventral & Lateral, Cranial & External[1]	100	100	100	100
Geometric[3] & Traditional	Ventral & Lateral, Cranial & External[1]	100	100	100	100

[1]Results were similar regardless of if the total length and tail length external characters were examined separately, or included as a ratio of tail length to head-body length.
[2]Non-allometry-minimized residuals.
[3]Allometry-minimized residuals.

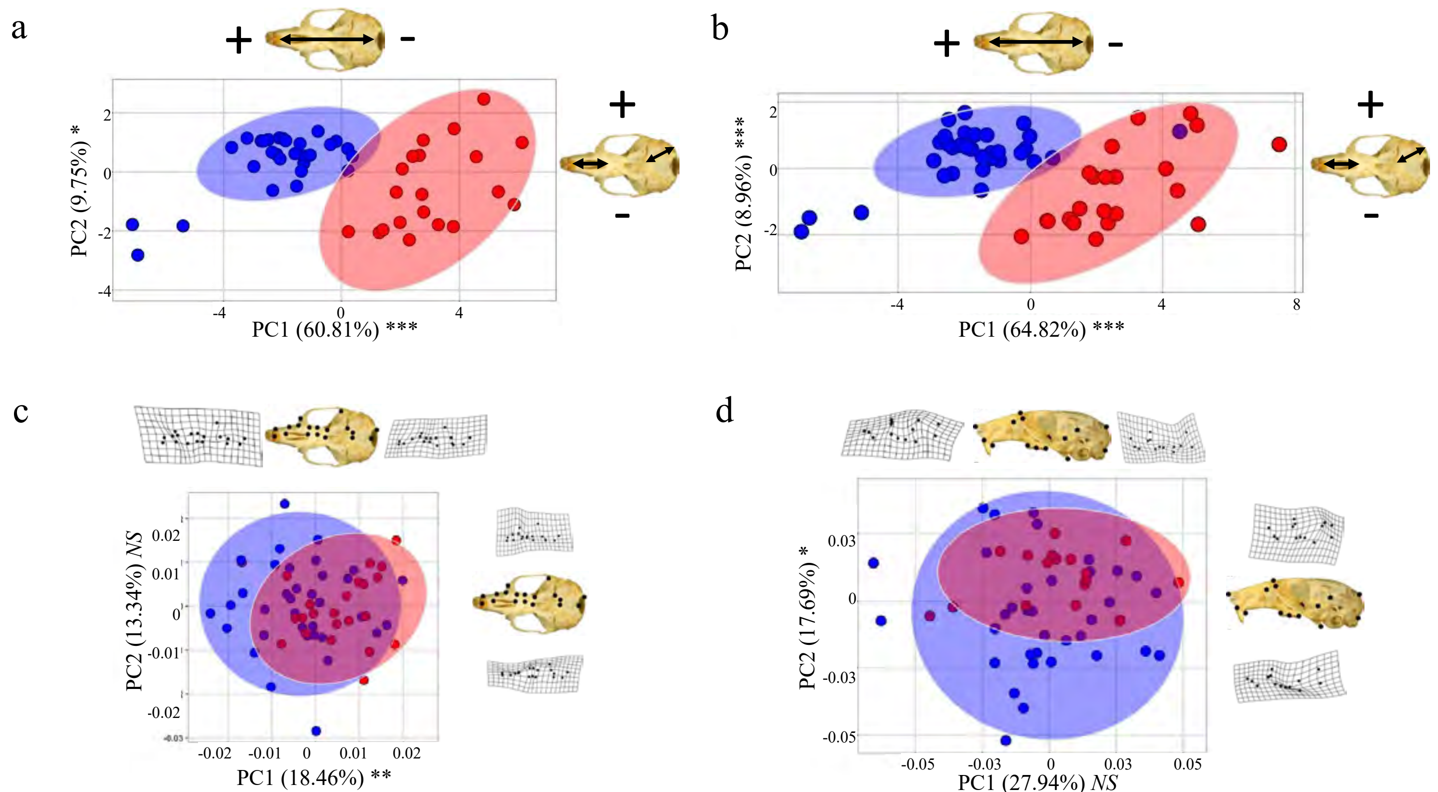

Figure 4. Principal components analysis of traditional and geometric morphometric analyses, where skull photos, arrows, and thin-plate splines represent the morphology of each PC axis. a) Traditional morphometric dataset including both cranial and external characters. b) Traditional morphometric dataset including cranial characters only. c) Ventral view geometric morphometric dataset. d) Lateral view geometric morphometric dataset. Blue = *Peromyscus leucopus* and red = *P. maniculatus*. Asterisks represent significant morphological differences at a PC axis as assessed with an ANOVA. *P*-value: 0.01-0.05*, 0.001-0.01**, 0-0.001*** where *NS* indicates non-significance. For the traditional morphometric analyses (Plates a and b), the morphology across each PC axis is associated with longer (plus sign corresponding to the skull photo) or shorter (minus sign) cranial trait characters (see Table S3 for complete PCA factor loadings). For the geometric morphometric analyses (Plates c and d), the morphology across each PC axis is represented by thin-plate splines depicting the relative configuration of the skull morphology at the extremes of each PC axis.

misclassified as a *P. maniculatus* and TCWC 46972 misclassified as a *P. leucopus*; however, all specimens were correctly classified with the inclusion of traditional morphological characters (Table 1). By far, the worst-performing dataset was that using allometry-minimized residuals of the combined ventral and cranial views (although all specimens were again correctly classified with the inclusion of traditional morphological characters). Results for the cross-validation linear discriminant function analyses (DFA-CVs) were similar to those of the DFAs, although sometimes with increased rates of misclassification (Table 1). When geometric and traditional morphometric data were combined in singular DFA and DFA-CV analyses, ventral and lateral cranial views had the highest factor loadings.

