

# An 1896 specimen helps clarify the phylogenetic placement of the Mexican endemic Hooper's deer mouse

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Hooper's deer mouse, *Peromyscus hooperi*, is the sole member of the *Peromyscus hooperi* species group. This species is endemic to México where it is restricted to the grassland transition zone in the states of Coahuila, Zacatecas, and San Luis Potosí. Previous studies using mitochondrial and nuclear genes (*Cytb*, *Adh1-12*, *Fgb-17* and *Rbp3*) did not resolve the phylogenetic relationships of this relatively poorly known species. It was hypothesized that *P. hooperi* is sister to *P. crinitus*, and these two taxa are related to *P. melanotis*, *P. polionotus*, *P. maniculatus*, *P. keeni*, *P. leucopus*, *P. gossypinus*, *P. eremicus*, *P. californicus*, and *Osgoodomys banderanus*. Based on morphological characters, karyotypes, and allozymes, *P. hooperi* does not align with either subgenera *Haplomylomys* or *Peromyscus*. However, its unique characteristics (e. g., phallus, karyotype) have been recognized, and therefore it has been retained as its own species group. To better resolve the phylogenetic placement of *P. hooperi*, we performed target-enrichment and high-throughput sequencing and obtained several thousand nuclear ultraconserved elements and a complete mitogenome from a specimen collected in 1896 by Nelson and Goldman in Coahuila, México. We compared these data with 21 other species of neotomines using genome-wide data. Contrary to previous studies, we found high nodal support for the placement of *P. hooperi* as sister to a clade that includes *Podomys floridanus*, *Neotomodon alstoni*, *Habromys simulatus*, *H. ixtlani*, *Peromyscus mexicanus*, *P. megalops*, *P. melanophrys*, *P. perfulvus*, *P. aztecus*, *P. attwateri*, *P. pectoralis*, and *P. boylii*. We dated a Pliocene divergence of *P. hooperi* from its sister group at approximately 3.98 mya, and after the split of *P. crinitus* at ca. 4.31 mya from other peromyscines. We demonstrated that genome-wide data improve the phylogenetic signal, independently of taxon sampling, for a phylogenetically problematic species such as *P. hooperi*. We recommend that future genomic studies expand taxon sampling, including members of the subgenus *Haplomylomys*, to confirm the phylogenetic relationships of *P. hooperi* and the genetic status of its populations.

El ratón de Hooper *Peromyscus hooperi*, es el único miembro del grupo de especies que lleva su mismo nombre. Es una especie endémica de México que se encuentra restringida a las zonas de transición de pastizales en los estados de Coahuila, Zacatecas y San Luis Potosí. Estudios previos en los que se han analizado genes mitocondriales y nucleares (*Cytb*, *Adh1-12*, *Fgb-17* y *Rbp3*) no han podido resolver las relaciones filogenéticas de esta especie poco conocida. Sin embargo, se ha sugerido que *P. hooperi* podría ser la especie hermana de *P. crinitus*, y estar cercanamente relacionada con *P. melanotis*, *P. polionotus*, *P. maniculatus*, *P. keeni*, *P. leucopus*, *P. gossypinus*, *P. eremicus*, *P. californicus* y *Osgoodomys banderanus*. Con base en datos morfológicos, cariotipos y aloenzimas, no se ha podido determinar si esta especie se encuentra más estrechamente relacionada con el subgénero *Haplomylomys* o *Peromyscus*. Sin embargo, las características únicas de *P. hooperi* (e. g., falo, cariotipo) han sido reconocidas, por lo que se ha mantenido en su propio grupo de especies. Con el objetivo de proveer nueva evidencia sobre la posición filogenética de *P. hooperi*, utilizamos el método de captura por hibridación y secuenciación masiva para obtener miles de elementos ultraconservados y el genoma mitocondrial de un ejemplar colectado en 1896 por Nelson y Goldman en Coahuila, México. Además, analizamos datos genómicos de 21 especies de neotominos. Contrario a estudios previos, encontramos altos valores de soporte en el nodo que posiciona a *P. hooperi* como la especie hermana del clado que incluye a *Podomys floridanus*, *Neotomodon alstoni*, *Habromys simulatus*, *H. ixtlani*, *Peromyscus mexicanus*, *P. megalops*, *P. melanophrys*, *P. perfulvus*, *P. aztecus*, *P. attwateri*, *P. pectoralis* y *P. boylii*. Datamos la divergencia de *P. hooperi* de su grupo hermano hace aproximadamente 3.98 millones de años, después de la divergencia de *P. crinitus* y de otros peromiscinos hace aproximadamente 4.31 millones de años, ambos eventos durante el Plioceno. Nuestro estudio es un claro ejemplo de que analizar datos a nivel del genoma mejoran la señal filogenética, independientemente del número de taxones, para especies cuyas relaciones filogenéticas son conflictivas o se encuentran poco resueltas como en el caso de *P. hooperi*. Sin embargo, recomendamos que futuros estudios genómicos incluyan un muestreo taxonómico más amplio, sobre todo de miembros del subgénero *Haplomylomys*, para confirmar las relaciones filogenéticas de *P. hooperi* y el estatus genético de sus poblaciones.

**Keywords:** Historical DNA; genomics; mitogenomes; museum specimens; *Peromyscus*, Pliocene-Pleistocene; ultraconserved elements.

## Introduction

Two of the most important naturalists from the turn of the 20<sup>th</sup> Century were Edward William Nelson and Edward Alphonso Goldman. They contributed greatly to our knowledge, understanding, and documentation of the biota in the United States and México (López-Medellin and Medellín 2016, <https://sova.si.edu/record/SIA.FARU7364>). The scientific material collected by both naturalists continues to be used as a rich resource in the systematic revision of many groups of birds and mammals (López-Medellin and Medellín 2016). Nelson and Goldman's biological surveys encompassed all of the states in México and lasted 14 years (1892 to 1906). In 1896, Nelson and Goldman conducted field work in Coahuila, México where they collected three individuals, later recognized as *Peromyscus hooperi*. These specimens were deposited and remain housed at the Smithsonian Institution's National Museum of Natural History in Washington DC.

*Peromyscus hooperi* is a monotypic species, endemic to México and only known from portions of the states of Coahuila, Zacatecas, and San Luis Potosí (Álvarez-Castañeda 2002). This species is sympatric with *P. eremicus*, *P. melanophrys*, and *P. pectoralis* in the states of Coahuila and Zacatecas (Schmidly et al. 1985). Its preferred habitat is the grassland transition zone, a mixture of desert scrub and grassland vegetation (Schmidly et al. 1985; Lee and Schmidly 1977). Its present fragmented and restricted distribution is considered a relict of a much larger historical distribution (Schmidly et al. 1985).

