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A new species in the *Peromyscus boylii* species group (Cricetidae: Neotominae) from Michoacán, México

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Specimens of the *Peromyscus boylii* species group occurring in the montane regions of Michoacán, México, historically have been assigned to *P. levipes*. However, previous studies have shown that some specimens from eastern Michoacán possessed mitochondrial DNA haplotypes and karyotypes that were distinct from *P. levipes* and other members of the *P. boylii* species group. Phylogenetic analyses (parsimony and likelihood) of additional DNA sequences obtained from the mitochondrial cytochrome-*b* gene indicated that specimens from central and eastern Michoacán and western Morelos formed a monophyletic clade that was sister to a clade containing representatives of *P. beatae*. Estimations of genetic divergence for members of these 2 sister clades exceeded 5% and were greater than most pairwise comparisons reported for other members of the *P. boylii* species group. Collectively, there are no discernable morphological differences between those specimens and other cryptic species in the *P. boylii* species group. Together, these results indicated that specimens from the Sierra Madre del Sur region of Michoacán, Morelos, and likely throughout the Neovolcanic Axis of the Estado de México represent an undescribed species of *Peromyscus* for which we propose the name *Peromyscus kilpatricki*.

Especímenes del grupo especies de *Peromyscus boylii* que ocurren en las regiones montañosas de Michoacán, México, han sido tradicionalmente asignadas a *P. levipes*. Sin embargo, estudios previos indican que algunos especímenes del Este de Michoacán tienen haplotipos mitocondriales y cariotipos que son distintos a los de *P. levipes* y a los de otros miembros del grupo de especies de *P. boylii*. Análisis filogenéticos (parsimonia y verosimilitud) de secuencias adicionales de ADN obtenidas del gen mitocondrial citocromo-b indicaron que los ejemplares del centro y este de Michoacán y oeste de Morelos formaron un clado hermano a un clado que contiene representantes de *P. beatae*. Estimaciones de divergencia genética de miembros de estos 2 clados hermanos exceden del 5% y fueron mayores que la mayoría de comparaciones pareadas reportadas para otros miembros del grupo de especies de *P. boylii*. Colectivamente, no hay diferencias morfológicas entre los especímenes del este de Michoacán y otras especies crípticas del grupo de *P. boylii*. En conjunto, estos resultados indicaron que los especímenes de la región de Sierra Madre del Sur de Michoacán, Morelos, y posiblemente la región central del Estado de México representan una especie no descrita de *Peromyscus*, para la cual proponemos el nombre *Peromyscus kilpatricki*.

Key words: cryptic species, cytochrome-*b* gene, karyotype, morphology, *Peromyscus*, *P. boylii* species group

Resolving the systematic and taxonomic relationships within the *Peromyscus boylii* species group has been an active endeavor over the last 35 years. Since 1977, 2 species were described (*P. schmidlyi* Bradley et al. 2004, and *P. carletoni* Bradley et al. 2014); 5 taxa previously recognized as subspecies of *P. boylii* were elevated to the species level (*P. madrensis*, *P. simulus*, and

P. spicilegus [see Carleton 1977], *P. beatae*, and *P. levipes* [see Houseal et al. 1987; Rennert and Kilpatrick 1987; Schmidly et al. 1988]); and 2 subspecies were reassigned to other species (*P. boylii ambiguus* to *P. levipes* [see Castro-Campillo et al. 1999], and *P. boylii sacarensis* to *P. beatae* [see Bradley et al. 2000]). Despite these advances, several taxonomic issues remain unresolved in this species group, especially in the poorly studied regions of eastern and southwestern México.

Houseal et al. (1987) summarized the karyotypic diversity within the *P. boylii* species group and based on number of autosomal chromosomes in the karyotype (FN) identified 3 potentially undescribed taxa from Michoacán, México. Those taxa had karyotypes (FN = 54, FN = 56, and FN = 65–66, 68, respectively) that were either distinct from other members of the *P. boylii* species group (*beatae*, *boylii*, *levipes*, *madrensis*, and *simulus*) or their distinct geographic distribution precluded an association with named taxa. Allozymic data (Rennert and Kilpatrick 1987) revealed that the Michoacán populations (corresponding to the FN = 56 and FN = 65–66, 68 forms) were genetically divergent from each other and from other members of the *P. boylii* species group; however, no fixed genic differences were apparent. More recently, Tiemann-Boege et al. (2000) reported a unique mitochondrial cytochrome-*b* (*Cytb*) haplotype for specimens from western Michoacán and suggested that those specimens represented an undescribed species. Despite this relatively high level of genetic differentiation, there has been a low level of accompanying morphological divergence reported among members of the *P. boylii* species group (Schmidly et al. 1988; Castro-Campillo et al. 1999; Bradley et al. 2004, 2014). In addition, given the confluence of diverse morphotectonic provinces in this region, such as the Trans-Mexican Volcanic Belt and Sierra Madre del Sur (Ferrusquía-Villfranca 1993), it would not be surprising that these genetically distinct populations might prove to be cryptic species, hidden within the overall conservative morphology that exemplifies the *P. boylii* species group.

In this study, efforts were focused on determining the taxonomic status of populations distributed in the high-elevation (> 1,900 m) pine-oak forests (*Pinus* spp. and *Quercus* spp.) of Michoacán, Estado de México, and Morelos. In addition, phylogenetic relationships of these populations were examined relative to other members of the *P. boylii* species group.

Materials and Methods

Samples.—Eight individuals were obtained from 3 naturally occurring populations in Michoacán, México and 1 locality in Morelos, México (Localities 16–19; Fig. 1). *Cytb* DNA sequences from 36 individuals, representing 15 species and 2 presumably undescribed taxa, reported in Bradley et al. (2000,

