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Patterns of phenotypic and genetic variation in three species of endemic Mesoamerican *Peromyscus* (Rodentia: Cricetidae)

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Three species of Mexican deer mice of the *Peromyscus mexicanus* species group (*P. grandis*, *P. guatemalensis*, and *P. zarhynchus*) were characterized morphologically and genetically to test hypothesized concepts of species limits. We investigated if previously proposed phenetic relationships among these 3 taxa were supported by morphometric and genetic data. Analyses of nongeographic and geographic variation for individuals from 36 localities in Guatemala and southeastern Mexico were conducted to assay morphologic and geographic boundaries. In addition, 35 mitochondrial cytochrome-*b* gene sequences were analyzed using maximum-parsimony and Bayesian inference methods to determine relationships among the 3 taxa. This study based on comparisons to type specimens provided support for the presence of 3 morphologically and genetically distinct units. Our analyses suggest that *P. grandis* and *P. guatemalensis* are more closely related to each other than either is to *P. zarhynchus*, rejecting existing hypotheses that suggest that *P. zarhynchus* and *P. grandis* are phenetically more similar. The results of this study depict relationships among other members of the *P. mexicanus* group and patterns of speciation and biogeography and allow identification of regionally important phylogeographic units in Mesoamerica. DOI: 10.1644/09-MAMM-A-167.1.

Key words: cytochrome *b*, morphometrics, *Peromyscus*, *Peromyscus mexicanus* species group

The mountains of Mesoamerica currently are recognized as a region with high levels of diversification and endemism for many mammalian taxa (León-Paniagua et al. 2007). Among the most diversified of these is the rodent genus *Peromyscus* (deer mice). Relationships and taxonomic membership within *Peromyscus* have been revised at least 3 times over the past century (Carleton 1989; Hooper 1968; Osgood 1909), results of which were summarized by Musser and Carleton (2005). In addition, the monophyly of *Peromyscus* has been questioned several times in the last 30 years (Carleton 1980; Rogers 1983; Schmidly et al. 1985; Stangl and Baker 1984; Yates et al. 1979). Several recent molecular studies (Bradley et al. 2007; Miller and Engstrom 2008; Rogers et al. 2005) have provided support for a monophyletic *Peromyscus* if *Isthmomys* is removed and recognized as a separate genus. However, most studies have supported conceptualizing species groups as proposed by Osgood (1909), who arranged phenetically similar taxa into 7 ''species groups.'' In the last taxonomic summary (Musser and Carleton 2005) 13 species groups were recognized, most of which have been supported by recent molecular evidence (Bradley et al. 2007).

Peromyscus mexicanus and its allies form 1 of the best morphologically characterized species groups. The *P. mexicanus* species group, centered in Guatemala and southern Mexico, comprises 7 species, 5 of which presumably are closely related (*P. grandis*, *P. guatemalensis*, *P. gymnotis*, *P. mexicanus*, and *P. zarhynchus*) and whose morphological recognition involves almost imperceptible size and color gradations (Carleton 1989). However, an assessment by Carleton (1989) differed from that of Huckaby (1980), who identified discrete size differences among allopatric populations. In addition, Bradley et al. (2007) used molecular data to refer *P. nudipes* to the *mexicanus* group. Several members of this species group are restricted to 1 or a few habitat types (e.g., *P. grandis* is found exclusively in the mountains of central Guatemala), whereas others range widely through diverse environments and have evolved numerous subspecies (6 recognized subspecies of *P. mexicanus*, located from central Mexico to Costa Rica—Hall 1981).

It has been difficult to define relationships among members of the *P. mexicanus* group (Rogers and Engstrom 1992; Van

Coeverden De Groot 1995). Huckaby (1973), in the 1st attempt to delineate species boundaries for the *P. mexicanus* group, concluded that the 3 species forming the core group (*P. grandis*, *P. guatemalensis*, and *P. zarhynchus*) were fragmented populations of the single species, *P. zarhynchus*. However, Huckaby (1980) later elevated these 3 taxa to species based on characters of the skull, male reproductive structures, and external morphology (average in size and color). Van Coeverden De Groot (1995) failed to recover *P. guatemalensis* and *P. grandis* as distinct taxonomic units based on a study of restriction fragment–length polymorphism data because each of these 2 taxa appeared to be paraphyletic. Based on an analysis of mitochondrial DNA (mtDNA) gene sequences, Wade (1999) found that several species in the *P. mexicanus* species group were likely polyphyletic, including *P. guatemalensis*. Because of the patchy distribution of *Peromyscus* in the highlands of Mesoamerica, morphological and genetic variation are expected to be partitioned geographically, although it is unclear as to which taxonomic hierarchy these populations should be ascribed. The objectives of this study were to characterize *P. grandis*, *P. guatemalensis*, and *P. zarhynchus* morphometrically and molecularly to test hypotheses concerning their taxonomic validity, species limits, and distributions; to use mitochondrial cytochrome-*b* gene (*Cytb*) sequences to infer phylogenetic relationships of these species within the *P. mexicanus* group; and to assess whether the phenetic relationships among these 3 taxa, as proposed by Huckaby (1980), can be supported.

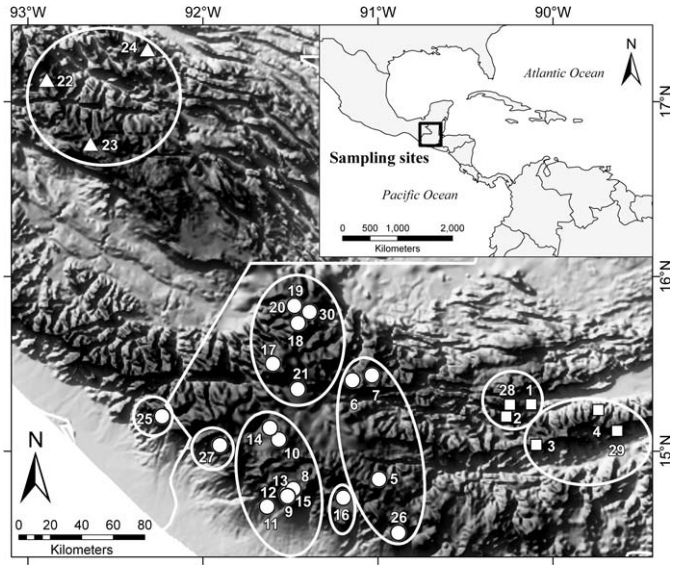

Fig. 1.—Map of Mesoamerica depicting sampling localities of *Peromyscus guatemalensis*, *P. grandis*, and *P. zarhynchus*. Sampling localities for *P. grandis* in eastern Guatemala include localities 1–4 and 28–29 (type locality is 1). Sampling localities for *P. guatemalensis* in southwestern Guatemala and southern Chiapas, Mexico, include localities 5–21 and 25–27, 30 (type locality is 17). Sampling localities for *P. zarhynchus* in northeastern Chiapas, Mexico, include localities 22–24 (type locality is 24). Ellipses represent operational taxonomic units (OTUs, based on perceived geographic proximity) used in the data analyses.

