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Mitochondrial DNA indicates paraphyletic relationships of disjunct populations in the *Neotoma mexicana* species group

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Woodrats (genus *Neotoma*) comprise 24 species found primarily in the United States and México. The *Neotoma mexicana* species group reaches its southernmost distribution in the highlands of southern México and Central America. Previous research suggested that *N. mexicana* has a discontinuous distribution, whereas *N. ferruginea* and *N. picta* have allopatric distributions around the lowlands of the Isthmus of Tehuantepec. However, these hypotheses were suggested with incomplete subspecific sampling near the isthmus. We used samples of *N. m. parvidens* from the Sierra Sur de Oaxaca and *N. m. tropicalis* from the Sierra Norte de Oaxaca to assess their taxonomic affinity. Our phylogenetic analyses of the mitochondrial cytochrome-b gene place both subspecies in *N. ferruginea*. Therefore, we suggest that *N. mexicana* is continuously distributed from the United States to the Transmexican Volcanic Belt, *N. picta* inhabits the Guerreran Sierra Madre del Sur, and *N. ferruginea* ranges from the Oaxacan Sierra Madre del Sur to Central America. Our findings also indicate that the Isthmus of Tehuantepec did not promote speciation in these woodrats.

Las ratas de campo (género *Neotoma*) incluyen 24 especies que principalmente habitan en Estados Unidos de América y México. El grupo de especies *Neotoma mexicana* alcanza su distribución más sureña en las zonas montañosas del sureste de México y Centro América. Previas investigaciones sugirieron que *N. mexicana* presenta una distribución discontinua, mientras que *N. ferruginea* y *N. picta* tienen distribuciones alopátricas alrededor de las tierras bajas del Istmo de Tehuantepec. Sin embargo, estas hipótesis fueron sugeridas con un muestreo sub-específico incompleto cerca del istmo. Utilizamos muestras de *N. m. parvidens* de la Sierra Sur de Oaxaca y *N. m. tropicalis* de la Sierra Norte de Oaxaca para evaluar su afinidad taxonómica. Nuestros análisis filogenéticos del gen mitocondrial citocromo b revelaron que ambas subespecies pertenecen a *N. ferruginea*. Por lo tanto, sugerimos que *N. mexicana* se distribuye de manera continua desde Estados Unidos hasta la Faja Volcánica Transmexicana, N. picta habita en la Sierra Madre del Sur en Guerrero, y *N. ferruginea* se distribuye desde la Sierra Madre del Sur en Oaxaca hasta Centro América. Nuestros resultados también indican que el Istmo de Tehuantepec no promovió procesos de especiación en estas ratas de campo.

Keywords: cytochrome b; Isthmus of Tehuantepec; molecular phylogeny; *Neotoma ferruginea*; Sierra Madre del Sur.

Introduction

The heterogenous topography of southern México, and Pleistocene climatic changes, generated complex biogeographic patterns and high species diversity in vertebrates (León-Paniagua and Morrone 2009; Morrone 2017), especially in small mammals (Vallejo and González-Cózatl 2012; Guevara and Cervantes 2014; León-Paniagua and Guevara 2019). Many of the region's mammals possess conservative morphologies; therefore, the number of species and their phylogenetic relationships are not entirely understood (Sullivan *et al.* 1997; Ordóñez-Garza *et al.* 2014; Pérez-Consuegra and Vázquez-Domínguez 2017). Without an adequate taxonomy, it is impossible to understand fundamental aspects of the processes that generate and maintain biodiversity (Upham *et al.* 2019).

Woodrats of the genus *Neotoma* comprise at least 24 species, distributed across portions of southern Canada and most of the continental United States and México, reaching Central America (Edwards and Bradley 2002a; Longhofer and Bradley 2006; Pardiñas *et al.* 2017). Although *Neotoma* has been studied for almost 200 years, phylogenetic relationships and species limits are not entirely resolved (Edwards and Bradley 2002a; Longhofer and Bradley 2006; Matocq *et al.* 2007; Ordóñez-Garza *et al.* 2014) because some species and subspecies are rare and/or have restricted distributions that are poorly sampled (Rogers *et al.* 2011; Fernández 2014).

Taxonomic revisions (Merriam 1894; Goldman 1910) divided woodrats into several species groups, with only the *N. mexicana* species group reaching southern México and Central America (Pardiñas *et al.* 2017). The *N. mexicana* species group, as defined by Goldman (1910), included eight species: *N. chrysomelas*, *N. distincta*, *N. ferruginea* (with subspecies *N. f. ferruginea*, *N. f. chamula*, *N. f. isthmica*, *N. f. ochracea*, *N. f. picta*, *N. f. solitaria*, and *N. f. tenuicauda*), *N. mexicana* (subspecies *N. m. mexicana*, *N. m. bullata*, *N. m. fallax*, *N. m. madrensis*, *N. m. pinetorum*, and *N. m. sinaloae*), *N. navus*, *N. parvidens*, *N. torquata*, and *N. tropicalis*. Subsequently, *N. f. griseoventer* Dalquest, 1951; *N. f. vulcani* Sanborn, 1935; *N. m. atrata* Burt, 1939; *N. m. eremita* Hall, 1955;

N. m. inopinta Goldman, 1933; *N. m. inornata* Goldman, 1938; and *N. m. scopulorum* Finley, 1953 were also described. However, all species and subspecies in the *N. mexicana* species group, except *N. chrysomelas*, were relegated to subspecific status within *N. mexicana* by Hall (1955), and later, Anderson (1972) synonymized the subspecies *N. m. madrensis* with *N. m. mexicana*. As defined by these revisions, the *N. mexicana* species group inhabits montane areas from northern Colorado throughout much of New México and western Arizona south to western Nicaragua (Edwards and Bradley 2002b; Ordóñez-Garza *et al.* 2014).