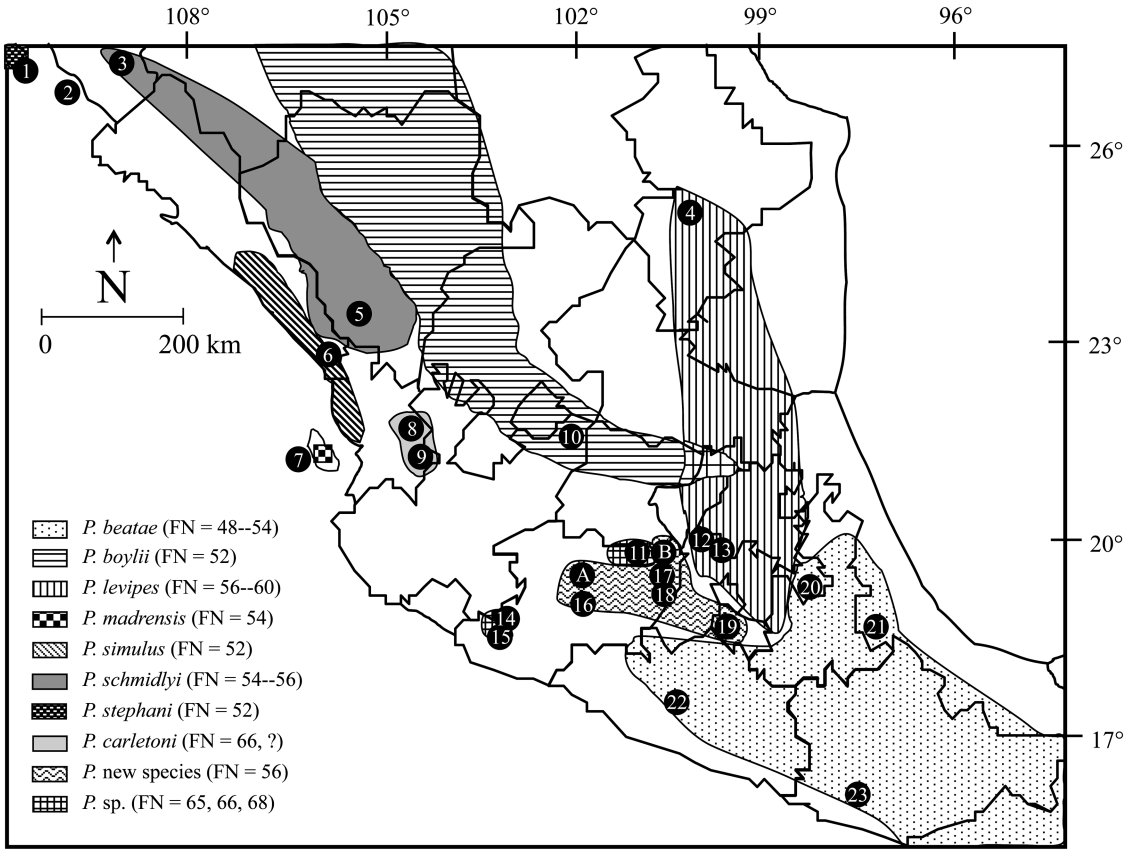

Fig. 1.—Distribution of selected populations and species of the *Peromyscus boylii* species group from western Mexico and surrounding states. Emphasis was placed on depicting the newly described species and its closest phylogenetic allies or taxa with similar karyotypes. Closed circles represent collecting localities and numbers refer to samples listed in Appendix I. Localities A, B, and 16–19 represent the new species (*P.* species novum).

2004, 2007, 2014), Tiemann-Boege et al. (2000), and this study, were included as internal references and outgroup comparisons. Specimens were collected following methods outlined in the ASM Guidelines (Sikes et al. 2016) and approved by the Texas Tech University Animal Care and Use Committee. Specimen numbers and collection localities are listed in Appendix I.

Karyotypic data.—One individual of *Peromyscus* from Zitácuaro, Michoacán, was karyotyped following the bone marrow method of Baker and Qumsiyeh (1988). At least 5 metaphase spreads were examined and photographed. Karyograms were constructed based on chromosomal morphology presented in Committee for Standardization of Chromosomes of *Peromyscus* (1977) and Greenbaum et al. (1994) and were compared to karyotypes and FNs (Table 1) previously reported by Lee et al. (1972), Schmidly and Schroeter (1974), Houseal et al. (1987), Smith (1990), and Bradley et al. (2014).

Sequence data.—Mitochondrial DNA was isolated from approximately 0.1 g of frozen liver tissue using the DNeasy kit (Qiagen, Valencia, California). The entire *Cytb* gene (1,143 bp) was amplified using the polymerase chain reaction (PCR) method (Saiki et al. 1988) and the following primers: MVZ05 (Smith and Patton 1993) and PERO3′ (Tiemann-Boege et al. 2000) or primer pairs L14724 with CBH3 (Irwin et al. 1991; Palumbi 1996) and F1 with H15915 (Irwin et al. 1991; Whiting et al. 2003). Thermal profiles for PCR were as follows: initial denaturation at 95°C for 2 min, followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 51°C for 1 min, and extension at 72°C for 2 min, with a final extension at 72°C for 7 min. Most PCR products were purified with either an ExoSAP-IT (Affymetrix, Santa Clara, California) or QIAquick PCR Purification Kit (Qiagen, Valencia, California) or the Millipore Multiscreen™ 96-Well Filtration System (Cat. No. MANU03050). Primers used to cycle sequence the products included: WDRAT1100, 400R, 700H, and NEO700L (Peppers and Bradley 2000), and 400F (Tiemann-Boege et al. 2000), H15915, L14724, F1, and CB3H. Cycle sequencing reactions were purified using isopropanol cleanup protocols and were analyzed with an ABI 3100-Avant automated sequencer and ABI Prism Big Dye version 3.1 terminator technology (Applied Biosystems, Foster City, California) or using Millipore Multiscreen™ Filter Plates for High Throughput Separations (Cat. No. MAHVN4510) with a Perkin-Elmer ABI Prism 377. Resulting sequences were aligned and proofed using Sequencher 4.0 or 4.1.2 software (Gene Codes, Ann Arbor, Michigan); chromatograms were examined to verify all base changes. All *Cytb* sequences obtained in this study were deposited in GenBank and are listed in Appendix I.

Using the phylogenetic relationships of the genus *Peromyscus* presented in Bradley et al. (2007), *P. gratus* was selected as the outgroup taxon for all sequence analyses. Representatives of all currently recognized members of the *P. boylii* species group (*beatae*, *boylii*, *carletoni*, *levipes*, *madrensis*, *schmidlyi*, *simulus*, and *stephani*) were included in analyses as internal standards. In addition, members of the *P. aztecus* species group (*aztecus*, *evides*, *hylocetes*, *oaxacensis*, *spicilegus*, and *winkelmanni*) and 1 undescribed taxon with apparent affiliations to the *P. boylli* species group were included based on their chromosomal similarities or geographic proximity.

A parsimony analysis (PAUP*—Swofford 2002) was conducted using equally weighted characters and variable nucleotide positions treated as unordered, discrete characters with 4 possible states: A, C, G, or T. Phylogenetically uninformative characters were excluded from analyses. The heuristic search and tree-bisection-reconnection options in PAUP* (version 4.0a149—Swofford 2002) were used to find the most-parsimonious trees. A strict consensus tree was generated from the available trees and the bootstrap (BS) analysis (Felsenstein 1985) with 1,000 iterations was used to evaluate nodal support.

Table 1.—Comparison of karyotypes for members of the *Peromyscus boylii* species group examined in this study. All chromosomal assessments are based on nondifferentially stained karyotypes as interpreted from comparisons to data presented in or cited by Houseal et al. (1987) and Smith (1990). Only chromosomes that have been identified as biarmed for the *P. boylii* species group are included. All karyotypes possessed a biarmed condition for chromosomes 1, 22, and 23 (except in some populations of *P. beatae*—see Davis et al. 1986). Abbreviations are as follows: a = acrocentric, b = biarmed, and p = polymorphic. References: 1 = Lawlor (1971), 2 = Lee et al. (1972), 3 = Schmidly and Schroeter 1974, 4 = Carleton et al. (1982), 5 = Houseal et al. (1987), 6 = Smith (1990), 7 = Bradley et al. (2004), 8 = Bradley et al. (2014), and 9 = this study.