Multiple logistic regressions did not detect a significant association between species misidentification and morphology in either the traditional or geometric morphometric analyses (including both allometry-minimized and

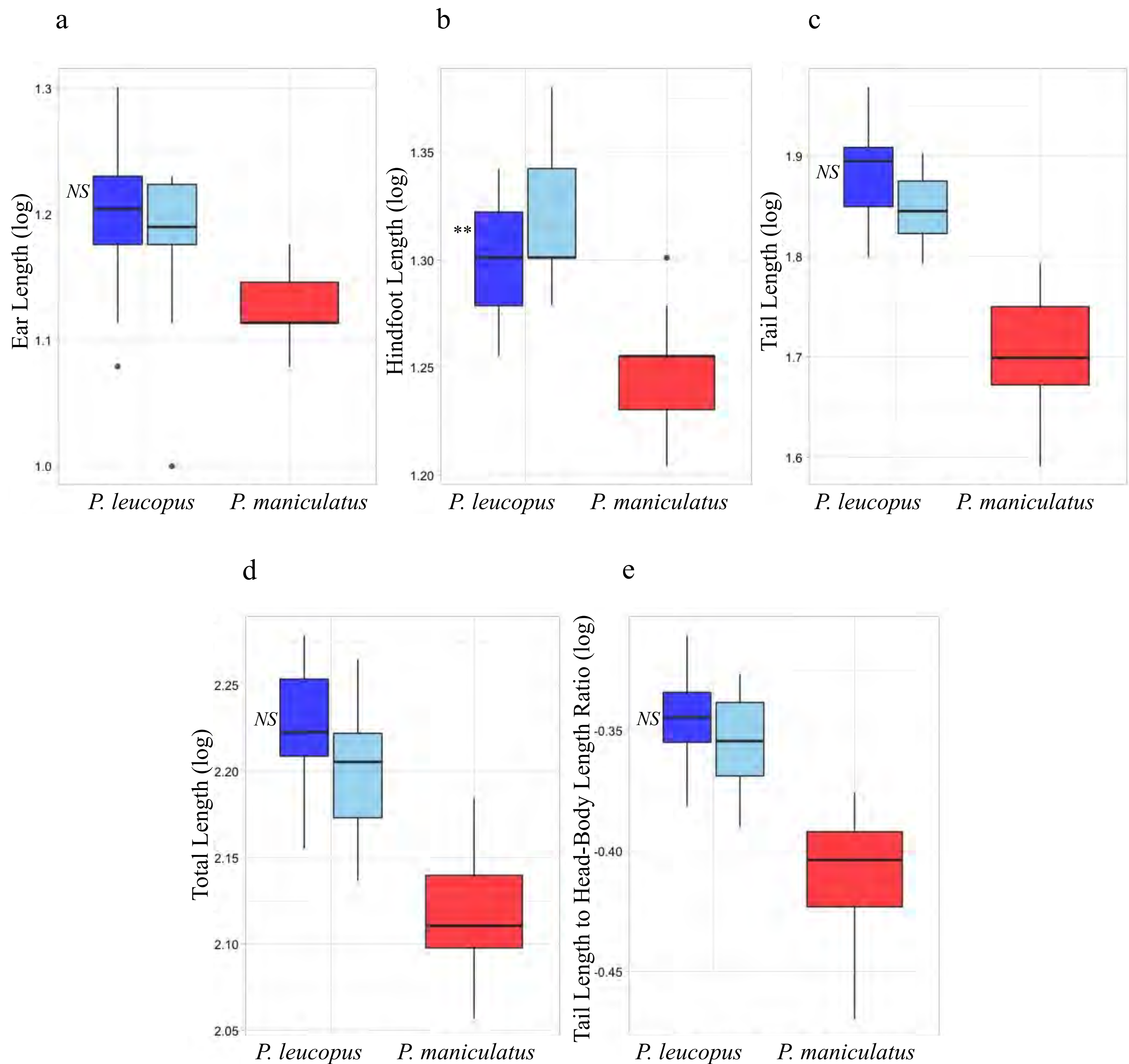

Figure 5. Specimen misidentification based on external traditional morphometric characters. a) Ear length, b) Hindfoot length, c) Tail length, d) Total length, and e) Tail to total length ratio; blue = *Peromyscus leucopus* and red = *P. maniculatus*. For *P. leucopus*, the darker shade of blue indicates specimens that are genetically *P. leucopus* and were correctly identified by the collector or natural history collection; light blue indicates specimens that are genetically *P. leucopus* but were misidentified in natural history collections as *P. maniculatus*; asterisks represent a significant association between morphology and misidentification as assessed with a Multiple Logistic Regression conducted with tail length and total length external characters considered separately (Plates a-d) and with tail length and total length external characters considered as a ratio (Plate e). *P*-value: 0.01-0.05*, 0.001-0.01**, 0-0.001*** where *NS* indicates non-significance.

non-minimized datasets). However, when conducted using exclusively traditional external morphometric characters (ToL, TL, HL, EL, and TL:TBL), multiple logistic regression detected a significant relationship between species misidentification and hindfoot length ($P < 0.01$), where specimens of *P. leucopus* with the longest hindfoot length were more likely to be misidentified (Figure 5).