Materials and Methods

Individuals of *Peromyscus* were captured in 2006 and 2007 in several highland localities in Guatemala. All specimens collected for this study were captured in accordance with animal welfare guidelines established by the American Society of Mammalogists (Gannon et al. 2007) and San Jose State University Institutional Animal Use and Care Protocol 851. Specimens were prepared as skins and skulls; tissue samples were stored in either liquid nitrogen or 99% ethanol. All specimens collected were deposited at the collections of the United States National Museum, or the Natural Sciences Research Laboratory, Museum of Texas Tech University (Appendix I).

Morphometric analyses.—Species identifications were verified by examination of cranial and pelage characters after careful comparison with original descriptions (Goodwin 1932; Merriam 1898) and type series. We examined 385 specimens collected from Guatemala and Mexico that are housed at the American Museum of Natural History, Field Museum of Natural History, Museum of Texas Tech University, Royal Ontario Museum, and United States National Museum (Appendix I), including 58 *P. grandis* (28 ♀, 30 ♂), 254 *P. guatemalensis* (103 ♀, 151 ♂), and 73 *P. zarhynchus* (20 ♀, 53 ♂). The specimens were grouped into 9 operational taxonomic units (OTUs) to reflect geographic distributions, barriers, proximity, and habitat assemblages: 2 for *Peromyscus grandis*, 6 for *P. guatemalensis*, and 1 for *P. zarhynchus* (Fig. 1). Specimens were assigned to 1 of 4 age classes (juvenile, subadult, adult, and old adult) based on degree of eruption of M3, condition of the occlusal surface of molars due to wear (Lorenzo et al. 2006; Monroy-Gamboa et al. 2005), and pelage coloration (Carleton 1977).

To assess phenetic similarities among taxa, 18 cranial measurements were taken to the nearest 0.01 mm with digital calipers. Crania were examined with a stereoscope to ensure that measurements were taken between homologous points. Cranial measurements included occipitonasal length, greatest zygomatic breadth, greatest lambdoidal breadth, height of braincase, breadth across occipital condyles, least interorbital breadth, greatest breadth of rostrum, length of rostrum, postpalatal length, length of bony palate, length of upper diastema, length of left incisive foramen, breadth of incisive foramen, breadth of palate between 1st molars, width of mesopterygoid fossa, breadth of zygomatic plate, coronal length of maxillary toothrow, and width of M1, defined in Carleton (1980). These measurements were chosen because they were useful in addressing questions about phenotypic relationships among *Peromyscus* species (Carleton 1979; Carleton et al. 1982; Castro-Campillo et al. 1999; Martínez-Coronel et al. 2006; Schmidly and Bradley 1995).

For descriptive and comparative purposes the mean and standard error (*SE*) of each character were calculated for each species (Table 1); for all further analyses, log-transformed characters were used. The complete analysis of data required

Table 1.—Summary statistics for the 18 characters examined in this study of *Peromyscus*.

	Character	*P. grandis*	*P. guatemalensis*	*P. zarhynchus*
		$\bar{X} \pm SE$	$\bar{X} \pm SE$	$\bar{X} \pm SE$
1	Occipitonasal length	36.09 ± 0.194	33.29 ± 0.160	36.43 ± 0.207
2	Greatest zygomatic breadth	17.22 ± 0.104	16.15 ± 0.054	16.86 ± 0.094
3	Greatest lambdoidal breadth	13.54 ± 0.099	13.12 ± 0.031	13.23 ± 0.073
4	Height of braincase	9.98 ± 0.056	9.39 ± 0.027	10.03 ± 0.058
5	Breadth across occipital condyles	7.82 ± 0.042	7.32 ± 0.022	7.70 ± 0.044
6	Least interorbital breadth	5.55 ± 0.035	5.26 ± 0.018	5.44 ± 0.072
7	Greatest breadth of rostrum	6.05 ± 0.044	5.74 ± 0.039	6.11 ± 0.044
8	Length of rostrum	14.93 ± 0.112	13.20 ± 0.067	14.71 ± 0.140
9	Postpalatal length	13.12 ± 0.095	12.24 ± 0.055	13.00 ± 0.103
10	Length of bony palate	5.18 ± 0.047	5.07 ± 0.027	5.16 ± 0.055
11	Length of upper diastema	9.06 ± 0.080	7.62 ± 0.051	9.55 ± 0.105
12	Length of left incisive foramen	7.26 ± 0.071	6.78 ± 0.041	7.82 ± 0.062
13	Breadth of incisive foramen	2.77 ± 0.035	2.60 ± 0.037	2.60 ± 0.025
14	Breadth of palate between the M1s	7.02 ± 0.039	6.62 ± 0.028	6.99 ± 0.046
15	Width of mesopterygoid fossa	2.22 ± 0.039	2.22 ± 0.018	2.13 ± 0.023
16	Breadth of zygomatic plate	3.22 ± 0.049	2.83 ± 0.027	2.53 ± 0.038
17	Coronal length of maxillary toothrow	5.53 ± 0.033	5.24 ± 0.016	5.46 ± 0.027
18	Width of M1	1.68 ± 0.010	1.58 ± 0.006	1.61 ± 0.012

an estimation of missing data (Strauss et al. 2003), accomplished by a maximum-likelihood expectation-maximization algorithm (Dempster et al. 1977). The amount of missing data (4.4%) was sufficiently low that the expectation-maximization algorithm is expected to provide accurate predicted values with no reduction in total variance (Strauss et al. 2003). If all specimens having missing data had been omitted from analysis, the sample size would have decreased from 385 to 262, and the number of localities represented by <10 specimens would have increased from 16 to 22.

Discriminant function analysis was used to distinguish among species and to identify the most discriminatory characters (dos Reis et al. 1990; Strauss 1985). Size-adjusted discriminant function analysis (dos Reis et al. 1990) was used to determine how species differ in traits independent of size variation. This approach was effective in accounting for differences among groups by eliminating potentially confounding effects of geographic and intrapopulational variation in size or ontogenetic growth. Bootstrapping (McLachlan 1992), which creates empirical sampling distributions of parameters, was used to estimate confidence limits for percentages of explained variance and for vector correlations. To assess group distinctiveness, overall levels of similarity and relationships among individual samples were estimated by Mahalanobis distances (Mahalanobis 1936) and summarized using the unweighted pair group method with arithmetic mean cluster analysis (Sokal and Sneath 1973). Levels of statistical significance were estimated using multivariate analysis of variance (MANOVA), randomized (1,000 permutations) to mitigate the conventional assumptions of multivariate normality and homogeneity of covariance matrices.