Taxon	FN	Chromosome										Reference
		2	3	4	5	6	7	8	9	10	13	
P. beatae	48–54	p	a	a	a	a	a	a	a	a	a	5, 6
P. boylii	52	a	a	a	a	a	a	a	a	a	a	5
P. carletoni	66[a]	b	b	a	b	b	b	a	b	b	a	4, 8
P. levipes	56–60	b	p	a	a	p	a	a	b	a	a	5, 6
P. madrensis	54	a	a	a	a	a	a	a	b	a	a	4
P. schmidlyi	54–56	p	a	a	a	a	a	a	p	a	a	2, 3, 7
P. simulus	52	a	a	a	a	a	a	a	a	a	a	4
P. stephani	52	a	a	a	a	a	a	a	a	a	a	1
P. new species (Zitácuaro, Michoacán; *n* = 1)	56	b	a	a	a	a	a	a	b	a	a	8, 9
P. new species (Los Azufres, Michoacán; *n* = 3)	56	b	a	a	a	a	a	a	b	a	a	5
P. new species (Pátzcuaro, Michoacán; *n* = 1)	56	b	a	a	a	a	a	a	b	a	a	5
P. sp2 (Dos Aguas, Michoacán; *n* = 5)	65, 66, 68	b	b	a	b	b	p	a	b	p	p	5
P. sp2 (Zinapécuaro, Michoacán; *n* = 4)	68	b	b	a	b	b	b	a	b	b	b	8, 9

[a]Bradley et al. (2014) discuss an aberrant karyotype for *P. carletoni*.

Fifty-six maximum likelihood models were evaluated using MODELTEST (Posada and Crandall 1998) in order to determine the model of DNA evolution best fitting the data. The Akaike information criterion identified HKY+I+G as being the most appropriate model, relative to complexity of model, for this data set. This model generated significantly better likelihood scores (–lnL = 5,305.4365) than all other models and included the following parameters: base frequencies (A = 0.3326, C = 0.3006, G = 0.1079, T = 0.2589), proportion of invariable sites (I = 0.5952), and gamma distribution (G = 1.2044).

Bayesian inference methods (MrBayes—Huelsenbeck and Ronquist 2001) were used in a maximum likelihood framework and to generate clade probabilities values indicative of nodal support. The HKY+I+G model was used and parameters were estimated within the analysis. The analysis was run with the following options: 4 Markov chains, 10 million generations, and sample frequency = every 1,000th generation. Following a visual inspection of likelihood scores, the 1st 1,000 trees were discarded and a consensus tree (50% majority rule) was constructed from the remaining trees.

The Kimura 2-parameter model of evolution (Kimura 1980) was used to calculate genetic distances between selected taxa. These values were used to assess levels of genetic divergence following criteria outlined in Bradley and Baker (2001) and Baker and Bradley (2006).

Morphometric data.—Eighteen cranial measurements (defined in Carleton et al. 1982) were recorded in millimeters (mm) from adults or were obtained from previous studies (Bradley et al. 2014). Only adults (age classes IV–VI), identified as such based on patterns of molar tooth wear (Schmidly 1973), were included in this study. Measurements are as follows: greatest length of skull (GLS), length of auditory bulla (LAB), postpalatal length (PPL), length of mesopterygoid fossa (LMF), palatal length (PL), length of incisive foramen (LIF), length of molar toothrow (LMT), greatest zygomatic breadth (ZB), mastoidal breadth (MB), greatest breadth across molars (GBM), postdental palatal breadth (PPB), greatest width of mesopterygoid fossa (WMF), depth of braincase (DB), breadth of braincase (BB), least interorbital width (LIW), rostral breadth (RB), nasal length (NL), and rostral length (RL).

Statistical analyses of morphologic data.—For the 18 cranial characters, measurements were included from specimens collected at the following localities in Michoacán: Zitacuaro (n = 6), Uruapan (n = 41), Los Azufres (n = 1), Opopeo (n = 2), Pátzcuaro (n = 1), Quiroga (n = 1), Sierra Carcalosa (n = 2), and Dos Aguas (n = 10). These populations were compared to other populations delimited by previous allozyme, karyotypic morphologic, and genetic studies (Houseal et al. 1987; Rennert and Kilpatrick 1987; Schmidly et al. 1988; Bradley et al. 2004, 2014) to represent samples of *P. beatae* (n = 32), *P. boylii* (n = 44), *P. carletoni* (n = 54), *P. levipes* (n = 85), *P. schmidlyi* (n = 9), and *P. simulus* (n = 15). For all analyses, sexes were combined following Schmidly et al. (1988).

For descriptive and comparative purposes, means, ranges, and *SE*s were calculated for each character and species; for all further analyses, characters were log-transformed (natural log). Specimens with missing measurements were excluded from analyses. The Shapiro–Wilk normality test (Shapiro and Wilk 1965) was performed to test for normality among the data. Not all the variables were normally distributed; therefore, a nonparametric 1-way analysis of variance (ANOVA), the Kruskal–Wallis test (Kruskal and Wallis 1952; 1-way ANOVA on ranks test), was performed to determine if statistically significant differences existed between 2 or more groups. A Dunn post hoc test (Dunn 1964) with adjustment for P-value using the Bonferroni correction method (Ury 1976) was then applied to the results of the Kruskal–Wallis test, and a probability level of < 0.05 values was selected to indicate statistical significance.

Among-group (localities) variation was examined using an ANOVA on the 18 morphological characters, followed by a Mann–Whitney (Ryan 1959) pairwise comparison using Bonferroni-corrected P-values (Ury 1976). To best explain the variation of the data, a principal component analysis (PCA) was performed comparing all the species included in the study. In addition, a discriminant function analysis (DFA) was used to produce a scatter plot of specimens along the 1st and 2nd axes producing maximal and 2nd to maximal separation among all groups derived from multigroup discriminant function. All variable loadings are expressed as a product of correlation coefficients of the extracted components of canonical variates with the log-transformed cranial measurements. Statistical tests were evaluated at $\alpha = 0.05$ and were performed using PAST (Hammer et al. 2001) or R software version 3.2.1 (R Core Team 2014).

Results

Karyotypic data.—A single karyotype (Fig. 2) was obtained from an individual collected at Zitácuaro, Michoacán. Additional karyotypes from closely related species and

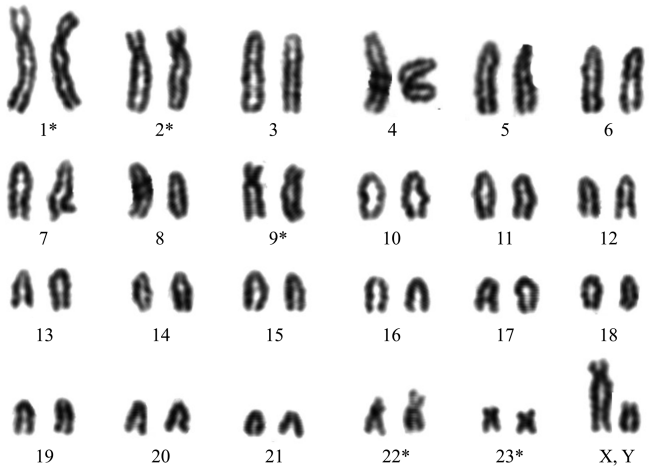

Fig. 2.—Karyogram of an individual from Zitácuaro, Michoacán (TTU104808). Chromosome presentation and numbering follows that presented in Committee for Standardization of Chromosomes of *Peromyscus* (1977). An asterisk (*) indicates chromosomes that are biarmed.

populations from nearby geographic localities were examined for comparison (Table 1). The karyotype of the specimen from Zitácuaro, Michoacán (TK150644), possesses a diploid number (2n = 48) and fundamental number (FN = 56) similar to that reported for specimens from Los Azufres and Pátzcuaro, Michoacán, by Houseal et al. (1987). This karyotype contained 2 large pairs of biarmed, 1 medium pair of biarmed, and 2 small pairs of biarmed chromosomes.

