Systematics of the *Neotoma mexicana* species group (Mammalia: Rodentia: Cricetidae) in Mesoamerica: new molecular evidence on the status and relationships of *N. ferruginea* Tomes, 1862

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Abstract.—Analyses of the mitochondrial cytochrome-*b* gene were used to determine levels of genetic differentiation and patterns of relationship among members of the *Neotoma mexicana* (Mexican woodrat) species group in Mesoamerica. Three well-supported clades were obtained that conform to the species *N. ferruginea* Tomes, 1862, *N. mexicana* Baird, 1855, and *N. picta* Goldman, 1904. *Neotoma ferruginea* is the senior name for the clade that contains samples from southern Mexico and Nuclear Central America previously identified as *N. isthmica* Goldman, 1904, or as subspecies of *N. mexicana* (*chamula* Goldman, 1909; *vulcani* Sanborn, 1935). The phylogeographic pattern observed within the *N. mexicana* species group resembles that reported for other vertebrates co-distributed in mountains to the west (Trans-Mexican Neovolcanic Belt, Oaxacan sierras) and east (highlands of Chiapas, Mexico, and Nuclear Central America) of the Isthmus of Tehuantepec.

Resumen.—Análisis filogenéticos del gen mitocondrial Citocromo *b* se utilizaron para determinar los niveles de diferenciación genética y los patrones de relaciones entre miembros del grupo de especies de *Neotoma mexicana* en Mesoamerica. Se obtuvieron tres clados bien soportandos que están conformados por las especies *Neotoma ferruginea* Tomes, 1862; *N. mexicana* Baird, 1855; y *N. picta* Goldman, 1904. *Neotoma ferruginea* es el nombre más antiguo para el clado que contiene muestras del sur de México y Centro América Nuclear que previamente fueron identificadas como *N. isthmica* Goldman, 1904, y una subespecie de *N. mexicana* (*chamula* Goldman, 1909; *vulcani* Sanborn, 1935). El patrón filogeográfico observado entre el grupo de especies de *N. mexicana* se asemeja al reportado para otros vertebrados co-distribuidos en las montañas del Oeste (Eje neovolcánico transversal, Sierras de Oaxaca) y el Este (tierras altas de Chiapas, México y Centro América Nuclear) del Itsmo de Tehuantepec.

Keywords: DNA sequences, Mesoamerica, *Neotoma*, taxonomy

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Members of the *Neotoma mexicana* (Mexican woodrat) species group are distributed from the western United States to western Nicaragua. Throughout this broad distribution *N. mexicana* generally occupies mid to high elevation montane habitats, especially rocky outcroppings in mesic, pine-oak forests (*Pinus* and *Quercus* spp.). Early studies of woodrats resulted in the description of 14 species (Baird 1855, Tomes 1862, Ward 1891, Merriam 1892, 1893, 1894a, 1894b, 1903; Allen 1898, 1908; Bangs 1903, Goldman 1904, 1905, 1909), culminating in the recognition of 8 species following Goldman's (1910) comprehensive revision of the genus *Neotoma*. Goldman (1910) erected the *N. mexicana* species group to include eight species: *N. chrysomelas*, *N. distincta*, *N. ferruginea* (7 subspecies—*chamula*, *ferruginea*, *isthmica*, *ochracea*, *picta*, *solitaria*, and *tenuicauda*), *N. mexicana* (6 subspecies—*bullata*, *fallax*, *madrensis*, *mexicana*, *pinetorum*, and *sinaloae*), *N. navus*, *N. parvidens*, *N. torquata*, and *N. tropicalis*. Following Goldman's (1910) revision, two additional subspecies of *N. ferruginea* were described from Mesoamerica: *N. f. griseoventer* Dalquest, 1951, and *N. f. vulcani* Sanborn, 1935. Based on previous studies by Dalquest (1951) and Hooper (1955), as well as his own investigations, Hall (1955) named *N. m. eremita* and uncritically relegated all members of the *N. mexicana* species group sensu Goldman (1910) to subspecific status within *N. mexicana*, with the exception of *N. chrysomelas*, which he retained as a species.

In the most recent assessment of the *Neotoma mexicana* complex, Edwards & Bradley (2002) demonstrated that, based on DNA sequences obtained from the mitochondrial cytochrome-*b* gene (*Cyt*b), *N. m. isthmica* and *N. m. picta* differed from populations of *N. mexicana* from other regions of Mexico and the United States by large divergence values, 9.7% and 9.2%, respectively. In addition, samples of *N. m. isthmica* and *N. m. picta* differed from one another by 7.8%. As a result, Edwards & Bradley (2002) recommended that the two subspecies, *isthmica* and *picta*, be elevated to specific status. The case for elevating *N. m. picta* Goldman, 1904 was straightforward, as this taxon is restricted to the montane regions of central Guerrero and southern Oaxaca, Mexico. The reasoning for elevating *N. m. isthmica*, distributed east and south of the Isthmus of Tehuantepec in Oaxaca and Chiapas, proved to be more difficult because samples representing an older available name, namely *N. ferruginea* Tomes, 1862, were then unavailable for study. Therefore, Edwards & Bradley (2002) tentatively elevated *isthmica* Goldman, 1904, to specific status, although they stipulated that the name *N. ferruginea* Tomes, 1862, has nomenclatorial priority if samples of that taxon eventually proved to be conspecific with *N. isthmica*. Musser & Carleton (2005) also recommended that a systematic revision was needed to assess the relevance of *N. ferruginea* and other older synonyms.

Samples of *N. m. ferruginea* sensu stricto were recently collected from El Salvador and Guatemala and now allow a critical assessment of the taxonomic affinity of *N. m. ferruginea* to *N. isthmica* and other *N. mexicana*-like populations from southern Mexico and Nuclear Central America. The objective of this study is to use DNA sequences from the *Cyt*b gene to resolve systematic and phylogenetic relationships within the *N. mexicana* species group.