All statistical tests were evaluated at $\alpha = 0.05$. Based on current distribution maps, taxa appear to replace each other geographically; therefore, the Mantel's test (Daniels 1944; Mantel 1967) was used to assess significant associations between morphological distinction and geographical distances. All statistical analyses were performed using MATLAB version 6.5.0.1 for Windows (The MathWorks, Inc. 2006) with functions and script files written by Strauss (2009).

Molecular analyses.—Thirty-five mitochondrial *Cytb* gene sequences were used to test the hypothesis that the taxa of this study form a monophyletic assemblage. Seventeen of the sequences were obtained from samples collected in natural populations from the type localities of *P. grandis* ($n = 7$) and from the type and additional localities of *P. guatemalensis* ($n = 10$). Eighteen additional DNA sequences were downloaded from GenBank.

Mitochondrial DNA was extracted from 0.1 g of liver or heart tissue preserved in 99% ethanol and isolated using the modified standard phenol–chloroform method (Chomczynski and Sacchi 1987) or a DNeasy blood and tissue kit (QIAGEN, Inc., Valencia, California). The entire *Cytb* gene (1,143 base pairs [bp]) was polymerase chain reaction–amplified with primers LH14115 and H15288 (Martin et al. 2000). Amplification reactions were performed in 50-µl volumes, purified used QIAquick polymerase purification kits (QIAGEN, Inc.), and visualized on a 1.5% agarose gel in 0.5 TAE buffer with GelRed (BioTium, Hayward, California). Sequencing used the following primers: WRAT400R and WRAT400F (Tiemann-Boege et al. 2000); 700H (Peppers and Bradley 2000); 700L (Bradley et al. 2000); F1 (Whiting et al. 2003); MVZ04 (Smith and Patton 1991); MVZ16 (Smith and Patton 1993); 870R (Sebald et al. 2003); and CB3-3 and CB1-5 (Palumbi 1996) combined with primers H15288 and LH14115 (Martin et al. 2000). Amplicons were sequenced with 90–440 ng/µl of DNA. Cycle-sequenced products were purified in an EdgeBiosystems Performa DTR gel filtration cartridge (EdgeBiosystems, Inc., Gaithersburg, Maryland) using an isopropanol precipitation reaction and electrophoresed on an ABI 3100-Aviant automated sequencer (Applied Biosystems, Inc., Foster City,

California). Sequences were edited and aligned using Clustal X (Thompson et al. 1997). All DNA sequences were deposited in GenBank, and accession numbers are provided in Appendix I.

Phylogenetic analyses were performed using maximum-parsimony optimality criteria in PAUP* (Swofford 2002), and Bayesian analysis was conducted in MRBAYES 3.1.2 (Huelsenbeck and Ronquist 2001). *Peromyscus mayensis*, *P. perfulvus*, *P. megalops*, *P. melanophrys*, *P. melanocarpus*, *P. boylii*, and *Neotomodon alstoni* were used as outgroup taxa in the maximum-parsimony analyses, based on relationships suggested by Carleton (1980), Huckaby (1980), and Bradley et al. (2007). In the maximum-parsimony analyses, all characters were treated as unordered and equally weighted. Analyses employed a heuristic search using tree bisection reconnection with 100 random stepwise additions of taxa. Nodal support was evaluated with PAUP*, using 5,000 bootstrap iterations (Felsenstein 1985) with 10 random stepwise additions of taxa. ModelTestServer (Posada 2006) was used to determine the model of DNA evolution that best fit the data. The GTR + I + G model was chosen using the Akaike information criterion (Akaike 1973). Bayesian analysis was used to generate support values (posterior clade probabilities). The Bayesian analysis consisted of 2 independent runs each with 3 heated and 1 cold Markov chains using *N. alstoni* as the outgroup taxon. Uniform interval prior probabilities were assumed for all parameters except base composition and GTR parameters, which assumed Dirichlet process priors. Runs were allowed to proceed for 10 million generations, and trees were sampled every 100 generations for each chain. To check that each run converged on a stable log-likelihood value the log-likelihood values were plotted against generation time for each run. The 1st 25,000 trees were discarded as burn-in, and the remaining trees were used to compute a 50% majority-rule consensus tree and to obtain posterior probability estimates for each clade. Genetic distances were calculated using the Kimura 2-parameter model (Kimura 1980) in PAUP*; haplotype diversity was calculated with DnaSP 4.50 (Rozas et al. 2003). Correlations (r) among genetic distances (Kimura 2-parameter) and geographic distances were assessed using a reduced major axis regression and a Mantel test as implemented in IBD version 1.52 (Bohonak 2002).

Results

Morphometric analyses.—Sexual dimorphism was statistically significant in all 3 species, although different variables were dimorphic among species. Sexes in *P. grandis* differed primarily in width of M1 (Wilks' $\lambda = 0.69$, $F_{1,56} = 3.06$, $P < 0.05$). Females and males of *P. guatemalensis* differed primarily in height of braincase (Wilks' $\lambda = 0.87$, $F_{1,252} = 2.10$, $P < 0.05$), and females and males of *P. zarhynchus* differed primarily in postpalatal length (Wilks' $\lambda = 0.03$, $F_{1,71} = 182.80$, $P < 0.05$).

Age classes of *P. grandis* differed primarily and significantly in occipitonasal length and length of the bony plate (Wilks' $\lambda = 0.37$, $F_{1,55} = 10.28$, $P < 0.05$). Age classes of *P. guatemalensis* differed primarily in breadth of incisive foramen (Wilks' $\lambda = 0.06$, $F_{3,250} = 29.83$, $P < 0.05$). Individuals of different age classes in *P. zarhynchus* differed significantly in postpalatal length (Wilks' $\lambda = 0.03$, $F_{3,69} = 4.66$, $P < 0.05$).

Geographic variation for *P. grandis* and *P. guatemalensis* (species with >1 OTU) was statistically significant. *P. grandis* showed significant geographical differences among OTUs in height of braincase (Wilks' $\lambda = 0.27$, $F_{1,56} = 16.20$, $P < 0.05$), whereas *P. guatemalensis* showed significant geographical variation among OTUs in coronal length of maxillary toothrow (Wilks' $\lambda = 0.47$, $F_{4,248} = 5.10$, $P < 0.05$).