Sequence data.—Sequences from 8 individuals representing the new species were combined with 28 samples representing members of the *P. boylii* species group, other closely related species groups, and the outgroup taxon. In all analyses (parsimony, likelihood, and Bayesian), the *P. boylii* species group as defined by Tiemann-Boege et al. (2000) and Bradley et al. (2004, 2014) was retrieved as monophyletic. Relationships of some taxa included as reference samples were peripheral to this study and are not discussed in detail.

The Bayesian inference analysis produced a topology (Fig. 3) in which the 8 individuals, representing the undescribed taxon (samples from Michoacán and Morelos), formed a strongly supported clade (clade probability value, CPV = 1.00). This clade, in turn, was sister to a clade containing 5 representatives of *P. beatae* (CPV = 1.00). The sister relationship between the new species and *P. beatae* was strongly supported (CPV = 0.95). The clade containing *P.* species novum and *P. beatae* then joined with samples of *carletoni*, *levipes*, *schmidlyi*, and samples representing an additional unrecognized taxon (reported as *Peromyscus* sp.) to form a large, well-supported clade (CPV = 1.00). Samples of *boylii*, *madrensis*, *simulus*, and *stephani* were added in a stepwise fashion to form a monophyletic *P. boylii* species group (CPV = 1.00).

The parsimony analysis generated 10 equally most-parsimonious trees (length = 651). A majority rule consensus tree was generated (not shown) that was similar in topology to the tree obtained in the Bayesian analysis. BS support values were obtained and superimposed onto Fig. 3. In this analysis, the 7 samples from Michoácan and the single sample from Morelos formed a strongly supported monophyletic clade (BS = 97). This clade was sister (BS = 78) to a clade containing the representatives of *P. beatae*. Strong support was obtained (BS = 97) for a sister relationship between the clade containing the new species and *P. beatae* and a clade (BS = 92) containing samples of *P. carletoni*, *P. levipes*, *P. schmidly*, and 2 presumably, undescribed *Peromyscus* from Michoácan. This large clade was sister (BS = 94) to a clade containing the remaining members of the *P. boylii* species group (*boylii*, *madrensis*, *simulus*, and *stephani*).

The genetic divergence values (Table 2), estimated using the Kimura 2-parameter model of evolution (Kimura 1980), among samples representing the new species averaged 1.48%. Collectively, these samples differed from their closest relatives (determined in this study), *P. beatae*, *P. carletoni*, *P. levipes*, and *P. schmidlyi*, by 5.31%, 5.16%, 5.38%, and 5.57%, respectively. Genetic divergence values between other closely related species in the *P. boylii* species group ranged from 3.25% (*P. levipes* and *P. schmidlyi*) to 8.50% (*P. boylii* and *P. levipes*). The undescribed taxon differs from *P. levipes*, to which it was considered conspecific, by a genetic divergence value of 5.38%.

Morphometric data.—Means, ranges, and *SE*s for the 18 cranial measurements for all species in the *P. boylii* group are presented in Table 3. Perusal of the data reveals that the undescribed taxon, compared to the other taxa, is smaller than *levipes*, *beatae*, and *P.* sp1 in most of the cranial measurements, whereas it averages larger or about similar in measurements compared with *simulus*, *schmidlyi*, *boylii*, and *carletoni* (see "Comparisons" for more details).

ANOVA for the 18 measurements revealed statistically significant differences ($P < 0.05$) among the taxa in each of the measurements. However, a Shapiro–Wilk normality test revealed that only 4 of the cranial measurements (LN, PPL, ZB, and LBP) were normally distributed. Application of the nonparametric Kruskal–Wallis test to indicate differences among groups after correction for non-normality revealed significant differences in only 7 measurements (LN, PPL, LIW, LMR, LAB, RB, and BAM).

Both the Mann–Withney and Kruskal–Wallis tests, with Bonferroni corrections, were used to assess pairwise comparisons between the undescribed taxon and the other taxa for the 18 cranial measurements. The results reveal several cranial measurements that are significantly different between the undescribed taxon and the other taxa (see "Comparisons" for more detail). However, there is no single cranial measurement that will distinguish the undescribed taxon from all other taxa in the *P. boylii* species group.

The multivariate analyses confirmed the close morphological similarity among the taxa. The 1st 3 components in the PCA accounted for 63% of the total variation (PC1 35%, PC2 17%, PC3 8%). The character loadings were all positive for the 1st component with the 5 highest being LMF, LMR, LR, LN, and LAB, respectively. For component II, all of the loadings were positive except for 4 negative ones (LR, LAB, DB, and LMF). The scatter plot for the 1st 2 components (not shown) revealed extensive overlap among specimens from all the taxa except for *P. schmidlyi* which overlapped with *P. carletoni* but none of the other taxa. The new undescribed taxon was overlapped in the scatter plot by all of the taxa except for *P. schmidlyi*.

The DFA (see Fig. 4) revealed a similar pattern with individuals of the undescribed taxon overlapping by all the other taxa except for individuals of *P. schmidlyi*. The loadings for DF1 were all negative except for 2 (MB and LIF) and the highest loadings were for LMF, LAB, and LR. DF2 had a mixture of positive and negative loadings with fewer positive high loadings (LMF, LN) than negative high loadings (LMR, LBP, LIF, LR, and LIW).

The classification analysis, associated with the DFA, reveals only 2 taxa (*P. schmidlyi* and *P. simulus*) for which all of the individuals are correctly classified; 91% (29 of 32) of the *P. beatae* specimens were correctly identified. For *P. carletoni* and *P. boylii*, the percent correctly classified was 76 (41 of 54 individuals) and 66% (29 of 44 individuals), respectively. Only 73% of the individuals representing the undescribed taxon were correctly classified (40 out of 55); of the 15 misclassifications,