*Peromyscus hooperi* is poorly represented in mammal collections and little is known about its current status in their restricted distribution; however, it is not protected by the Mexican government (Norma Oficial Mexicana – 059 – 2020, Secretaría de Medio Ambiente y Recursos Naturales 2010) and is classified as Least Concern by the International Union for Conservation of Nature – IUCN – (accessed on August 2022, Álvarez-Castañeda 2016). The species resembles *P. eremicus*, *P. merriami*, and *P. pectoralis* in cranial and external characters but differs in the karyotype (Lee and Schmidly 1977; Schmidly et al. 1985). Fuller et al. (1984) and Schmidly et al. (1985) found that the karyotype of *P. hooperi* is very similar to *P. crinitus*, *P. simulus*, *Osgoodomys banderanus* and northern populations of *P. boylii*. However, *P. hooperi* has been described as a medium size mouse for the genus, with a long and bicolored tail (light grayish brown above and whitish below) with short hair. The upper parts, including face and top of head, are grayish with faint to moderate wash brown; lateral line is faint and near light buff; underparts are cream; and hind feet and lower legs are whitish. The skull contains large auditory bullae, and the first two upper and lower molars lack mesolophes. The glans penis is small but wide with a long protractile tip, and the baculum is long and slender with a cartilaginous tip (Lee and Schmidly 1977). The karyotype (2n = 48, FN = 52) comprises three pairs of biarmed autosomes and 20 pairs of acrocentric acrosomes (Lee and Schmidly 1977; Schmidly et al. 1985).

The taxonomic affinity of Hooper's deer mouse has been problematic (Carleton 1989). Based on a series of morphological characters (*i. e.*, cranial characteristics, accessory lophs, and styles of the anterior molars, structure of the hyoid, and number and placement of the mammae) it was suggested to be closely related to the subgenus *Haplomylomys* (Lee and Schmidly 1977). However, based on the anatomy of the phallus, it was more similar to species representing the subgenus *Peromyscus* (Lee and Schmidly 1977; Schmidly et al. 1985). Therefore, *P. hooperi* was characterized as an intermediate form between these two subgenera (Lee and Schmidly 1977; Fuller et al. 1984; Schmidly et al. 1985). *Peromyscus hooperi* currently is recognized as the sole member of the *Peromyscus hooperi* species group (Schmidly et al. 1985; Carleton 1989), based on morphological characters, karyotypes, allozymes, and mtDNA – cytochrome b (*Cytb*; Carleton 1989; Musser and Carleton 1993, 2005; Hogan et al. 1993; Dawson 2005; Bradley et al. 2007).

Bradley et al. (2007) used *Cytb* sequence data to conduct a phylogenetic analysis of the genus *Peromyscus*. They recovered strong nodal support for a sister group relationship between *P. hooperi* and *P. crinitus* with Maximum Parsimony (MP), however, using Maximum Likelihood (ML) and Bayesian Inference (BI) they did not resolve this relationship. In turn, this clade was sister to a clade including *P. melanotis*, *P. polionotus*, *P. maniculatus*, *P. keeni*, *P. leucopus*, *P. gossypinus*, *P. eremicus*, *P. californicus*, and *Osgoodomys banderanus*. Platt et al. (2015), included *Cytb* and three nuclear genes – *Adh1-l2*, *Fgb-l7* and *Rbp3*, and concluded that the phylogenetic position of *P. hooperi* remains uncertain due to a lack of support values and the different placement between ML and BI analyses.

An additional problem for the systematic classification of the species within *Peromyscus* is the very definition of the genus. Several revisions and classifications have recognized subgenera – *sensu lato* – (Osgood 1909; Hooper and Musser 1964; Hooper 1968) and genera – *sensu stricto* – (Carleton 1980; Carleton 1989; Musser and Carleton 2005) within *Peromyscus*. However, the current resolution of this group does not fully adhere to either of those classifications. In addition, genetic and genomic studies have demonstrated the paraphyly of *Peromyscus* (Bradley et al. 2007; Miller and Engstrom 2008; Platt et al. 2015; Sullivan et al. 2017; Castañeda-Rico et al. 2022). While clarifying the definition of *Peromyscus* is beyond the scope and objective of this manuscript, it is important to point out that whether we align to the *sensu lato* or *sensu stricto* classification of the genus, the phylogenetic placement of *P. hooperi* has not been well-resolved. However, hereafter, we recognized the genus *Peromyscus* as paraphyletic, including *Habromys*, *Megadontomys*, *Neotomodon*, *Osgoodomys*, and *Podomys* at the generic level (*sensu stricto*).

Uncertainty of the phylogenetic position of *P. hooperi* based on previous studies necessitates a reevaluation using additional sequence data. To accomplish this, we used genome-wide data, including several thousand

nuclear ultraconserved elements and whole mitochondrial genomes from a museum voucher specimen of *P. hooperi* collected by Nelson and Goldman combined with data from previous studies. These data provide new evidence about the phylogenetic placement of *P. hooperi* and its time of divergence from other peromyscines.

## Materials and methods

**Sample collection and laboratory methods.** We used a museum specimen sample of *Peromyscus hooperi* – USNM 79619 – (ca. 2 mm<sup>2</sup> of dry skin) deposited at the Smithsonian Institution's National Museum of Natural History; and collected by E. W. Nelson and E. A. Goldman on August 14, 1896 from Carneros, Coahuila, México. We followed strict protocols to avoid contamination during sampling, as described in [McDonough et al. \(2018\)](#) and [Castañeda-Rico et al. \(2020\)](#). All pre-PCR steps were performed in a laboratory dedicated to processing ancient and historical DNA at the Center for Conservation Genomics, Smithsonian National Zoo and Conservation Biology Institute, Washington, DC. A silica column extraction protocol ([McDonough et al. 2018](#)) was used to extract DNA. We quantified DNA with a Qubit 4 fluorometer (Thermo Fisher, Waltham, MA) using a 1x dsDNA HS assay and visualized DNA with a TapeStation 4200 System (Agilent Technologies, Santa Clara, CA) using High Sensitivity D1000 reagents. A dual-indexed library was prepared using the SRSly PicoPlus NGS library prep kit (Claret Bioscience, LLC), according to the manufacturer's protocol. We performed dual indexing PCR with TruSeq-style indices ([Meyer and Kircher 2010](#)) using Kapa HiFi HotStart Uracil+ (Roche Sequencing), following the manufacturer's protocol. This library was amplified with 12 cycles of PCR. We then pooled three PCRs from the same library before cleaning to increase DNA fragment representation. We cleaned the indexed library using 1.6X solid-phased reversible immobilization (SPRI) magnetic beads ([Rohland and Reich 2012](#)), quantified concentration using a Qubit 4 fluorometer, and inspected size ranges and quality with a TapeStation 4200 System (conditions as mentioned above). Target-enrichment was performed to capture ultraconserved elements (UCE) and mitogenomes using the myBaits® UCE Tetrapods 5Kv1 kit (Daicel Arbor Biosciences) following the myBaits protocol v3, and the myBaits® Mito kit (Daicel Arbor Biosciences) for the house mouse *Mus musculus* panel, following the myBaits protocol v4. We amplified post-enrichment UCE and mitogenome libraries with 18 cycles of PCR using Kapa HiFi HotStart Ready Mix (Roche Sequencing), following the manufacturer's protocol. A 1.6X SPRI magnetic bead clean-up was performed subsequently. We again quantified and visualized the enriched libraries using a Qubit 4 fluorometer and a TapeStation 4200 System, respectively (conditions as mentioned above). Finally, captured libraries were sequenced on a partial lane of a NovaSeq 6000 SP PE 2 x 150 base pairs (bp; Illumina, Inc., San Diego, CA, US) at the Oklahoma Medical Research Foundation, Oklahoma City (combined with samples from unrelated projects).