Although several studies have investigated the phylogenetic relationships among *Neotoma* species (Edwards and Bradley 2002a; Longhofer and Bradley 2006; Matocq *et al.* 2007), only two have focused on the *N. mexicana* species group (Edwards and Bradley 2002b; Ordóñez-Garza *et al.* 2014). Using mitochondrial cytochrome b (*cyt-b*) sequences, Edwards and Bradley (2002b), and Ordóñez-Garza *et al.* (2014) concluded that this species group includes at least the species *N. mexicana* from the United States through northern and central México and south of the Transmexican Volcanic Belt in southeastern México and Central America, *N. picta* in the Sierra Madre del Sur from Guerrero, *N. ferruginea* from western portions of the Isthmus of Tehuantepec south to El Salvador, and *Neotoma chrysomelas*, which inhabits parts of Honduras and Nicaragua (Pardiñas *et al.* 2017). After these taxonomic changes, 19 subspecies of *N. mexicana* and four subspecies of *N. ferruginea* are recognized, whereas *N. picta* and *N. chrysomelas* are monotypic (Pardiñas *et al.* 2017).

Despite the progress on the systematics and phylogenetic relationships in the *N. mexicana* species group, no samples of some subspecies have been analyzed with genetic data. These include *N. m. parvidens*, *N. m. tropicalis* from Oaxaca, or *N. m. solitaria* from Central America, and these three subspecies, with disjunct geographic ranges, have remained in *N. mexicana* (Edwards and Bradley 2002b; Ordóñez-Garza *et al.* 2014; Pardiñas *et al.* 2017; Figure 1). Nevertheless, Edwards and Bradley (2002b) suggested that individuals from southeastern Oaxaca and east of the Isthmus of Tehuantepec (possibly including *N. m. solitaria* from Guatemala and Honduras) are *N. ferruginea*, specimens from the Sierra Madre del Sur in Guerrero (and possibly including *N. m. parvidens* from the Sierra Sur de Oaxaca) are *N. picta*, and all samples in northern Oaxaca (possibly including *N. m. tropicalis* from Sierra Norte de Oaxaca and hills near the Chiapas border) represent *N. mexicana*. These taxonomic hypotheses, which placed the boundaries among the ranges of *N. mexicana*, *N. ferruginea*, and *N. picta* near the Isthmus of Tehuantepec, relied on the biogeographic recognition of this lowland area as an essential barrier that has promoted speciation in many other highland mammals species (Woodman and Timm 1999; Arellano *et al.* 2005; León-Paniagua *et al.* 2007; Ordóñez-Garza *et al.* 2010).

Herein, we use samples of *N. m. parvidens* and *N. m. tropicalis* from the western Isthmus of Tehuantepec (Figure 1) to test the taxonomic affinity of these subspecies. *Neotoma m. parvidens* and *N. m. tropicalis* are geographically isolated from other populations of *N. mexicana* (Figure 1). The type locality of *N. m. parvidens* is "Juquila, Oaxaca, México" and the *N. m. parvidens* sample (MZFC 11029) is from the same location: La Yerbabuena, Santa Catarina Juquila, Oaxaca (Figure 1). The type locality of *N. m. tropicalis* is the northeastern Oaxacan mountains (Goldman 1910) in Totontepec (Goldman 1904). This subspecies only occurs in the Sierra Norte de Oaxaca and hills near the Chiapas border, and no other *Neotoma* inhabit this area (Ordóñez-Garza *et al.* 2014; Pardiñas *et al.* 2017). The *N. m. tropicalis* sample (MZFC 8088) is from Xiacaba, 6.5 km ESE de Santa María Yavesía, Santa María Yavesía, Oaxaca, in the Sierra Norte de Oaxaca, around 36 km west of the type locality (Figure 1).

We sequenced the mitochondrial *cyt-b* because of its availability from a broad range of *N. mexicana* samples (Edwards and Bradley 2002b; Ordóñez-Garza *et al.* 2014), and its proven utility to clarify relationships in *Neotoma* (Edwards and Bradley 2002a) and closely related genera (Amman and Bradley 2004; Arellano *et al.* 2005; Bradley *et al.* 2007; León-Tapia 2013; Rogers *et al.* 2007; Vallejo and González-Cózatl 2012).

Materials and methods

We sequenced 1,143 base pairs of the mitochondrial *cyt-b* in specimens of *N. m. parvidens* (n = 1), *N. m. tropicalis* (n = 1), *N. m. tenuicauda* (n = 2), and *N. leucodon* (n = 1). We examined the external and cranial morphology of these specimens to confirm their taxonomic identity (Goldman 1904, 1910). Voucher specimens are deposited in the mammal collection of the Museo de Zoología, Facultad de Ciencias, Universidad Nacional Autónoma de México, Ciudad de México, México (MZFC; Appendix I). We also downloaded twenty-five sequences from GenBank: *N. mexicana* (n = 15), *N. picta* (n = 2), *N. ferruginea* (n = 7), and *N. stephensi* (n = 1; Appendix I; Edwards and Bradley 2002b; Ordóñez-Garza *et al.* 2014).

Molecular protocols. We extracted whole genomic DNA using a Qiagen DNEasy Blood and Tissue kit (Qiagen, Germantown, Maryland), following the manufacturer's recommended protocols. Through polymerase chain reaction (PCR), we amplified the complete *cyt-b* 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, USA), 4.75 µL of H20, 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 the extension at 72°C. We included a 5-minute final extension step at 72°C. We visualized 3 µL of each PCR product using electrophoresis in 1% agarose gels, stained with SYBR Safe DNA Gel Stain (Life Technologies, Carlsbad, CA, USA). Each PCR product was then cleaned with 1 µL of a 20 % dilution