For the additional specimens downloaded from VertNet with county of collection and external measurement information, of the 56 counties of eastern Texas from which *P. maniculatus* has been recorded, 29 were represented by a single locality, and 17 of those 29 were represented by a single specimen. Twelve of these single sample, single site records are from south or considerably east of the fault zone indicating that *P. maniculatus* may not be common in this region of Texas. Four taxonomic groups were compared in the DFA based on external measurements only (Figure 6): *P. m. blandus* ($n = 99$), *P. m. luteus* ($n = 119$), and the reference groups of *P. m. pallescens* ($n = 22$) and *P. leucopus* ($n = 32$). All taxonomic groups were significantly different ($P < 0.01$) from one-another with the exception of *P. m. blandus* and *P. leucopus* ($P = 0.687$). Specimens of suspect identification as *P. m. pallescens* ($n = 30$) from 16 counties in East Texas were assigned to either *P. maniculatus* ($n = 20$ from eight counties) or *P. leucopus* ($n = 10$ from eight counties) with a posterior probability (pp) of ≥ 0.75. Specimens with suspect identification as *P. m. blandus* ($n = 17$) from six counties in the Río Grande Plains were assigned to *P. leucopus* (pp ≥ 0.90). However, it is not possible to distinguish this subspecies from *P. leucopus* based on external measurements alone, and these localities remain as undetermined species (Figure 6). An additional 13 specimens from four counties were assigned with a lower pp and were excluded from further consideration.

Discussion

This study resulted in several main findings: 1) genetically identified specimens of *P. leucopus* and *P. maniculatus* from east of the Balcones fault zone in Texas can be differentiated morphologically based on size; 2) both traditional and geometric morphometric techniques can be used to differentiate these species; 3) many of the specimens of *P. leucopus* used in this study were originally misidentified as *P. maniculatus*; and 4) *P. maniculatus* appears to be rare in East Texas.

Morphological species differentiation. Results from this study generally support that analysis of a suite of morphological characters can successfully differentiate *P. leucopus* and *P. maniculatus* (Figure 4; Table 1). Although multiple traditional morphological characters can be used to differentiate *P. leucopus* and *P. maniculatus* (Figure 3), it is unlikely that these two species can be consistently differentiated based on any one morphological character alone, similar to findings from previous studies (*e. g.*, Choate 1973; Choate *et al.* 1979; Stromberg 1979; Feldhamer *et al.* 1983; Thompson and Conley 1983; Rich *et al.* 1996; Kamler *et al.* 1998; Lindquist *et al.* 2003; Reed *et al.* 2004; Stephens *et al.* 2014; Millien *et al.* 2017). For example, although length of incisive foramen (IFL) and length of auditory bulla (LAB) were not significantly different between *P. leucopus* and *P. maniculatus* when assessed individually in the traditional morphometric analyses (Figure 3), these two characters were significant ($P < 0.05$) drivers of PC2, which differed significantly ($P < 0.05$) between the species (Figure 4).

Field researchers often rely on individual external characters such as hindfoot length, ear length, tail length, or the ratio tail length to head-body length for identification of *P. leucopus* and *P. maniculatus*. The utility of these characters, however, often varies with geography. Some studies have used external characters to successfully differentiate *P. leucopus* and *P. maniculatus* (*e. g.*, Kamler *et al.* 1998; Ridenhour and Cramer 2015) whereas other studies were not successful (*e. g.*, Feldhamer *et al.* 1983; Stromberg 1979; Palas *et al.* 1992; Stephens *et al.* 2014). Given the variation in the utility of external characters to differentiate *P. leucopus* and *P. maniculatus*, reliance on these characters may be associated with species misidentification (see below). Although this study shows genetically-identified *P. leucopus* and *P. maniculatus* can be correctly classified with greater than 80 % confidence when exclusively using external characters, low certainty of classification and misclassification of individual specimens still occurred (Table 1). This finding provides additional support for caution when using exclusively external characters to differentiate these two morphologically similar species.

Size appears to be especially important when differentiating *P. leucopus* and *P. maniculatus* in East Texas; there is minimal overlap of these species in principal component morphospace (Figures 4a and 4b) and all or nearly-all specimens were correctly classified in discriminant function analyses when including datasets that accounted for size (Table 1). Examination of centroid sizes for both ventral and lateral views from geometric morphometrics (the square root of the sum of squared distances between each landmark and the geometric center of the landmark scheme; Zelditch *et al.* 2012), which primarily examines size, revealed clear separation between *P. leucopus* and *P. maniculatus* (Appendix 6). However, when size is removed from principal component analyses, there is substantially more species overlap in morphospace. This can be seen when examining PC2 and PC3 of the traditional morphological characters (Appendix 7) and PC1 and PC2 of the geometric morphometric datasets (Figures 4c and 4d). Additionally, species misclassification when analyzing allometry-minimized residuals (which reduces the effect of size relative to shape) of ventral and lateral cranial data was substantially higher than when analyzing non-allometry minimized residuals (Table 1). Shape can still be used to differentiate *P. leucopus* and *P. maniculatus* (DFAs as well as PCs of the ventral and lateral views of the skull having the highest factor loadings when geometric and traditional morphometric data were combined in DFA and DFA-CV analyses), but it appears to be