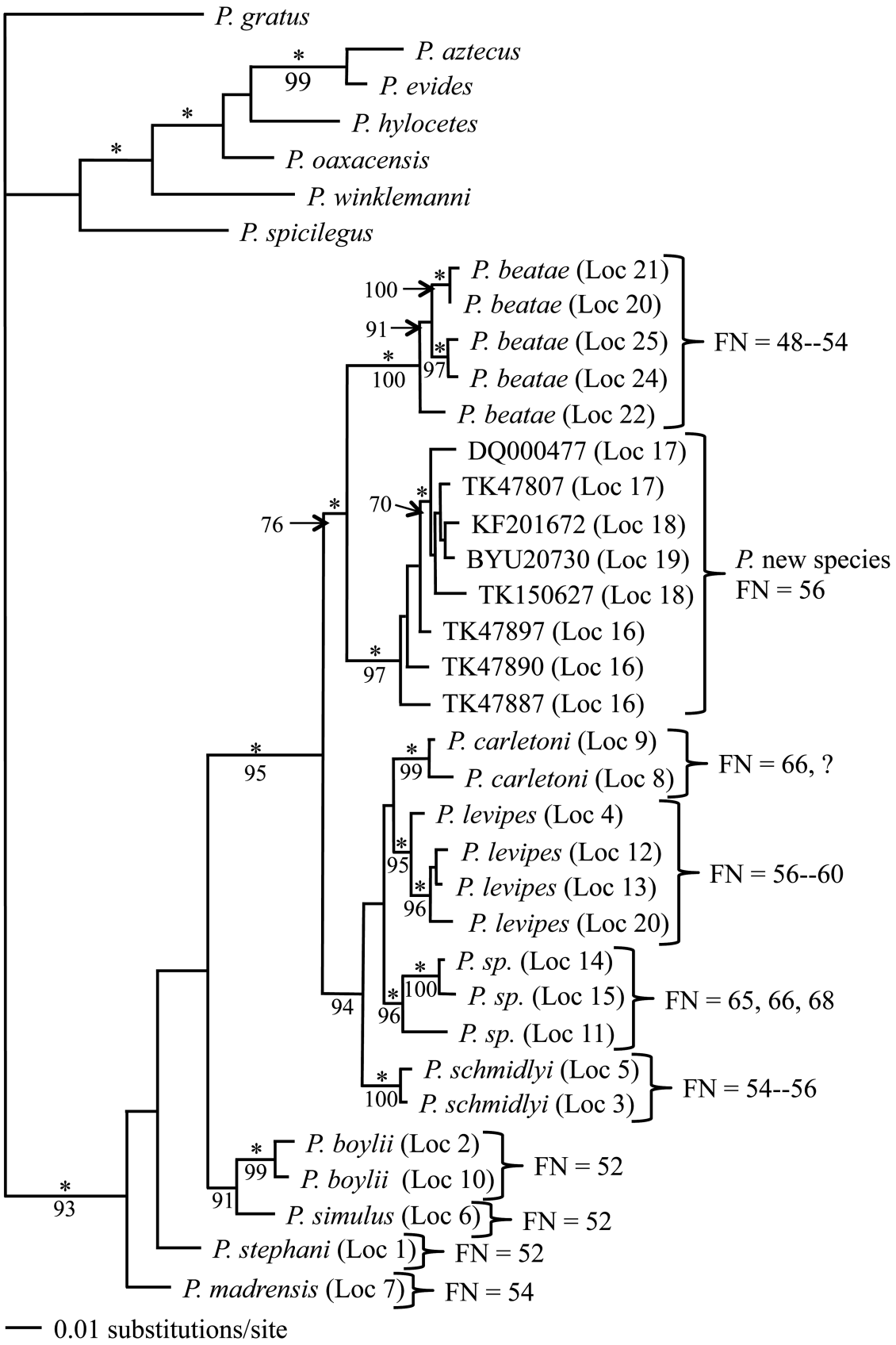

Fig. 3.—Phylogenetic tree generated using Bayesian methods (MrBayes—Huelsenbeck and Ronquist 2001) and the HKY+I+G model of evolution. Clade probability values (≥ 0.95) are indicated by an asterisk (*) and are shown above branches, bootstrap support values (≥ 70) obtained from the parsimony analysis are below branches.

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

Comparison	Average genetic distance
Within species	
P. beatae	1.24%
P. boylii	0.56%[a]
P. levipes	0.96%
P. schmidlyi	0.87%[a]
P. carletoni	0.65%[b]
P. new species	1.48%
Between *P.* species novum and closely related species in the *P. boylii* species group	
P. new species–*P. beatae*	5.31%
P. new species–*P. boylii*	8.09%
P. new species–*P. carletoni*	5.16%
P. new species–*P. levipes*	5.38%
P. new species–*P. schmidlyi*	5.57%
Between selected species in the *P. boylii* species group	
P. beatae–*P. boylii*	8.31%
P. beatae–*P. levipes*	5.65%
P. beatae–*P. schmidlyi*	5.63%
P. boylii–*P. levipes*	8.50%
P. boylii–*P. schmidlyi*	7.94%
P. carletoni–*P. levipes*	3.50%
P. carletoni–*P. schmidlyi*	3.40%
P. levipes–*P. schmidlyi*	3.25%

[a]Values obtained from Bradley et al. (2004).
[b]Values obtained from Bradley et al. (2014).

2 were identified as *P. beatae*, 5 as *boylii*, 6 as *levipes*, and 2 as *P.* sp1. Among the taxa, 16 *levipes* were incorrectly classified as the undescribed taxon, as were 5 *boylii* and 2 *carletoni*.

Discussion

Together, results from karyotypic data, DNA sequence data, and patterns of geographic distribution suggest that populations (historically referred to *P. levipes*) from Michoácan, Morelos, and presumably Estado de México represent an undescribed species of *Peromyscus*. Below, these populations are formally described as a new species.

Peromyscus kilpatricki, new species

Holotype.—Museum of Texas Tech University MoTTU (TTU104808); adult male; skin, skull, and skeleton. Original number Steve R. Hoofer 1179; TK150644 identifies tissue samples deposited in Natural Sciences Research Laboratory, MoTTU.

Type locality.—México: Michoácan; 13.5 km SW Zitácuaro (UTM 14Q-352122-2140934); collected 25 July 2006.

Paratype.—One female (TTU104799, TK150627) deposited in the Museum of Texas Tech University.

Diagnosis.—A species of *Peromyscus* with the following characters: size medium for the genus; tail slightly longer than head and body; hind foot medium; ear medium; dorsal coloration dark (Sepia at tips, Blackish Slate at base; color nomenclature following Ridgway [1912]); sides Dresden Brown; venter pelage White at tips, Blackish Slate at base; feet with Clove Brown strip extending slightly past ankle; toes White; tail slightly bicolored, Clove Brown above and White below, scantily haired at base and tufted at tip; ears Dark Mouse Gray; vibrissae Black; skull elongate, twice as long as wide; braincase slightly rounded; NL 114.6% of RL and RL 35% of greatest skull length; molar tooth row about 14% of skull length; interorbital constriction a smooth outline, not angular; zygomatic arches nearly parallel; auditory bullae medium; karyotype with 2n = 48 and 5 pairs of biarmed chromosomes.

Distribution.—Occurs in the high-elevation mesic, montane, pine-oak forests of the Sierra Madre del Sur in central Michoácan eastward to Morelos. Although undocumented at this time, this species presumably occurs in the montane regions of Estado de México, because they form part of the same mountain ranges. Additional collecting efforts are needed to better refine the distribution of this taxon.

Measurements.—External measurements of the holotype as taken in the field (in mm) are: total length, 205; tail length, 106; hindfoot, 22; and ear, 21. Cranial measurements were obtained using dial calipers (in mm), accurate at 0.5 mm, and are as follow: GLS, 27.7; LAB, 4.8; PPL, 9.4; LMF, 4.5; PL, 4.5; LIF, 5.6; LMT, 4.0; ZB, 13.5; MB, 11.5; GBM, 5.7; PPB, 4.3; WMF, 3.4; DB, 9.2; BB, 13.0; LIW, 4.4; RB, 4.8; NL, 11.0; and RL, 9.6. Mean measurements, ranges, and *SE*s for additional specimens are presented in Table 3.

Comparisons.—A species in the *P. boylii* species group, resembling the other 7 species in external and cranial size and coloration, however, distribution (allopatric), karyotype, and DNA sequence divergence (*Cytb* gene) preclude confusion. The cryptic nature of the various species in the *P. boylii* group is confirmed by the morphological analysis.

