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36. PHYLOGENETIC RELATIONSHIPS AMONG PEROMYSCINE RODENTS: ALLOZYME EVIDENCE

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Abstract

To assess phylogenetic relationships among peromyscine rodents, we examined 25 presumptive genetic loci in *Habromys ixtlani, Isthmomys pirrensis, Megadontomys nelsoni, Neotomodon alstoni, Onychomys leucogaster, Osgoodomys banderanus*, 11 species of *Peromyscus* (*sensu stricto – P. boylii, P. crinitus, P. eremicus, P. leucopus, P. megalops, P. melanocarpus, P. melanotis, P. mexicanus, P. ochraventer, P. perfulvus,* and *P. truei*), *Reithrodontomys megalotis,* and *R. mexicanus. Baiomys musculus, Neotoma albigula, Ototylomys* sp., *Scotinomys xerampilinus,* and *Sigmodon hispidus* were used as outgroup taxa. The most parsimonious phylogenetic trees were constructed using the step matrix option of PAUP. All tree building algorithms were consistent in demonstrating that the genus *Peromyscus* is not monophyletic. This finding is consistent with other studies based on allozymes, micro-complement fixation, mtDNA sequences, and differenetially stained chromosomes, but does not follow results based on morphological analyses. *I. pirrerensis*, together with *Reithrodontomys* are sister groups to *Peromyscus* (sensu lato – which includes *Habromys, Megadontomys, Neotomo*don, and *Podomys*).

Key words: Allozymes, cladistic analysis, peromyscine rodents, monophyly, *Peromyscus,* step matrix.

Resumen

Para estudiar las relaciones filogenéticos entre roedores "peromyscinidos" examinamos la variación genética de 25 presuntos loci en las especies; *Habromys ixtlani, Isthmomys pirrensis, Megadontomys nelsoni, Neotomodon alstoni, Onychomys leucogaster, Osgoodomys banderanus*, 11 especies de *Peromyscus* (sensu stricto – *P. boylii, P. crinitus, P. eremicus, P. leucopus, P. megalops, P. melanocarpus, P. melanotis, P. mexicanus, P. ochraventer, P. perfulvus,* and *P. truei*), *Reithrodontomys megalotis,* y *R. mexicanus.* Las especies *Baiomys musculus, Neotoma albigula, Ototylomys* sp., *Scotinomys xerampilinus,* y *Sigmodon hispidus* fueron utilizados como grupos externos. Los árboles filogenéticos fueron reconstruidos usando la opción matriz de pasos (Step Matriz) en PAUP. Todos los algoritmos de reconstrucción filogenética coincidieron en demostrar que el género *Peromyscus* no es monofilético. Este hallazgo es consistente con otros estudios basados en aloenzimas, fijación de microcomplementos, secuencias de ADN mitocondrial y tinción cromosómica diferencial, pero no con los resultados obtenidos a partir de análisis morfológicos. Finalmente, *I. pirrensis* y *Reithrodontomys* aparecen como grupos hermanos de *Peromyscus* (sensu lato; que incluye *Habromys, Megadontomys, Neotomo*don, y *Podomys*).

Palabras clave:

Well corroborated estimates of phylogenetic relationships form the basis for all comparative biology. Among mammals, the genus *Peromyscus* has been likened to *Drosophila* in terms of importance to development of systematic biology in North America (Carleton 1989; Dewey and Dawson 2001; Musser and Carleton 1993). Nevertheless, phylogenetic relationships among the majority of species of *Peromyscus* (and among genera of peromyscine rodents-*sensu* Carleton 1989) remain largely controversial. Despite the hope expressed by Carleton (1980) that his compendium on morphological relationships among the Neotomine-Peromyscine rodents would serve as an incentive for further investigations, few authors have examined phylogenetic relationships within the group as a whole. As a result, taxonomically comprehensive data sets amenable to cladistic analysis are available for only certain morphological characters (Carleton 1980) and banded chromosomes (Rogers *et al.* 1984; Stangl and Baker 1984).