less important than size in differentiating these two species in this region. Given that *P. leucopus* and *P. maniculatus* last shared a common ancestor approximately 2.5 million years ago (Platt *et al.* 2015; Bradley *et al.* 2019) and are separated by at least eight speciation events (Greenbaum *et al.* 2019), the overall morphological similarity in shape observed between these two species is consistent with a hypothesis of remarkable convergent evolution between these two species. Mitochondrial DNA data from *P. maniculatus sensu lato* has been hypothesized to represent multiple, cryptic species across its geographic range (reviewed in Bradley *et al.* 2019 and Greenbaum *et al.* 2019) and it is unknown if there are reliable and consistent morphological differences among these putative species.

Traditional and geometric morphometrics. Both traditional and geometric morphometric techniques can be used to differentiate these species, primarily based on size as described above. Traditional morphometrics are by far the more common methodology used to morphologically differentiate mammalian species and continue to be a reliable and efficient way to examine morphological differentiation. Geometric morphometric techniques are primarily used to examine the inter-relationship across multiple landmark locations, reducing the influence of rotation, location, and scale to explore shape exclusive of size (Lawing and Polly 2010; Zelditch *et al.* 2012), thereby offering a novel way to examine morphological shape. In addition to our study, geometric morphometric analyses of other cranial and mandibular views have been useful in differentiating *P. leucopus* and *P. maniculatus* in other geographic regions (*e. g.*, width of skulls and size of braincases in Berens 2015; length and width of the rostrum and the position of the anterior margin of the tooth row; Millien *et al.* 2017) and with other rodent species (*e. g.*, expanded crania in Camargo *et al.* 2019; thickness of mandibles and shapes of mandibular processes in Rowsey *et al.* 2019). Future workers attempting to differentiate *P. leucopus* and *P. maniculatus* across their geographic range therefore have options regarding types of data to collect and analyses to perform.

Specimen misidentification and P. maniculatus *distribution in Texas*. Over a third of the specimens examined in this study initially were misidentified. This is alarming given the use of *Peromyscus* specimens in a wide variety of ecological and evolutionary studies as well as the economical and medical importance of *P. leucopus* and *P. maniculatus* as reservoirs for disease-causing pathogens. Some of these misidentifications are apparently the result of over-reliance on certain traits, such hindfoot length (Figure 5). Specimens of *P. leucopus* with relatively long hindfoot measurements were more likely to be misidentified, a surprising result given that *P. maniculatus* has comparatively shorter hindfeet. These results imply that specimens measured incorrectly or with unusual body proportions may be more likely to be misidentified.

This study resulted in the reassignment of specimens of *P. maniculatus* to *P. leucopus* from localities in 19 Texas counties, five of which were among the few supposed records of *P. maniculatus* located east of the Balcones fault zone (Figure 6). These corrections were based on either molecu-