External measurements average smaller in *P. kilpatricki* compared to *P. beatae*, *P. levipes*, and *P.* sp1. Compared to *P. boylii*, *kilpatricki* is similar in the total length and tail length, is slightly smaller in body and ear length, but larger in hind foot length. Relative to *P. carletoni*, *kilpatricki* has smaller total and hind foot lengths but has larger tail, body, and ear lengths; from *P. simulus*, it is larger in total, tail, and ear lengths but smaller in body and hind foot lengths; from *P. schmidlyi*, it is larger in total, hind foot, and ear lengths but smaller in total and body lengths.

In cranial measurements, compared to *P. beatae*, *kilpatricki* averages smaller or similar in all measurements but LBP; from *P. levipes*, it is decidedly smaller in all measurements except WMF; from *P. boylii*, it averages larger, or about the same in all measurements except 3 (LR, LN, and LAB); from *P. carletoni*, it averages about the same in 5 measurements (ZB, LIW, RB, PPB, and WMF), is smaller in 4 (LN, PPL, MB, and LIF), and larger in the other 9; from *P. simulus*, it averages similar in 4 measurements (PPL, LAB, RB, and PPB), is smaller in 2 (ZB and RB), and larger in the other 13; from *P.* sp1, *kilpatricki* averages similar in 3 measurements (LBP, PPB, and WMF) and is smaller in the other 15.

Table 3.—Summary of univariate statistics for *Peromyscus* samples.

	P. new species (*n* = 55)			*P. beatae* (*n* = 32)			*P. boylii* (*n* = 44)		
	Mean	Range	*SE*	Mean	Range	*SE*	Mean	Range	*SE*
Greatest length of skull	27.24	26.05–29.00	0.094	28.37	27.35–29.75	0.59	27.10	25.80–28.35	0.76
Length of rostrum	10.94	10.20–12.20	0.054	11.85	10.10–12.75	0.44	11.01	9.80–12.20	0.51
Length of nasal	9.81	8.77–11.66	0.072	10.61	9.98–11.48	0.38	9.89	8.86–11.20	0.58
Postpalatal length	9.07	8.20–10.00	0.054	9.46	8.80–10.45	0.38	9.08	8.40–9.70	0.37
Zygomatic breadth	13.64	12.80–10.00	0.059	14.18	13.60–15.30	0.38	13.32	12.30–14.95	0.53
Breadth of braincase	12.70	11.80–14.45	0.054	12.87	12.40–13.60	0.27	12.46	11.75–13.35	0.32
Mastoid breadth	11.64	10.95–12.60	0.046	12.04	11.55–12.75	0.28	11.62	11.15–12.50	0.29
Least interorbital width	4.32	3.95–4.70	0.022	4.42	4.20–4.70	0.13	4.32	4.00–4.60	0.15
Length of molar row	4.42	4.00–5.23	0.025	4.41	3.95–4.67	0.14	4.19	3.73–5.38	0.30
Length of incisive foramen	5.14	4.55–5.80	0.038	5.55	5.15–6.10	0.22	4.94	4.15–5.65	0.39
Length of auditory bulla	5.22	4.75–5.88	0.027	5.53	5.13–5.88	0.19	5.30	4.95–5.69	0.18
Depth of braincase	9.74	9.20–10.60	0.036	10.06	9.60–10.45	0.21	9.68	9.10–10.25	0.31
Length of mesopterygoid fossa	4.85	4.40–5.50	0.033	4.90	4.30–5.40	0.24	4.62	4.00–5.20	0.27
Length of bony palate	4.41	3.65–4.95	0.035	4.23	3.90–4.85	0.20	4.20	3.75–4.70	0.22
Rostral breadth	4.59	4.15–5.15	0.029	4.59	4.20–4.90	0.19	4.55	4.20–4.90	0.19
Breadth across molars	5.40	5.10–5.70	0.021	5.57	5.30–6.00	0.17	5.30	4.45–5.70	0.23
Postdental palatal breadth	3.97	3.60–4.50	0.024	4.11	3.70–4.50	0.20	3.97	3.20–4.35	0.22
Width of mesopterygoid fossa	2.38	2.00–2.85	0.020	2.46	2.20–2.70	0.13	2.33	2.10–4.35	0.15

	P. carletoni (*n* = 54)			*P. levipes* (*n* = 85)			*P. schmidlyi* (*n* = 9)		
	Mean	Range	*SE*	Mean	Range	*SE*	Mean	Range	*SE*
Greatest length of skull	27.15	25.40–28.70	0.10	28.20	25.20–31.15	0.96	26.80	26.30–27.80	0.49
Length of rostrum	10.32	9.00–12.00	0.13	11.37	10.14–13.00	0.57	10.58	9.70–11.30	0.48
Length of nasal	10.44	8.86–11.70	0.10	10.34	8.86–12.65	0.63	9.01	8.40–9.60	0.43
Postpalatal length	9.28	8.40–10.00	0.06	9.58	8.60–11.10	0.48	8.98	8.60–9.40	0.27
Zygomatic breadth	13.66	12.70–14.60	0.05	14.20	13.05–15.60	0.48	13.28	12.60–13.80	0.38
Breadth of braincase	12.57	11.80–13.80	0.04	12.97	12.20–13.85	0.39	12.61	12.40–12.80	0.13
Mastoid breadth	11.85	11.00–12.60	0.06	11.95	11.10–13.15	0.38	12.10	11.70–12.40	0.24
Least interorbital width	4.37	4.00–4.70	0.02	4.45	4.05–4.95	0.18	4.53	4.40–4.80	0.14
Length of molar row	4.15	3.73–4.60	0.03	4.51	3.92–5.13	0.24	4.14	4.00–4.40	0.14
Length of incisive foramen	5.32	4.70–5.90	0.04	5.34	4.35–6.05	0.33	5.54	5.20–5.90	0.25
Length of auditory bulla	4.95	3.90–5.69	0.06	5.39	4.67–5.97	0.24	4.57	4.10–4.90	0.23
Depth of braincase	9.66	9.30–10.20	0.03	9.79	5.40–10.60	0.59	9.37	8.70–9.90	0.39
Length of mesopterygoid fossa	4.35	3.80–4.90	0.04	4.95	4.10–5.90	0.33	3.82	3.50–4.20	0.20
Length of bony palate	4.25	3.70–4.80	0.03	4.44	3.90–5.25	0.26	4.39	4.00–4.70	0.19
Rostral breadth	4.58	4.05–5.20	0.04	4.75	4.30–5.40	0.23	4.47	4.10–4.80	0.25
Breadth across molars	5.34	3.80–5.80	0.04	5.54	5.05–6.40	0.24	5.26	5.10–5.50	0.13
Postdental palatal breadth	3.96	3.30–4.80	0.04	4.07	3.55–4.50	0.23	3.93	3.80–4.20	0.15
Width of mesopterygoid fossa	2.35	1.90–2.80	0.03	2.36	2.05–2.70	0.12	2.48	2.30–2.70	0.16