Materials and Methods

Throughout the Materials and Methods and Results (Table 1), our employment of taxonomic names and their rank, as species or subspecies, within the *Neotoma mexicana* group follows Hall (1955, 1981) and Edwards & Bradley (2002).

Samples.—DNA sequences were either generated herein or obtained from Edwards & Bradley (2002) and GenBank.

Table 1.—Mesoamerican taxa assigned to the *Neotoma mexicana* species group, listed as originally described, together with their taxonomic rank as observed in Goldman's (1910) revision, Hall's (1981) influential classification of North American mammals, and recent molecular studies (Edwards & Bradley 2002, this study). Taxa that have not been gene-sequenced are indicated with –.

Original taxon	Goldman (1910)	Hall (1981)	Edwards & Bradley (2002)	This study
N. chrysomelas Allen, 1908	*N. chrysomelas*	*N. chrysomelas*	–	–
N. distincta Bangs, 1903	*N. distincta*	*N. m. distincta*	–	–
N. ferruginea Tomes, 1862	*N. f. ferruginea*	*N. m. ferruginea*	–	*N. f. ferruginea*
N. ferruginea chamula Goldman, 1909	*N. f. chamula*	*N. m. chamula*	–	*N. f. chamula*
N. ferruginea griseoventer Dalquest, 1951	–	*N. m. griseoventer*	–	–
N. ferruginea ochracea Goldman, 1905	*N. f. ochracea*	*N. m. ochracea*	–	–
N. ferruginea solitaria Goldman, 1905	*N. f. solitaria*	*N. m. solitaria*	–	–
N. ferruginea vulcani Sanborn, 1935	–	*N. m. vulcani*	–	*N. f. vulcani*
N. isthmica Goldman, 1904	*N. f. isthmica*	*N. m. isthmica*	*N. isthmica*	*N. f. isthmica*
N. mexicana Baird, 1855	*N. m. mexicana*	*N. m. mexicana*	*N. m. mexicana*	*N. m. mexicana*
N. mexicana eremita Hall, 1955	–	*N. m. eremita*	–	–
N. mexicana inopinata Goldman, 1933	–	*N. m. inopinata*	*N. m. inopinata*	*N. m. inopinata*
N. mexicana scopulorum Finley, 1953	–	*N. m. scopulorum*	*N. m. scopulorum*	*N. m. scopulorum*
N. parvidens Goldman, 1904	*N. parvidens*	*N. m. parvidens*	–	–
N. picta Goldman, 1904	*N. f. picta*	*N. m. picta*	*N. picta*	*N. picta*
N. pinetorum Merriam, 1893	*N. m. pinetorum*	*N. m. pinetorum*	–	*N. m. pinetorum*
N. tenuicauda Merriam, 1892	*N. f. tenuicauda*	*N. m. tenuicauda*	*N. m. tenuicauda*	*N. m. tenuicauda*
N. torquata Ward, 1891	*N. torquata*	*N. m. torquata*	–	*N. m. torquata*
N. tropicalis Goldman, 1904	*N. tropicalis*	*N. m. tropicalis*	–	–

Samples included four individuals representing three subspecies of *N. mexicana* (*chamula*, *ferruginea*, and *vulcani*), three individuals representing *N. isthmica*, and two representatives of *N. picta* distributed in southern Mexico and Mesoamerica (see Fig. 1). In addition, 16 individuals representing six subspecies of *N. mexicana* distributed throughout the United States and northern and central Mexico were included as reference samples. *Hodomys alleni* and *Neotoma cinerea* of the subgenus *Teonoma*, a basal clade to members of the subgenus *Neotoma* (e.g., Planz et al. 1996, Edwards & Bradley 2002), were used as outgroup taxa. DNA sequences from *N. albigula*, *N. angustapalata*, *N. goldmani*, *N. leucodon*, and *N. micropus* were included as ingroup references given their geographic occurrence within Mesoamerica and proximity to the taxa of interest. Specimen numbers and collection localities are listed in the Appendix.

Sequence data.—Mitochondrial DNA was isolated from approximately 0.1 g of liver tissue stored in 95% ethanol using the Wizard Miniprep kit (Promega Corp., Madison, Wisconsin). The entire *Cyt*b gene (1143 bp) was amplified using the polymerase chain reaction method (PCR, Saiki et al. 1988) and the following primers: LGL765 Forward (Bickham et al. 1995), and LGL766 Reverse (Bickham et al. 2004). Thermal profiles for PCR were as follows: initial denaturation at 95°C for 2 min, followed by 40 cycles of denaturation at 95°C for 40 sec, annealing at 49.5°C for 45 sec, ramped at 0.6°C /sec at 73°C and extension at 73°C for 1 min 20 sec, with a final extension at 73°C for 10 min. PCR
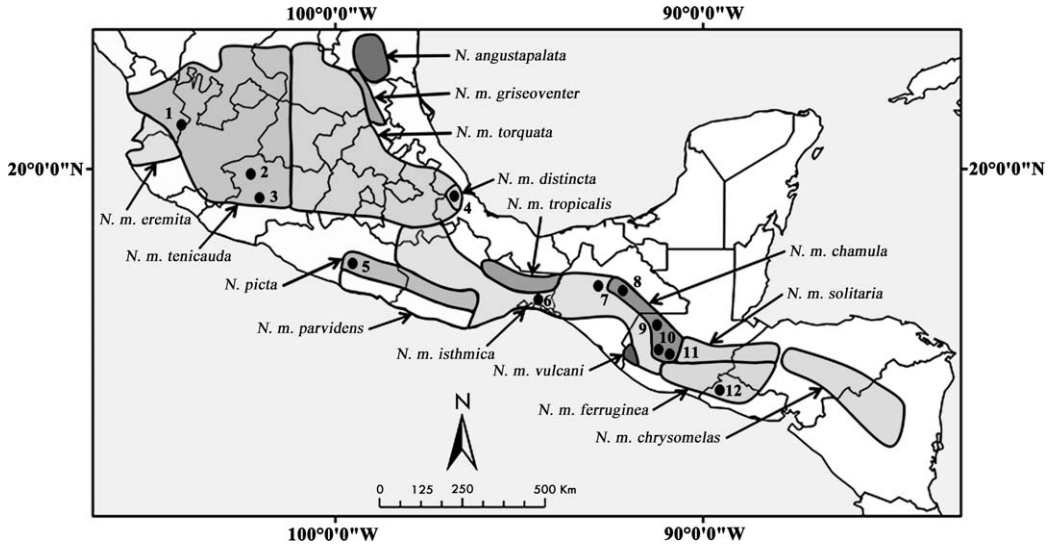
Fig. 1. Map depicting distribution of taxa assigned to the *Neotoma mexicana* species group occurring in Mexico and Central America (adapted from Hall 1981). Numbered dots represent localities where samples were obtained for this study (see Appendix for key to numbered localities). Taxonomic designations follow Hall (1955, 1981), except for *N. isthmica* and *N. picta* as recognized by Edwards & Bradley (2002).