With the ascendency of protein electrophoresis in the 1970s and 1980s as an alternate technique for examining systematic relationships, peromyscine rodents were an important proving ground for the utility of molecular data as an alternate to morphological analyses (see review by Carleton 1989). Several comparative studies above the species level appeared during this period, most of which employed phenetic distance analysis of allozyme frequencies (for example, Avise *et al.* 1974; 1979; Kilpatrick and Zimmerman 1975; Schmidly *et al.* 1985; Zimmerman *et al.* 1975; 1978). Given that electromorphs are inherently discrete characters, however, they also can be analyzed by strict phylogenetic parsimony. Following the paper by Patton *et al.* (1980), several more recent studies used cladistic methodologies to examine relationships among subsets of peromyscine taxa (Arellano *et al.* 2003; Rogers and Engstrom 1992; Sullivan *et al.* 1991; Werbitsky and Kilpatrick 1987). Few of these studies, however, focused on phylogenetic relationships among higher taxa, and none was comprehensive in scope. Likewise, relatively recent studies (Dickerman 1992; Engel *et al.* 1998; Hogan *et al.* 1993; Sullivan *et al.* 1997) have used DNA-DNA hybridization or mitochondrial DNA sequence data to assess relationships among certain peromyscine taxa, but these also were limited in taxonomic coverage.

The purposes of this study were to examine electromorphic variation among higher taxa of peromsycine rodents and to evaluate the utility of these data in estimating phylogenetic relationships at this level. Even though DNA sequences have eclipsed allozymes as one of the premier methods of estimating phylogenetic relationships, allozymes offer the advantage of assessing dozens of nuclear markers relatively rapidly and inexpensively. Because allozyme mobilities are a secondary and sometimes indirect product of underlying DNA sequences, we present this analysis as an initial hypothesis recognizing that a refined estimate of phylogeny awaits DNA sequence data from several genetic loci and a synthetic analysis of multiple data sets. This overview of protein divergence yielded a data set suitable for analysis from a rigorous, phylogenetic approach in a model taxon. To that end, we examined phylogenetic patterns in allozymic divergence among representatives of all recognized peromyscine genera, subgenera, and 10 of the 13 species groups within the genus *Peromyscus* (*sensu* Carleton 1989). In addition, we used representatives of the allied Tribes Baiomyini, Neotomini, and Tylomyini (*sensu* Carleton 1989) as outgroup taxa (Watrous and Wheeler 1981).

Materials and Methods

Tissue samples were examined from 143 individuals representing 14 genera and 25 species of sigmodontine rodents using horizontal starch-gel electrophoresis. Localities and museum deposition of voucher specimens are listed in the Appendix.

Twenty-five genetic loci were evaluated from liver or combined kidney and heart homogenate (Murphy *et al.* 1996; Selander *et al.* 1971) for all individuals examined. Protocols for buffers and stains were prepared following Selander *et al.* (1971); Harris and Hopkinson (1976) or Murphy *et al.* (1996). Enzyme abbreviations are as listed by Murphy *et al.* (1996). Enzymes examined and buffer systems are as follows: Lithium hydroxide: purine-nucleoside phosphorylase, Enzyme Commission (E.C.) 2.4.2.1 (PNP). Phosphoglucose isomerase phosphate: glucose-6-phosphate isomerase, E.C. 5.3.1.9 (GPI); adenosine deaminase, E.C. 3.5.4.4 (ADA). Poulik: peptidases, E.C. 3.4.13 (PEPA, PEPB1, PEPB2, PEPD). Tris-citrate, pH 8.0: glyceraldehyde-3-phosphate dehydrogenase, E.C. 1.2.1.12 (GAPDH); glycerol-3-

phosphate dehydrogenase, E.C. 1.2.1.8 (G3PDH); isocitrate dehydrogenase, E.C. 1.1.1.42 (IDH1 and IDH2); L-lactate dehydrogenase, E.C. 1.1.1.27 (LHDA and LDHB); malate dehydrogenase, E.C. 1.1.1.37 (MHD1 and MDH2); L-iditol dehydrogenase, E.C. 1.1.1.14 (IDDH); aspartate aminotransferase, E.C. 2.6.1.1 (AAT1 and AAT2); fructose-biphosphatase, E.C. 3.1.3.11 (FBP). Tris-citrate, pH 7.0: mannose-6-phosphate isomerase, E.C. 5.3.1.8 (MPI); superoxide dismutase, E.C. 1.15.1.1 (SOD1). Tris-malate, pH 7.4: phosphoglucomutase, 5.4.2.2 (PGMA and PGMB); phosphogluconate dehydrogenase, E.C. 1.1.1.44 (PGDH). Tris ethylenediaminetetracetic acid borate I, pH 8.0: malate dehydrogenase (NADP+), E.C. 1.1.1.40 (MDHP).