In addition to the data generated in this study, we also reanalyzed previously published data including the following: UCEs and full mitogenomes from [Castañeda-Rico et al. \(2020, 2022\)](#), as well as *Cytb* gene sequences from [Bradley et al. \(2007\)](#), [Platt et al. \(2015\)](#), and [Sullivan et al. \(2017\)](#); Table 1 and Appendix 1).

**Ultraconserved elements.** We analyzed the raw data following the PHYLUCe v1.6.7 pipeline with the default parameters (Faircloth 2016 <https://github.com/faircloth-lab/phyluce>). Illuminaprocessor 2.10 ([Faircloth 2013](#)) and Trim Galore 0.6.5 (<https://github.com/FelixKrueger/TrimGalore>) were used to trim adapters, barcode regions and low-quality bases. Reads were assembled into contigs using Trinity 2.8.5 ([Grabherr et al. 2011](#)), and identified contigs matching UCE loci in the 5K UCE probe set (<https://github.com/faircloth-lab/uce-probe-sets>). A monolithic FASTA file was produced to extract sequences from each sample. We aligned FASTA sequences using MAFFT 7.4 ([Katoh and Standley 2013](#); [Nakamura et al. 2018](#)) and performed edge trimming. The resulting alignments were filtered to test them for various degrees of missing data (matrix completeness): 65 % matrix (35 % of the taxa missing for each UCE locus), 75 % matrix (25 % of taxa missing), 85 % matrix (15 % of taxa missing), 90 % matrix (10% of taxa missing), and 95 % matrix (5 % of taxa missing). Samples included in this dataset are shown in Table 1. We quantified informative sites with the PHYLUCe script *phyluce\_align\_get\_informative\_sites.py*. All of these analyses were performed on the Smithsonian Institution High Performance Computing Cluster (Smithsonian Institution, <https://doi.org/10.25572/SIHPC>).

We conducted a Maximum Likelihood (ML) analysis using RAxML 8.12 ([Stamatakis 2014](#)) with a GTRGAMMA site rate substitution model and 20 ML searches for the phylogenetic tree for each of the aforementioned data matrices (*i. e.*, 65 % to 95 % matrices). Nonparametric bootstrap replicates were generated using the -N autoMRE option which runs until convergence was reached. We reconciled the best fitting ML tree with the bootstrap replicate to obtain the final phylogenetic tree with support values using the -f b command.

We estimated the best evolutionary model of nucleotide substitution in jModelTest 2.1.1 ([Guindon and Gascuel 2003](#); [Darriba et al. 2012](#)) using the Akaike Information Criterion (AIC). The TVW+G model was selected as the best fitting model with the following parameters: base frequencies A = 0.2988, C = 0.2013, G = 0.2026, T = 0.2972; nst = 6; and gamma shape = 0.1070. A Bayesian Inference analysis (BI) using MrBayes 3.2.6 ([Huelsenbeck and Ronquist 2001](#); [Ronquist and Huelsenbeck 2003](#)) was performed on the 90 % matrix. The BI analyses comprised two independent runs with 50 million generations, sampling trees and parameters every 1,000 generations with four Markov-chains Monte Carlo (MCMC), three heated and one cold. We visualized output parameters using Tracer v1.7.1 ([Rambaut et al. 2018](#)) to check for convergence between runs and we discarded the first 25 % of the trees as burn-in.

A species tree analysis under the multispecies coalescent (MSC) model with ASTRAL-III v.5.7.8 (Zhang *et al.* 2018) was performed on the 90 % matrix. The local posterior probability – LPP – (Sayari and Mirarab 2016) was used as branching support. We used the uce2speciestree pipeline script (Campana 2019 <https://github.com/campanam/uce2speciestree>) to generate input files for ASTRAL. This script uses RAxML to infer individual gene trees under the GTRGAMMA substitution model, and 100 bootstrap replicates.

**Mitogenomes.** FASTQ files were analyzed using FastQC v0.11.5 (Andrews 2010, [www.bioinformatics.babraham.ac.uk/projects/fastqc](http://www.bioinformatics.babraham.ac.uk/projects/fastqc)). Adapter sequences and low-quality reads were removed using the default parameters (Phred:20, mean min-len:20) in Trim Galore 0.6.5 (<https://github.com/FelixKrueger/TrimGalore>). Exact duplicates were removed (-derep1,4) using Prinseq-lite v0.20.4 (Schmieder and Edwards 2011). We mapped the resulting high-quality reads to the closest available reference genome (*Peromyscus crinitus* – GenBank accession number KY707308), using the Geneious algorithm in Geneious Prime® 2021.2.2 (<https://www.geneious.com>) with default parameters (Medium-Low sensitivity, Maximum mismatches = 20 %, Maximum gaps = 10 %). A consensus sequence was generated with Geneious Prime® 2021.2.2 (<https://www.geneious.com>), using 4X as the lowest coverage to call a base, and aligned them using MAFFT 7.45 plug-in (Katoh and Standley 2013). We transferred annotations from the reference to rule out

the presence of nuclear copies of mitochondrial genes (NUMTs), and translated all protein-coding genes to check for frame shifts or stop codons.

We aligned sequences with MAFFT 7.45 plug-in (Katoh and Standley 2013) in Geneious Prime® 2021.2.2 (<https://www.geneious.com>). Samples included in this dataset are listed in Table 1. A BI analysis was conducted on a partitioned dataset using MrBayes 3.2.6 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). The best model and partition scheme were estimated using PartitionFinder 2.1.1 (Lanfear *et al.* 2016). Our search was limited to the models available in MrBayes, with linked, corrected Akaike Information Criterion (AICc) and greedy parameters. The data block was defined by gene, tRNA, rRNA and D-loop selection. We conducted two independent runs with 50 million generations, sampling trees and parameters every 1,000 generations with four MCMC and parameters as mentioned above, to perform the BI analysis. Convergence between runs was checked using Tracer v1.7.1 (Rambaut *et al.* 2018), and the first 25 % of the trees was discarded as burn-in.

We performed a ML analysis using the concatenated dataset in RAxML 8.12 (Stamatakis 2014) with a GTRGAMMA site rate substitution model. Clade support was assessed by bootstrapping with the -N autoMRE option for a bootstrap convergence criterion. The -f b option was used to reconcile the best fitting ML tree with the bootstrap rep-

**Table 1.** Specimens examined in this study using UCE and mitogenomes with species name, accession number collection/ID study (Smithsonian Institution’s National Museum of Natural History USNM, Museum of Texas Tech University TK, and TTU associated, Museo de Zoología “Alfonso L. Herrera” Facultad de Ciencias UNAM MZFC, and University of Michigan Museum of Zoology –UMMZ), reference (the study from which the sequences were obtained or reanalyzed), GenBank BioProject, and GenBank accession numbers.