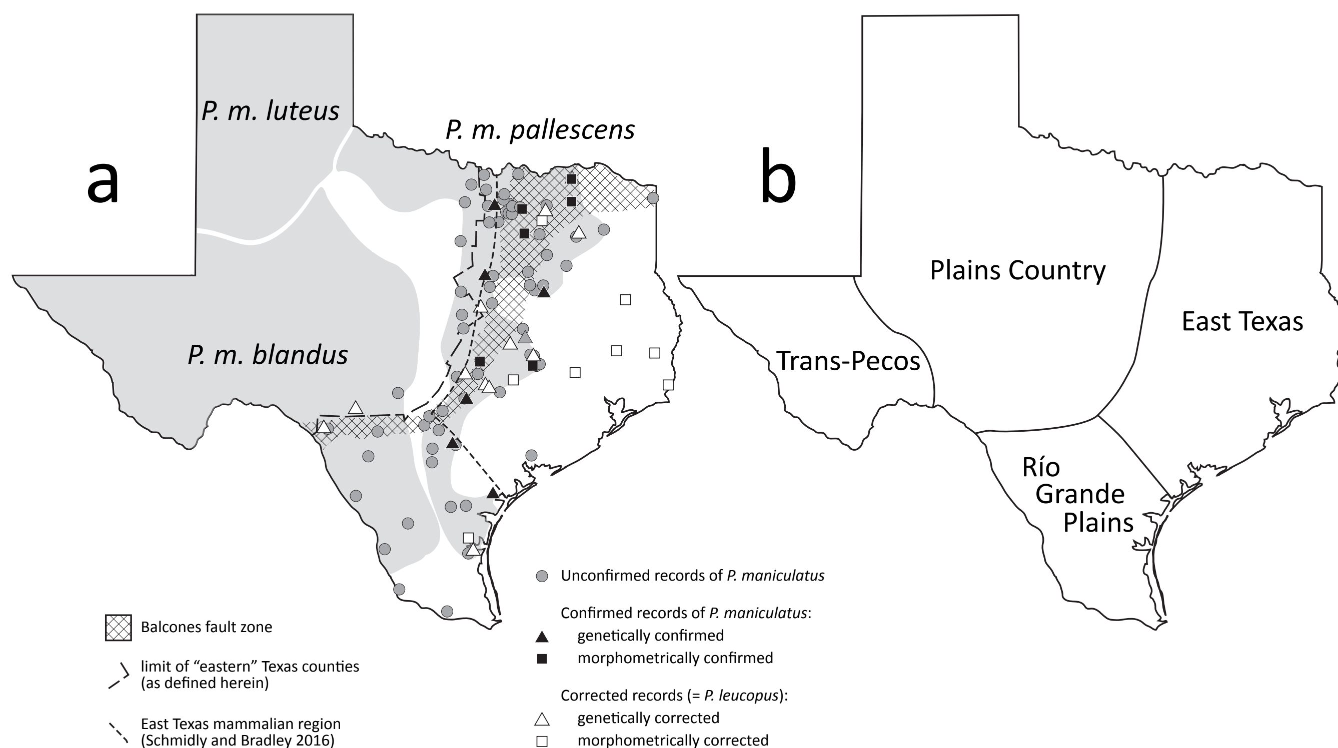

Figure 6. a) Geographic distribution of *Peromyscus maniculatus* (*sensu lato*) in Texas (shading), modified from Hall (1981), Bradley *et al.* (2019), and Greenbaum et al. (2019) based on VertNet localities. b) Mammalian regions of Texas (Schmidly and Bradley 2016).

lar sequences (12 localities in 11 counties; Supplementary material 1) or the significantly larger external measurements observed in VertNet specimens from *P. leucopus* versus *P. maniculatus* (8 localities in 8 counties; Kleberg, Lee, Montgomery, Nagadoches, Orange, Polk, Rockwall, and Tyler Cos.). Because only external characters from VertNet were examined, juvenile *P. leucopus* (being smaller) might be mistaken for *P. maniculatus*, and localities that could not be assigned to species may represent a mixture of juvenile and adult *P. leucopus*, mixtures of the two species, or simple errors in measurements. Additional research should examine skull and dental morphology of these specimens to determine appropriate age classes such that external characters can be more confidently used in future analyses (but see results above recommending use of a suite of morphological characters to differentiate *P. leucopus* and *P. maniculatus*).

It is clear that *P. maniculatus* is far less common east of the Balcones fault zone than was previously thought (Figure 6). Museum records previously indicated that *P. maniculatus* occurred in 21 counties east of the Balcones fault zone; records from 10 of those 21 counties have been reidentified as *P. leucopus,* four based on genetics (Bastrop, Brazos, Kenedy, and Milam Cos.) and six based on external morphology (Kleberg, Montgomery, Nagadoches, Orange, Polk, and Tyler Cos.). In addition to the remaining unconfirmed species records from 11 counties east of the fault zone, there are several specimens from localities from southern Texas (Figure 6) that may eventually be determined to be either *P. leucopus* or *P. maniculatus*. Thus, the Balcones fault zone may limit the distribution of *P. maniculatus* in Texas, as it does for many other taxa and that different climates, flora, and fauna across the four major regions in Texas (Trans-Pecos, Plains Country, East Texas, and Rio Grande Plains), and may additionally delimit the distribution of cryptic species within *P. maniculatus* (Bradley *et al.* 2019; Greenbaum *et al.* 2017, 2019). Future research with increased sampling is needed to determine the geographic range of "*P. maniculatus*" species in Texas.

The level of specimen misidentification observed herein is also of concern to natural history collections and researchers using specimens from these collections; large numbers of specimens in collections may be misidentified. Researchers, curators, and collections managers could use the same morphometric methods as described in this study to verify the species identification. Care should be taken, however, to recognize that *P. leucopus* and *P. maniculatus* are morphologically variable across their geographic range and the methodologies used in this study may not result in similar findings if used in different geographic areas even though use of a suite of morphological characters has repeatedly been shown to accurately differentiate these species (*e. g.*, this study; Choate 1973; Choate *et al.* 1979; Stromberg 1979; Feldhamer *et al.* 1983; Thompson and Conley 1983; Rich *et al.* 1996; Kamler *et al.* 1998; Lindquist *et al.* 2003; Reed *et al.* 2004; Stephens *et al.* 2014; Millien *et al.* 2017). Researchers trying to assess identification of unknown specimens will need to adjust their analyses accordingly (*e. g.*, use a base dataset including genetically-known specimens such as used in this study and include "unknown" specimens in PCAs and DFAs). Field ecologists and others working with specimens of *Peromyscus* in the field should consider recording additional data at the site of capture, such as external measurements in the field as well as habitat of collection, because *P. leucopus* and *P. maniculatus* are known to differ in their habitat preferences. To be truly confident in species identifications of *P. leucopus* and *P. maniculatus* in East Texas and possibly throughout their range, genetic or molecular tools are likely to be the most accurate methodology.

Determination of the distribution and relationships of the taxa within *P. maniculatus sensu lato* in Texas will depend on additional genetic sampling and responsible collecting efforts, possibly via novel collaborations with field courses and wildlife agencies (McLean *et al.* 2016; Cook and Light 2019; Miller *et al.* 2020). Newly collected specimens accessioned into natural history collections are vital to the future of organism-based research. These specimens can be invaluable for a variety of disease ecology, evolutionary, and distributional studies, especially those examining fairly common species such as *P. leucopus* and *P. maniculatus* in eastern Texas.

