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What Is *Peromyscus*? Evidence from nuclear and mitochondrial DNA sequences suggests the need for a new classification

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The evolutionary relationships between *Peromyscus*, *Habromys*, *Isthmomys*, *Megadontomys*, *Neotomodon*, *Osgoodomys*, and *Podomys* are poorly understood. In order to further explore the evolutionary boundaries of *Peromyscus* and compare potential taxonomic solutions for this diverse group and its relatives, we conducted phylogenetic analyses of DNA sequence data from alcohol dehydrogenase (*Adh*1-I2), beta fibrinogen (*Fgb*-I7), interphotoreceptor retinoid-binding protein (*Rbp*3), and cytochrome-*b* (*Cytb*). Phylogenetic analyses of mitochondrial and nuclear genes produced similar topologies although levels of nodal support varied. The best-supported topology was obtained by combining nuclear and mitochondrial sequences. No monophyletic *Peromyscus* clade was supported. Instead, support was found for a clade containing *Habromys*, *Megadontomys*, *Neotomodon*, *Osgoodomys*, *Podomys*, and *Peromyscus* suggesting paraphyly of *Peromyscus* and confirming previous observations. Our analyses indicated an early divergence of *Isthmomys* from *Peromyscus* (approximately 8 million years ago), whereas most other peromyscine taxa emerged within the last 6 million years. To recover a monophyletic taxonomy from *Peromyscus* and affiliated lineages, we detail 3 taxonomic options in which *Habromys*, *Megadontomys*, *Neotomodon*, *Osgoodomys*, and *Podomys* are retained as genera, subsumed as subgenera, or subsumed as species groups within *Peromyscus*. Each option presents distinct taxonomic challenges, and the appropriate taxonomy must reflect the substantial levels of morphological divergence that characterize this group while maintaining the monophyletic relationships obtained from genetic data.

Key words: *Habromys*, *Megadontomys*, *Neotomodon*, *Osgoodomys*, *Peromyscus*, phylogeny, *Podomys*, species group, systematics, taxonomy

What is *Peromyscus*? More than 100 years since Osgood's (1909) monograph the question remains unsolved. A historical perspective and overview of the taxonomic challenges affiliated with *Peromyscus* are provided in Bradley et al. (2007), Carleton (1980, 1989), and Miller and Engstrom (2008). At conflict is the taxonomic status of *Habromys*, *Isthmomys*, *Megadontomys*, *Neotomodon*, *Osgoodomys*, and *Podomys*. At various times, these taxa have been recognized at the generic (sensu stricto) or subgeneric (sensu lato) level, though most major classifications generally fall into 1 of 2 categories. No single historical classification fits perfectly into the sensu stricto or sensu lato categories, though most major classifications tend to reflect one interpretation over the other. Carleton (1980, 1989) and Musser and Carleton (2005) are most closely aligned with a *Peromyscus* (sensu lato) taxonomy, whereas Hooper (1968) is a variation of a *Peromyscus* (sensu stricto) classification. Most current classifications recognize *Peromyscus* (sensu stricto).

Bradley et al. (2007) completed the most comprehensive molecular study of *Peromyscus* and its allies in which DNA sequences from the entire mitochondrial cytochrome-*b* gene

(*Cytb*) were examined. They found 5 genera (*Habromys*, *Megadontomys*, *Neotomodon*, *Osgoodomys*, and *Podomys*) to be embedded within a monophyletic clade containing *Peromyscus* (sensu stricto); *Isthmomys* was sister to *Reithrodontomys* and basal to this group. In order to recognize *Habromys*, *Megadontomys*, *Neotomodon*, *Osgoodomys*, and *Podomys* as genera, Bradley et al. (2007) stated that at least 5 additional genera would have to be recognized to accommodate strongly supported clades under the rules of monophyly and phylogenetic principles.

Miller and Engstrom (2008) added to the molecular data set by obtaining DNA sequences from *Cytb* as well as 2 nuclear genes: interphotoreceptor retinoid-binding protein (*Rbp3*) and growth hormone receptor (*Ghr*). Their results were similar to Bradley et al. (2007) in that *Habromys*, *Megadontomys*, *Neotomodon*, *Osgoodomys*, and *Podomys* were placed inside of *Peromyscus* (sensu stricto) and *Isthmomys* was sister to *Reithrodontomys*. Further, Miller and Engstrom (2008) agreed with Bradley et al. (2007) that additional groups would have to be elevated to avoid paraphyly in a sensu stricto interpretation of *Peromyscus*.

The primary objective in this study was to examine the phylogenetic relationships within the genus *Peromyscus* using a combination of mitochondrial and nuclear markers. Although the studies of Bradley et al. (2007) and Miller and Engstrom (2008) recovered paraphyly within *Peromyscus*, each study had limitations that impact phylogenetic interpretation. Bradley et al. (2007) had a more broad taxonomic sampling scheme but was based on a single genetic marker (*Cytb*). Miller and Engstrom (2008) lacked representation of some species groups but included multiple genetic markers. Herein, we seek to expand upon these molecular data sets by including representatives from all species groups and to examine DNA sequences from 4 markers, 2 of which were not used in the previous studies: intron 2 of the alcohol dehydrogenase (*Adh*1-I2) and intron 7 of the beta fibrinogen gene (*Fgb*-I7). We selected these markers based on their previous use in rodent phylogenetics (Amman et al. 2006; Longhofer and Bradley 2006; Reeder and Bradley 2007). We combined nuclear and mitochondrial DNA sequence data from *Adh*1-I2, *Fgb*-I7, *Rbp*3, and *Cytb* to test the monophyly of *Peromyscus* (sensu stricto versus sensu lato) and to ascertain whether *Habromys*, *Isthmomys*, *Megadontomys*, *Neotomodon*, *Osgoodomys*, and *Podomys* are paraphyletic within *Peromyscus*. The genetic evidence presented herein support the need for a formal taxonomic revision of *Peromyscus*. Below we identify 3 potential taxonomic solutions that are consistent with the evidence in hand.

Materials and Methods

Samples.—Tissue samples obtained from individuals collected in naturally occurring populations or through museum loans were used to generate DNA sequences for the 4 genetic markers described below. In some cases, DNA sequences were obtained directly from GenBank. A single representative was examined from the following taxa: *Baiomys*, *Habromys*, *Isthmomys*, *Megadontomys*, *Neotoma*, *Neotomodon*, *Ochrotomys*, *Onychomys*, *Osgoodomys*, and *Podomys*; 4 representatives were included for *Reithrodontomys*. For the subgenus *Peromyscus*, 2 representatives of each of the species groups were examined (except the *crinitus*, *furvus*, *hooperi*, *megalops*, and *melanophrys* species groups—1 sample each). Likewise, for the subgenus *Haplomylomys*, 1 sample each from the *californicus* and *eremicus* species groups were examined. An attempt was made to obtain mitochondrial and nuclear sequences from a single individual, but in a few instances, this was not possible. In these cases, sequences from conspecific individuals within close geographic proximity were used to complete the data set. Concatenation of sequence data from conspecifics to represent a composite species rather than a single individual has been successfully used in various taxa (Campbell and Lapointe 2009; Townsend et al. 2011; Haddrath and Baker 2012). This strategy guaranteed at least 1 individual per species group was sampled. Specimens used in this study are listed in Table 1.