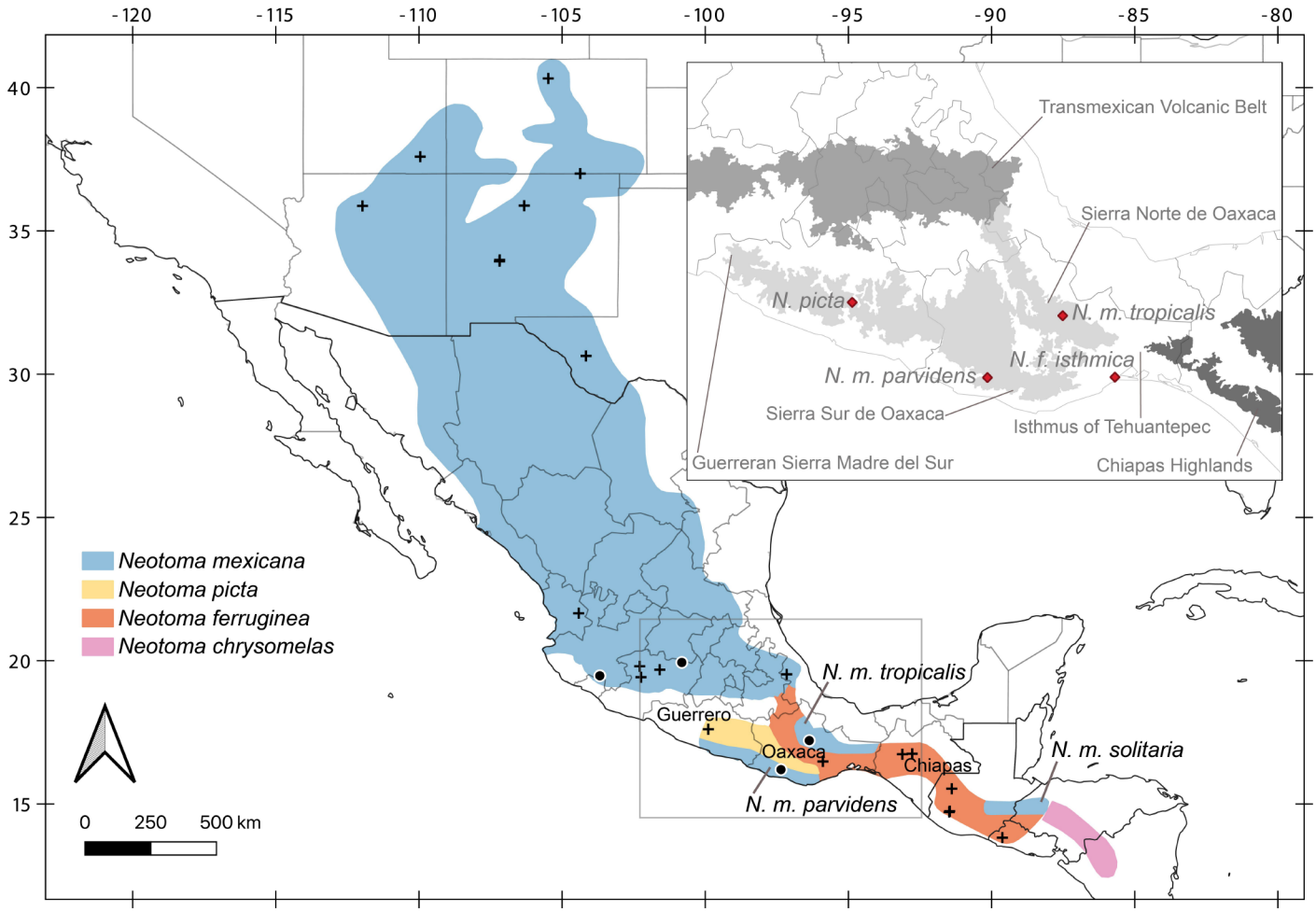

Figure 1. Specimens analyzed in this study. Black circles represent samples sequenced in this work, whereas black crosses indicate previously published sequences. Map colors show previously suggested geographic ranges for the *N. mexicana* species group (Edwards and Bradley 2002b; Pardiñas *et al.* 2017). The inset shows all type localities from Guerrero and Oaxaca (red diamonds) and the main biogeographic regions. Localities of samples included in this work: *N. m. parvidens*, México: Oaxaca; Santa Catarina Juquila, La Yerbabuena (MZFC 11029); *N. m. tenuicauda*, México: Colima; Comala, La Yerbabuena (MZFC 11989); Michoacán; Zinapécuaro, Araró, Campo Alegre (MZFC 12327); *N. m. tropicalis*, México: Oaxaca; Santa María Yavesía, Xiacaba, 6.5 km ESE de Santa María Yavesía (MZFC 8088).

of ExoSAP-IT (GE Healthcare Bio-Sciences Corp. Piscataway, NJ, USA) incubated for 30 minutes at 37°C followed by 15 minutes at 80°C. Samples were cycle-sequenced using 6.1 µL of H20, 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, USA), and 1 µL of the cleaned 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 the 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, USA). DNA sequences were edited, aligned, and visually inspected using MEGA X (Kumar *et al.* 2018) and FINCHTV 1.4 (Patterson *et al.* 2004).

Phylogenetic relationships. We used maximum likelihood (ML) and Bayesian inference (BI) to estimate the *N. mexicana* species group's phylogenetic relationships. We analyzed a total of 28 individuals in the *N. mexicana* species group with *N. leucodon* and *N. stephensi* as outgroups. We used both external groups because it is not clear if *N. stephensi*, or the clade that includes the species groups *N. floridana* + *N. lepida* + *N. micropus* (that includes *N. leucodon*), is sister to the *N. mexicana* species group (Matocq *et al.* 2007). In PARTITIONFINDER 2 (Lanfear *et al.* 2016), we selected the best model and partition scheme (maximally divided by codon position) among all available models in MRBAYES 3.2 (Ronquist *et al.* 2012), using the Bayesian Information Criterion (BIC). We used this result for both ML and BI. In IQ-TREE 1.6.12 (Nguyen *et al.* 2015), we estimated the ML gene tree, with branch support estimated by 1,000 replicates of nonparametric bootstrap. In MRBAYES 3.2 we used three hot chains and one cold chain in two independent runs of 10 million generations, sampling data every 1,000 iterations. We checked for convergence of MCMC results 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 test whether our inferred best topologies are statistically superior to past taxonomic hypotheses, we constrained topologies to fit taxonomy (forcing the monophyly of *N. m. parvidens* or *N. m. tropicalis* and all other *N. mexicana* samples) and analyzed these in MRBAYES 3.2 (same settings as above). To compare the unconstrained BI and the constrained topologies, we used the Shimodaira-Hasegawa

test (Shimodaira and Hasegawa 1999) as implemented in the package PHANGORN 2.5.5 (Schliep 2011) for R 3.6.2 (R Core Team 2014). We compared the likelihood fits assuming an HKY+G substitution model and 10,000 bootstrap replicates. We performed analyses with and without optimizing the rate matrices and base frequencies.