Species	Number Scientific Collection/ID	Reference	UCE (GenBank BioProject)	Mitogenome (GenBank number)
<i>Peromyscus hooperi</i>	USNM79619/USNM79619	This study	PRJNA880321	OP432689
<i>Peromyscus boylii</i>		This study		MZ433362
<i>Peromyscus maniculatus</i>		This study		MH260579
<i>Peromyscus leucopus</i>		This study		BK010700
<i>Peromyscus megalops</i>	USNM340233/USNM340233	Castañeda-Rico <i>et al.</i> (2022)	PRJNA838631	ON528115
<i>Peromyscus attwateri</i>	TTU143738/TK185663	Castañeda-Rico <i>et al.</i> (2022)	PRJNA838631	ON528112
<i>Peromyscus aztecus</i>	USNM569848/USNM569848	Castañeda-Rico <i>et al.</i> (2022)	PRJNA838631	ON528113
<i>Peromyscus polionotus</i>	USNM585473/USNM585473	Castañeda-Rico <i>et al.</i> (2022)	PRJNA838631	ON528117
<i>Peromyscus crinitus</i>	TTU146966/TK193714	Castañeda-Rico <i>et al.</i> (2022)	PRJNA838631	ON528114
<i>Podomys floridanus</i>	TTU97866/TK92501	Castañeda-Rico <i>et al.</i> (2022)	PRJNA838631	ON528118
<i>Neotomodon alstoni</i>	TTU82668/TK93098	Castañeda-Rico <i>et al.</i> (2022)	PRJNA838631	ON528110
<i>Onychomys leucogaster</i>	TTU146304/TK171574	Castañeda-Rico <i>et al.</i> (2022)	PRJNA838631	ON528111
<i>Reithrodontomys mexicanus</i>	TTU138428/TK178510	Castañeda-Rico <i>et al.</i> (2022)	PRJNA838631	ON528119
<i>Isthmomys pirrensis</i>	USNM565924/USNM565924	Castañeda-Rico <i>et al.</i> (2022)	PRJNA838631	ON528108
<i>Neotoma mexicana</i>	TTU104969/TK150189	Castañeda-Rico <i>et al.</i> (2022)	PRJNA838631	ON528109
<i>Peromyscus mekisturus</i>	UMMZ88967/UMMZ88967	Castañeda-Rico <i>et al.</i> (2020)	PRJNA606805	MT078818
<i>Peromyscus melanophrys</i>	MZFC3907/MQ1229	Castañeda-Rico <i>et al.</i> (2020)	PRJNA606805	MT078816
<i>Peromyscus perfulvus</i>	– /MCP119	Castañeda-Rico <i>et al.</i> (2020)	PRJNA606805	MT078817
<i>Peromyscus pectoralis</i>	MZFC10465/FCR176	Castañeda-Rico <i>et al.</i> (2020)	PRJNA606805	MT078819
<i>Peromyscus mexicanus</i>	MZFC11150/MRM030	Castañeda-Rico <i>et al.</i> (2020)	PRJNA606805	
<i>Habromys simulatus</i>	MZFC10104/HBR031	Castañeda-Rico <i>et al.</i> (2020)	PRJNA606805	

licate to obtain the final phylogenetic tree (as mentioned above). DNA damage patterns were evaluated for the *P. hooperi* sample with mapDamage2.0 (Jónsson et al. 2013) using --rescale option.

**Cytochrome b.** We analyzed *Cytb* sequences extracted from the mitogenome that was generated in this study and from mitogenomes published by Sullivan et al. (2017) and Castañeda-Rico et al. (2020, 2022). We also used the *Cytb* sequences published by Bradley et al. (2007) and Platt et al. (2015) in order to compare the phylogenetic position of *P. hooperi* using genome-wide data as well as a single mitochondrial gene. The *Cytb* dataset allowed us to include more species within the genus *Peromyscus* and representatives of the genera *Habromys*, *Megadontomys*, *Neotomodon*, *Osgoodomys*, *Podomys*, *Isthmomyss*, *Onychomys*, *Reithrodontomys*, *Neotoma*, *Ochrotomys*, *Baiomys*, *Ototylomys*, *Tylomys*, *Nyctomys*, *Oryzomys* and *Sigmodon*. Samples included in this dataset are shown in Table 1 and Appendix 1.

The *Cytb* dataset was analyzed as follows: we performed alignment using MAFFT 7.45 plug-in (Katoh and Standley 2013) in Geneious Prime® 2021.2.2 (<https://www.geneious.com>). We estimated the best evolutionary model of nucleotide substitution in jModelTest 2.1.1 (Guindon and Gascuel 2003; Darriba et al. 2012) using the AIC method. The TPM3uf+I+G model was selected as the best fitting model with the following parameters: base frequencies A = 0.3896, C = 0.3336, G = 0.0500, T = 0.2267; nst = 6; proportion of invariable sites = 0.4080; and gamma shape = 0.6220. A BI analysis was run using MrBayes 3.2.6 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) as mentioned above for UCE and mitogenome datasets. We used Tracer v1.7.1 (Rambaut et al. 2018) to check for convergence between runs, and the first 25 % of the trees was discarded as burn-in.

**Divergence times estimation.** Molecular dates of divergence were estimated in BEAST2 v2.6.6 (Bouckaert et al. 2019) using the mitogenome dataset. First, we obtained the best model and partition scheme in PartitionFinder 2.1.1 (Lanfear et al. 2016). The search was limited to the models available in BEAST, linked branch lengths, AICc model selection, and greedy schemes search. The data block was defined by codon position, tRNA, rRNA and D-loop selection, and the result was incorporated in the dating analysis. The BEAST analysis was performed under an uncorrelated lognormal relaxed molecular clock model. The calibrated Yule speciation processes model (Heled and Drummond 2012) with a randomly generated starting tree were set up as priors. We used the same three calibration points with a lognormal distribution from Castañeda-Rico et al. (2022). Calibrations were based on fossil records of 1) *Reithrodontomys* (mean = 1.8 million years ago [mya], stdev = 1.076, offset = 1.63), as used by Steppan and Schenk (2017); 2) *Onychomys* (mean = 4.9, stdev = 1.169, offset = 4.753), as used by Steppan and Schenk (2017); and 3) the most recent common ancestor of *P. attwateri* (mean = 2.7, stdev = 0.9, offset = 2.4 [Dalquest 1962; Karow et al. 1996;