DNA isolation and PCR.—DNA was isolated from liver samples (0.1 g) using 2 methods. Mitochondrial DNA was extracted and purified using a Wizard Miniprep kit (Promega, Madison, Wisconsin), whereas total genomic DNA was extracted from liver using DNeasy Blood and Tissue kits (Qiagen, Valencia, California) following the method of Smith and Patton (1999). The complete mitochondrial *Cytb* gene (1,143 bp) was amplified following methods outlined in Bradley et al. (2007) and Tiemann-Boege et al. (2000) using primers MVZ05 (Smith and Patton 1993), H15915 (Irwin et al. 1991), and CB40 (Hanson and Bradley 2008). Intron 2 of the alcohol dehydrogenase gene (*Adh1*-I2, 598 bp) was amplified following the methods of Amman et al. (2006) using primers 2340-I, 2340-II, Exon II-F, and Exon III-R. The complete intron of the beta-fibrinogen gene (*Fgb*-I7, 674 bp) was amplified following the methods of Carroll et al. (2005) and Wickliffe et al. (2003) using primers *Fgb*-17U-*Rattus*, *Fgb*-17L-*Rattus* (Wickliffe et al. 2003), B17-mammU, and B17-mammL (Matocq et al. 2007). Exon I of interphotoreceptor retinoid-binding protein gene (*Rbp3*, 924 bp) was amplified following the methods of Chambers et al. (2009) and Jansa and Voss (2000) using primers A and B (Stanhope et al. 1992).

Sequencing.—PCR products were purified using the QIAquick PCR Purification kit (Qiagen) or ExoSAP-IT (USB Products, Cleveland, Ohio) and PCR amplicons were sequenced using ABI Prism Big Dye Terminator v3.1 ready reaction mix (Applied Biosystems, Foster City, California). Nucleotide sequences were resolved on an ABI 3100 Avant automated sequencer (Applied Biosystems) with the following primers: *Cytb*—PERO3′ and 752R (Tiemann-Boege et al. 2000), CWE-1 and 400F (Edwards et al. 2001), and 700L and WDRAT400R (Peppers and Bradley 2000); *Adh1*-I2—Exon II-F, Exon III-R, Adh350F, and Adh350R (Amman et al. 2006); *Fgb*-I7—*Fgb*-17U-*Rattus* and *Fgb*-17L-*Rattus* (Wickliffe et al. 2003) and bFIB-I7U and bFIB-I7L (Carroll et al. 2005); and *Rbp3*—A, B, and D (Stanhope et al. 1992), E2 (Weksler 2003), and 125F (DeBry and Sagel 2001). Sequencher 5.0 software

Table 1.—Specimens examined in this study are listed by taxon and genetic marker (*Adh1*-I2—intron 2 of alcohol dehydrogenase, *Cytb*—cytochrome-*b*, *Fgb*-I7—intron of the beta-fibrinogen, and *Rbp3*—interphotoreceptor retinoid-binding protein) and grouped by tribe, genus, and species group. GenBank accession (left of slash) and museum catalog (right of slash) numbers are given for each specimen. Museum acronyms are as follows: ASNHC (Angelo State Natural History Collection), BYU (Brigham Young University), CNMA (Colección Nacional de Mamíferos, Universidad Nacional Autónoma de México), PGSC (*Peromyscus* Genetic Stock Center), ROM (Royal Ontario Museum), TCWC (Texas Cooperative Wildlife Collection), and TTU (Museum of Texas Tech University). If museum catalog numbers were unavailable, specimens were referenced with the corresponding collector's numbers or TK (special number of the Museum of Texas Tech University).

Taxon	*Adh*1-I2	*Cytb*	*Fgb*-I7	*Rbp*3
Tribe Baiomyini				
Baiomys				
B. taylori	AY994205/TTU82642	AF548469/TTU54633	AY274213/TTU54633	EF989838/ROM114886
Tribe Ochrotomyini				
Ochrotomys				
O. nutalli	JX910114/TCWC31929	AY195798/TCWC31929	AY274203/TCWC31929	EF989862/ROM113008
Tribe Neotomini				
Neotoma				
N. mexicana	AY817646/TTU79129	AF294345/TTU79129	AY274200/TTU79129	JX910120/TTU79129
Tribe Reithrodontomyini				
Habromys				
H. ixtlani	AY994239/TK93160	DQ000482/TK93160	FJ214701/TTU82703	EF989842/CNMA29849
Isthmomys				
I. pirrensis	FJ214668/TTU39162	FJ214681/TTU39162	FJ214692/TTU39162	EF989846/ROM116308
Neotomodon				
N. alstoni	AY994210/TK45309	AY195796/TK45302	AY274202/TK45309	EF989851/ASNHC1595
Megadontomys				
M. thomasi	AY994208/TK93388	AY195795/TK93388	FJ214693/TK93388	EF989849/CNMA29186
Onychomys				
O. arenicola	JX910115/TTU67559	AY195793/TTU67559	AY274204/TTU67559	EF989855/ROM114904
Osgoodomys				
O. banderanus	AY994209/TK45952	DQ000473/TK45952	FJ214694/TK45952	EF989857/ASNHC2664
Peromyscus				
aztecus group				
P. evides	FJ214670/TTU82696	FJ214685/TTU82696	FJ214700/TTU82696	JX910121/TTU82696
P. spicilegus	AY994234/TK45255	FJ214669/TK45255	FJ214695/TK45255	JX910122/TK47888
boylii group				
P. boylii	AY994227/TTU82688	AF155388/TTU81702	AY274208/TTU81702	EF989871/ASNHC3449
P. levipes	AY994224/TK47819	DQ000477/TK47819	FJ214707/TTU105150	JX910123/TK47819
californicus group				
P. californicus	AY994211/TTU83292	AF155393/TTU81275	FJ214697/TTU83291	EF989873/PGSCIS1590
crinitus group				
P. crinitus	AY994213/DSR6171	FJ214684/TK119629	FJ214698/TK119629	EF989874/BYU16629
eremicus group				
P. eremicus	AY994212/TTU81850	AY322503/TTU83249	FJ214699/TTU83249	EF989876/BYU17952
furvus group				
P. furvus	JX910116/FXG1168	AF271032/CNMA32298	JX910113/FXG1168	JX910124/FXG1168
hooperi group				
P. hooperi	FJ214672/TTU104425	DQ973103/TTU104425	FJ214704/TTU104425	JX910125/TTU104425
leucopus group				
P. gossypinus	FJ214671/TTU80682	DQ973102/TTU80682	FJ214702/TTU80682	JX910126/TTU80682
P. leucopus	AY994240/TTU75694	DQ000483/TTU101645	FJ214706/TTU101645	EF989880/ROM101861
maniculatus group				
P. maniculatus	AY994242/TTU97830	DQ000484/TTU38739	FJ214708/TTU97830	EF989884/ROM98941
P. melanotis	FJ214673/TK70997	AF155398/TK70997	FJ214711/TK70997	EF989891/PGSC25
megalops group				
P. megalops	AY994217/TTU82712	DQ000475/TTU82712	FJ214709/TTU82712	JX910127/TTU82712
melanophrys group				
P. melanophrys	AY994216/TTU75509	AY322510/TTU75509	FJ214710/TTU75509	EF989890/PGSCXZ1073
mexicanus group				
P. mexicanus	AY994236/TTU97013	JX910118/TTU105005	AY274210/TTU82759	EF989895/ROM113250
P. nudipes	AY994238/TTU96972	FJ214687/TTU96972	FJ214713/TTU96972	EF989893/ROM113216
truei group				
P. attwateri	AY994220/TTU55688	AF155384/TTU55688	AY274207/TTU55688	JX910128/TTU55688
P. gratus	AY994218/TK46354	AY376421/TK46354	FJ214703/TK46354	JX910129/TK46354