Electrophoretic results were summarized in the form of individual genotypes by locus for each individual. Alleles for each locus were designated alphabetically in order of decreasing mobility as determined from side-by-side comparisons and the data were analyzed using BIOSYS-1 (Swofford and Selander 1989).

Sample sizes in this study were relatively small. However, use of small samples can be justified when values for heterozygosity and percentage of polymorphism are low, and allele frequencies are equal or very close to 0 or 1, indicating that alleles move toward fixation. In this study, heterozygosity and polymorphism values were not high and the majority of samples were distinguished by fixed allelic differences. Therefore, estimates of phylogenetic relatedness developed from these data likely approximate those derived from larger sample sizes (Hafner *et al.* 1994).

In the cladistic analysis, data were subjected to parsimony analyses using PAUP (Phylogenetic Analysis Using Parsimony) software of Swofford (1999), version 4.07b. We used the step matrix option in which each locus was considered as a single character and alleles and each possible combination of them were considered as character states (Arellano *et al.* 2003; Harris and Rogers 1999; Mabee and Humphries 1993). Uninformative characters (autapomorphies) were not used in the original data matrix. Although fixed characters provide the most phylogenetic signal (Wiens 1995), we also included polymorphic characters because they also are phylogenetically informative (Wiens 1995; Wiens and Servedio 1997). Characters, as defined in Table 1, were treated as reticulate (unordered) assuming that all character state transformations were possible instead of imposing a specific pathway. The combinations of alleles we used were those inferred to be present in ancestral nodes to reduce the dimensions of the step matrix (Mabee and Humphries 1993; Mardulyn and Pasteels 1994). We used PAUP* version 4.07b (Swofford 1999) to reconstruct the array of plesiomorphic character states consistent with the most-parsimonious tree(s), based on the character matrix (Table 1) and distances stored in the step matrix (Table 2—Arellano *et al.* 2003; Harris and Rogers 1999; Mardulyn and Pasteels 1994).

The most parsimonious trees were found using the heuristic search including stepwise addition sequence, 10 replications, and TBR swapping algorithm of PAUP* version 4.07b (Swofford 1999). Consensus trees using the 50%-majority-rule were generated when more than one parsimonious tree resulted from the analysis.

Results

All 25 genetic loci were variable across the 25 taxa examined (Table 1). Average polymorphism was 9.1% (range 0.0% in *Isthmomys pirrensis* to 20.0% in *Neotoma albigula, Osgoodomys banderanus, Peromyscus eremicus*, and *P. mexicanus*), mean number alleles per locus was 1.1 (range of 1.0 in *I. pirrensis* to 1.2 in *P. eremicus, P. megalops,* and *P. mexicanus)*, and mean heterozygosity (H; direct-count method) was 3.6% (range 0.0% in *I. pirrensis* to 12.0% in *Sigmodon hispidus*). Rogers (1972) genetic distance values (complete matrix available upon request) ranged from 0.21 between *P. boylii* and *P. mexicanus* to 0.91 between *I. pirrensis* and *Ototylomys* sp.

A data matrix with 28 informative characters (Table 2) was subjected to phylogenetic analysis to resolve relationships among peromyscine taxa included in this study. A step matrix was used to establish the number of steps required in a transition between any 2 character states (Table 1). Cladistic reconstruction using *Sigmodon hispidus* as the outgroup taxon (sensu Watrous and Wheeler 1981) produced 917 equally parsimonious trees. In the resulting consensus tree (Fig. 1), all samples of *Peromyscus* as well as the genera *Habromys, Megadontomys, Neotomodon, Osgoodomys, and Podomys* formed a single clade (Clade I) with poor internal

Table 1. Data matrix with 35 polymorphic charaacters coded for the 25 Samples evaluated in the phylogenetic analysis. Characters 1-26 represent genetic loci MPI-ALS, respectively. Characters codes proceed from 1-9 and then by letters of the alphabet according to Table 2.