Acknowledgments

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Dedication. The authors dedicate this manuscript in honor of David J. Schmidly in recognition of his lifelong commitment and prolific contributions to the science and history of mammalogy. To one of us (IFG), David Schmidly has been a mentor, role model, academic and field colleague, NSF co-investigator and friend since his undergraduate days. David remains an inspiration to all of us. We gratefully thank Robert and Lisa Bradley for the invitation to participate in this festschrift honoring Professor Schmidly.

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Supplementary material 1

List of specimens genetically-identified to species and used in traditional and geometric morphometric analyses. Collection and catalog number are indicated (ASNHC = Angelo State University Natural History Collections; TCWC = Biodiversity Teaching and Research Collections at Texas A&M University; TTU = Natural Science Research Laboratory, the Museum at Texas Tech University) as is tissue type, GenBank number ("N/A" indicates those specimens from which genetic data were not collected), identification based on the collector or museum (Museum ID), identification based on molecular laboratory work (Genetic ID), sex (F=female, M=male, U=unknown), year collected, state, county, and locality. Raw data for cranial and external morphological characters are listed for each specimen. Four main datasets were analyzed in this study: traditional morphological analyses including and excluding external characters (Traditional Cranial and External and Traditional Cranial Only, respectively) and geometric morphometics of the ventral and laterial cranial views (Geometric Morphometrics Ventral and Geometric Morphometrics Lateral, respectively). Specimens included in each dataset are indicated with an "X".

https://www.revistas-conacyt.unam.mx/therya/index.php/THERYA/downloadFile/1116/882

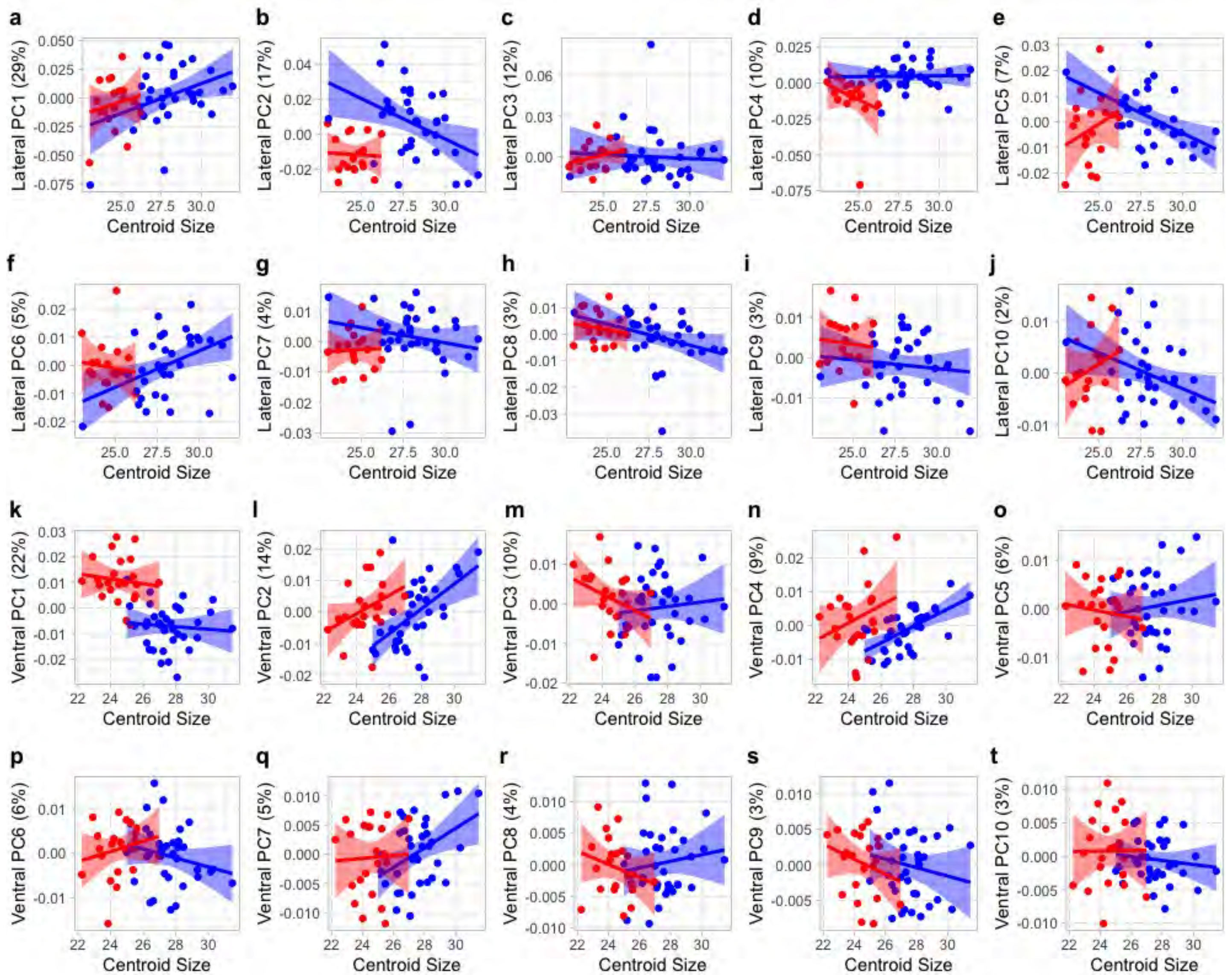
Appendix 1

Anatomical definitions of geometric morphometric landmark locations. See Figure 2 for placement of landmark locations.