products were purified with a QIAquick PCR Purification Kit (Qiagen, Valencia, California). Primers used for cycle sequencing included: LGL 765 Forward (Bickham et al. 1995), 700H (Peppers & Bradley 2000), WDRAT400F (Tiemann-Boege et al. 2000), LGL766 Reverse (Bickham et al. 2004), 700L (Peppers & Bradley 2000). Cycle sequencing reactions were purified using isopropanol cleanup protocols. Purified products were sequenced with an ABI 3100-Avant automated sequencer and ABI Prism Big Dye version 3.1 terminator technology (Applied Biosystems, Foster City, California). Resulting sequences were aligned and proofed using Sequencer 4.10 software (Gene Codes, Ann Arbor, Michigan); chromatograms were examined to verify all base changes. All *Cyt*b sequences obtained in this study were deposited in GenBank and are listed in the Appendix.

Phylogenetic analyses.—A parsimony analysis (PAUP*, Swofford 2002) was conducted using equally-weighted characters. The variable nucleotide positions within the data set were treated as unordered, discrete characters with four possible states: A, C, G, or T. All phylogenetically uninformative characters were excluded from these analyses. The heuristic search and tree-bisection-reconnection options in PAUP* were used to find the most-parsimonious trees and a strict consensus tree was generated from the available trees.

Bayesian inference (MrBayes version 3.1.2; Huelsenbeck & Ronquist 2001) was used to analyze the DNA sequence data under a maximum likelihood framework. The best-fit model of evolution was determined using Modeltest version 3.06 (Posada & Crandall 1998). The Akaike Information Criterion (Akaike 1974) identified GTR+Γ as having a significantly better likelihood score ($-\ln L = 4943.3472$) than all other models examined and consequently was determined as being the most appropriate model for the dataset. A GTR+G model with a site-specific gamma ($\Gamma = 0.2016$) distribution and base frequencies (A = 0.3215, C = 0.3114, G = 0.1223, and T = 0.2448) was used with the following options: eight MCMC chains, 10 million generations, and sample frequency equals every 1000 generations. Default

priors were used for model parameters. After a visual inspection of the likelihood scores, the first 1000 trees were discarded and a consensus tree (50% majority rule) was constructed from the remaining trees.

For the parsimony analysis, the bootstrap analysis (Felsenstein 1985) as implemented in PAUP* (Swofford 2002) with 1000 iterations, was used to evaluate nodal support. Bootstrap support values (BS) were superimposed on the topology recovered from the Bayesian analysis (Fig. 2). For the Bayesian analysis, posterior probabilities ≥0.95 were considered as indicators of significant nodal support (Huelsenbeck & Ronquist 2001), and such nodes are annotated on Fig. 2. Alternative tree topologies and phylogenetic hypotheses were tested using the Shimodaira-Hasegawa test (SH; Shimodaira & Hasegawa 1999) with restricted likelihood as implemented in PAUP* (Swofford 2002). The SH test was performed using constrained and unconstrained topologies and 1000 replicates of the log-likelihood scores under the RELL model (Kishino et al. 1990).

The Kimura 2-parameter model of evolution (Kimura 1980) was used to estimate genetic distances. This model was selected so that distance values could be compared to results of other rodent studies. These values were then used to assess levels of genetic divergence among individual clades of *Neotoma* following the criteria outlined in Bradley & Baker (2001) and Baker & Bradley (2006).

Divergence dating.—Divergence dates for species of *Neotoma* were estimated from *Cyt*b sequences (obtained in this study and from GenBank), using the program BEAST v1.7 (Drummond et al. 2012). Because we were interested only in estimating interspecific divergence, when multiple sequences were available for a species, a single representative sequence was chosen at random to represent the taxon. *Hodomys alleni* was used as the outgroup taxon. To define the appropriate molecular clock, the molecular clock test in the program MEGA 5.05 (Tamura et al. 2011) was used to determine whether to accept or reject a strict molecular clock. A Yule tree prior was used for the BEAST analysis, implying that the *Cyt*b gene tree was representative of the *Neotoma* species tree. A prior lognormal distribution was placed on root height to constrain the divergence date estimates of the overall tree but to allow for uncertainty in available fossil dates. The lognormal distribution was offset at 2.7 million years ago (mya) following a divergence date estimate for populations of *N. cinerea* (Hornsby & Matocq 2012), allowing for the fossil date ($\sim$ 6.6 mya) of the most recent common ancestor to the outgroup taxon. The same distribution was placed on the node for the common ancestor of *Neotoma*, given that *N. cinerea* is the basal lineage of the genus (Planz et al. 1996). Test runs of 1.0×10^7 generations with a 10% burn-in were used to optimize for the final analysis. Initial test runs using the GTR+G model of substitution (per previous model selection results) yielded low values of effective sample size, necessitating the selection of a simpler model. Therefore, HKY+I+G was chosen to minimize the effects of over-parameterization on effective sample size. Two final runs of 2.0×10^7 generations were analyzed with log and tree files combined for final divergence date estimates, producing a maximum clade credibility tree. Results were examined for sufficient mixing, convergence stability, and effective sample size >200 for all parameters using the program Tracer v1.5.