Table 1.—*Continued*

Taxon	*Adh*1-I2	*Cytb*	*Fgb*-I7	*Rbp*3
Unknown group				
P. ochraventer	FJ214676/TTU104930	JX910119/TTU104930	FJ214715/TTU104930	JX910130/TTU104930
P. pectoralis	AY994221/TK48645	AY376427/TK48642	FJ214716/TK48645	JX910131/TK48645
Podomys				
P. floridanus	AY994214/TTU97867	DQ973109/TTU97867	FJ214723/TTU97867	EF989878/TTU97866
Reithrodontomys				
R. fulvescens	AY994207/TTU54898	AF176257/TTU54898	AY274211/TTU54898	EF989901/ASNHC3465
R. sumichrasti	JX910117/TTU54952	AF176256/TTU54952	AY274212/TTU54952	EF989924/ROM98383
R. megalotis	AF176248/TTU40942	AF176248/ TTU40942	KJ697789/TTU40942	EF989909/ASNHC2133
R. mexicanus	KJ697791/TTU85234	AY859453/ROM101508	—	EF989911/ROM98468

(Gene Codes Corporation 2013) was used to align and proof individual sequencing reads into contigs representing each gene. Conflicting base calls were verified against the associated chromatograms. For nuclear intron sequences, all heterozygous sites were designated following the International Union of Biochemistry polymorphic code. All DNA sequences were deposited in GenBank and accession numbers are provided in Table 1.

Phylogenetic analysis.—Nucleotide positions were treated as unordered, discrete characters with 6 possible states: A, C, G, T, gaps (–), or missing (?) for each marker. For nuclear intron sequences, polymorphic sites were designated following the International Union of Biochemistry polymorphic code. However, because these polymorphisms could be the result of heterozygosity or sequencing error, to be conservative, these nucleotides were excluded from downstream analyses. Alignment of *Adh1*-I2 and *Fgb*-I7 sequences required hypothesized gaps (inserted based on homology) to represent insertion or deletion events, but gaps were not included in the phylogenetic analysis. Analyses were conducted using 3 data sets: nuclear (*Adh1*-I2, *Fgb*-I7, and *Rbp*3), mitochondrial (*Cytb*), and combined (*Adh1*-I2, *Cytb*, *Fgb*-I7, and *Rbp3*). *Neotoma mexicana* was used as the outgroup taxon for all analyses (Bradley et al. 2004b).

MrModeltest and the Akaike information criterion (AIC—Nylander 2004) were used to estimate the most appropriate model of evolution for each gene region. Bayesian inference (BI) was conducted to estimate a phylogeny and generate posterior probability values for the mitochondrial, nuclear, and combined data sets using MRBAYES v3.2.1 (Ronquist et al. 2012). Each analysis included the appropriate model identified by MrModeltest (Nylander 2004), 2 simultaneous runs of 4 Markov-chains, 10×10^9 generations, and a sample frequency of every 1,000th generation. The number of invariable sites and gamma distribution were estimated from the data. After a visual inspection of likelihood score distributions in Tracer v1.5 (Drummond and Rambaut 2008), the first 10,000 trees were discarded and a consensus tree (50% majority rule) was constructed from the remaining trees. Values ≥ 95% were viewed as supportive following Alfaro et al. (2003), Douady et al. (2003), and Huelsenbeck et al. (2002). For ML analyses, RaxML (Stamatakis et al. 2005) was used to generate trees from each data set. In these analyses, the GTR+G substitution model was used since the less parameter rich HKY+G model, identified by MrModeltest (Nylander 2004) as the most appropriate model, is unavailable. Nodal support was estimated with 10,000 bootstrap replicates using the "fast bootstrapping" option (Felsenstein 1985).

Topological tests.—Maximum likelihood (ML) trees from RAxML (Stamatakis et al. 2005) were used to test the difference between competing taxonomic hypotheses. Site likelihood scores generated in RAxML (Stamatakis et al. 2005) were used to score several constrained topologies. *P*-values were generated in Consel (Shimodaira and Hasegawa 2001) for each topology using the approximately unbiased test (Shimodaira 2002). In particular, *Peromyscus* (sensu stricto) versus (sensu lato) were tested against the ML topology from the combined data analysis as well as other alternative hypothetical taxonomic groupings.

Molecular dating.—BEAST v1.7 (Drummond et al. 2012) was used to estimate divergence dates for the sampled taxa. Sequence data from each gene were used in the analysis but were partitioned to allow modeling of each data set. Models of substitution were the same as those used in previous Bayesian analyses (see above). The program MEGA 5.05 (Tamura et al. 2011) was used to determine whether to accept or reject a strict molecular clock for each data set. Given all data sets contained a single individual from each species sampled, a Yule tree prior was chosen for the BEAST analysis. Fossil limits were used to calibrate the *leucopus/maniculatus* group (~0.3 million years ago [mya]—Dalquest 1962; Karow et al. 1996) and *Reithrodontomys* (~1.8 mya—Cassiliano 1999). To account for the uncertainty in the fossil record, a prior lognormal distribution was used for both calibrations with means and standard deviations adjusted to create an upper bound of 14.8 mya to reflect the closest dated fossil outside of the taxa sampled (Behrensmeyer and Turner 2013). Test runs of 2.5×10^7 generations with a 10% burn-in were used to optimize for the final analysis. Bayes factors (Kass and Raftery 1995; Suchard et al. 2001) were calculated to compare the results of test runs to determine final parameters. Two final runs of 1.0×10^8 generations were analyzed with log and tree files combined for final divergence date estimates. Results were examined for sufficient mixing, convergence stability, and effective sample size > 200 for all parameters using the program Tracer.