Genetic differentiation and genetic diversity. To evaluate differentiation levels among members of the *N. mexicana* species group, we calculated p-distances in MEGA X, using the pairwise deletion option and the Kimura 2-parameter model (Kimura 19804). These settings were chosen to facilitate comparisons with previous works (Bradley and Baker 2001; Baker and Bradley 2006; Ordóñez-Garza *et al.* 2014). To clarify whether intraspecific variation was correlated with geography, we performed a Mantel test on genetic distances (previously calculated in MEGA) and Euclidean geographic distances in the R package ADEGENET 2.1.3 (Jombart 2008; Jombart and Ahmed 2011). The Mantel test's significance was assessed using 99,999 permutations, and plots were colored by 2-dimensional kernel density estimation in the R package MASS 7.3-51.4 (Venables and Ripley 2002). To further characterize genetic diversity, we used DNASP 5.10 (Librado and Rozas 2009) to calculate the number of segregating sites, the number of haplotypes, haplotype diversity (*Hd*), and nucleotide diversity (π) for each species.

Results

Our alignment covered 100 % in > 97 % of positions, contained 290 variable characters, and 196 parsimony-informative characters. The best evolutionary model scheme was K80+G, HKY+I, and GTR+I applied to the first, second, and third codon positions, respectively. Topologies from ML and BI trees were similar (Figure 2), revealing well-supported sister relationships between *N. picta* (from the Guerreran Sierra

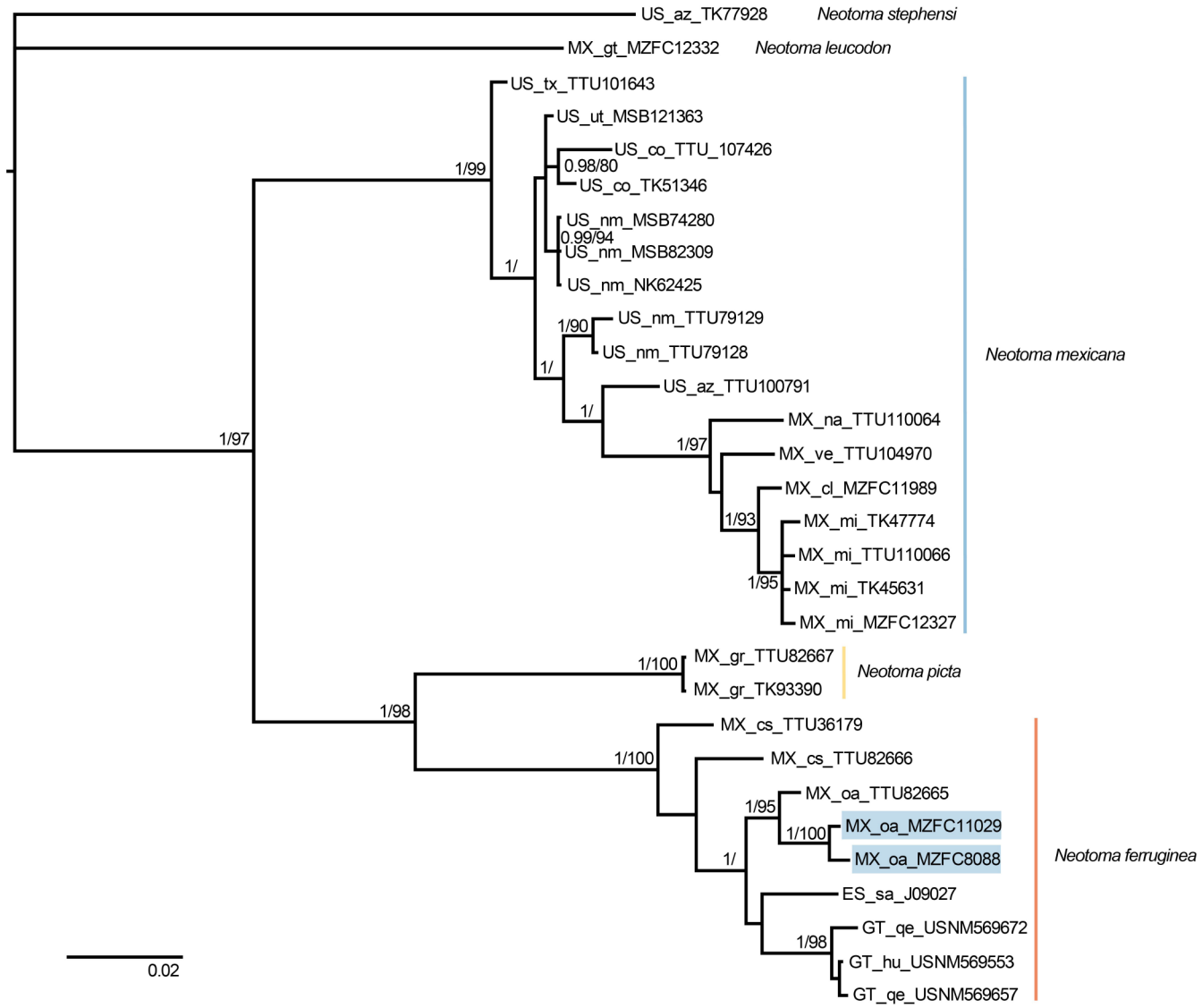

Figure 2. Majority rule consensus tree of the *Neotoma mexicana* species group, obtained from Bayesian analysis of cytochrome b sequences. Support values are shown as posterior probabilities followed by bootstrap values from a maximum likelihood analysis. Support values < 0.8/80 are not shown. Samples of *N. m. parvidens* (MZFC 11029) and *N. m. tropicalis* (MZFC 8088) are denoted with blue boxes within *N. ferruginea*. Tip labels show country (ES = El Salvador, GT = Guatemala, MX = México, US = the United States), states/provinces (sa = Santa Ana; hu = Huehuetenango, qe = Quetzaltenango; cl = Colima, cs = Chiapas, gr = Guerrero, gt = Guanajuato, mi = Michoacán, na = Nayarit, oa = Oaxaca, ve = Veracruz; az = Arizona, co = Colorado, nm = New Mexico, tx = Texas, ut = Utah), and catalog number.