Results

The parsimony analysis generated three equally most-parsimonious trees (length = 737). A strict consensus tree was generated (not shown) from the set of most-parsimonious trees, and the bootstrap support
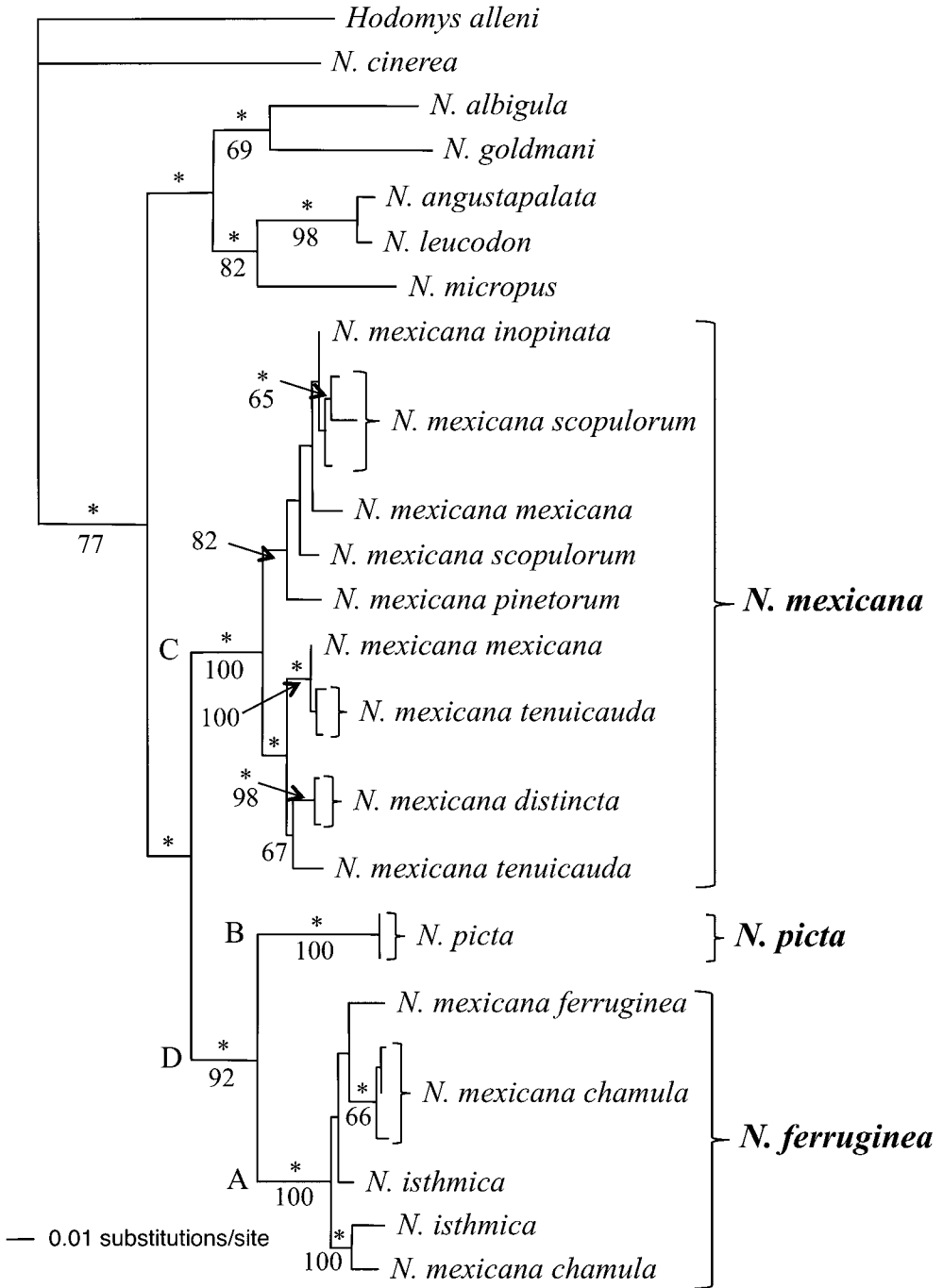
Fig. 2.   Phylogenetic tree resulting from the Bayesian analyses of mitochondrial cytochrome-*b* sequences. Values above branches indicate posterior probabilities and those below branches indicate bootstrap support as generated from parsimony analysis; only posterior probability values ≥0.95 (indicated by an asterisk) and bootstrap values ≥65 are shown. Alphabetic clades A–D as discussed in the Results are labeled at their basal nodes and terminal taxa are bracketed according to our taxonomic conclusions on specific status (see Discussion).

Table 2.—Average genetic distances (AGD) estimated using the Kimura two-parameter model of evolution (Kimura 1980) for selected taxa of *Neotoma* listed according to our taxonomic conclusions. Alphabetic cladal designations as used in the Results and in Fig. 2 are also indicated in parentheses. Some estimates were obtained from Edwards & Bradley (2001) and Edwards & Bradley (2002), as designated by an * or **, respectively.