Genetic divergence.—To compare rates of genetic divergence between taxa recognized at various taxonomic ranks, Kimura 2-parameter (K2P—Kimura 1980) genetic distance values were compared among currently recognized genera (*Habromys*,

Isthmomys, *Megadontomys*, *Neotomodon*, *Osgoodomys*, and *Podomys*). The K2P model was selected based on its utility as a distance metric in rodent phylogenetics (Bradley and Baker 2001).

Results

Phylogenetic analyses.—Twenty-seven species of *Peromyscus* (sensu lato) and 8 additional taxa (outgroup and reference samples), representing taxonomic diversity within the Neotominae, were sampled for the nuclear introns *Adh*1-I2 and *Fgb*-I7, nuclear exon *Rbp3*, and the mitochondrial gene *Cytb*. The entire 560 bp of *Adh1*-I2, 590 bp of *Fgb*-I7, and 921 bp of *Rbp3* were analyzed for 23 of the 27 *Peromyscus* (sensu lato) species. Full gene sequences were not available for *Peromyscus californicus* (*Rbp3*: 833 of 921 bp), *P. eremicus* (*Rbp3*: 907 of 921 bp), *P. furvus* (*Cytb*: 540 of 1,143 bp), or *P. ochraventer* (*Rbp3*: 914 of 921 bp). To analyze the most complete data set possible, other gene sequences available through GenBank, including *Ghr*, were not used due to lack of sequence data for many taxa. MrModeltest (Nylander 2004) identified the substitution models HKY+G for *Adh1*-I2 (AIC = 6577.2827, −lnL = 3283.6414, G = 1.1092), GTR+G for *Fgb*-I7 (AIC = 6931.1782, −lnL = 3456.5891, G = 1.3322), and GTR+I+G for *Rbp3* (AIC = 6213.5400, −lnL = 3096.7700, I = 0.4097, G = 0.8473) as the best-fit models.

Individual *Adh1*-I2, *Cyt*b, *Fgb*-I7, and *Rbp*3 sequences were combined to generate a single concatenated sequence (3,283 bp). The ML phylogeny (−lnL = 22631.507197) with bootstrap values and Bayesian clade probability values are shown in Fig. 1. Specific placement of some taxa varied between the ML and BI analyses, specifically in the placement of *Megadontomys thomasi*, *Osgoodomys banderanus*, and *P. hooperi*. Despite uncertain placement of these taxa, placement of terminal taxa within well-supported nodes did not conflict between markers. Significant nodal support was obtained throughout the phylogeny with most basal and terminal nodes garnering support. Middle regions of the phylogeny were less likely to receive nodal support. Both BI and ML analyses were unable to recover a monophyletic *Peromyscus* (sensu stricto) or *Peromyscus* (sensu lato) clade.

Constrained topologies reflecting various taxonomic groupings were tested using the approximately unbiased test in CONSEL (Shimodaira and Hasegawa 2001) with 10,000 replicates per test. A generalized *Peromyscus* (sensu lato) was unable to be rejected ($P = 0.406$), but *Peromyscus* (sensu stricto) was strongly rejected ($P = 0.014$) by the approximately unbiased test. An additional taxonomic scheme uniting *Peromyscus*

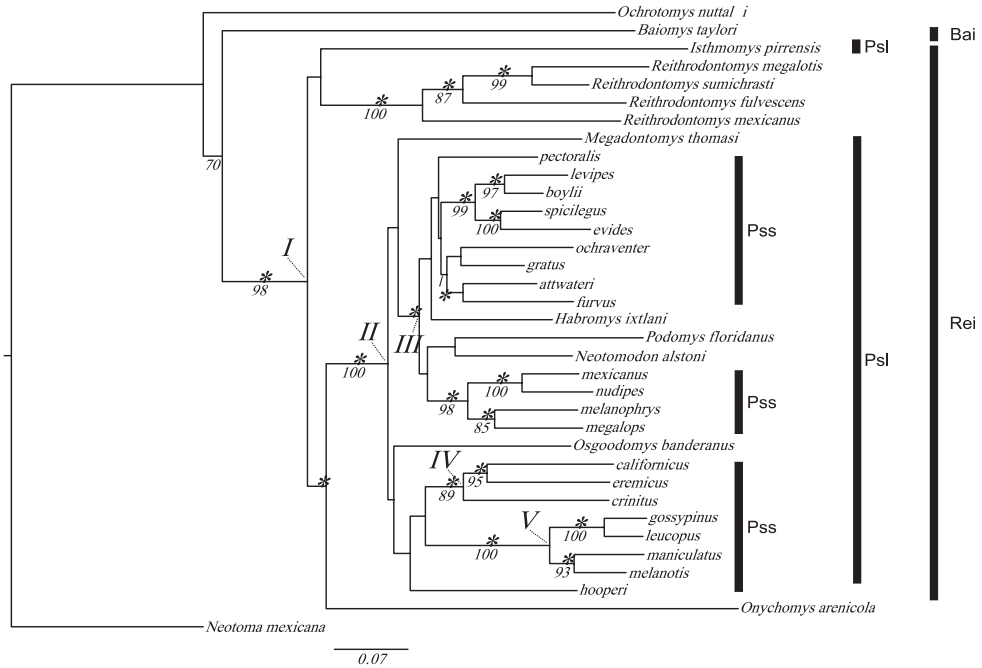

Fig. 1.—Phylogenetic tree obtained from maximum likelihood analysis of the combined mitochondrial cytochrome-*b* gene (*Cytb*) and 3 nuclear genes (alcohol dehydrogenase—*Adh*1-I2, beta fibrogen—*Fgb*-I7, and interphotoreceptor retinoid-binding protein—*Rbp3*). Taxonomic groups of interest are designated as follows: Pss (*Peromyscus* [sensu stricto]), Psl (*Peromyscus* [sensu lato]), Rei (Reithrodontomyini), and Bai (Baiomyini). Nodal support values are superimposed on the maximum likelihood tree topology. Support values are as follows: 10,000 bootstrap replicates of the maximum likelihood analysis (below branch) and clade probability values for the Bayesian inference analysis (above branch). Statistically significant clade probability values (≥ 0.95) are designated with an asterisk. All bootstrap support values ≥ 50 are shown. For members of *Peromyscus* (sensu stricto) only, species epithets are given. *Peromyscus* (sensu lato) affiliated genera are indicated in bold. Major nodes are indicated with roman numerals.