The discriminant function analysis (Fig. 2A) incompletely discriminated the 3 species, although the differences among multivariate means were statistically significant (Wilks' $\lambda = 0.25$, $F_{2,382} = 22.81$, $P < 0.01$). This analysis includes both size and shape effects, as indicated by *P. grandis* possessing larger values, on average, for almost all characters. However, the size-adjusted discriminant analysis (Fig. 2C) completely discriminated among the species groups (Wilks' $\lambda = 0.01$, $F_{2,382} = 166.00$, $P < 0.01$). The most discriminatory size-independent characters were breadth of zygomatic plate (character 11) and length of upper diastema (character 16; Fig. 2D).

The Mahalanobis distance (D^2) value between *P. grandis* and *P. guatemalensis* was 28.9, between *P. grandis* and *P. zarhynchus* 193.2, and between *P. guatemalensis* and *P. zarhynchus* 362.7. Mantel's test of the relationship between Mahalanobis distances among samples and geographic distances among localities was positive but nonsignificant ($r = 0.061$, $n = 385$, $P = 0.12$).

Molecular analyses.—Most (33 of 35) mtDNA sequences included complete sequence of the *Cytb* gene. One downloaded sequence (AY041200, *P. nudipes*) was only 727 bp long and 1 sequence (AY376425, *P. mexicanus*) was missing 24 bp in the middle of the gene. The best evolutionary model included the following base frequencies: 0.3262 (A), 0.2996 (C), 0.1240 (G), and 0.2503 (T) with the proportion of invariable sites = 0.5792 and variable sites with a gamma distribution shape parameter = 1.5124.

Characters that were parsimony informative totaled 287. The parsimony analysis generated 8 most-parsimonious trees, each 888 steps in length with the following statistical indices: consistency index of 0.4032, retention index of 0.695, and homoplasy index of 0.5968. The strict consensus tree of the 8 most-parsimonious trees had a topology matching that of the Bayesian analyses (see below); however, *P. gymnotis* was embedded within *P. mexicanus*. Topologically, none of the 8 most-parsimonious trees recovered *P. zarhynchus* as a sister taxon to *P. grandis* or *P. guatemalensis* or both.

The Bayesian analysis generated a tree similar in topology to that obtained in the parsimony analysis (Fig. 3). Support values were significant (posterior probability > 0.95, parsimony bootstrap > 75%) for many clades, including those of *P. guatemalensis*, *P. grandis*, and *P. zarhynchus*. Similar to the parsimony analysis, the *P. mexicanus* species

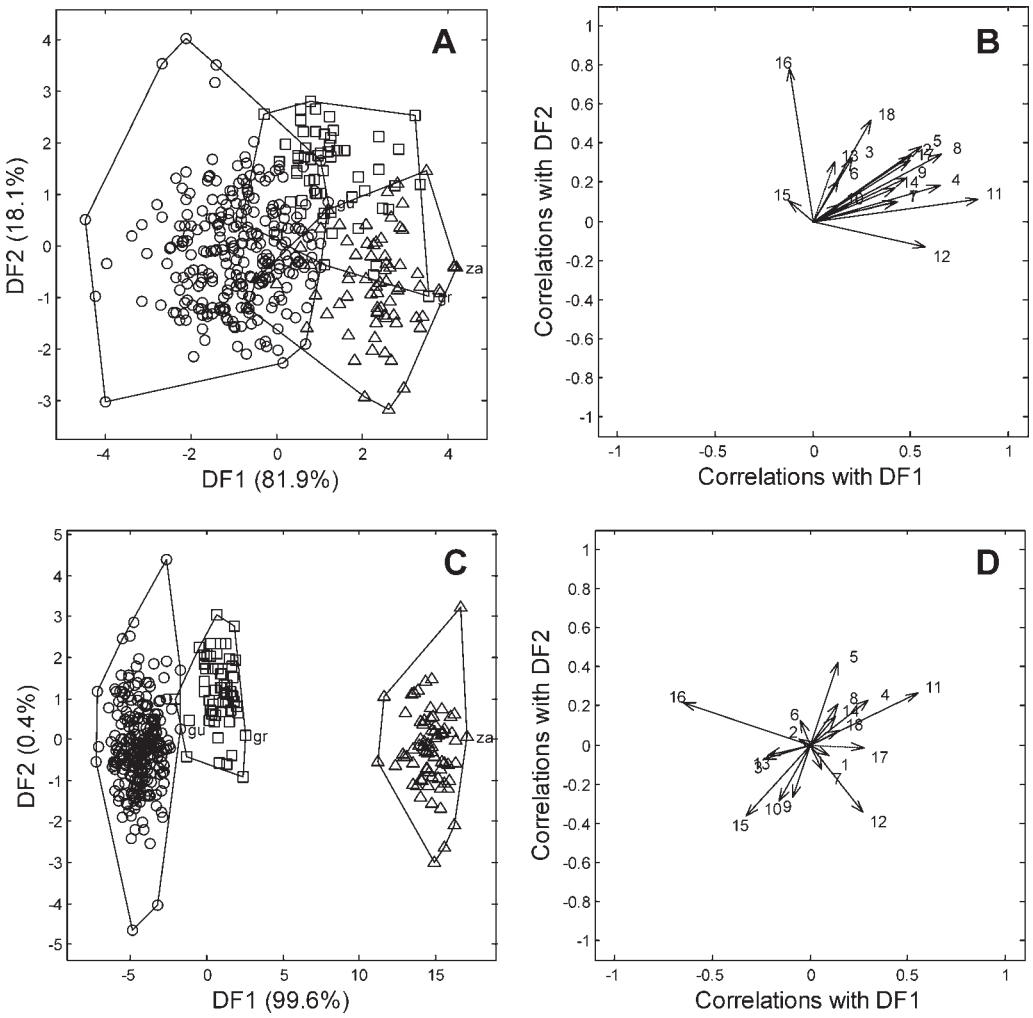

Fig. 2.—Results of discriminant function analysis performed on 18 log-transformed cranial variables measured on 385 specimens representing 9 operational taxonomic units of *Peromyscus*. *P. grandis* is represented by squares, *P. guatemalensis* by circles, and *P. zarhynchus* by triangles. A) Projection of 1st 2 discriminant function analyses, including all information about size and shape within the 3 taxa. B) Corresponding correlations of mensural characters (solid vectors) for discriminant function analysis. C) Projection of 1st 2 size-adjusted discriminant function analyses within the 3 taxa. D) Corresponding correlations of mensural characters (solid vectors) with corresponding principal components for size-adjusted discriminant function analysis. Polygons enclose individuals of each species.

group formed a statistically supported clade with the exclusion of *P. stirtoni*. Genetic distances, calculated using the Kimura 2-parameter method (Kimura 1980), ranged between 4.5% and 6.6% in the ingroup and from 9% to 13.1% among the ingroup and outgroup species for the members of the *P. mexicanus* species group (Table 2).