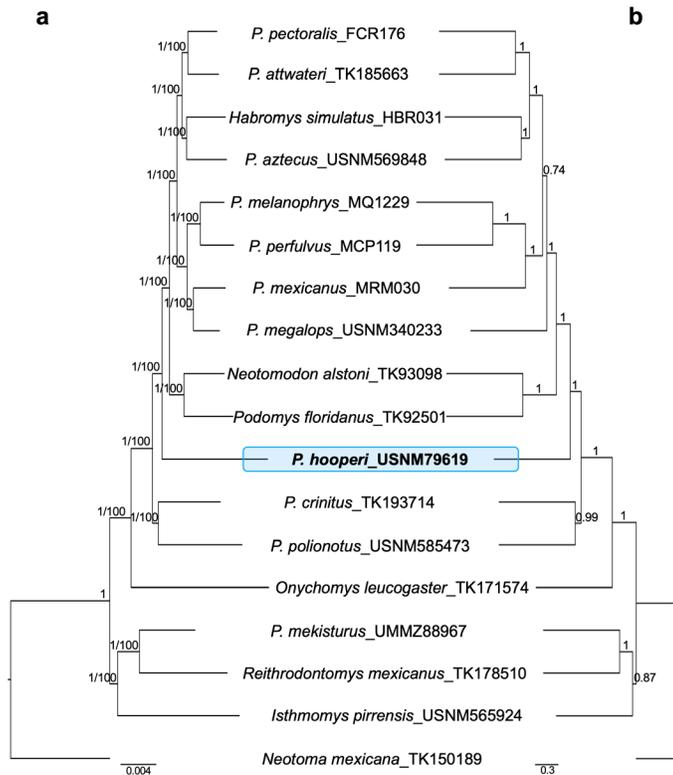
Wright et al. 2020]). Two independent runs of 50 million iterations were performed, each was sampled every 1,000 iterations. We checked convergence statistics for Effective Sample Sizes (ESS) using Tracer v1.7.1 (Rambaut et al. 2018) and a 25 % of burn-in was performed on each run. We used LogCombiner v2.6.6 to combine trees and TreeAnnotator v2.6.2 to get a consensus tree with node height distribution (both packages available in BEAST). All phylogenetic and ultrametric dated trees from the UCE, mitogenome and *Cytb* datasets were visualized in FigTree 1.4.4 (<http://tree.bio.ed.ac.uk/software/figtree/>). All analyses were performed on the Smithsonian Institution High Performance Computing Cluster (Smithsonian Institution <https://doi.org/10.25572/SHPC>).

## Results

Following the PHYLUCE v1.6.7 pipeline, we recovered 1,087 UCE loci (raw data are available in GenBank under BioProject PRJNA880321), and a complete mitogenome of 16,288 bp (GenBank accession number OP432689) from the *P. hooperi* sample. The average number of paired-end reads and fragment size after trimming were 13,075,112 reads and 67 bp long, respectively. The lowest-quality bases were detected at the end of the reads. We also recovered between 1,353 and 3,859 UCE loci from the reanalyzed samples. The average number of paired-end reads and fragment size after trimming for those samples ranged from 1,811,856 to 21,093,430 reads, and from 94 to 222 bp, respectively.

**Ultraconserved Elements phylogenies.** We recovered 9,840 contigs for *P. hooperi* after Trinity assemblies. The mean, minimum, and maximum length for contigs were 242, 201, and 3,784 bp, respectively. The incomplete matrix contained 4,406 UCE loci ( $n = 18$ , average = 3,136, min = 1,087, max = 3,859). A total of 1,087 UCE loci were obtained for *P. hooperi* with a mean, minimum, and maximum length of 235, 201, and 636 bp, respectively. The 65 % matrix contained 3,681 UCE loci (UL) with an average of 13.80 informative sites per locus (IS), the 75 % matrix (UL = 2,974, IS = 14.18, the 85 % matrix (UL = 1,514, IS = 14.29), the 90 % matrix (UL = 677, IS = 14.07), and the 95 % matrix (UL = 168, IS = 14.30).

The datasets representing various levels of matrix completeness yielded the same ML topology with high support values for all branches (Figure 1, phylogenetic trees obtained from the 65 %, 75 %, 85 %, and 95 % matrices are not shown). The BI tree topology, based on the 90 % matrix, showed the same topology with high posterior probability values for all branches (Figure 1). Both, ML and BI trees placed *P. hooperi* as sister to the clade containing *Podomys floridanus*, *Neotomodon alstoni*, *P. mexicanus*, *P. megalops*, *P. melanophrys*, *P. perfulvus*, *P. aztecus*, *Habromys simulatus*, *P. attwateri*, and *P. pectoralis*. The species tree supported, with high LPP values, the same phylogenetic position of *P. hooperi* (Figure 1, based on the 90 % matrix). The only difference among the species tree and the concatenated ML and BI trees, was the relationship between *P. mexicanus* and



**Figure 1.** (a) Bayesian Inference and Maximum Likelihood phylogenies based on a 90 % matrix UCE with 677 loci. Nodal support is provided with posterior probability/ bootstrap values. (b) Species tree based on a 90 % matrix UCE with 677 loci. Nodal support is provided with local posterior probability values. The blue block highlights the phylogenetic position of *Peromyscus hooperi*.

*P. megalops*. These two species are sisters in the ML and BI trees but not in the species tree, where *P. megalops* is sister to the clade containing *P. mexicanus*, *P. melanophrys*, *P. perfulvus*, *P. aztecus*, *Habromys simulatus*, *P. attwateri*, and *P. pectoralis*.

**Mitogenome phylogenies.** The final alignment included 21 taxa and was 16,272 bp in length. BI and ML analyses, with six partitions, provided slightly different topologies (Figure 2). However, both analyses supported ( $pp = 1$ , bootstrap = 76) the placement of *P. hooperi* as sister to the clade including *Podomys floridanus*, *Neotomodon alstoni*, *P. mexicanus*, *P. megalops*, *P. melanophrys*, *P. perfulvus*, *P. boylii*, *P. aztecus*, *Habromys ixtlani*, *P. attwateri*, and *P. pectoralis*. The phylogenetic position of *P. crinitus* changed across phylogenies (Figure 2), as did the position of the clade containing *Podomys floridanus* + *Neotomodon alstoni*. However, the BI tree yielded higher support values. The DNA damage analysis showed a weak signal of damage, typical of historical DNA (Appendix 2).

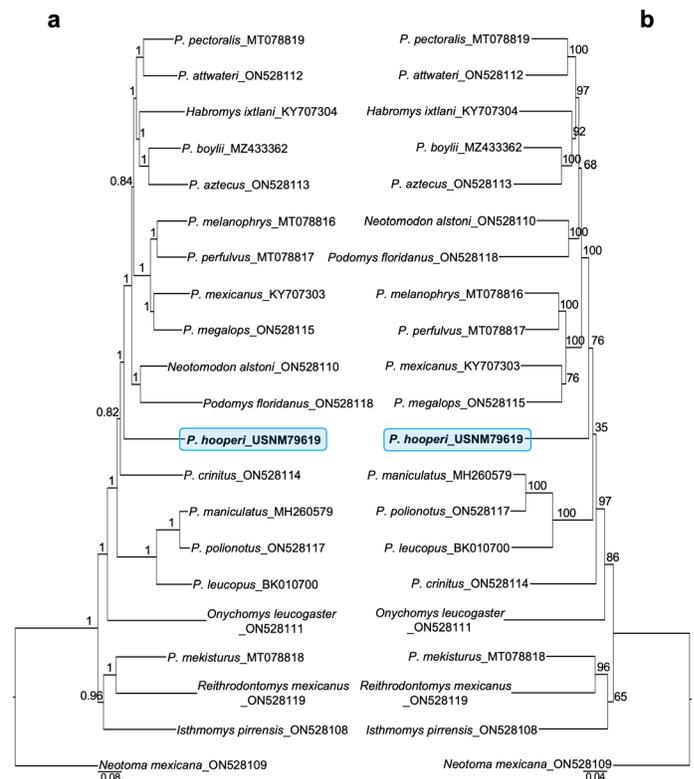
**Cytochrome b phylogeny.** The alignment included 64 taxa, 154 sequences, and was 1,143 bp in length. The BI analysis placed *P. hooperi* sister to the clade containing *P. maniculatus*, *P. polionotus*, *P. keeni*, *P. melanotis*, *P. leucopus*, and *P. gossypinus* (Appendix 3). However, the branch support value for this phylogenetic position was low ( $pp = 0.53$ ). The two samples of *P. hooperi*, one sequenced in this study (USNM 79619) and the other by [Bradley et al. \(2007;](#)

TTU 104425, GenBank accession number DQ973103) clustered together with high support ( $pp = 1$ ).