Madre del Sur) and *N. ferruginea* (Oaxaca to Central America; ML and BI), with this clade sister to *N. mexicana* (from the United States to the Transmexican Volcanic Belt in central México; BI only). Our samples of *N. m. parvidens* (MZFC 11029) and *N. m. tropicalis* (MZFC 8088) were closely related to *N. ferruginea* rather than *N. mexicana*. The constrained analyses, which forced these subspecies to be members of *N. mexicana*, produced significantly worse likelihoods in both cases, with the optimized ($P < 0.001$ for each subspecies) and not optimized ($P < 0.001$ for each subspecies) data. Hence, the Shimodaira-Hasegawa test strongly rejected the placement of *N. m. parvidens* and *N. m. tropicalis* in *N. mexicana* (Table 1). In the following analyses, we included both specimens (MZFC 11029 and 8088) in *N. ferruginea*.

Table 1. Results of Shimodaira-Hasegawa tests of alternative phylogenetic hypotheses (unconstrained = obtained in this work from BI, constrained = monophyly of *N. m. parvidens* or *N. m. tropicalis* forced with all other *N. mexicana* samples), with and without optimizing the rate matrices and base frequencies. Asterisks indicate statistical rejection of topological equivalence (α = 0.05).

	No optimization			Optimization		
	ln L	**∂ L**	**P**	**ln L**	**∂ L**	**P**
Unconstrained	-4568.9	0.000	0.4965	-4089.1	0.000	0.4967
Constrained (*N. m. parvidens*)	-4684.6	115.665	0.0000*	-4137.5	48.405	0.0000*
Constrained (*N. m. tropicalis*)	-4683.2	114.291	0.0000*	-4136.8	47.736	0.0001*

Table 2. Genetic diversity summary statistics for species in the *Neotoma mexicana* species group. n = sample size, S = number of segregating sites, h = number of haplotypes, *Hd* = haplotype diversity, π = nucleotide diversity, SD = standard deviation.

	n	**S**	**h**	**Hd**	**SD (Hd)**	**π**	**SD (π)**
Neotoma mexicana	17	99	15	0.978	0.031	0.025	0.002
Neotoma picta	2	0	1	0	0	0.000	0.000
Neotoma ferruginea	9	72	9	1	0.052	0.023	0.003

The average mitochondrial distance between *N. mexicana* and *N. picta* was 9.68 % (range = 8.97 to 10.1), between *N. mexicana* and *N. ferruginea* was 9.46 % (range = 8.02 to 10.81), and between *N. picta* and *N. ferruginea* was 7.94 % (range = 7.79 to 7.98). Within species, the average genetic distance between the Chiapan and all other samples in *N. ferruginea* was 2.91% (range = 2.05 to 3.3), and between the Mexican and the United States *N. mexicana* samples was 3.86 % (range = 3.15 to 4.83; Figure 3). The Mantel tests revealed significant isolation by distance among *N. mexicana* ($P = 0.00001$, $R^2 = 0.8351$) and *N. ferruginea* ($P = 0.00844$, $R^2 = 0.1907$; Figure 4). Finally, in *N. mexicana* and *N. ferruginea* we found high haplotype diversity values (*Hd* = 0.978 and 1, respectively), but within each species, all haplotypes were similar ($\pi = 0.025$ and 0.023, segregating sites = 99 and 72, respectively). In *N. picta* the two analyzed specimens had the same haplotype (Table 2).