High haplotype diversity (Hd) was present in the small sample of *P. grandis* ($n = 7$, Hd = 1.00, $\pi = 0.0325$), whereas our larger sample of *P. guatemalensis* showed lower levels of haplotype diversity ($n = 12$, Hd = 0.78, number of haplotypes = 5, $\pi = 0.01173$). Although the genetic distance between *P. grandis* and *P. guatemalensis* was the lowest among any pair of species (4.5%), they did not share any mtDNA haplotypes. Notably, 28 fixed nucleotide differences were found between the 2 taxa. Based on Mantel's test, the pairwise correlation between genetic and geographic distances was low and not significant ($r = 0.2343$, $n = 10$, $P = 0.92$).

Discussion

Patterns of morphometric variation.—Significant differences existed between sexes within the 3 taxa examined; however, these differences were limited to only a few characters. Similarly, patterns of geographic variation were evident for *P. grandis* and *P. guatemalensis*, as supported by the MANOVA. Individuals of *P. grandis* from the type locality (OTU1) were significantly different from individuals representing OTU2 from the cloud forest of Sierra de las Minas; these OTUs represent localities between 25 km and 50 km apart, but in 2 distinct ecological regions. Differences in color among individuals of OTU1 and OTU2 also were apparent; rich brown pelage characterizes individuals representing OTU1. Van Coeverden De Groot (1995) suggested that some specimens he assigned to *P. grandis* from Sierra de las Minas (OTU2) had unique haplotypes that were embedded within *P. mexicanus*, which led him to

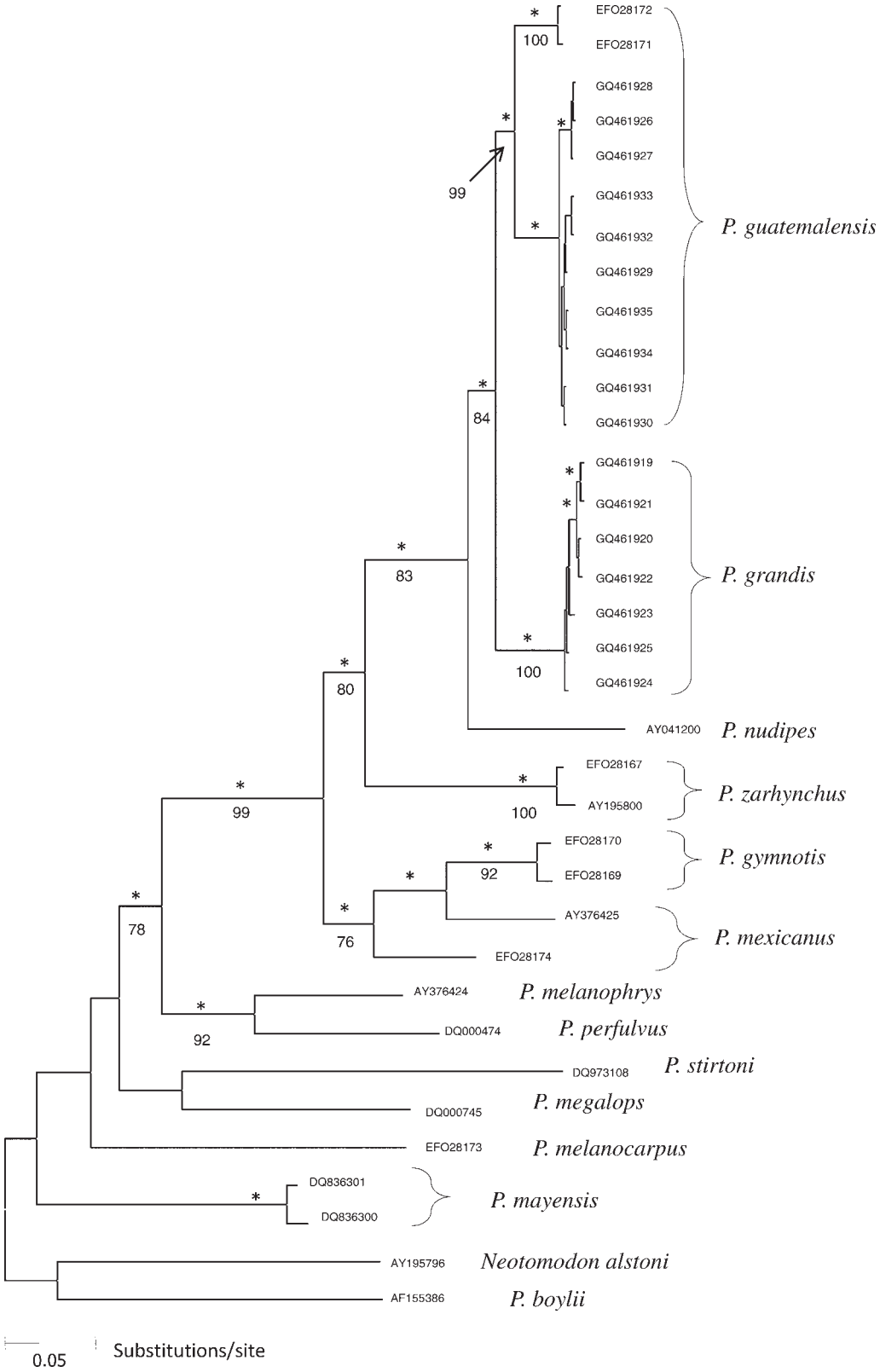

Fig. 3.—Tree topology calculated using Bayesian inference methods from analysis of the cytochrome-*b* gene for samples of *Peromyscus grandis*, *P. guatemalensis*, and *P. zarhynchus* in the context of the *P. mexicanus* species group. Posterior clade probability values > 0.95 are marked with an asterisk above the branches and bootstrap values $> 75\%$ from the parsimony analysis are below the branches.

Table 2.—Average genetic distances (AGDs) for the cytochrome-*b* data estimated using the Kimura 2-parameter model of evolution (Kimura 1980) for selected taxa of *Peromyscus*.