Taxon	AGD
Within *N. ferruginea* (Clade A)	2.09%
Within *N. mexicana* (Clade C)	2.76%
N. m. inopinata/ N. m. mexicana/ N. m. pinetorum/ N. m. scopulorum vs. *N. m. torquata/ N. m. tenuicauda*	3.89%
N. f. chamula vs. *N. f. ferruginea*	2.56%
N. f. chamula vs. *N. f. isthmica*	2.01%
N. f. chamula vs. *N. f. vulcani*	1.56%
N. f. ferruginea vs. *N. f. isthmica*	2.42%
N. f. ferruginea vs. *N. f. vulcani*	2.51%
N. f. isthmica vs. *N. f. vulcani*	2.19%
N. ferruginea (Clade A) vs. *N. mexicana* (Clade C)	9.45%
N. ferruginea (Clade A) vs. *N. picta* (Clade B)	7.84%
N. mexicana (Clade C) vs. *N. picta* (Clade B)	9.75%
N. floridana vs. *N. magister*	7.90% *
N. floridana vs. *N. micropus*	14.20% **
N. leucodon vs. *N. albigula*	12.32% **
N. leucodon vs. *N. floridana*	11.14% **
N. leucodon vs. *N. micropus*	9.18% **

values obtained were superimposed onto the tree derived from the Bayesian analysis (Fig. 2). In the parsimony analysis, the seven individuals of *N. mexicana* from southern Mexico (Chiapas and Oaxaca), El Salvador, and Guatemala formed a strongly supported monophyletic clade (BS = 100). This clade then formed a strongly supported sister relationship with samples of *N. picta* from Guerrero, Mexico (BS = 92). A second strongly supported clade (BS = 100) containing samples from central and northern Mexico and the United States was recovered. These two major clades then formed a sister relationship, although they were weakly supported (BS = 60).

The Bayesian analysis produced a topology (Fig. 2) containing four major clades (A–D) among forms of the *N. mexicana* group surveyed. Each of these clades received highly significant nodal support (*P* = 0.98–1.00), and all are identical in composition to those generated in the parsimony analysis. Clade A contained the seven individuals representing

samples from southern Mexico (Chiapas and Oaxaca), El Salvador, and Guatemala. This clade (A) was sister to clade B, which contained samples of *N. picta*. The samples from central and northern Mexico and the United States formed a third clade (C). Clade C was then sister to the larger clade (D) formed by clades A and B.

The genetic divergence values (Table 2), estimated using the Kimura 2-parameter model of evolution (Kimura 1980) revealed that members of clade A differed from members of clades B and C by values of 7.84% and 9.45%, respectively. Average genetic distances estimated between members within clade A (*N. m. chamula, N. m. ferruginea, N. isthmica*, and *N. m. vulcani*) was 2.09%. Genetic divergence values between other currently recognized species of *Neotoma* range from 7.90% (*N. floridana* and *N. magister*) to 14.20% (*N. floridana* and *N. micropus*).

The molecular clock model test indicated rates of genetic change in accordance with a strict molecular clock. BEAST analyses estimated a mean rate of evolu-
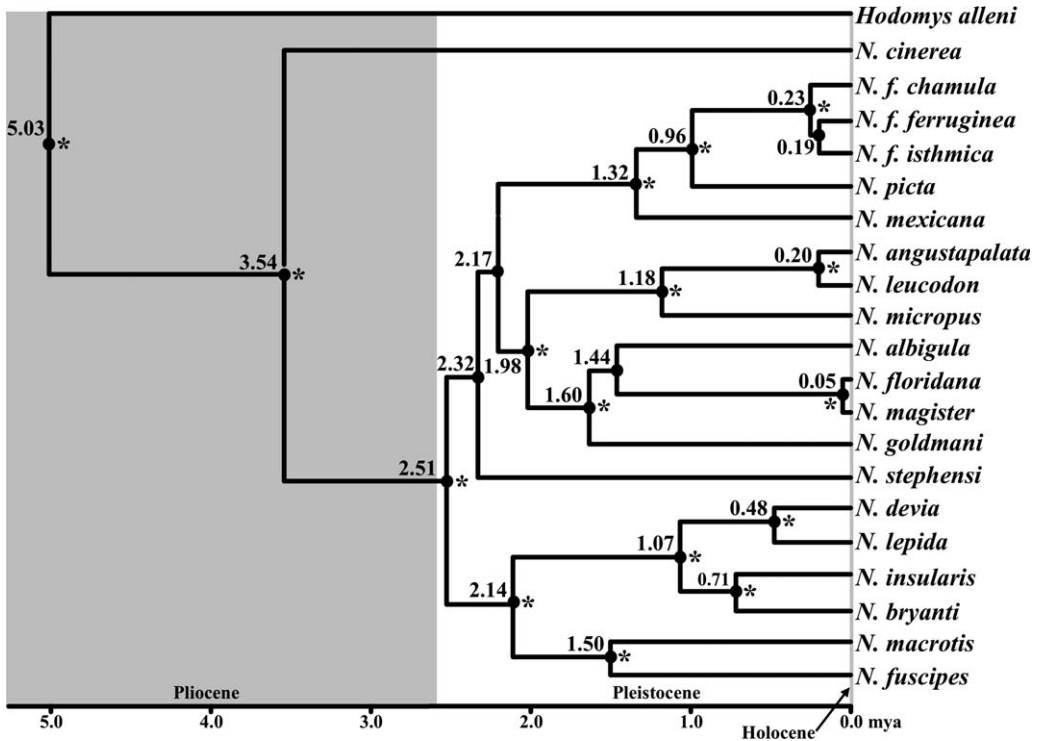
Fig. 3. Maximum clade credibility tree based on mitochondrial cytochrome-*b* sequence data showing dates of divergence estimates within *Neotoma*. All but three extant species within the genus that have been genetically sampled are represented, using *Hodomys alleni* as the outgroup taxon. Estimates of divergence dates, as million years ago, are indicated next to corresponding nodes (enclosed circles). Asterisks (*) next to these nodes represent Bayesian posterior probabilities ≥95%. Relevant epochs of the geological timescale are indicated on the tree.

tion of 0.06 substitutions per site per million years (95% highest posterior density—HPD = 0.04–0.08); the Yule birth rate was estimated to be 0.61 (95% HPD = 0.33–0.90). Divergence date estimates indicated that the initial divergence of *Neotoma* (*Teonoma*) from *Neotoma* (*Neotoma*) began approximately 3.54 mya (95% HPD = 2.99–4.32), occurring in the Late Pliocene (Fig. 3). However, the subsequent radiation of the majority of *Neotoma* species from the basal lineage, *N. cinerea*, began approximately 2.51 mya (95% HPD = 1.93–3.22), prior to the dramatic cooling trend of the Pleistocene. All other species-level differentiation occurred during the Pleistocene, including the divergence of the *N. mexicana* species group in the middle Pleistocene (ca. 1.31 mya—95% HPD =

0.94–1.78). In this context, the estimated divergence of the samples referred to *ferruginea* and *isthmica* is only 0.19 mya (95% HPD = 0.14–0.32), indicating a very recent separation of these two taxa.