**Divergence time estimation of *Peromyscus hooperi*.** The mitochondrial divergence dating analysis, with six data partitions, estimated a Pliocene divergence time for *P. hooperi* around 3.98 mya (95 % HPD: 3.57 to 4.47 mya; Figure 3). The divergence of *P. crinitus* was dated ca. 4.31 mya (95 % HPD: 3.80 to 4.70 mya), the split of the clade including *P. leucopus* + (*P. polionotus* + *P. maniculatus*) at ca. 4.49 mya (95 % HPD: 4.03 – 5.02 mya), and the divergence of the genus *Peromyscus* was dated ca. 5.21 mya (95 % HPD: 4.79 – 5.71 mya).

## Discussion

The biological expeditions undertaken by Nelson and Goldman in México were arguably among the most important ever achieved by two naturalists for a single country ([López-Medellin and Medellin 2016](#); [Guevara 2021](#); <https://sova.si.edu/record/SIA.FARU7364>). To our knowledge, this is one of a few studies in which genome-wide data were obtained and analyzed from a specimen collected by these two naturalists (see [McDonough et al. 2022](#)). Our results not only provide new evidence about the phylogenetic position of *P. hooperi* but also joins a short list of mammal studies within the blooming field of Museomics (see [Card et al. 2021](#) for a review) that have successfully analyzed specimens collected before 1900 within a phylogeny (e. g., [Abreu-Jr et al. 2020](#); [Sacks et al. 2021](#); [Roycroft et al. 2021, 2022](#); [Castañeda-Rico et al. 2022](#); [McDonough et al. 2022](#); [Tavares et al. 2022](#)).



**Figure 2.** Mitogenome phylogenies based on Bayesian Inference (a) and Maximum Likelihood (b). Nodal support is provided with posterior probability and bootstrap values, respectively. The blue block highlights the phylogenetic position of *Peromyscus hooperi*.



tion is not resolved. In conclusion, we confirmed that the phylogenetic position of the Hooper's deer mouse cannot be resolved using only *Cytb* sequences or a few genes, as [Platt et al. \(2015\)](#) documented. Our results demonstrate that genome-wide data allow a better resolution of the phylogenetic relationships of phylogenetically problematic species.

Our divergence times estimations indicated that the crown of *Peromyscus* was estimated *ca.* 5.21 mya (95 % HPD: 4.79 to 5.71 mya), and the diversification of the genus occurred *ca.* 4.49 mya (95 % HPD: 4.03 to 5.02 mya), both events during the Pliocene. We dated the split of *P. hooperi* during the Pliocene at *ca.* 3.98 mya (95 % HPD: 3.57 to 4.47 mya), following the split from *P. crinitus* at *ca.* 4.31 mya (95 % HPD: 3.80 to 4.70 mya). These dates coincide with previously dated phylogenies obtained from genome-wide data of peromyscines (*e. g.*, [Castañeda-Rico et al. 2022](#)). They estimated the crown of the genus *Peromyscus* during the Pliocene at *ca.* 5.32 mya (95 % HPD: 4.85 to 5.98 mya), and the origin of *P. crinitus* at *ca.* 4.62 mya (95 % HPD: 4.05 to 5.28 mya), using mitogenomes. Our results also show that the *Peromyscus hooperi*, *crinitus*, *maniculatus*, and *leucopus* species groups were among the first to diverge within the genus *Peromyscus* (Figure 3), followed by the *Peromyscus megalops*, *mexicanus*, *melanophrys*, *boylii*, *aztecus*, and *truei* species groups, together with *Neotomodon*, *Podomys*, and *Habromys*. Based on our results and those of previous studies (*e. g.*, [Hibbard 1968](#); [Riddle et al. 2000](#); [Dawson 2005](#); [Castañeda-Rico et al. 2014, 2022](#); [Platt et al. 2015](#); [Sawyer et al. 2017](#); [León-Tapia et al. 2021](#)), we suggest the Pliocene and Pleistocene as the time when speciation and diversification events took place within peromyscines, potentially associated with climatic cycles related to numerous vicariant and dispersal events.

Previous phylogenetic studies of the genus *Peromyscus* that analyzed single or few genes, provided older divergence times estimations (*e. g.*, [Castañeda-Rico et al. 2014](#); [Platt et al. 2015](#); [Cornejo-Latorre et al. 2017](#); [Bradley et al. 2019](#)). For example, [Platt et al. \(2015\)](#), using *Cytb*, estimated the origin of *Peromyscus* and its diversification, during the Miocene, at approximately 8 mya and 5.71 mya, respectively; and the divergence of *P. hooperi* at *ca.* 5.2 mya, during the early Pliocene. However, estimates of the time to the most recent common ancestor (TMRCA) calculated from individual or few genes can be overestimated ([Duchêne et al. 2011](#)).

The evolutionary uniqueness of *P. hooperi* is supported by our results and previous studies by [Fuller et al. \(1984\)](#) and [Schmidly et al. \(1985\)](#) who found that this species does not fit well with either of the subgenera *Haplomyiomys* or *Peromyscus*. We hypothesize that *P. hooperi* will remain the sole member of the *Peromyscus hooperi* group as first proposed by [Schmidly et al. \(1985\)](#) and later supported by [Carleton \(1989\)](#) based on the morphological, karyotypic, and allozyme evidence.

The genetic and morphological uniqueness of *P. hooperi*, as well as its restricted distribution to grassland transition zones should make this a species of special concern for conservation. In addition, [Schmidly et al. \(1985\)](#) stated that *P. hooperi* is a relictual, monotypic species without close living relatives, and its survival is jeopardized/threatened by the fragile conditions of its habitat in central Coahuila as a result of overgrazing. During the last 21 years, habitat shifts from native grasslands to crops zones have increased with agricultural intensification, grain-fed cattle feedlots, and new land use policies in the Mexican states of Durango, Sinaloa, Chihuahua, Nuevo León, and particularly Coahuila where *P. hooperi* is mostly distributed ([Galván-Miyoshi et al. 2015](#); [Bonilla-Moheno and Aide 2020](#)). We recommend that future studies conduct population genetic analyses to determine the genetic diversity and structure of the different populations of *P. hooperi*. This species remains poorly known and potentially threatened by habitat loss, therefore new information is needed to determine an appropriate conservation strategy and category.

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## Appendix 1

Specimens examined in this study using *Cytb* gene. We show the name of the species, reference (the study from which the sequences were obtained or reanalyzed), and GenBank accession number.