Orientation	Number	Definition
Ventral	1	Medial I1 alveolus
Ventral	2	Lateral I1 alveolus
Ventral	3	Anterior edge of incisive foramen
Ventral	4	Suture of premaxilla and maxilla bones
Ventral	5	Maxilla-rostral connection point
Ventral	6	Anterior margin of zygomatic arch
Ventral	7	Posterior edge of incisive foramen
Ventral	8	Anterior edge of M1 alveolus
Ventral	9	Posterior edge of M1 alveolus
Ventral	10	Anterior edge of posterior foramen palatine
Ventral	11	Posterior edge of M3 alveolus
Ventral	12	Posterior edge of palatine bone
Ventral	13	Anterior curvature of squamosal
Ventral	14	Medial, anterior edge of foramen ovale
Ventral	15	Lateral suture of basisphenoid and basioccipital at tympanic bulla
Ventral	16	Medial suture of basisphenoid and basioccipital bones
Ventral	17	Medial posterior edge of foramen magnum
Ventral	18	Lateral edge of foramen magnum
Lateral	1	Posterior edge of 11 alveolus
Lateral	2	Anterior edge of I1 alveolus
Lateral	3	Anterior-most tip of nasal bone
Lateral	4	Ventral-most edge of zygomatic arch
Lateral	5	Suture of the nasal and frontal bones
Lateral	6	Dorsal-most edge of zygomatic arch
Lateral	7	Anterior edge of M1 alveolus
Lateral	8	Posterior edge of M1alveolus
Lateral	9	Posterior edge of M3 alveolus
Lateral	10	Ventral tip of pterygoid process
Lateral	11	Posterior edge of zygomatic arch, concave-most point
Lateral	12	Ventral-most tip of squamosal and parietal bone suture
Lateral	13	Suture of the interparietal and occipital bones
Lateral	14	Concave-most point of the occipital condyle, posterior-most point

Appendix 2

Allometric size-shape relationship in the geometric morphometric datasets across PC1-10 of the lateral view (a-j) and the ventral view (k-t). Percents labeled on the y-axis indicate the amount of total variation explained by each PC. Blue = *Peromyscus leucopus* and red = *P. maniculatus.*



Appendix 3

Welch's two-sample t-test results of traditional morphometric trait differences between *P. leucopus* and *P. maniculatus* (see text for definitions of abbreviations). P-values represent Bonferroni-corrections. *P*-value: 0.01-0.05*, 0.001-0.01**, 0-0.001***

	Trait	t-value	P-value
Cranial Traits	DB	8.28	1.85e-9***
	DIA	6.64	5.90e-7***
	IFL	0.05	1
	IOC	6.43	3.42e-6***
	LAB	0.57	1
	MB	7.63	1.25e-7***
	MTR	5.34	7.08e-5***
	NL	4.58	4.63e-4***
	OIL	9.84	2.47e-11***
	ONL	10.74	5.04e-13***
	PPL	6.04	4.08e-6***
	RW	6.60	4.84e-7***
	ZB	6.68	7.57e-7***
External Traits	EL	5.01	9.66e-5***
	HL	7.62	1.63e-8***
	TL:TBL	9.59	2.20e-10***

Appendix 4

Traditional morphometric principal component factor loadings. Percentages represent the proportion of variance associated with each PC axis. See text for definitions of abbreviations of cranial and external traits. Factor loadings in bold indicate high loading values.

		PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12	PC13	PC14	PC15	PC16
Percent of Variation		60.81	9.75	5.51	3.75	3.72	3.23	2.56	2.35	2.05	1.71	1.38	1.15	1.08	5.8e-3	2.7e-3	9.1e-4
Eigenvalues		9.73	1.56	0.88	0.60	0.60	0.52	0.41	0.38	0.33	0.27	0.22	0.18	0.17	0.09	0.04	0.01
Cranial Traits	DB	-0.27	-0.03	-0.29	0.14	-0.10	-0.07	0.21	-0.35	0.24	0.48	-0.20	0.28	0.10	-0.47	0.07	0.03
	DIA	-0.26	0.15	0.28	-0.16	-0.41	0.12	0.05	-0.45	-0.13	-0.01	-0.13	0.24	0.14	0.50	0.23	-0.01
	IFL	-0.13	**-0.57**	0.20	-0.23	-0.47	-0.26	-0.19	0.38	0.13	0.22	0.13	0.06	-0.02	0.01	-0.02	4.7e-3
	IOC	-0.26	-0.15	-0.33	0.05	0.15	0.31	-0.03	0.14	0.59	-0.22	0.11	0.04	0.26	0.41	-0.08	0.06
	LAB	-0.14	**-0.59**	0.16	0.05	0.37	0.12	-0.38	-0.33	-0.25	-0.21	-0.24	-0.15	-0.09	0.05	-0.07	-0.02
	MB	-0.28	-0.01	-0.22	0.16	-0.23	0.05	0.16	-0.15	0.08	0.07	0.08	-0.32	-0.77	0.13	-0.07	0.01
	MTR	-0.23	-0.18	-0.44	0.34	-0.19	-0.01	0.17	0.15	-0.56	-0.02	0.11	-0.16	0.35	0.02	0.21	0.03
	NL	-0.23	-0.02	0.38	0.42	0.09	-0.45	0.35	-0.05	0.29	0.04	-0.27	-0.27	0.21	4.2e-3	-0.03	0.05
	PPL	-0.28	-0.12	0.16	-0.14	-3.7e-3	0.14	0.33	0.02	0.09	-0.52	0.14	0.21	-0.12	-0.48	0.38	0.05
	OIL	**-0.31**	0.06	0.03	-0.16	0.02	0.02	0.17	0.03	-0.23	-0.03	-0.02	0.21	0.04	-0.07	-0.67	0.54
	ONL	**-0.31**	0.05	0.02	-0.07	0.05	0.01	0.14	0.06	-0.10	-0.01	0.03	0.13	0.05	-0.06	-0.38	-0.83
	RW	-0.26	0.18	-0.02	-0.56	4.2e-3	0.14	-0.06	0.03	0.02	0.10	-0.14	-0.67	0.21	-0.16	0.09	0.02
	ZB	-0.27	0.03	-0.07	-0.19	0.48	-0.14	0.11	0.36	-0.13	0.31	-0.28	0.27	-0.24	0.25	0.35	0.05
External Traits	EL	-0.20	0.22	0.47	0.40	-0.02	0.53	-0.27	0.36	-0.08	0.17	0.10	-0.05	-0.04	-0.06	0.03	0.04
	HL	-0.26	0.14	0.08	-0.03	0.29	-0.34	-0.24	-0.23	-0.03	0.10	0.75	-0.02	0.05	0.04	0.09	0.05
	TL:TBL	-0.22	0.34	-0.15	0.09	-0.18	-0.38	-0.54	0.18	3.8e-3	-0.46	-0.27	0.06	-0.08	-0.03	4.0e-3	-3.3e-3