Discussion

Phylogenetic analyses of DNA sequences from the mitochondrial *Cytb* gene indicated that seven samples of *Neotoma mexicana* from southern Mexico and Nuclear Central America are genetically highly divergent from their more western and northern counterparts (Fig. 2). These samples, currently assigned to *N. mexicana chamula*, *N. m. ferruginea*, and *N. isthmica*, formed a monophyletic clade that was

sister to a clade containing samples of *N. picta*, not to individuals of *N. mexicana*. The taxon *N. mexicana*, as currently defined (i.e., Edwards & Bradley 2002), is thus rendered as polyphyletic on trees produced by both parsimony and Bayesian analyses. In addition, levels of genetic divergence revealed by the Kimura 2-parameter distances (Kimura 1980) indicated that these seven southern samples differed from the northern forms by 9.45% and from *N. picta* by 7.84% (Table 2). Such magnitudes of genetic differentiation approximate that observed between other pair-wise interspecific comparisons within *Neotoma* (Table 2) and exceed distances reported for most species of mammals (see summaries by Bradley & Baker 2001 and Baker & Bradley 2006).

To test the hypothesis that samples from southern Mexico and Nuclear Central America should be retained within *N. mexicana* or alternatively recognized as a separate species, a maximum likelihood analysis was conducted in which all taxa currently recognized as *N. mexicana* (clades A and C) were constrained to be monophyletic. The SH test (Shimodaira & Hasegawa 1999) was used to compare the constrained topology to that obtained from the Bayesian analysis (Fig. 2); according to the SH test, the constrained topology produced a significantly worse (P = 0.016) log-likelihood score (–ln L 5818.8362) relative to that obtained from the unconstrained topology (–ln L 5795.0135). We therefore rejected the hypothesis that members of clade A (samples from Chiapas and Oaxaca, Mexico, El Salvador, and Guatemala) should be included within *N. mexicana*.

Given the presence of reciprocal monophyly and levels of genetic divergence between samples of *N. mexicana* from the United States and northern Mexico (clade C) and those residing in southern Mexico and Nuclear Central America (clades A and B), members of clade A should be recognized as a separate species. Edwards

& Bradley (2002) reached similar conclusions based on a much smaller geographic sampling. In their study, samples from Oaxaca and Chiapas, Mexico proved to be genetically distinct from samples of *N. mexicana* from Guerrero, Mexico, and samples from northern Mexico and the United States. Edwards & Bradley (2002) were able to assign material from Guerrero, Mexico, to *N. picta* but were unable to unequivocally assign the samples from Oaxaca and Chiapas, Mexico, due to the absence of some nominal taxa (especially *ferruginea*) from Nuclear Central America. Consequently, Edwards & Bradley (2002) tentatively used the name *N. isthmica*, the oldest name available for samples examined in their study, until fresh material from Nuclear Central America would become available (also see Musser & Carleton 2005:1058). Although several taxa were not available for this study, placement of the taxon *ferruginea* as a member of clade A establishes *Neotoma ferruginea* Tomes, 1862, as the oldest available name for the *N. mexicana*-like samples from southern Mexico and Nuclear Central America. *Neotoma ferruginea* has priority over all recognized taxa of Mesoamerican woodrats, except for the senior taxon *Neotoma mexicana* Baird, 1855. Therefore, *N. ferruginea* should be recognized as the proper species to include those taxa that have been genetically sampled to date (i.e., *chamula*, *ferruginea*, *isthmica*, and *vulcani*). This specific epithet may also encompass other taxa that Goldman (1910) formally included in *N. ferruginea* and that occur in close geographical proximity in mountains to the east of the Isthmus of Tehuantepec (i.e., *solitaria* and *tropicalis*). The status of *N. chrysomelas* Allen, 1908, described from Nicaragua and provisionally retained as a species by Hall (1981) and Musser & Carleton (2005), especially deserves resolution.

Members of the *Neotoma mexicana* group are distributed from northern Colorado through western and central Mex-
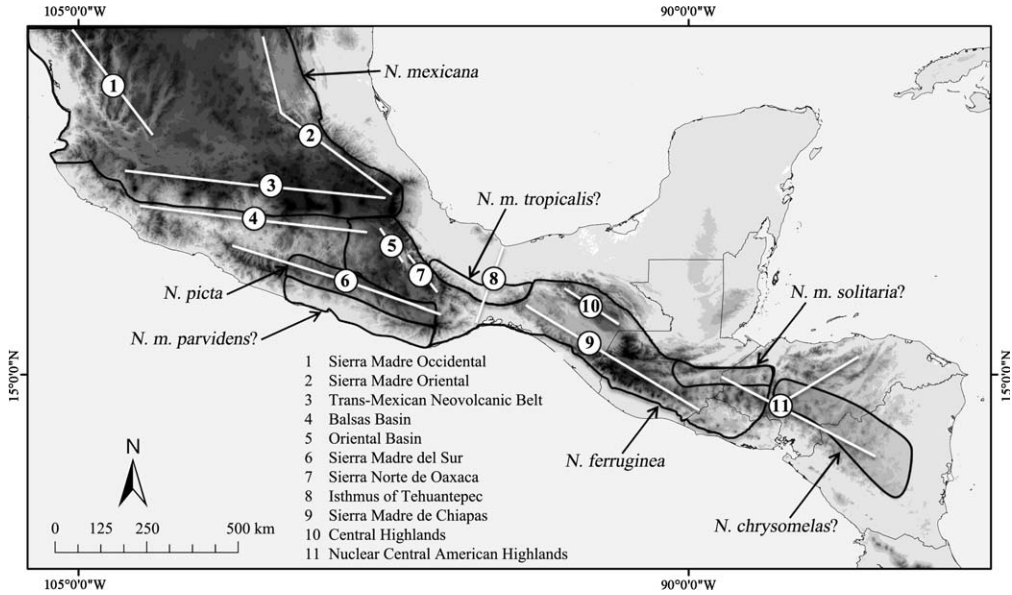
Fig. 4. Map depicting major physiographic regions in southern Mexico and Central America that are discussed in the text. Also shown are general taxonomic distributions and assignments based on the results of this study and on the revisions of Goldman (1910) and Hall (1955, 1981).