Comparison	AGD (%)
Within taxa	
P. grandis	0.3
P. zarhynchus	0.4
P. guatemalensis	1.2
Among taxa	
P. grandis versus *P. guatemalensis*	4.5
P. grandis versus *P. nudipes*	6.9
P. guatemalensis versus *P. zarhynchus*	9.0
P. guatemalensis versus *P. nudipes*	9.4
P. grandis versus *P. zarhynchus*	9.5
Within clades	
P. gymnotis–P. mexicanus	6.6
P. melanophrys–P. perfulvus	9.0
P. megalops–P. melanocarpus	13.1

treat these specimens as a separate population in his analyses.

Similar to the case of *P. grandis*, patterns of geographic and nongeographic variation in *P. guatemalensis* were not sufficient to overwhelm the levels of differentiation among the 3 species. For *P. zarhynchus* an insufficient number of sample localities was used to adequately assess geographic variation patterns. However, Lorenzo et al. (2006) observed little geographic variability in multivariate space in this species except for that associated with the greatest skull length (which presumably reflects variation in overall body size), diastemal length, and palatine bridge length (presumably reflecting feeding habits). Mahalanobis distance analyses indicated that *P. guatemalensis* and *P. grandis* are phenetically close to each other and that *P. zarhynchus* is not equally distant from both. This finding contradicts the analysis of Huckaby (1980), which clustered *P. zarhynchus* and *P. grandis*.

Patterns of genetic variation.—Patterns of phylogenetic relationships showed that *P. guatemalensis* and *P. grandis* were sister taxa followed by *P. nudipes* from Costa Rica and *P. zarhynchus* as successively more basal species. This does not support the sister-group relationship of *P. zarhynchus* to either *P. grandis* or *P. guatemalensis* as previously suggested by Huckaby (1980). In addition, constraining individuals of *P. grandis* and *P. zarhynchus* to form a monophyletic group resulted in a significantly worse tree ($P = 0.18$) based on the test of Shimodaira and Hasegawa (1999) than by having *P. grandis* and *P. guatemalensis* as sister taxa. Hierarchical levels of sequence divergence showed similar patterns of variation in these and other groups of Mesoamerican rodents (Bradley et al. 2007) to those previously seen with the *Cytb* marker. The interspecific variation between *P. grandis* and *P. guatemalensis* is the smallest in the data set (4.5%). However, this genetic distance value is within the limits observed between sister taxa in *Peromyscus* (2.8–10.2%—Baker and Bradley 2006). For example, interspecific variation is 2.7% between *P. simulus* and *P. boylii*, 3.8% between *P. simulus* and *P. madrensis*, and 4.2% between *P. boylii* and *P. stephani* (Tiemann-Boege et al. 2000).

Neither of our parsimony or Bayesian analyses group *P. nudipes* with *P. mexicanus* as Huckaby (1980) suggested based on morphology. Our data support the position of Osgood (1909) and Hooper (1968) that *P. nudipes* is a species separate from *P. mexicanus*. In addition, our topology of *P. nudipes* as the sister species to a clade of *P. grandis* and *P. guatemalensis* supports the relationships proposed by Bradley et al. (2007).

The *P. mexicanus* and *P. gymnotis* clade was highly supported, but these species were not reciprocally monophyletic. This clade appears as a sister clade to the one formed by *P. zarhynchus*, *P. nudipes*, *P. grandis*, and *P. guatemalensis*, and was similar to that of Bradley et al. (2007). *P. gymnotis* was initially classified as a subspecies of *P. mexicanus* (Osgood 1909), but was elevated to species level in subsequent years (Musser 1971). Later, Huckaby (1980) expanded the southern range of *P. gymnotis* to include southern Nicaragua. In addition, Rogers and Engstrom (1992) found that samples of *P. mexicanus* from Chiapas were genetically similar to *P. gymnotis* and also found that these taxa were consistently grouped together.

Initially, *P. stirtoni* was placed in the *P. mexicanus* group (Hall and Kelson 1959). Although Hooper (1968) questioned the specific recognition of *P. stirtoni*, Huckaby (1980) substantiated its distinctive morphology. The association of *P. stirtoni* with the *P. mexicanus* group was considered tentative (Carleton 1989), even though the G-banded karyotype appeared identical to that reported for the core members of the *P. mexicanus* group (Peppers et al. 1999). However, mtDNA analysis suggested that *P. stirtoni* be placed with the *P. megalops* species group (Bradley et al. 2007). This placement was corroborated with mtDNA data in this study.

Isolation by distance.—One explanation for the patterns observed for the 3 taxa examined herein, as proposed by Baker and Bradley (2006) for speciation in *Peromyscus*, is that genetic isolation was accompanied by minimal morphological evolution and divergence. An alternative interpretation of our results may be that the patterns of morphometric and genetic variation within these species are explained by geographic distance and not by the classic case of allopatric isolation. We would expect significant and positive correlations between the morphometric and genetic distance matrices to that of geographical distance. To test this hypothesis with morphometric data we regressed a matrix of Mahalanobis distances among samples against a corresponding matrix of geographic distances among localities. The correlation coefficient was not significant. The pairwise correlation between genetic and geographic distances was low and not significant.

Biogeographic context.—The mountains of Mesoamerica currently are recognized as a region with high levels of diversification and endemism for small mammals (Amman and Bradley 2004; Carleton et al. 2002; Harris et al. 2000; León-Paniagua et al. 2007; Ordóñez-Garza 2004; Rogers et al. 2007; Sullivan et al. 2000; Woodman and Timm 1999). Genetic studies additionally have documented extensive

intraspecific differentiation and high levels of genetic variation in rodents from highlands in the region, including the genus *Peromyscus* (Bradley et al. 1996, 2000, 2004; Castro-Campillo et al. 1999; Rogers and Engstrom 1992; Sullivan et al. 1997; Zimmerman et al. 1978).

Carleton (1989) argued that the regional differentiation for the members of the *P. mexicanus* group, including *P. grandis*, *P. guatemalensis*, and *P. zarhynchus*, presumably was due to a response to the topographic and climatic diversity of their environments in Mesoamerica. At 1st glance the molecular analysis in this study suggests a progression from north to south among the major clades (Fig. 3). This observation is seen in the basal clade containing *P. gymnotis* and *P. mexicanus* found in Mexico, the clade containing individuals of *P. zarhynchus* in southern Mexico and, moving farther south to more derived Guatemalan clades, *P. grandis* and *P. guatemalensis*. However, the clade containing individuals of *P. nudipes* suggests that no such geographical progression exists. This observation also is supported by the low and nonsignificant correlations between morphometric, genetic, and geographic distances observed in this study.