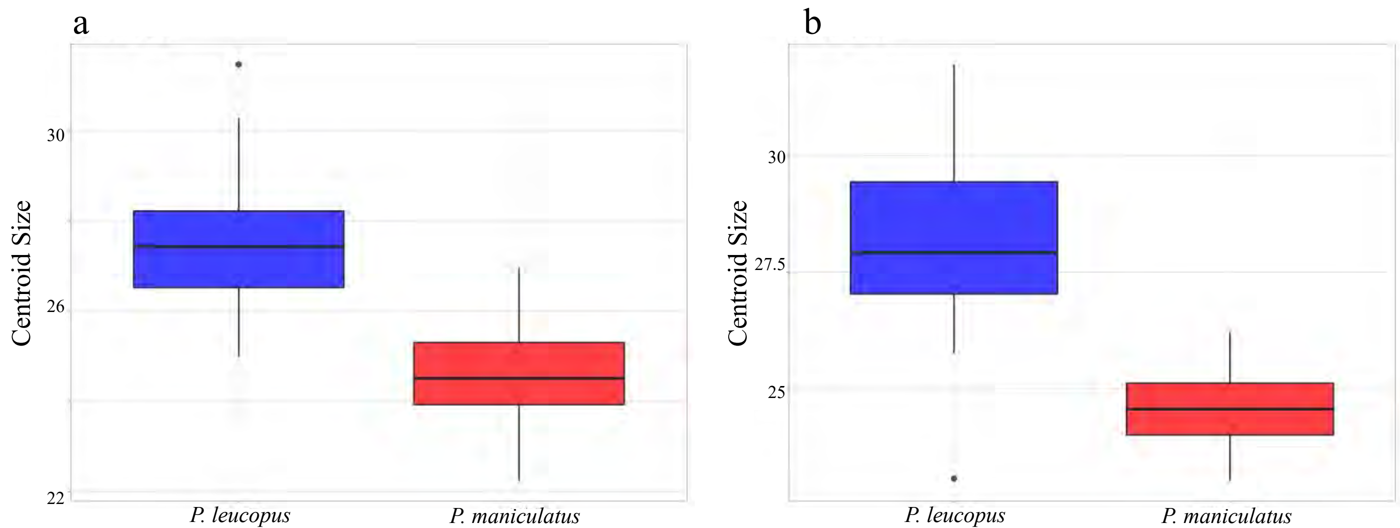
Appendix 5

Complete MANOVA results assessing the morphological differentiation between *Peromyscus leucopus* and *P. maniculatus* using allometry-minimized and non-minimized geometric morphometric analyses of the ventral and lateral views of the crania.

	Df	Wilks	F-Statistic	p-value
Ventral View (Allometry minimized)	1	0.63	0.34	0.99
Ventral View (Non-allometry minimized)	1	0.07	8.27	1.9e-6***
Lateral View (Allometry minimized)	1	0.64	0.49	0.97
Lateral View (Non-allometry minimized)	1	0.12	6.44	8.1e-6***
P-value significance: 0.01-0.05*, 0.001-0.01**, 0-0.001***				

Appendix 6

Centroid sizes of *P. leucopus* and *P. maniculatus*. a) Ventral view geometric morphometric dataset, b) Lateral view geometric morphometric dataset. Blue = *Peromyscus leucopus* and red = *P. maniculatus*.



Appendix 7

Principal Component Analysis of traditional morphometric characters depicting PC2 and PC3. a) Traditional morphometric dataset including both cranial and external characters, b) Traditional morphometric dataset including cranial characters only. Blue = *Peromyscus leucopus* and red = *P. maniculatus*; asterisks represent significant morphological differences at a PC axis as assessed with an ANOVA. *P*-value: 0.01-0.05*, 0.001-0.01**, 0-0.001*** where NS indicates non-significance.