Species	Study	Mitogenome (GenBank number)	<i>Cytb</i> (GenBank number)
<i>Onychomys leucogaster</i>	Castañeda-Rico et al. (2020)	KU168563 (To extract <i>Cytb</i> )	
<i>Habromys ixtlani</i>	Sullivan et al. (2017)	KY707304 (To extract <i>Cytb</i> )	
<i>Isthmomys pirrensis</i>	Sullivan et al. (2017)	KY707312 (To extract <i>Cytb</i> )	
<i>Neotoma mexicana</i>	Sullivan et al. (2017)	KY707300 (To extract <i>Cytb</i> )	
<i>Neotomodon alstoni</i>	Sullivan et al. (2017)	KY707310 (To extract <i>Cytb</i> )	
<i>Peromyscus attwateri</i>	Sullivan et al. (2017)	KY707299 (To extract <i>Cytb</i> )	
<i>Peromyscus aztecus</i>	Sullivan et al. (2017)	KY707306 (To extract <i>Cytb</i> )	
<i>Peromyscus crinitus</i>	Sullivan et al. (2017)	KY707308 (To extract <i>Cytb</i> )	
<i>Peromyscus megalops</i>	Sullivan et al. (2017)	KY707305 (To extract <i>Cytb</i> )	
<i>Peromyscus mexicanus</i>	Sullivan et al. (2017)	KY707303 (To extract <i>Cytb</i> )	
<i>Peromyscus pectoralis</i>	Sullivan et al. (2017)	KY707309 (To extract <i>Cytb</i> )	
<i>Peromyscus polionotus</i>	Sullivan et al. (2017)	KY707301 (To extract <i>Cytb</i> )	
<i>Podomys floridanus</i>	Sullivan et al. (2017)	KY707302 (To extract <i>Cytb</i> )	
<i>Reithrodontomys mexicanus</i>	Sullivan et al. (2017)	KY707307 (To extract <i>Cytb</i> )	
<i>Sigmodon hispidus</i>	Sullivan et al. (2017)	KY707311 (To extract <i>Cytb</i> )	
<i>Baiomys taylori</i>	Bradley et al. (2007)		AF548469
<i>Habromys ixtlani</i>	Bradley et al. (2007)		DQ861395
			DQ000482
<i>Habromys ixtlani</i>	Bradley et al. (2007)		DQ973099
<i>Isthmomys pirrensis</i>	Bradley et al. (2007)		DQ836299
<i>Megadontomys cryophilus</i>	Bradley et al. (2007)		DQ861373
<i>Megadontomys thomasi</i>	Bradley et al. (2007)		AY195795
<i>Neotoma mexicana</i>	Bradley et al. (2007)		AF294345
<i>Neotomodon alstoni</i>	Bradley et al. (2007)		AY195796
			AY195797
			DQ861374
<i>Nyctomys sumichrasti</i>	Bradley et al. (2007)		AY195801
<i>Ochrotomys nuttalli</i>	Bradley et al. (2007)		AY195798
<i>Onychomys arenicola</i>	Bradley et al. (2007)		AY195793
<i>Oryzomys palustris</i>	Bradley et al. (2007)		DQ185382
<i>Osgoodomys banderanus</i>	Bradley et al. (2007)		AF155383
			DQ000473
<i>Ototylomys phyllotis</i>	Bradley et al. (2007)		AY009789
<i>Peromyscus attwateri</i>	Bradley et al. (2007)		AF155384
			AF155385
<i>Peromyscus aztecus</i>	Bradley et al. (2007)		U89968
<i>Peromyscus beatae</i>	Bradley et al. (2007)		AF131921
			AF131922
			AF131914
<i>Peromyscus boylii</i>	Bradley et al. (2007)		AF155386
			AF155392
			AF155388
<i>Peromyscus californicus</i>	Bradley et al. (2007)		AF155393
<i>Peromyscus crinitus</i>	Bradley et al. (2007)		AY376413
			DQ861378
<i>Peromyscus crinitus</i>	Bradley et al. (2007)		EF028168
<i>Peromyscus difficilis</i>	Bradley et al. (2007)		AY376419 AY376415
			AY387488

## Appendix 1

Continuation

Species	Study	Mitogenome (GenBank number)	Cytb (GenBank number)
<i>Peromyscus eremicus</i>	Bradley <i>et al.</i> (2007)		AY195799
			AY322503
<i>Peromyscus eremicus</i>	Bradley <i>et al.</i> (2007)		DQ973100
<i>Peromyscus evides</i>	Bradley <i>et al.</i> (2007)		U89970
<i>Peromyscus furvus</i>	Bradley <i>et al.</i> (2007)		AF271032
			AF271012
			AF271005
<i>Peromyscus gossypinus</i>	Bradley <i>et al.</i> (2007)		DQ973101
			DQ973102
<i>Peromyscus gratus</i>	Bradley <i>et al.</i> (2007)		AY322507
			AY376421
			AY376422
<i>Peromyscus guatemalensis</i>	Bradley <i>et al.</i> (2007)		EF028171
			EF028172
<i>Peromyscus gymnotis</i>	Bradley <i>et al.</i> (2007)		EF028169
			EF028170
			EF028169
<i>Peromyscus hooperi</i>	Bradley <i>et al.</i> (2007)		DQ973103
<i>Peromyscus hylocetes</i>	Bradley <i>et al.</i> (2007)		U89976
			DQ000481
<i>Peromyscus keeni</i>	Bradley <i>et al.</i> (2007)		X89787
			AF119261
<i>Peromyscus leucopus</i>	Bradley <i>et al.</i> (2007)		AF131926
			DQ000483
<i>Peromyscus leucopus</i>	Bradley <i>et al.</i> (2007)		DQ973104
<i>Peromyscus levipes</i>	Bradley <i>et al.</i> (2007)		AF131928
			AY322509
			AF155396
<i>Peromyscus madrensis</i>	Bradley <i>et al.</i> (2007)		AF155397
<i>Peromyscus maniculatus</i>	Bradley <i>et al.</i> (2007)		DQ000484
			AY322508
<i>Peromyscus maniculatus</i>	Bradley <i>et al.</i> (2007)		DQ973111
<i>Peromyscus mayensis</i>	Bradley <i>et al.</i> (2007)		DQ836300
			DQ836301
<i>Peromyscus megalops</i>	Bradley <i>et al.</i> (2007)		DQ000475
<i>Peromyscus melanocarpus</i>	Bradley <i>et al.</i> (2007)		EF028173
<i>Peromyscus melanophrys</i>	Bradley <i>et al.</i> (2007)		AY322510
			AY376424
<i>Peromyscus melanophrys</i>	Bradley <i>et al.</i> (2007)		DQ973105
<i>Peromyscus melanotis</i>	Bradley <i>et al.</i> (2007)		AF155398
<i>Peromyscus mexicanus</i>	Bradley <i>et al.</i> (2007)		AY376425
<i>Peromyscus mexicanus</i>	Bradley <i>et al.</i> (2007)		EF028174
<i>Peromyscus nasutus</i>	Bradley <i>et al.</i> (2007)		AF155399
			AY376426
<i>Peromyscus nudipes</i>	Bradley <i>et al.</i> (2007)		AY041200
<i>Peromyscus oaxacensis</i>	Bradley <i>et al.</i> (2007)		U89972
<i>Peromyscus ochraventer</i>	Bradley <i>et al.</i> (2007)		DQ973106