	P. simulus (*n* = 15)			*P.* sp1 (*n* = 10)		
	Mean	Range	*SE*	Mean	Range	*SE*
Greatest length of skull	27.12	25.30–28.40	0.78	28.37	27.75–29.50	0.18
Length of rostrum	10.73	10.00–11.40	0.41	11.64	10.75–12.35	0.14
Length of nasal	9.47	8.40–10.30	0.50	10.67	9.70–11.85	0.20
Postpalatal length	9.08	8.00–9.60	0.39	9.61	9.20–10.10	0.10
Zygomatic breadth	13.89	13.20–14.50	0.41	14.37	13.65–15.35	0.14
Breadth of braincase	12.35	11.10–11.80	0.24	13.02	12.45–13.60	0.11
Mastaoid breadth	11.46	3.90–4.40	0.20	12.11	11.25–13.00	0.14
Least interorbital width	4.18	3.60–4.20	0.14	4.38	4.10–4.90	0.06
Length of molar row	3.83	4.50–5.50	0.16	4.62	4.29–4.85	0.06
Length of incisive foramen	5.03	5.20–5.90	0.30	5.47	5.15–5.90	0.07
Length of auditory bulla	5.17	4.10–4.90	0.13	5.49	5.23–5.69	0.05
Depth of braincase	9.47	8.70–9.90	0.22	10.04	9.60–10.45	0.08
Length of mesopterygoid fossa	4.80	3.50–4.20	0.24	5.02	4.80–5.25	0.05
Length of bony palate	4.02	3.70–4.40	0.21	4.43	4.10–4.90	0.06
Rostral breadth	4.77	4.50–5.00	0.15	4.80	4.55–5.40	0.08
Breadth across molars	5.31	4.70–5.90	0.28	5.63	5.40–6.00	0.07
Postdental palatal breadth	3.95	3.70–4.30	0.15	3.94	3.75–4.30	0.05
Width of mesopterygoid fossa	2.32	2.10–2.50	0.10	2.35	2.20–2.50	0.03

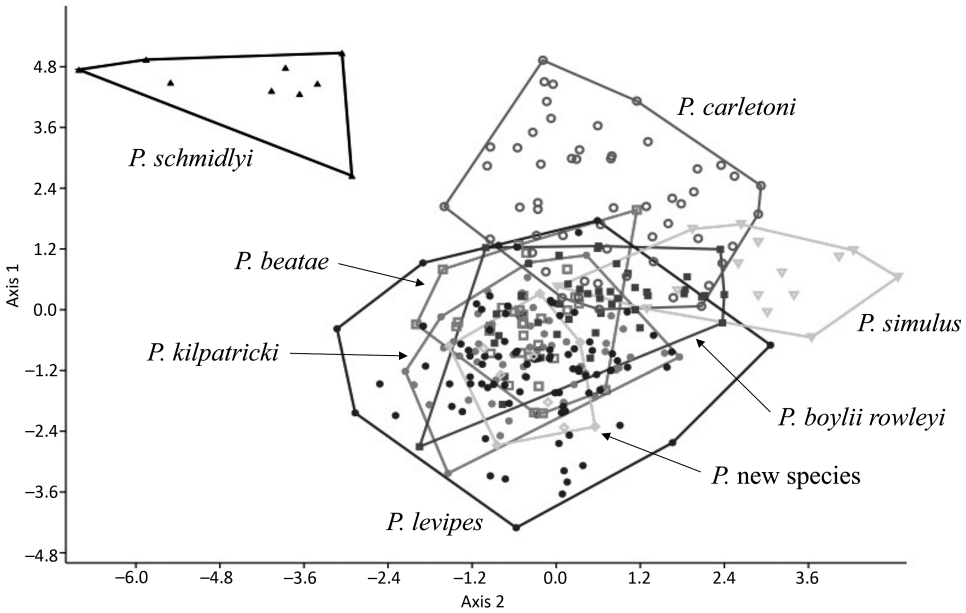

Fig. 4.—Plots of the 1st 2 discriminant function axes extracted from a discriminant function analysis of 8 taxa of *Peromyscus*. This analysis was performed on specimens with complete craniodental measurements. Polygons enclose maximal dispersion of individual specimen scores around centroids for each taxa.

The results of the pairwise comparisons, using Mann–Withney and Kruskal–Wallis tests, revealed that *P. kilpatricki* is significantly smaller ($P < 0.05$) than *P. levipes* in 13 of the measurements (all except for DB, LMF, LBP, PPB, and WMF), in 12 of the 18 measurements from *P. beatae* (all except for BB, LIW, LMR, LMF, RB, and WMF), and 11 of the measurements for *P.* sp1 (all except for BB, DB, LMF, LBP, RB, PPB, and WMF). Compared to *P. boylii*, *P. kilpatricki* was significantly larger in 4 measurements (LMR, LMF, ZB, and BB) and smaller in only 1 (LN); from *P. carletoni*, it was significantly larger in 4 (LMR, LMF, LR, and LAB) and smaller in 1 (LN); from *P. simulus*, it was significantly larger in 3 (BB, LMR, and LBP) but not significantly smaller in any of the measurements; from *P. schmidlyi*, it was significantly larger in 4 measurements (LAB, LMF, LN, and LMR) but smaller in 3 others (MB, LIW, and LIF).

Peromyscus kilpatricki is most distinct from *P. schmidlyi*, particularly in the multivariate analyses. In the univariate analysis of cranial measurements, the 2 species are similar for 2 measurements (LBP and PPB), smaller in 5 (LN, MB, LIW, LIF, and WMF), and larger in the other 11 measurements. Especially noteworthy is the relatively shorter but wider mesopterygoid fossa, smaller auditory bullae, and relatively larger braincase in *kilpatricki* compared to *schmidlyi*.

Peromyscus kilpatricki differs genetically, based on *Cytb* sequences, from other members of the *P. boylii* species group (*P. beatae*, *P. boylii*, *P. carletoni*, *P. levipes*, *P. madrensis*, *P. schmidlyi*, *P. simulus*, and *P. stephani*) by 5.31%, 8.09%, 5.16%, 5.38%, 7.22%, 5.57%, 7.41%, and 8.17% sequence divergence, respectively. The sister taxon of *P. kilpatrick* appears to be *P. beatae*.

Peromyscus kilpatricki differs from other members of the genus *Peromyscus* and the *P. boylii* species group by the karyotype with a diploid number of (2n) 56. The karyotype has 5 pairs of biarmed chromosomes that are similar in size and morphology to those reported by Houseal et al. (1987) for populations in Pátzcuaro and Los Azufres, Michoácan. This karyotype is distinguishable from the FN = 52 forms (*P. boylii* and *P. simulus*), FN = 52–54 forms (*P. beatae*), FN = 54 forms (*P. madrensis*), by the presence of additional biarmed chromosomes, and differs from *P. carletoni* by having fewer biarmed chromosomes (Table 2). Although the karyotype of *P. kilpatricki* is similar to the FN = 54–56 forms (*P. schmidlyi*), and FN = 56–60 forms (*P. levipes*), genetic differentiation in DNA sequences suggests they represent different taxa.

Etymology.—This species is named in honor of Dr. C. William Kilpatrick (Zadock Thompson Museum of Natural History, University of Vermont) for his many contributions to the systematic studies of the *P. boylii* species group and overall passion for rodent systematics and taxonomy.

Nomenclatural statement.—A life science identifier (LSID) number was obtained for the new species *Peromyscus kilpatricki*: urn:lsid:zoobank.org:pub:D6B494C4-1608-472F-9087-FEEBD35D7240.