(sensu lato), but excluding *Isthmomys pirrensis*, was unable to be rejected ($P = 0.556$).

Molecular dating.—Molecular clock tests (Tamura et al. 2011) indicated a strict molecular clock for the *Cytb* and *Fgb-I7* data sets but a relaxed molecular clock for the *Adh1-I2* and *Rbp3* data sets. BEAST analyses estimated a Yule birth rate of 0.23 (95% highest posterior density [HPD]: 0.11–0.37). Mean rates of evolution (as substitutions per site per million years) were 0.007 for *Adh1-I2* (95% HPD: 0.004–0.01), 0.06 for *Cytb* (95% HPD: 0.03–0.09), 0.006 for *Fgb-I7* (95% HPD: 0.003–0.009), and 0.003 for *Rbp3* (95% HPD: 0.001–0.004). Divergence date estimates (Fig. 2) suggested that the split of Reithrodontomyini and Baiomyini began approximately 9.56 mya (95% HPD: 5.65–15.27), during the Late Miocene. The split between the *Isthmomys*/*Reithrodontomys* clade and *Onychomys*/*Peromyscus* (senus lato) clade was estimated to occur approximately 7.93 mya (95% HPD: 4.67–12.59), also in the Late Miocene. In addition, the divergence of *Peromyscus* (sensu lato) was estimated to occur during the Late Miocene but near the Miocene/Pliocene boundary (approximately 5.71 mya, 95% HPD: 3.37–9.08). However, most species-level divergence within *Peromyscus* (sensu lato) occurred during the Blancan North American land mammal age (1.8–4.9 mya).

Genetic distances.—K2P (Table 2) genetic distances were used to compare taxa and provide additional information on the phylogenetic utility of each marker. Data obtained from comparisons of *Isthmomys*, *Onychomys*, and *Reithrodontomys* to other genera and species groups indicated the highest levels of genetic divergence among taxa. Comparison of the 5 genera (*Habromys*, *Megadontomys*, *Neotomodon*, *Osgoodomys*, and *Podomys*) to all other genera resulted in genetic distances ranging from 2.58% (*Rbp3*) to 15.4% (*Cytb*). Values obtained from comparisons of these 5 genera to currently recognized species groups within *Peromyscus* (sensu stricto) ranged from 1.9% (*Rbp3*) to 14.62% (*Cytb*) and were similar in magnitude to comparisons of species groups to each other. By comparing the genetic distance between all taxa examined for each respective marker, their relative rates of evolution could be compared. Overall, *Adh*1-I2 and *Fgb*-I7 exhibited rates of evolution slower than *Cytb*; however, *Rbp3* was substantially slower than all of the other markers.

Discussion

Use of a combined data set often increases resolution at different hierarchical levels with one data set providing resolution at deep nodes and others resolving shallow nodes. More quickly evolving mitochondrial markers tend to depict more resolution at terminal nodes, whereas nuclear markers generally resolve relationships at the base of a phylogeny. Therefore, combined data sets are often advantageous in studies whose phylogenetic relationships have been debated due to inconsistencies

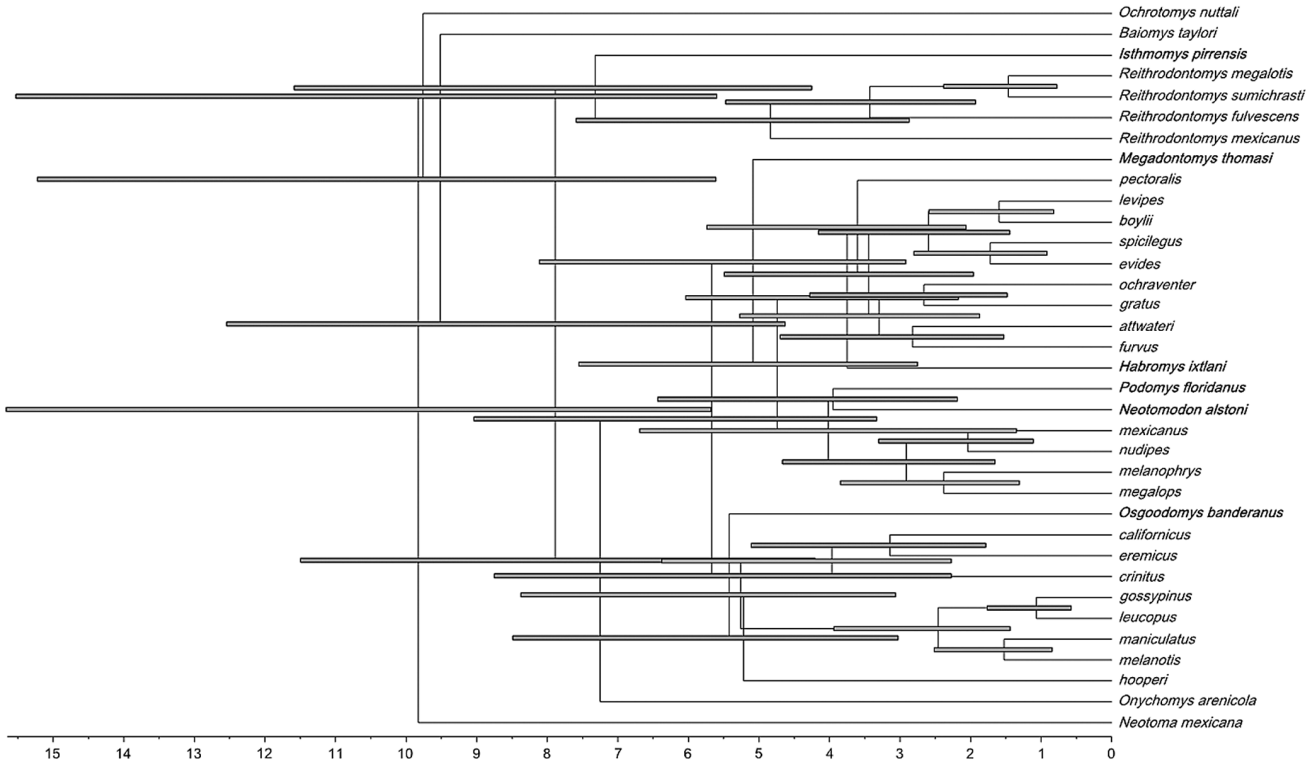

Fig. 2.—Maximum clade credibility tree showing divergence date estimates based on a combined analysis of the mitochondrial cytochrome-*b* gene (*Cytb*) and 3 nuclear genes (alcohol dehydrogenase—*Adh*1-I2, beta fibrogen—*Fgb*-I7, and interphotoreceptor retinoid-binding protein—*Rbp3*). Divergence date estimates are indicated along the *x*-axis in millions of years. Error bars represent the 95% highest posterior density for node height. *Peromyscus* (sensu lato) affiliated genera are indicated in bold.