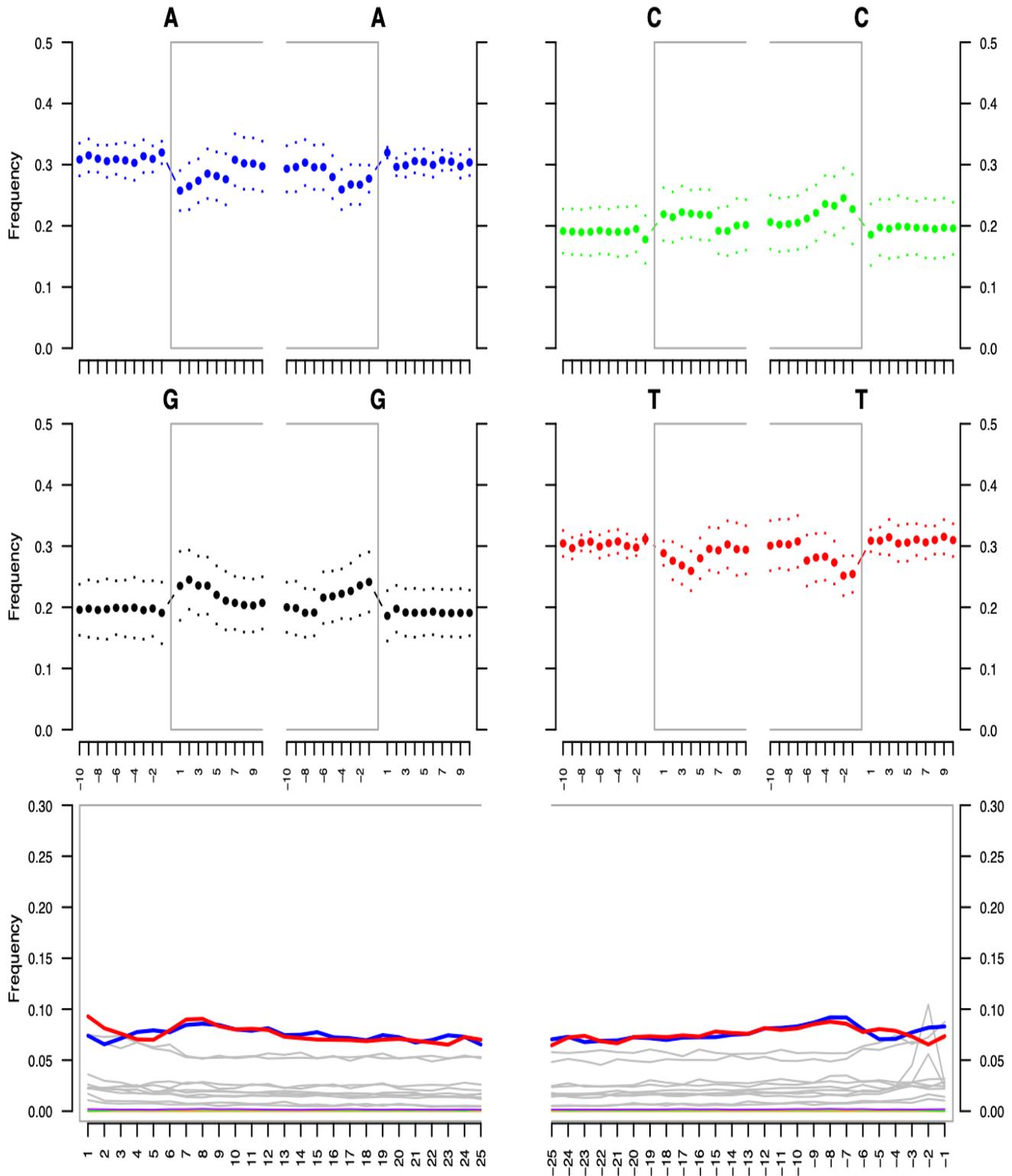
## Appendix 1

### Continuation

Species	Study	Mitogenome (GenBank number)	Cytb (GenBank number)
<i>Peromyscus pectoralis</i>	Bradley et al. (2007)		AF155400
			AY322511
			AY376427
<i>Peromyscus perfulvus</i>	Bradley et al. (2007)		DQ000474
<i>Peromyscus polionotus</i>	Bradley et al. (2007)		X89792
<i>Peromyscus polius</i>	Bradley et al. (2007)		AF155403
<i>Peromyscus sagax</i>	Bradley et al. (2007)		AF155404
<i>Peromyscus schmidlyi</i>	Bradley et al. (2007)		AY322520
			AF155405
			AY370610
<i>Peromyscus simulus</i>	Bradley et al. (2007)		AF131927
<i>Peromyscus spicilegus</i>	Bradley et al. (2007)		AY322512
			DQ000480
			DQ973107
<i>Peromyscus stephani</i>	Bradley et al. (2007)		AF155411
<i>Peromyscus stirtoni</i>	Bradley et al. (2007)		DQ973108
<i>Peromyscus truei</i>	Bradley et al. (2007)		AY376433
			AF108703
			AY376428
<i>Peromyscus winkelmanni</i>	Bradley et al. (2007)		AF131930
			U89983
			AY195800
<i>Peromyscus zarhynchus</i>	Bradley et al. (2007)		AY195800
<i>Podomys floridanus</i>	Bradley et al. (2007)		DQ973109
			DQ973110
			AF176248
<i>Reithrodontomys megalotis</i>	Bradley et al. (2007)		AF176248
<i>Reithrodontomys mexicanus</i>	Bradley et al. (2007)		AY859447
<i>Sigmodon hispidus</i>	Bradley et al. (2007)		AF155420
<i>Tylomys nudicaudatus</i>	Bradley et al. (2007)		AF307839
<i>Isthmomys pirrensis</i>	Platt II et al. (2015)		FJ214681
<i>Peromyscus crinitus</i>	Platt II et al. (2015)		FJ214684
<i>Peromyscus eremicus</i>	Platt II et al. (2015)		AY322503
<i>Peromyscus evides</i>	Platt II et al. (2015)		FJ214685
<i>Peromyscus levipes</i>	Platt II et al. (2015)		DQ000477
<i>Peromyscus mexicanus</i>	Platt II et al. (2015)		JX910118
<i>Peromyscus nudipes</i>	Platt II et al. (2015)		FJ214687
<i>Peromyscus ochraventer</i>	Platt II et al. (2015)		JX910119
<i>Peromyscus spicilegus</i>	Platt II et al. (2015)		FJ214669
<i>Reithrodontomys fulvescens</i>	Platt II et al. (2015)		AF176257
<i>Reithrodontomys sumichrasti</i>	Platt II et al. (2015)		AF176256
<i>Reithrodontomys mexicanus</i>	Platt II et al. (2015)		AY859453

## Appendix 2

Comparison of C → T terminal deamination patterns of *Peromyscus hooperi* (USNM 79619).



### Appendix 3

Bayesian phylogenetic tree based on mtDNA *Cytb* sequence data. Nodal support is provided with posterior probability values. The blue block highlights the phylogenetic position of *Peromyscus hooperi*.