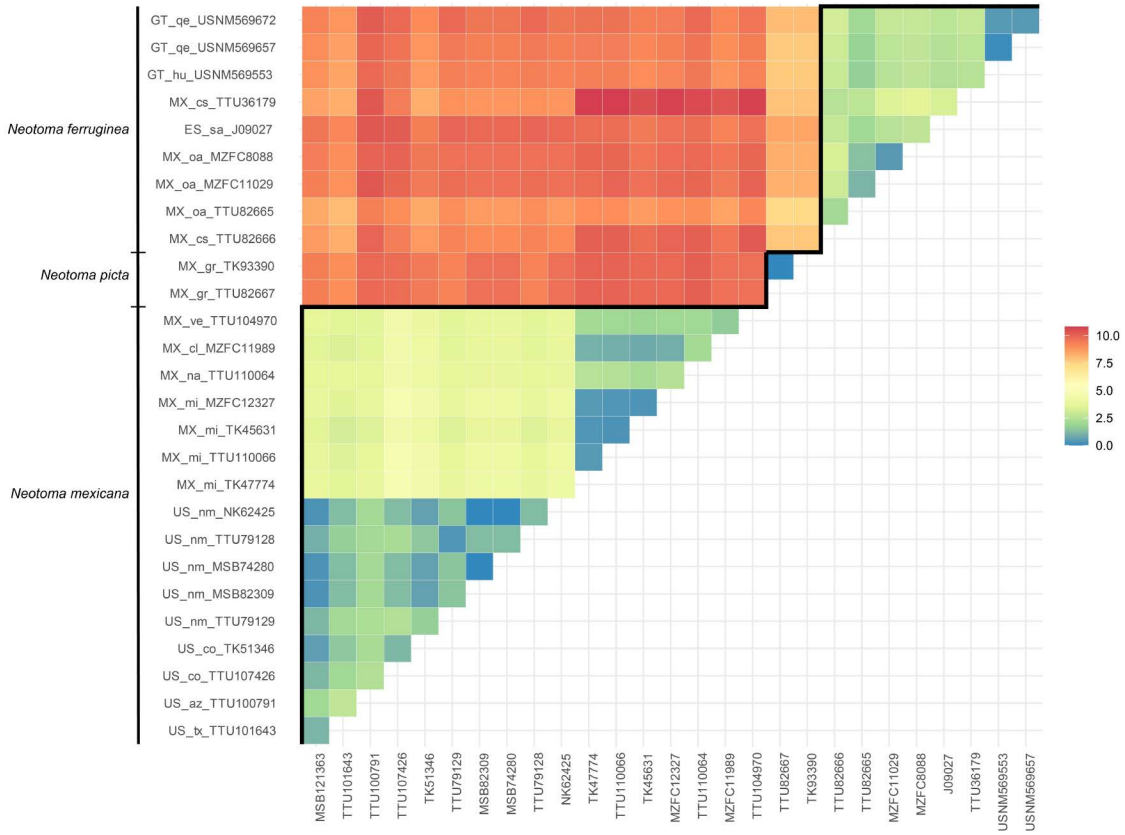

Figure 3. Heat map showing Kimura 2-parameter genetic distances in the *N. mexicana* species group. Interspecific and intraspecific comparisons are shown above and below the black line, respectively. Geographic information is shown on the y-axis (ES = El Salvador, GT = Guatemala, MX = México, US = the United States; sa = Santa Ana; hu = Huehuetenango, qe = Quetzaltenango; cl = Colima, cs = Chiapas, gr = Guerrero, gt = Guanajuato, mi = Michoacán, na = Nayarit, oa = Oaxaca, ve = Veracruz; az = Arizona, co = Colorado, nm = New Mexico, tx = Texas, ut = Utah).

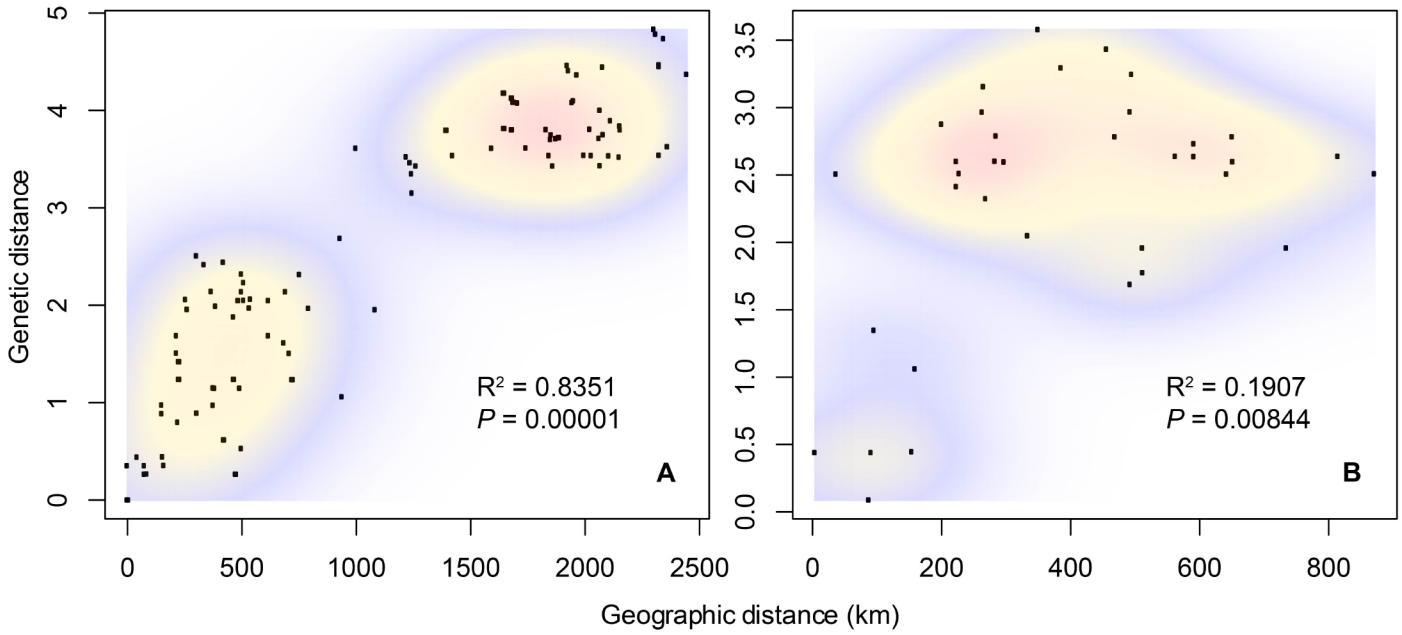

Figure 4. Bi-variate plots of geographic and genetic distances of A) *Neotoma mexicana*, and B) *Neotoma ferruginea*. Warmer colors indicate higher point densities. Mantel test results are shown.

Discussion

Although woodrats are regionally typical, taxa in the *N. mexicana* group are poorly known regarding their systematics and ecology (Edwards and Bradley 2002b). Previous analyses pointed out the possibility that the Isthmus of Tehuantepec promoted diversification in this species group because individuals from the eastern Isthmus were assigned to *N. ferruginea*, those from the Guerreran Sierra Madre del Sur and, possibly from Sierra Sur the Oaxaca, were referred to *N. picta*, and individuals from northern Oaxaca were designated *N. mexicana* (Edwards and Bradley 2002b). However, our results reject these taxonomic hypotheses. We found that *N. ferruginea* is paraphyletic, both *N. m. parvidens* from the Sierra Sur de Oaxaca and *N. m. tropicalis* from Sierra Norte de Oaxaca are related to *N. ferruginea* rather than *N. mexicana* or *N. picta*. For taxonomy to reflect evolutionary history, the parvidens and tropicalis subspecies should be considered populations of *N. ferruginea*. With these taxonomic modifications, species boundaries in the *N. mexicana* species group no longer lie near the Isthmus of Tehuantepec, and *N. ferruginea* spans this biogeographic barrier. As such, we find no evidence that the isthmus promoted speciation or maintains long-term geographic isolation in this species group. Our conclusions are based on high levels of mitochondrial DNA divergence and backed by morphological evidence (see below), but should be further tested in future works using independently sorting nuclear loci.