Table 2.—Estimated genetic distances (K2P—Kimura 1980) for selected taxonomic groups based on sequences from the 4 genetic markers (*Adh1*-I2—intron 2 of alcohol dehydrogenase, *Cytb*—cytochrome-*b*, *Fgb*-I7—intron of the beta-fibrinogen, and *Rbp3*—interphotoreceptor retinoid-binding protein) examined in this study.

Taxon	*Adh1*-I2	*Cytb*	*Fgb*-I7	*Rbp3*
Reithrodontomys and *Onychomys* versus all other groups	8.6/13.07	18.0/19.7	7.5/7.5	4.3/2.7
Isthmomys versus all other groups	14.0	16.5	8.9	3.6
Habromys versus other "genera"	5.2	15.0	6.0	2.0
Megadontomys versus other "genera"	5.2	15.0	6.7	2.9
Neotomodon versus other "genera"	5.3	14.7	6.1	2.6
Osgoodomys versus other "genera"	4.6	15.8	6.0	2.6
Podomys versus other "genera"	6.2	16.3	6.6	2.8
Habromys versus *Peromyscus* (sensu stricto)	4.0	14.0	5.2	1.3
Megadontomys versus *Peromyscus* (sensu stricto)	4.0	14.4	5.7	2.2
Neotomodon versus *Peromyscus* (sensu stricto)	3.19	13.9	5.2	2.0
Osgoodomys versus *Peromyscus* (sensu stricto)	3.5	15.1	5.0	2.0
Podomys versus *Peromyscus* (sensu stricto)	5.0	15.7	5.7	2.2
All species groups versus each other	4.4	14.2	5.1	1.6
aztecus species group versus other species groups	4.7	14.3	5.0	1.2
boylii species group versus other species groups	3.5	13.5	3.7	1.2
californicus species group versus other species groups	4.3	15.0	6.8	2.7
crinitus species group versus other species groups	4.4	14.2	5.8	1.8
eremicus species group versus other species groups	5.4	14.7	6.8	1.7
furvus species group versus other species groups	3.3	12.7	4.4	1.4
hooperi species group versus other species groups	5.9	12.7	4.4	1.4
leucopus species group versus other species groups	4.4	14.3	5.9	2.1
maniculatus species group versus other species groups	5.9	14.0	6.3	2.0
megalops species group versus other species groups	3.6	13.9	4.7	1.3
melanophrys species group versus other species groups	3.8	13.7	3.8	1.8
mexicanus species group versus other species groups	4.2	15.3	4.0	1.2
truei species group versus other species groups	3.9	14.2	4.5	1.3

among studies or data sets. In addition, increasing the number of characters allows phylogenetic signal to assert itself over noise (homoplasy), resulting in a more accurate estimate of relationships.

Of the data analyzed, the combined data set provided the greatest resolution and nodal support (Fig. 1). Additionally, the combined data set provided resolution at several levels throughout the topology. Therefore, we use the topology from the BI analysis, as well as statistical support from ML analyses (Fig. 1) of the combined data set, to discuss the phylogenetic relationships of *Peromyscus*. We begin by discussing *Peromyscus* using the current taxonomy based on the sensu stricto framework unless indicated otherwise (Carleton 1980; Musser and Carleton 2005).

Clade I contains members of *Peromyscus* (sensu lato), *Onychomys*, and *Reithrodontomys* (Fig. 1). This relationship agrees with a Reithrodontomyini tribal definition as proposed by Miller and Engstrom (2008) and Musser and Carleton (2005) as well as relationships recovered in Bradley et al. (2004b, 2007), Carleton (1980), McKenna and Bell (1997), and Reeder and Bradley (2004, 2007). The pairing of *Isthmomys* and *Reithrodontomys* in the combined analyses garnered no statistical support but generally agrees with analyses using allozymic (Rogers et al. 2005) and multiple combinations of DNA sequence data (Bradley et al. 2007; Miller and Engstrom 2008).

Clade II most closely represents *Peromyscus* (sensu lato) as interpreted by Hooper (1968), with the exclusion of *Isthmomys*. Analyses of the nuclear and combined data sets each formed a well-supported clade similar to clade II. Although some basal branching patterns within this clade receive little to no support, it is apparent that the taxa recognized as separate genera (*Habromys*, *Megadontomys*, *Neotomodon*, *Osgoodomys*, and *Podomys*) by Carleton (1980, 1989) and Musser and Carleton (2005) are embedded within an assemblage containing *Peromyscus* (sensu stricto). Subclades within clade II support *Peromyscus* (sensu stricto) paraphyly.

Clade III contains the majority of peromyscine species examined and generally agrees with the findings of Bradley et al. (2007). However, the current study differs from Bradley et al. (2007) in the placement of certain taxonomic groups although the terminal branching patterns are similar. Two strongly supported subclades recovered composed of the *aztecus* (*Peromyscus evides* and *P. spicilegus*) and *boylii* (*Peromyscus boylii* and *P. levipes*) species groups (Rennert and Kilpatrick 1986, 1987; Sullivan et al. 1991, 1997; Sullivan and Kilpatrick 1991; Tiemann-Boege et al. 2000; Bradley et al. 2004b) as well as a clade containing the *megalops* (*Peromyscus megalops*), *melanophrys* (*Peromyscus melanophrys*), and *mexicanus* (*Peromyscus mexicanus* and *P. nudipes*) species groups, which agrees with the study by Bradley et al. (2007). Clade IV depicts the relationship among *P. californicus*, *P. crinitus*, and *P. eremicus*, and *P. californicus* and *P. eremicus* are members of the subgenus *Haplomylomys*, whereas *P. crinitus* is the sole member of the *crinitus* species group. The relationship between these 2 species groups was supported in the combined Bayesian analysis. Clade V included members of the *leucopus*

(*Peromyscus gossypinus* and *P. leucopus*) and *maniculatus* (*Peromyscus maniculatus* and *P. melanotis*) species groups. Strong support existed for the sister relationship between these species groups.

Several clades did not receive support in any of the analyses. This includes the unresolved placement of *M. thomasi*, *Neotomodon alstoni*, *O. banderanus*, and *Podomys floridanus* although their inclusion within Clade II is supported. In addition, no support was recovered for a monophyletic group containing all members of the *P. attwateri* (*truei* species group) or for the relationships of several species groups (e.g., *hooperi*). A sister relationship between *Reithrodontomys* and *Peromyscus* (sensu lato, excluding *Isthmomys*) received strong support.