Other studies agree with a pattern of expansion of the ancestral species of *Peromyscus* from North America throughout Mexico to Central America and subsequent speciation in the Mesoamerican highlands (Dawson 2005). A similar pattern was suggested by Huckaby (1973), who noticed fragmented populations of *P. zarhynchus* occurring in the highlands of Mexico and might have served as a Guatemalan ancestor. However, Huckaby (1980) maintained that montane populations were a separate species occupying habitats with disjunctive ranges, yet he could not discern whether these species shared a common ancestor.

At least 2 stages figure into the speciation of *P. grandis*, *P. guatemalensis*, *P. nudipes*, and *P. zarhynchus*. First, the Isthmus of Tehuantepec served as a vicariant barrier resulting in isolation of the ancestral stock for *P. grandis*, *P. guatemalensis*, *P. nudipes*, and *P. zarhynchus* in the south from other members of the *P. mexicanus* species group in the north. This barrier has been well documented for other montane species (Carleton et al. 2002; Castoe et al. 2009; Watson and Peterson 1999) and is thought to have impacted the differentiation of shrews (Woodman and Timm 1999), *Habromys* (León-Paniagua et al. 2007; Rogers et al. 2007), *Reithrodontomys* (Arellano et al. 2005), and some *Peromyscus* (Sullivan et al. 1997). Second, *P. grandis*, *P. guatemalensis*, *P. nudipes*, and *P. zarhynchus* then differentiated within the region as a result of isolation on separate mountain systems within the Mesoamerican highlands, which provided a disjunctive, cool-adapted highland environment in an otherwise tropical ecosystem (Harris et al. 2000). *P. nudipes*, although a member of this species group, appears to have been isolated from *P. grandis* and *P. guatemalensis* and now occupies montane regions south of the Mesoamerican highlands. The effects of these mountain ranges have been suggested to impact different taxa that may have adapted to dramatic changes in climate during the Pleistocene (Van Coeverden De Groot 1995) and had considerable impact on phylogeographic patterns within and among closely related species (Avise et al. 1998). For example, climatic fluctuations during the Pleistocene, the process of isolation, allopatric differentiation in refugia, and subsequent range expansion have been suggested as an explanation and could have been responsible for the current distribution and diversity of many neotropical taxa (Haffer 1969, 1997), including *Orthogeomys* (Hafner 1991), *Glaucomys* (Braun 1988), *Neotoma mexicana* (Edwards and Bradley 2002), and members of the *Peromyscus aztecus* species group (Sullivan et al. 1997).

For peromyscines, Dawson (2005) proposed that both vicariant and dispersal events occurred during interglacial periods, which influenced and gave shape to the actual distribution of peromyscines. This hypothesis also implies that Mexico is a speciation center for the group because of the interaction between topography and glacial advances and retreats. Climate changes resulted in expansion and retraction of moist premontane and montane vegetation (Van Coeverden De Groot 1995). Therefore, during the warm intervals following glacial maxima some species were isolated in refugia resembling montane islands after the last glacial maximum 18,000–2,500 years ago (Bush et al. 1990). The regional conditions became consistently warmer and drier, and these frost-hardy species migrated upslope to cool-temperate, upper elevations (Toledo 1982). By 10,000 years ago, these forests were restricted to the high mountain ranges of Mesoamerica, and their distribution essentially has been unchanged (Watson and Peterson 1999).

As reported by León-Paniagua et al. (2007), no direct evidence exists for a link between these geographic events and the speciation events in Mesoamerica for *Habromys*. However, the unique geographic structure of Mesoamerica is reflected in the patterns of distribution and speciation of several groups of rodents, especially peromyscines (Carleton et al. 2002; Dawson 2005). León-Paniagua et al. (2007) suggested that *Megadontomys*, *Osgoodomys*, and *Podomys* have experienced events of expansion and differentiation and that they likely represent relicts of earlier events. Actual patterns of diversification in other taxa, for example, salamanders and lizards (García-París et al. 2000; Hasbún et al. 2005; Parra-Olea et al. 2004), suggest that patterns likely are due to repeated colonization of Mesoamerica from source populations during fluctuations in vegetation assemblages associated with climatic oscillations. The actual distribution of *P. grandis*, *P. guatemalensis*, *P. nudipes*, and *P. zarhynchus* might have resulted from repeated colonization in the highlands of Mesoamerica, which could have contributed to the establishment of the observed nonlinear phylogeographic pattern. These recurrent phylogeographic patterns of clear genetic structure seen in Mesoamerica suggest that it has been a center for speciation and that much of its biodiversity has originated in situ (Dawson 2005; Harris et al. 2000; León-Paniagua et al. 2007), including differentiation for groups of small mammals (Carleton et al. 2002) such as *P. grandis*, *P. guatemalensis*, *P. nudipes*, and *P. zarhynchus*.

Resumen

En Mesoamérica se han reportado altos niveles de variación entre especies del grupo *Peromyscus mexicanus*. Tres especies de este grupo, *P. grandis*, *P. guatemalensis*, y *P. zarhynchus*, se caracterizaron morfológica y genéticamente, con el objetivo de poner a prueba hipótesis sobre límites de especies. Por otra parte, se pusieron a prueba hipótesis previas sobre relaciones fenéticas entre estas especies, para responder si estas hipótesis eran respaldadas por nuestros datos. Los resultados incluyeron análisis de variación geográfica y no geográfica de especímenes de 36 localidades de Guatemala y del sureste de México. Adicionalmente, se analizaron 35 secuencias del gen citocromo-*b*, aplicando métodos de parsimonia e inferencia bayesiana, que concordaron con los datos morfométricos. Nuestros análisis sugieren que existen 3 unidades morfológicas y genéticas distintas, y que *P. grandis* y *P. guatemalensis* están más relacionadas entre ellas que alguna de estas con *P. zarhynchus*. Además este estudio incluye el análisis de las relaciones filogenéticas entre miembros del grupo de especies de *P. mexicanus*, patrones de especiación y biogeográficos, que ayudarán a la identificación de unidades regionales filogeográficas importantes en Mesoamérica.

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Appendix I

Specimens examined.—The 385 specimens analyzed are deposited in the following collections: American Museum of Natural History (AMNH), Field Museum of Natural History (FMNH), Royal Ontario Museum (ROM), Texas Tech University Museum (TTU), and United States National Museum (USNM). Species names are followed by locality numbers and locality name; all are referred to in Fig. 1. Museum acronyms and museum catalogue numbers are provided after locality name. GenBank accession numbers are provided in parentheses.