Our placement of parvidens and tropicalis in *N. ferruginea* (Figures. 2, 3, 4, Table 1) is consistent with Goldman's (1910) conclusions. Although *N. f. parvidens* and *N. f. tropicalis* were considered independent species in his monograph, he described both taxa as members of the "ferruginea section" inhabiting mountain slopes of southwestern and northeast Oaxaca, respectively (Goldman 1910). The geographic ranges we suggest herein eliminate some of the previously proposed geographic disjunctions, and they align well with some common biogeographic boundaries. Firstly, the southern geographic limit of *N. mexicana* is located in the Transmexican Volcanic Belt (Figure 5), a biogeographic barrier to many other Nearctic species (Morrone 2019). We detected intraspecific genetic variation consistent with isolation by distance (Figures 3 and 4A). Secondly, in southern México, *Neotoma picta*, *N. f. parvidens*, and *N. f. tropicalis* inhabit the eastern Sierra Madre del Sur sub-province, because the Sierra Sur de Oaxaca and the Sierra Norte de Oaxaca are also part of the eastern Sierra Madre del Sur. A recent biogeographical study of the eastern Sierra Madre del Sur suggested that it comprises two areas, the Guerreran and the Oaxacan Highlands districts, each one supported by many local endemic taxa (Santiago-Alvarado *et al.* 2016; Morrone 2017). We suggest that *N. picta* inhabits the Guerreran district of the Eastern Sierra Madre del Sur sub-province, whereas *N. ferruginea* inhabits a large area from the Oaxacan highlands district across the Isthmus of Tehuantepec to Central America (Figure 5).

A previous dated phylogenetic analysis inferred Late Pleistocene diversification in the *N. mexicana* group and suggested that habitat expansion and contraction promoted diversification (Ordóñez-Garza *et al.* 2014). We detected low levels of nucleotide diversity but high levels of haplotype diversity (Table 2), a pattern consistent with recent demographic expansions (Hedrick 2011), so the hypothesized effect of Pleistocene habitat cycles on this species group is consistent with our results. Additionally, we found intraspecific genetic differentiation from 1.68 to 3.58 % in *N. ferruginea* across the Isthmus of Tehuantepec. These lowlands are a minimally 200-km-wide valley at approximately 250 meters above sea level (Barrier *et al.* 1998), representing a significant barrier for many montane species (Peterson *et al.* 1999). However, the Isthmus of Tehuantepec did not

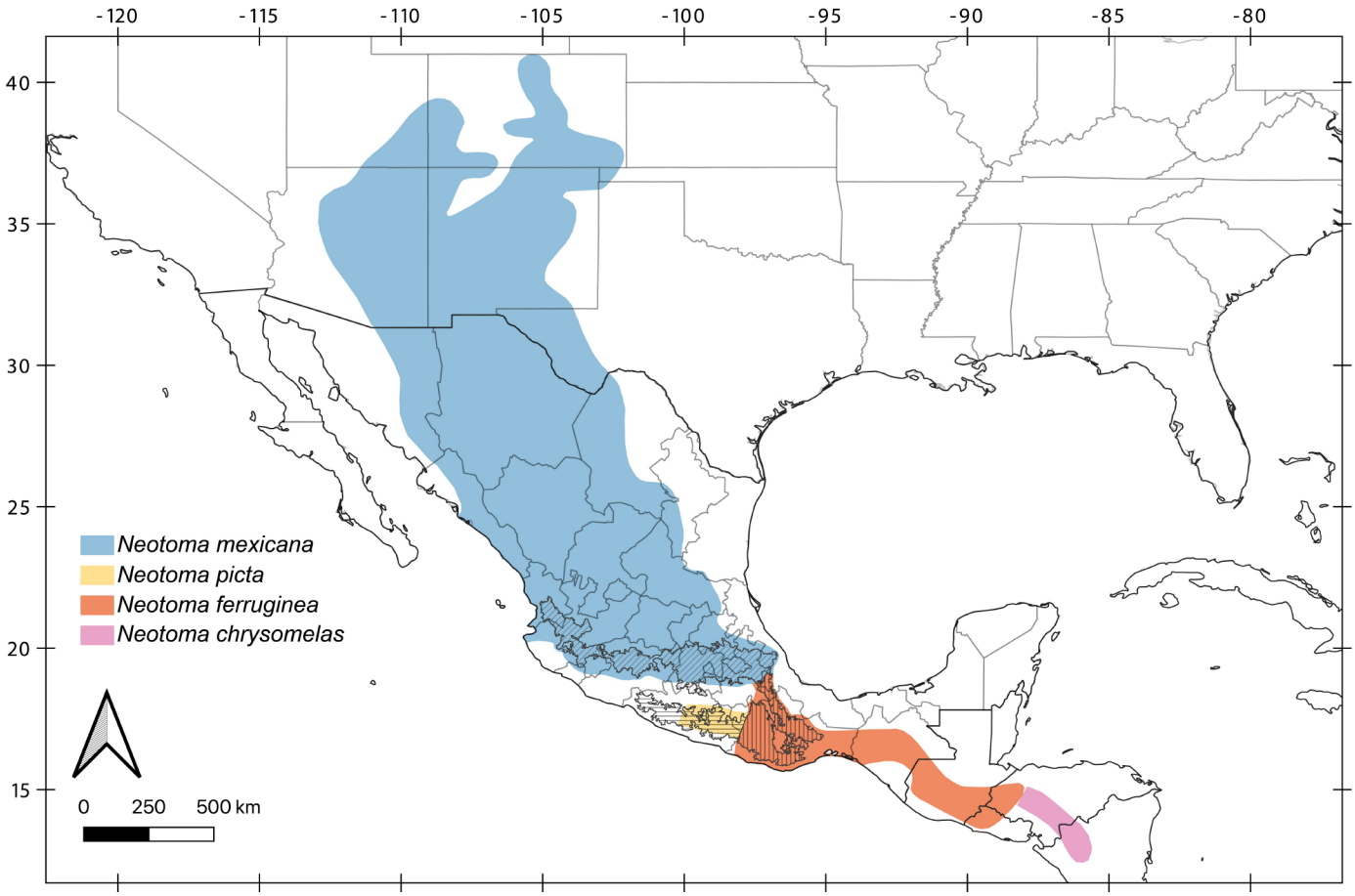

Figure 5. Revised geographic ranges within the *N. mexicana* species group. Guerreran Sierra Madre del Sur district is shown as horizontal hashes, Oaxacan highlands district as vertical hashes, and Transmexican Volcanic Belt with diagonal hashes (Morrone 2017).