The origin of *Peromyscus* (sensu lato) began approximately 8 mya (Fig. 2); however, the radiation of *Peromyscus* (sensu lato) excluding *Isthmomys* appears to have been focused around 5.71 mya (95% HPD: 3.37–9.08). During this time, *Habromys*, *Megadontomys*, *Neotomodon*, *Osgoodomys*, and *Podomys* originated as well as several major *Peromyscus* lineages including *Haplomylomys* (*P. californicus*, *P. eremicus*, and *P. crinitus*), the *mexicanus* (*P. mexicanus* and *P. nudipes*), and *boylii* (*P. boylii* and *P. levipes*) species groups, and *P. pectoralis*. These lineages emerged between the minimum and maximum dates from León-Paniagua et al. (2007) and are further evidence for an origination date of *Peromyscus* (sensu lato), excluding *Isthmomys*, around 6 mya followed by a rapid diversification.

Estimation of the genetic distance values among selected taxa (genera and species groups) allowed for a gross-level comparison of genetic divergence among said groups (Table 2). For example, *Isthmomys*, *Onychomys*, and *Reithrodontomys* depicted substantially higher levels of genetic divergence than any other comparison. Comparisons of genetic divergence among the 5 genera (*Habromys*, *Megadontomys*, *Neotomodon*, *Osgoodomys*, and *Podomys*) to other currently recognized species groups within *Peromyscus* (sensu stricto) produced values similar in magnitude to comparisons of the species groups to each other.

Although several recent studies have focused on developing phylogenies for *Peromyscus* (sensu lato and sensu stricto) and its affiliated genera, for a variety of reasons, none have been able to offer unambiguous taxonomic recommendations. First, *Peromyscus* is a large genus with new species still being described (Bradley et al. 2004a, 2014); complete taxonomic sampling is often difficult. Second, because several character systems have been studied and analyzed, it presents a challenge to resolve discrepancies when there are conflicting data. Third, *Peromyscus* may have undergone a rapid radiation that makes it difficult to reconstruct phylogenetic relationships (Fig. 1) with the available data. Fourth, and perhaps most important, is the occurrence of genetic conservation between taxa that exhibit substantial levels of morphological differences. For example, Carleton's (1980) decision to elevate *Habromys*, *Megadontomys*, *Neotomodon*, *Osgoodomys*, and *Podomys* to generic status was based on the occurrence of substantial morphological differentiation among taxa; yet these same taxa do not exhibit comparable levels of genetic divergence. Other morphological studies involving the glans penes and bacula (Hooper 1958; Hooper and Musser 1964) also have indicated high levels of morphological divergence among these same genera. Although recent genetic studies (i.e., herein; Rogers et al. 2005; Miller and Engstrom 2008) did not examine genes coding for the external morphological characters examined in Carleton's (1980) study, one would assume a concomitant rate of evolution. Resolving the incongruence between morphological and genetic data may be an exciting development of its own. A cursory analysis of the molecular topology produced herein and the morphological characters analyzed in Carleton (1980) failed to recover a fixed, derived character that unites *Peromyscus*.

No phylogenetic analysis, herein, recovered a clade that corresponded to *Peromyscus* (sensu stricto). In our analyses, *Habromys*, *Megadontomys*, *Neotomodon*, *Osgoodomys*, and *Podomys* continually were placed inside of *Peromyscus* (sensu stricto), producing a paraphyletic assemblage. When the molecular topology was constrained to reflect a *Peromyscus* (sensu stricto) framework, a significantly worse ($P < 0.05$) topology was recovered. Further, we were unable to reject a *Peromyscus* (sensu lato) relationship. When these results are combined with available genetic data (Reeder and Bradley 2004, 2007; Rogers et al. 2005; Reeder et al. 2006; Bradley et al. 2007; Miller and Engstrom 2008), it is clear that *Peromyscus* (sensu stricto) as currently recognized should be abandoned based on its paraphyletic nature. Providing a new, more accurate *Peromyscus* taxonomy is difficult because its placement can be interpreted in multiple ways due to the paraphyletic inclusion of *Habromys*, *Megadontomys*, *Neotomodon*, *Osgoodomys*, and *Podomys* in most analyses.

Similarly, *Peromyscus* (sensu lato) requires revision, as one of its members (*Isthmomys*) forms a paraphyletic assemblage with *Reithrodontomys* or groups outside of a monophyletic *Peromyscus*. However, the *Peromyscus* (sensu lato) moniker can be recovered by simply removing *Isthmomys* and recognizing it as a separate genus (regardless of its affinities outside of *Peromyscus*), following Bradley et al. (2007), Miller and Engstrom (2008), and Rogers et al. (2005). BI and ML analyses both recovered topologies that supported exclusion of *Isthmomys* from a *Peromyscus*/*Onychomys* clade, yet an approximately unbiased topology test did not produce a significantly worse topology when monophyly was enforced on a *Peromyscus* (sensu lato) and *Isthmomys* clade. The inability of the approximately unbiased topology test to support exclusion of *Isthmomys* from *Peromyscus* (sensu lato) is likely due to the inclusion of only 4 of more than 20 species of *Reithrodontomys* and a single representative for *Isthmomys*. It is expected that increased sampling will further support the exclusion of *Isthmomys* from *Peromyscus* (sensu lato). Even with the removal of *Isthmomys*, paraphyly in the subgenera *Peromyscus* and *Haplomylomys* produced by the inclusion of *Habromys*, *Megadontomys*, *Neotomodon*, *Osgoodomys*, and *Podomys* would remain problematic as discussed above.

It is possible to resolve monophyly of *Peromyscus* with taxonomies that broadly recognize groups at the generic,

subgeneric, or species group level. Monophyletic clades from within *Peromyscus* (sensu stricto), as well as *Habromys*, *Megadontomys*, *Neotomodon*, *Osgoodomys*, and *Podomys*, could each be recognized as genera. Similarly, *Habromys*, *Megadontomys*, *Neotomodon*, *Osgoodomys*, and *Podomys* could be subsumed to subgenera within *Peromyscus*. Finally, *Habromys*, *Megadontomys*, *Neotomodon*, *Osgoodomys*, and *Podomys* could be subsumed to species groups within *Peromyscus*. Each option presents specific taxonomic challenges that are discussed below and summarized in Table 3.