Peromyscus grandis.—Locality 1, GUATEMALA: Alta Verapaz; Tucurú, Finca Concepción, AMNH 79342, 79341, USNM 570098–57100 (GQ461919–GQ461921), USNM 570118–570121 (GQ461922–GQ461925). Locality 2, GUATEMALA: Baja Verapaz; 12.5 miles N Salamá, USNM 460246, 391964. Locality 3, GUATEMALA: El Progreso; San Agustín Acasaguastlán, Finca la Piedad, USNM 565236. Locality 4, GUATEMALA: Zacapa; Río Hondo, Finca Montes de Morán, 17 km NE Road Ca-9, km 143; Sierra De Las Minas, USNM 565194–565235, 565275. Locality 28, GUATEMALA: Baja Verapaz; 5 km E of Purulhá, ROM 98514. Locality 29, GUATEMALA: Zacapa; San Lorenzo, Sierra de las Minas, ROM 99863.

Peromyscus guatemalensis.—Locality 5, GUATEMALA: Chimaltenanago; 5 miles N Tecpán, USNM 391968, 391969, 392026, 392027. Locality 6, GUATEMALA: Quiché; Nebaj, USNM 275528–275534, 275544, 275545. Locality 7, GUATEMALA: Quiché; San Juan Cotzal, USNM 275547–275552. Locality 8, GUATEMALA: Quetzaltenango; Zunil, USNM 76865–76872, 76874, 76875. Locality 9, GUATEMALA: Quetzaltenango; Zunil, Volcán Santa María, USNM 76876–76886. Locality 10, GUATEMALA: Quetzaltenango; Calel, USNM 76887–76905, 77268, 77269, 77374. Locality 11, GUATEMALA: Quetzaltenango; El Palmar, Finca Helvetia, USNM 275498–275502, 275505–275507. Locality 12, GUATEMALA: Quetzaltenango; 4 km SE Zunil, Finca La Chingada, USNM 569619, 569626–569633, 69635–569639, 569651, 569653, 569654, 569658–569665, 569676, 569677, 569682–569687. Locality 13, GUATEMALA: Quetzaltenango; 6.5 km SW Zunil, bosque Zunil, USNM 569690–569695, 569697–569708. Locality 14, GUATEMALA: Quetzaltenango; 5 km ENE Cabricán, bosque Ojo de Agua, USNM 569721, 569726–569731, 569745, 569746, 569754–569756. Locality 15, GUATEMALA: Quetzaltenango; 1 mile S Quetzaltenango, USNM 391962, 391963. Locality 16, GUATEMALA: Sololá; Panajachel, 3.2 miles E, Panajachel, USNM 460248. Locality 17, GUATEMALA: Huehuetenango; Todos Santos Cuchumatán, USNM 76850–76855, 76858–76860, 76863, 76864, TTU 109060 (GQ461928), 109076 (GQ461926), 109080 (GQ461927). Locality 18, GUATEMALA: Huehuetenango; Chiantla, 27 miles N Chiantla, USNM 391965, 391966, 391970, 460247. Locality 19, GUATEMALA: Huehuetenango; 6 km NW Santa Eulalia, Yaiquich, USNM 569354–569357, 569364, 569365, 569369, 569370, 569379–569382. Locality 20, GUATEMALA: Huehuetenango; 5 km SW San Mateo Ixtatán, USNM 569452, 569406–569417, 569427–569430, 569437–569439, 569451, 569393, 569395, 569398–569401, 569404, 569405, 569492, 569498–569502, 569516, 569518, 569531–569534, 569549–569552, 569514, 569515, 569517, 569535–569537. Locality 21, GUATEMALA: Huehuetenango; 22 km NNE Chiantla, Laguna Magdalena, USNM 569557, 569558, 569561, 569587, TTU 109059 (GQ461929), 109062 (GQ461931), 109063 (GQ461933), 109065 (GQ461930), 109066 (GQ461934), 109067 (GQ461932), 109068 (GQ461935). Locality 25, MÉXICO: Chiapas; Pinabete, Cerro Mozotol, USNM 77614, 77615, 77617, 77620–77623, 77923 (EFO28171, EFO28172). Locality 26, GUATEMALA: Chimaltenanago; Santa Elena, FMNH 41682, 41688, 41680, 41690, 41692. Locality 27, GUATEMALA: San Marcos; Volcán Tajumulco, FMNH 41694, 41696, 41698, 74078, 74082, 74094, 74096. Locality 30, GUATEMALA: Huehuetenango; 10 km NW of Santa Eulalia, ROM 98288.

Peromyscus zarhynchus.—Locality 22, MÉXICO: Chiapas; Pueblo Nuevo Solstihuacán, AMNH 172589–172596, 172598, 172600–172604, 172606–172608, 172610–172612, 172614–172622, 172624–172634, 177337, 172498. Locality 23, MÉXICO: Chiapas; San Cristóbal de las Casas, USNM 76096–76099, 76101–76108, 76110, 76131–76134, AMNH 174774–174777. Locality 24, MÉXICO: Chiapas; Tumbala, USNM 76115–76118, 76120, 76136, 76137, 76139, 76140, 76142. MÉXICO: Chiapas; Chamula, Cerro Tzotenhuitz, 13 km NE San Cristóbal de las Casas (EFO28167). MÉXICO: Chiapas; Yalentay (AY195800).

Peromyscus boylii.—UNITED STATES: California; Monterey Co., Hastings Natural History Reservation (AF155386).

Peromyscus gymnotis.—MÉXICO: Chiapas; 21 km NE Mapastepec (EFO28170, EFO28169).

Peromyscus mayensis.—GUATEMALA: Huehuetenango; 16 km NW Santa Eulalia (DQ836301, DQ836300).

Peromyscus megalops.—MÉXICO: Guerrero; 6.4 km SSW Filo de Caballo (DQ000475).

Peromyscus melanocarpus.—MÉXICO: Oaxaca; Municipio Santiago Comaltepec, La Esperanza, 11 km SW Hacienda San Isidro (EFO28173).

Peromyscus melanophrys.—MÉXICO: Jalisco; 30 km W Huejuqilla del Alto (AY376424).

Peromyscus mexicanus.—MÉXICO: Chiapas; 14.4 km N Ocozocoautla (AY376425). MÉXICO: Veracruz; 10 km SE Zongolica (EFO28174).

Peromyscus nudipes.—COSTA RICA: Heredia; 2 km NE Getzemaní (AY041200).

Peromyscus perfulvus.—MÉXICO: Michoacán; Túnel de Riego, 2 km E Cerro Colorado (DQ000474).

Peromyscus stirtoni.—NICARAGUA: Masaya; Parque Nacional Volcán Masaya (DQ973108).

Neotomodon alstoni.—MÉXICO: Michoacán; Ladera, Cerra del Burro, 3 km W Opopeo-Tamborro (AY195796).