Habitat.—Found in mesic pine-oak forest (*Quercus* spp. and *Pinus* spp.) habitat at elevations greater than 1,600 m. Typically associated with rock outcroppings, fallen logs, and moist soils. Collected sympatrically with *Baiomys taylori*, *Liomys pictus*, *Osgoodomys banderanus*, and *P. spicilegus* at the type locality.

Remarks.—The cryptic nature of the various species in the *P. boylii* group is confirmed by the morphological analysis. There are no cranial measurements that uniquely distinguish *P. kilpatricki* from the other taxa in the species group. There is overlap in cranial measurements between *kilpatricki* and all of the other species. The results of pairwise comparisons between *kilpatricki* and the other species indicate that *kilpatricki* is

significantly smaller than *beatae*, *levipes*, and *P.* sp1 in most cranial measurements and that it is not significantly larger than these taxa in any cranial measurement. *P. kilpatricki* is significantly larger than *P. boylii*, *P. carletoni*, *P. simulus*, and *P. schmidlyi* in several (3 or 4) but not most of the cranial measurements. Both the PCA and DFA (with classification analysis) confirm these results. *P. kilpatricki* is broadly included within the character space of all of the other taxa except *P. schmidlyi*. Only 73% of the specimens of *kilpatricki* were correctly identified in the classification analysis. It appears that despite being significantly different from some of the other taxa in a few univariate comparisons, when variation across all morphometric characters is considered (multivariate analyses) these significant differences are overridden by the total morphological variation. The only reliable way to identify with certainty specimens of *kilpatricki* is to rely on chromosomal or molecular genetic characters.

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Appendix I

Specimens examined in the DNA sequencing portion of this study. For each specimen, the collection locality, museum catalog number (abbreviations for museum acronyms follow Hafner et al. [1997]), and GenBank accession number are provided in parentheses. Abbreviations are as follows: Monte L. Bean Life Science Museum (BYU), Museum of Texas Tech University (TTU), Texas Cooperative Wildlife Collection (TCWC), Universidad Nacional Autónoma de México (UNAM), University of Michigan, Museum of Zoology (UMMZ), and Smithsonian's National Museum of Natural History (USNM). If museum catalog numbers were unavailable, specimens were referenced with the corresponding TK number (special number of the Museum of Texas Tech University). GenBank sequences not generated in this study and were deposited by Bradley et al. (2000, 2004, 2007), Sullivan et al. (1997), and Tiemann-Boege et al. (2000). Localities corresponding to Fig. 1 are provided (in parentheses) only for select members of the *P. boylii* species group.

Peromyscus aztecus.—MEXICO: Veracruz; 8.8 km N Huatusco (TCWC47976, U89968).

Peromyscus beatae.—GUATEMALA: Huehuetenango; 10 km NW Sta. Eulalia (Locality 24; ROM98290, AF131919). HONDURAS: Francisco Morazán; 3.2 km NE El Hatillo (Locality 25; TCWC52288, AF131914). MEXICO: Guerrero; 6.4 km SW Filo de Caballo (Locality 22; TCWC45222, AF131922). Oaxaca; 6.4 km E Juquila (Locality 23; TCWC 45324, AF131920). Veracruz; Xometla (Locality 21; TCWC48060, AF131921).

Peromyscus boylii.—MEXICO: Jalisco; 2 km NW El Mesconcitos (Locality 10; TTU82688, AY322504); Sonora; Isla San Pedro Nolasco (Locality 2; UMMZ117347, AF155387).

Peromyscus carletoni.—MEXICO: Nayarit; Ocota de la Sierra, 21°50′N, 104°13′W (Locality 8; TCWC45206, KF201659); 70 km N Santa María del Oro, UTM 13Q-559922-2395306 (Locality 9; TTU110122, KF201662).

Peromyscus evides.—MEXICO: Guerrero; 6.4 km SSW Filo de Caballo (TTU82696, FJ214685).

Peromyscus gratus.—MEXICO: Michoacán; Las Minas, 3 km SW Tuxpan (Cat. No. TK47810, KF201656).

Peromyscus hylocetes.—MEXICO: Michoacán; Estación Cerro Burro, Microondas; 3,270 m (UNAM—catalog number unavailable TK45309, DQ000481).

Peromyscus levipes.—MEXICO: México; 12 km S Acambay (Locality 12; TTU82707, AY322509); 14.1 km NW Villa del Carbon (Locality 13; TTU90321, KX523178). Nuevo León; Cola de Caballo (Locality 4; TCWC47956, AF131928); Tlaxcala; 2 km W Teacalco, 2,710 m (Locality 20; TCWC48331, AF131929).

Peromyscus madrensis.—MEXICO: Nayarit; Isla María Madre (Locality 7; USNM512599, AF155397).

Peromyscus kilpatricki.—MEXICO: Michoacán; km marker 81 between Ario de Rosales and La Huacana, 1,602 m,

19°10′59″N, 101°43′42″W (Locality 16, catalog number not available—TK47887, KX523179; catalog number not available—TK47890, KX523180; catalog number not available—TK47897, KX523181); Las Minas, 3 km SW Tuxpan (Locality 17, catalog number not available—TK47819, DQ000477; catalog number not available—TK47807, KX523182); 13.5 km SW Zitácuaro, UTM 14Q-352122-2140934 (Locality 18; TTU104808, KF201672; TTU104799, KX523183). Morelos; Cuernavaca, 18°59.142′N, 99°14.130′W, 2,210 (Locality 19; BYU20730, KX523184).

Peromyscus oaxacensis.—GUATEMALA: Alta Verapaz, Yalijux Mountain, Chelemha Reserve, 15°23′09″N, 90°03′44″W, 2,090 m (USNM569872, KF201657).

Peromyscus sp1.—MEXICO: Michoacán; 11.8 km WSW Dos Aguas (Locality 14; TCWC45304, AF155409); 3 km NW Aguilla, 780 m, 18°46.238′N, 102°45.747′W (Locality 15; catalog number not available—TK45857, KX523185); 3.5 km S, 4.8 km E Zinapécuaro, 14Q-311971-2194257, 2,012 m (Locality 11; TTU110119, KF201673).

Peromyscus schmidlyi.—MEXICO: Durango; 6.1 km W Coyotes, UTM 13-465908E-2634281N (Locality 5; TTU81617, AY370610); Sonora; 3 km E Yecura, Colegio Yecura (Locality 3; TTU110286, KF201658).

Peromyscus simulus.—MEXICO: Sinaloa; 6.4 km E Concordia, Highway 40 (Locality 6; TCWC45592, AF131927).

Peromyscus spicilegus.—MEXICO: Durango; San Juan de Camarones, UTM 13-356961E-2757448N (TTU81640, AY322512).

Peromyscus stephani.—MEXICO: Sonora; Isla San Esteban (Locality 1; UMMZ117385, AF155411).

Peromyscus winkelmanni.—MEXICO: Michoacán; 6.9 mi WSW Dos Aguas (TCWC45621, AF131930).

Specimens examined in the karyotypic portion of this study. Previously published karyotypes used as references are listed in Table 1.

Peromyscus kilpatricki.—MEXICO: Michoacán; 13.5 km SW Zitácuaro, UTM 14Q-352122-2140934 (Locality 18; TTU104808, KF201672).