By retaining *Habromys*, *Megadontomys*, *Neotomodon*, *Osgoodomys*, and *Podomys* at the generic level, the paraphyly within *Peromyscus* must be resolved by elevating monophyletic clades to the generic level (Table 3). Unfortunately, many of these clades originate at unsupported nodes within the phylogeny produced herein (Fig. 1). Further studies may be better able to resolve these relationships. Based on the current phylogeny, the elevation of a minimum of 2 new genera would be necessary to resolve paraphyly within *Peromyscus* (Genus A—*P. pectoralis*, *P. levipes*, *P. boylii*,

Table 3.—Three potential taxonomic solutions for *Habromys*, *Isthmomys*, *Megadontomys*, *Neotomodon*, *Osgoodomys*, *Peromyscus*, and *Podomys*. Generic designations were identified by supported monophyletic clades within Fig. 1. Only species included in phylogenetic analyses are presented.

Generic taxonomy	Subgeneric taxonomy	Species group taxonomy
Genus *Isthmomys*	Genus *Isthmomys*	Genus *Isthmomys*
I. pirrensis	*I. pirrensis*	*I. pirrensis*
Genus *Habromys*	Genus *Peromyscus*	Genus *Peromyscus*
H. ixtlani	Subgenus *Habromys*	Species group *lepturus*
Genus *Megadontomys*	*H. ixtlani*	*H. ixtlani*
M. thomasi	Subgenus *Megadontomys*	Species group *thomasi*
Genus *Neotomodon*	*M. thomasi*	*M. thomasi*
N. alstoni	Subgenus *Neotomodon*	Species group *alstoni*
Genus *Osgoodomys*	*N. alstoni*	*N. alstoni*
O. banderanus	Subgenus *Osgoodomys*	Species group *banderanus*
Genus *Podomys*	*O. banderanus*	*O. banderanus*
P. floridanus	Subgenus *Podomys*	Species group *floridanus*
Genus *Haplomylomys*	*P. floridanus*	*P. floridanus*
P. californicus	Subgenus *Haplomylomys*	Species group *californicus*
P. crinitus	*P. californicus*	*P. californicus*
P. eremicus	*P. crinitus*	Species group *crinitus*
Genus *Peromyscus*	*P. eremicus*	*P. crinitus*
P. gossypinus	Subgenus *Peromyscus*	Species group *eremicus*
P. leucopus	*P. gossypinus*	*P. eremicus*
P. maniculatus	*P. leucopus*	Species group *aztecus*
P. melanotis	*P. maniculatus*	*P. evides*
New Genus A	*P. melanotis*	*P. spicilegus*
P. megalops	New Subgenus A	Species group *boylii*
P. melanophrys	*P. megalops*	*P. boylii*
P. mexicanus	*P. melanophrys*	*P. levipes*
P. nudipes	*P. mexicanus*	Species group *furvus*
New Genus B	*P. nudipes*	*P. furvus*
P. boylii	New Subgenus B	Species group *hooperi*
P. evides	*P. boylii*	*P. hooperi*
P. levipes	*P. evides*	Species group *leucopus*
P. spicilegus	*P. levipes*	*P. gossypinus*
New Genus C	*P. spicilegus*	*P. leucopus*
P. attwateri	New Subgenus C	Species group *maniculatus*
P. furvus	*P. attwateri*	*P. maniculatus*
P. gratus	*P. furvus*	*P. melanotis*
P. ochraventer	*P. gratus*	Species group *megalops*
New Genus D	*P. ochraventer*	*P. megalops*
P. pectoralis	New Subgenus D	Species group *melanophrys*
New Genus E	*P. pectoralis*	*P. melanophrys*
P. hooperi	New Subgenus E	Species group *mexicanus*
	P. hooperi	*P. mexicanus*
		P. nudipes
		Species group *truei*
		P. attwateri
		P. gratus
		P. ochraventer
		Species group *pectoralis*
		P. pectoralis

P. spicilegus, *P. evides*, *P. ochraventer*, *P. gratus*, *P. attwateri*, and *P. furvus*; Genus B—*P. mexicanus*, *P. nudipes*, *P. melanophrys*, and *P. megalops*). This option is unfeasible due to lack of statistical support in the phylogeny. A 2-genus option will need to be continually evaluated as new data and data types become available. If genera are designated only at supported monophyletic nodes, then up to 4 new genera would require elevation from *Peromyscus* (Genus A—*P. megalops*, *P. melanophyrs*, *P. mexicanus*, and *P. nudipes*; Genus B—*P. evides*, *P. boylii*, *P. levipes*, and *P. spicilegus*; Genus C—*P. attwateri*, *P. furvus*, *P. gratus*, and *P. ochraventer*; Genus *Peromyscus*—*P. gossypinus*, *P. leucopus*, *P. maniculatus*, and *P. melanotis*) with uncertain placement of *P. hooperi* and *P. pectoralis*. Using the subgeneric option, a genus taxonomically similar to *Peromyscus* can be retained by subsuming *Habromys*, *Megadontomys*, *Neotomodon*, *Osgoodomys*, and *Podomys* (Table 3). The elevation of subgenera within *Peromyscus* would be necessary, and newly elevated subgenera would be similar in species content to the genera created using the generic option. Finally by removing higher taxonomic ranks (genus or subgenus), paraphyletic assemblages can be resolved while continuing to recognize morphological variation and account for clades identified with genetic data (Table 3). Species groups have proven to be valuable units to study evolution within *Peromyscus* (Riddle et al. 2000; Bradley et al. 2004b; Durish et al. 2004) and perhaps their usage would serve as a viable solution until the phylogenetic relationships of unresolved taxa are determined. Additionally, monophyly of most species groups has been somewhat resolved (except *mexicanus* and *furvus*—Bradley et al. 2007). The species group option, however, fails to recognize degrees of morphological variation that the generic and subgeneric options could offer if additional subrankings were established. Taxonomic changes would still be required in the recognition of the species groups; however, this option requires minimal changes relative to recognizing additional genera or subgenera.

In developing a revised classification for *Peromyscus*, standards must be agreed upon that designate distinction at a genetic level yet accommodate morphological variation. Some of these standards already are understood such as achieving monophyly and cohesion within the group. However, determining how much variation warrants generic recognition is difficult. For example, Helgen et al. (2009) and Weksler (2003) recently revised the genera formerly recognized as *Spermophilus* and *Oryzomys*, respectively. Their revisions produced monophyly and clarification of groups by the naming of additional genera to accommodate monophyletic clades produced in their analyses. Based on the data herein, it is clear that the current taxonomy of *Peromyscus* (sensu stricto) should be abandoned as well. However, to resolve the paraphyly within *Peromyscus*, at least 3 different taxonomic options are available and should be considered. More diverse data types, including morphology, karyology, and ecology, as well as additional genetic data, will be required to develop the taxonomy that properly recognizes the diversity and distinction within *Peromyscus*.

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