ico, through Guatemala, El Salvador, Honduras, and into western Nicaragua (Hall 1981). In the northern part of their distribution, subspecies of *N. mexicana* (*atrata*, *bullata*, *fallax*, *inopinata*, *pinetorum*, and *scopulorum*) are restricted to montane forests, including *Pinus-Juniper* or *Quercus-Pinus* forest associations in the United States (Cornely & Baker 1986). In central Mexico, subspecies of *N. mexicana* inhabit various habitats in the Sierra Madre Oriental (coniferous forest—*distincta*, *griseoventer*, and *torquata*) and Sierra Madre Occidental (tropical thorn forest—*tenuicauda*). These subspecies are disjunct and isolated, apparently limited to the south by the Trans-Mexican Neovolcanic Belt and the arid depression of the Balsas River (Cornely & Baker 1986).

Biogeographic factors (Fig. 4) that have affected the diversification of the *Neotoma mexicana* species group to the south of the Trans-Mexican Neovolcanic Belt are more complex, principally involving the Balsas Basin (including the Tepalcatepec Depression), Oriental Basin, the Sierra Madre del

Sur, and the Isthmus of Tehuantepec (Bryson et al. 2011). These major physiographic features have undoubtedly influenced speciation and subsequent distribution of species of woodrats within this region. For example, *N. picta* is endemic to the cloud forest regions (Marshall & Liebherr 2000) of Guerrero and western Oaxaca, *N. mexicana tropicalis* occurs only in the Sierra Norte de Oaxaca and lowland mountains near the Chiapas border, and *N. m. parvidens* is distributed in lower elevations of the pine and oak forest associated with the Sierra Madre del Sur.

The divergence date of *Hodomys* and *Neotoma*, as estimated from the DNA sequence data (ca. 6.05 mya), concurs with early fossil evidence of neotomine rodents that indicates a late Miocene origin for *Neotoma* (Zakrzewski 1993, Korth 1994). Furthermore, diversification within the *N. mexicana* species group (ca. 1.32 mya, Fig. 3) and within *N. ferruginea* (ca. 0.19 mya, Fig. 3) appears to agree with various Pleistocene glaciation events that produced colder temperatures across isolated mon-

tane regions inhabited by these woodrats. During interglacial periods, montane forests returned to high elevations and occasionally were fragmented by intervening river valleys (Weir 2009) resulting in these montane regions serving as refuges and centers of differentiation for small mammals. For example, Sullivan et al. (1997) indicated that members of the *Peromyscus aztecus* complex experienced similar range expansions and contractions during the Pleistocene, presumably due to climatic fluctuations and changes in the distribution of floral communities in Middle America–Nuclear Central America and south of Mexico. In addition, there is considerable evidence that the Isthmus of Tehuantepec acted as a lowland barrier promoting population differentiation in vertebrate species (Weir 2009 and citations therein), including *Ototylomys phyllotis* (Gutiérrez-García & Vázquez-Domínguez 2012, 2013). Several examples of classically defined "Mexican" and "Central American" species (populations distributed west and southeast of the Isthmus of Tehuantepec) have been reported in mammals (Sullivan et al. 2000, Carleton et al. 2002, Arellano et al. 2003, 2005, 2006; León-Paniagua et al. 2007) and birds (Pérez-Emán 2005, García-Moreno et al. 2006, Bonaccorso et al. 2008). In regard to the phyletic separation of *N. ferruginea* and *N. picta* from a common ancestor, the divergence data (Fig. 3) suggest a time frame during the Late Pleistocene (ca. 0.96 mya). Presumably, habitat expansion and contraction occurred, with the Sierra Madre del Sur acting as a refuge and ultimately resulting in the isolation of *N. picta* from *N. ferruginea*.

Herein, we assign all genetically sampled subspecies formerly assigned to *N. mexicana*—those distributed along the foothills and montane regions of the Sierra Sur de Oaxaca, Sierra Madre de Chiapas (Chiapas massif), Chiapas Modern Volcanic Arch, and Nuclear Central American Highlands (see Fig. 4)—to *N. ferruginea*: namely, *chamula* Goldman, 1909; *ferruginea* Tomes, 1862; *isthmica* Goldman, 1904; and *vulcani*

Sanborn, 1935. This arrangement, in general, supports the revisionary conclusions of Goldman (1910), who earlier recognized *N. mexicana* and *N. ferruginea* as separate species. It appears that *N. ferruginea* is distributionally separated from *N. picta* and *N. mexicana*. Nonetheless, given the limited geographic sampling in this study of taxa described from southern Mexico and Nuclear Central America, it is premature to assess species distributions, the possible occurrence of sympatry, and biogeographic scenarios responsible for these distributions. Eight of the 19 Mesoamerican taxa assignable to the *N. mexicana* species group have yet to be sampled for DNA analyses (Table 1). Incorporation of additional samples from such critical taxa throughout this region will allow a more thorough examination of hypotheses pertaining to potential divergence times and associated patterns of biogeographic events.

Acknowledgments

ments on previous versions of this manuscript, and improving its final content.

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Associate Editor: Michael D. Carleton.