promote speciation in these woodrats because its genetic differentiation seems more related to geographic distances rather than geographic barriers (Figure 4B), there is not a clear and supported geographic structure in the phylogenetic inferences (Figure 2), and because the most different individuals were detected in Chiapas and not between eastern and westernmost populations (Figure 3). Interestingly, a Chiapan Pleistocene refugium has been suggested in other mammal studies (Guevara-Chumacero *et al.* 2010; Gutiérrez-García and Vázquez-Domínguez 2012). Future phylogeographic studies on *N. ferruginea* could test for signals of a Pleistocene refuge in the highlands of Chiapas, which could have served as a source for the Oaxacan and Central American populations.

Finally, *N. m. solitaria* from Guatemala and Honduras's uncertain placement, as a subspecies of *N. mexicana* or *N. ferruginea* has been previously mentioned (Ordóñez-Garza *et al.* 2014; Pardiñas *et al.* 2017). *Neotoma m. solitaria* was initially described as a subspecies of *N. ferruginea* with a small body size, and short, bright fur (Goldman 1905), but it was relegated to subspecific status within *N. mexicana* by Hall (1955) without a formal analysis. Subsequent revisions on the *N. mexicana* species group showed that the lumping of its members obscured the real diversity and evolutionary history of these woodrats (Edwards and Bradley 2002b; Ordóñez-Garza *et al.* 2014). Although we did not analyze samples of *N. m. solitaria*, previous morphological descriptions (Goldman 1910), and the geographic ranges of the *N. mexicana* species group members (Figure 5) suggest the best available option is to re-assign *N. m. solitaria* to *N. ferruginea*.

Although our results rely on a small data set, the inclusion of novel samples from type localities improved resolution of the evolutionary history and geographic limits of *N. mexicana* species group members. The species ranges we propose are geographically coherent and separated by standard biogeographic boundaries. A continued sampling of wild populations is needed to provide a rigorous understanding of southern Mexican mammals' diversity and endemism.

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Appendix I

Specimens analyzed in this work

Taxon	GenBank	Catalog #	Tissue #	Country	State/Province	Lat	Long
Neotoma leucodon	MW419110	MZFC 12332	3814	México	Guanajuato	21.583	-100.993
Neotoma stephensi	AF308867	TTU 78505	TK 77928	US	Arizona	34.737	-110.043
Neotoma mexicana inopinata	AF298841	MSB 121363	NK 36282	US	Utah	37.592	-109.955
Neotoma mexicana mexicana	AF294346	TTU 101643	TK 90038	US	Texas	30.639	-104.166
Neotoma mexicana pinetorum	FJ716222	TTU 100791		US	Arizona	35.874	-111.972
Neotoma mexicana scopulorum	FJ716223	TTU 107426		US	Colorado	40.321	-105.484
Neotoma mexicana scopulorum	AF186821	DMNH 8577	TK 51346	US	Colorado	37.002	-104.369
Neotoma mexicana scopulorum	AF294345	TTU 79129	TK 78350	US	New Mexico	35.883	-106.324
Neotoma mexicana scopulorum	AF298848	MSB 74280	NK 62439	US	New Mexico	33.990	-107.181
Neotoma mexicana scopulorum	AF298849	TTU 79128	TK 78349	US	New Mexico	35.883	-106.324
Neotoma mexicana scopulorum	AF298846	MSB 82309	NK 62415	US	New Mexico	33.944	-107.187
Neotoma mexicana scopulorum	AF298847		NK 62425	US	New Mexico	33.943	-107.186
Neotoma mexicana tenuicauda	MW419114	MZFC 11989	4442	México	Colima	19.478	-103.683
Neotoma mexicana tenuicauda	AF298843		TK 47774	México	Michoacán	19.809	-102.290
Neotoma mexicana tenuicauda	KF772877	TTU 110066		México	Michoacán	19.427	-102.244
Neotoma mexicana tenuicauda	AF298842		TK45631	México	Michoacán	19.689	-101.591
Neotoma mexicana tenuicauda	MW419113	MZFC 12327	4581	México	Michoacán	19.942	-100.820
Neotoma mexicana tenuicauda	KF772878	TTU 110064		México	Nayarit	21.660	-104.421
Neotoma mexicana torquata	KF801364	TTU 104970		México	Veracruz	19.527	-97.156
Neotoma picta	AF305568	TTU 82667	TK93384	México	Guerrero	17.612	-99.896
Neotoma picta	AF305569		TK 93390	México	Guerrero	17.612	-99.896
Neotoma ferruginea chamula	AF305567	TTU 82666	TK 93296	México	Chiapas	16.755	-92.773
Neotoma ferruginea chamula	KF772876	USNM 569553		Guatemala	Huehuetenango	15.535	-91.393
Neotoma ferruginea ferruginea	KF772873	JGO 9027		El Salvador	Santa Ana	13.827	-89.625
Neotoma ferruginea isthmica	AF298840	TTU 36179	TK 20551	México	Chiapas	16.738	-93.117
Neotoma ferruginea isthmica	AF329079	TTU 82665	TK 93257	México	Oaxaca	16.486	-95.893
Neotoma ferruginea parvidens	MW419111	MZFC 11029	4123	México	Oaxaca	16.203	-97.355
Neotoma ferruginea tropicalis	MW419112	MZFC 8088	2604	México	Oaxaca	17.216	-96.367
Neotoma ferruginea vulcani	KF772874	USNM 569657		Guatemala	Quetzaltenango	14.752	-91.463
Neotoma ferruginea vulcani	KF772875	USNM 569672		Guatemala	Quetzaltenango	14.721	-91.481