Appendix

Specimens examined.—The specimens examined, listed following our taxonomic conclusions, are given below in parentheses by museum identification numbers and GenBank accession numbers (to the right of the comma, prefixed by AF-, DQ-, FJ-, and HM-). An asterisk (*) distinguishes DNA sequences generated in this study from sequences obtained in GenBank. For some specimens, a TK (a special tissue number of the Museum of Texas Tech University) number is provided due to the inability to have museum numbers. Museum collections are abbreviated as follows (per Hafner et al. 1997): BYU – Brigham Young University, Provo, Utah; DMNH – Delaware Museum of Natural History, Greenville, Delaware; JGO – private collection of James G. Owen; MSB – The Museum of Southwestern Biology, University of New Mexico, Albuquerque, New Mexico; MVZ – Museum of Vertebrate Zoology, University of California, Berkeley; TTU – Museum of Texas Tech University, Lubbock, Texas; UCLA – University of California, Los Angeles; and USNM – United States National Museum of Natural History, Washington, D.C. Locality numbers correspond to the sampling sites depicted in Fig. 1.

Hodomys alleni.—MEXICO: Michoacán; 2 km NW Caleta de Campos (TK 45042, AF186801).

Neotoma albigula.—MEXICO: Chihuahua; 14 km E Ciudad de Chihuahua (MSB 60812, AF186804).

Neotoma angustapalata.—MEXICO: Tamaulipas; 5 km W El Carrizo, Fortuna Mine (BYU 27733, HM989966).

Neotoma bryanti.—UNITED STATES: California; Monterey County, Arroyo Seco (MVZ 186296, DQ781160).

Neotoma cinerea.—UNITED STATES: Utah; San Juan County, 0.32 km S Owaehamo Bridge (MSB 121427, AF186799).

Neotoma devia.—UNITED STATES: Arizona; Maricopa County, 1.2 mi. E Black Gap (MVZ 200714, DQ781302).

Neotoma ferruginea chamula.—GUATEMALA: Huehuetenango; 22 Km NNE Chiantla, Laguna Magdalena (Locality 9, USNM 569553, KF772876*). MEXICO: Chiapas; Yalentay (Locality 8, TTU 82666, AF305567).

Neotoma ferruginea ferruginea.—EL SALVADOR: Santa Ana, Parque Nacional Los Andes, Cerro Verde (Locality 12, JGO 9027, KF772873*).

Neotoma ferruginea isthmica.—MEXICO: Chiapas; 1.6 km S Tuxtla Gutierrez (Locality 7, TTU 36179, AF298840). Oaxaca; Las Minas (Locality 6, TTU 82665, AF329079).

Neotoma ferruginea vulcani.—GUATEMALA: Quetzaltenango; 4 km SE Zunil, Finca La Chingada (Locality 11, USNM 569657, KF772874*); 6 km SW Zunil, Bosque Zunil (Locality 10, USNM 569672, KF772875*).

Neotoma floridana.—UNITED STATES: Oklahoma; Major County, 2 mi. N Seiling (TTU 54755, AF186823).

Neotoma fuscipes.—UNITED STATES: California; Monterey County, 5.2 mi. NE King City (MVZ 195212, DQ781303).

Neotoma goldmani.—MEXICO: Nuevo León; 1 km S Providencia (TTU 45227, AF186829).

Neotoma insularis.—MEXICO: Baja California Sur; Isla Aìngel de la Guarda (UCLA 19911, DQ781161).

Neotoma lepida.—UNITED STATES: Utah; Emery County, Huntington Canyon, 13.2 km NW Huntington (BYU 18153, DQ781256).

Neotoma leucodon.—MEXICO: San Luis Potosí; 19.2 km W Cuidad del Maíz (TTU 44923, AF186805).

Neotoma macrotis.—UNITED STATES: California; Riverside County, Rancho Capistrano Ortega Mountains (TK 83707, AF376479).

Neotoma magister.—UNITED STATES: Virginia; Madison County, Shenandoah National Park (MSB 74952, DQ179856).

Neotoma mexicana inopinata.—UNITED STATES: Utah; San Juan County (MSB 121363, AF186841)

Neotoma mexicana mexicana.—UNITED STATES: Texas; Jeff Davis County, Mount Livermore Preserve (TTU 101643, AF294346).

Neotoma mexicana pinetorum.—UNITED STATES: Arizona; Coconino County, Skinner Tank (TTU 100791, FJ716222).

Neotoma mexicana scopulorum.—UNITED STATES: Colorado; Larimer County, Sylvan Dale Guest Ranch near Mouth of Big Thompson Canyon (TTU 107426, FJ716223); Las Animas County, Lake Dorothey State Wildlife Area, (DMNH 8577, AF186821). New Mexico; Los Alamos County, Los Alamos (TTU 79129, AF294345); Socorro County, 19.2 km S, 4.8 km E Magdalena (MSB 74280, AF298848).

Neotoma mexicana tenuicauda.—MEXICO: Michoacán; Repetidora Urascato, 20 km (by road) Los Zamora (Locality 2, TK 47774, AF298843); 22.8 km W, 6.6 km NW Uruapan (Locality 3, TTU 110066, KF772877*); 8 km W Quiroga (TK45631, AF298842); Nayarit; 70 km N Santa María del Oro (Locality 1, TTU 110064, KF772878*).

Neotoma mexicana torquata.—MEXICO: Veracruz; 6.7 km NE, 13.5 km SE Perote (Locality 4, TTU 104970, KF801364*; TTU 104969, KF801365*).

Neotoma micropus.—MEXICO: Coahuila; 32 km S Morelos (TTU 35383, AF186824).

Neotoma picta.—MEXICO: Guerrero; 6.4 km SSW Filo de Caballo (Locality 5, TTU 82667, AF305568; TK 93390, AF305569).

Neotoma stephensi.—UNITED STATES: Arizona; Coconino County, Woodhouse Mesa, south edge Wupatki National Monument (MVZ 197170, DQ781305).