	Charaters																									
	MPI	PGA	PGB	ID1	ID2	GAP	MD1	MD2	LDA	LDB	AT1	AT2	IDH	PGD	PB1	PB2	PPA	PPD	G3P	SOD	GPI	ADA	FBP	PNP	MDP	ALB
Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Habromys	5	9	3	4	5	4	1	3	3	1	GG	3	2	1	5	2	CC	1	3	3	H	GG	7	2	5	3
Isthmomys	1	8	3	5	5	3	3	3	3	4	8	4	4	3	1	3	6	5	2	1	8	5	1	6	3	7
Megadontomys	7	8	3	4	5	4	1	3	3	1	8	3	4	2	O	3	7	1	7	3	8	5	3	7	5	6
Neotomodon	Q	6	4	5	5	4	1	3	3	1	8	3	4	5	5	2	5	1	2	3	8	2	2	3	2	6
Onychomys	4	8	3	5	1	4	1	3	3	4	B	1	4	3	4	N	A	3	C	3	5	4	4	6	2	3
Osgoodomys	BB	5	O	W	5	1	1	3	3	1	8	3	2	5	U	2	Y	1	3	3	7	3	4	DD	3	6
P. boylii	4	9	3	5	5	4	1	3	3	1	7	3	4	L	5	2	7	2	7	3	6	5	4	U	2	6
P. crinitus	7	C	3	5	5	4	1	3	3	1	8	3	4	1	5	2	Q	1	7	3	A	8	4	FF	2	2
P. eremicus	A	9	3	X	4	4	1	3	J	1	8	3	S	S	5	E	4	2	7	3	6	5	4	7	2	2
P. leucopus	CC	C	4	5	5	4	1	3	3	1	9	1	6	9	5	2	1	1	8	4	M	5	2	D	2	6
P. megalops	7	9	3	5	5	4	1	3	3	1	GG	3	4	5	K	2	7	2	7	3	A	V	4	P	2	3
P. melanocarpus	T	9	G	4	5	4	1	3	3	1	9	3	4	8	5	2	2	2	7	3	8	HH	4	T	2	3
P. melanotis	7	7	2	5	5	4	1	3	3	1	9	1	4	A	5	2	7	1	8	3	8	X	6	D	2	6
P. mexicanus	7	R	3	4	O	4	1	F	3	1	GG	3	4	5	5	2	7	2	7	3	A	S	4	6	2	6
P. ochraventer	7	9	3	4	5	4	1	3	3	1	8	3	4	5	5	2	7	1	W	3	6	5	4	D	2	7
P. perfulvus	7	1	3	5	5	4	1	3	3	1	4	3	4	9	5	2	3	1	7	3	6	S	4	6	3	6
P. truei	7	9	3	F	4	4	1	3	3	1	8	3	4	6	5	2	7	2	7	3	6	5	4	9	2	2
Podomys	7	8	3	S	5	4	1	3	N	1	8	3	4	5	5	3	5	1	7	2	A	5	2	6	3	3
R. megalotis	4	5	3	5	4	4	3	3	3	4	8	5	4	7	5	5	7	4	EE	1	4	5	8	6	4	4
R. mexicanus	7	4	3	5	4	4	3	3	1	4	GG	5	4	3	5	3	9	4	6	1	4	X	8	AA	4	5
Baiomys	3	A	2	9	4	5	1	3	3	5	1	1	1	B	2	3	7	5	A	3	3	7	8	1	3	3
Neotoma	3	2	4	L	5	5	1	2	3	4	5	2	7	2	K	1	8	5	4	1	9	O	7	9	F	3
Ototylomys	S	D	2	8	2	4	1	2	3	4	2	3	5	B	2	1	8	4	1	3	4	G	6	4	3	1
Scotinomys	2	B	3	U	4	3	1	3	3	4	3	1	4	C	K	1	A	2	7	5	3	5	5	Z	3	3
Sigmodon	3	9	3	5	6	2	2	2	3	4	6	3	5	9	2	4	II	3	L	4	7	2	Y	B	4	7

Table 2. Step matrix used to code character states used in the phylogenetic analysis and listed in Table 1. A total of 44 characters (1-9) and (A-II) was identified. Numbers in the matrix represent steps required for every character transformation. For example, the number of steps required to change from character 9 (presence of alleles a and b) to character 1 (presence of allele a) is 1 (loss of allele b). Likewise, it requires 2 steps to move from character 1 (presence of allele a) to character 2 (presence of allele b-gain of allele b and loss of allele a).

	1	2	3	4	5	6	7	8	9	A	B	C	D	E	F	G	H	I	J	K	L	M
[1-a]	—	2	2	2	2	2	2	2	2	2	2	2	2	1	1	1	1	1	3	3	3	3
[2-b]	2	—	2	2	2	2	2	2	2	2	2	2	2	1	3	3	3	3	1	1	1	1
[3-c]	2	2	—	2	2	2	2	2	2	2	2	2	2	3	1	3	3	3	1	3	3	3
[4-d]	2	2	2	—	2	2	2	2	2	2	2	2	2	3	3	1	3	3	3	1	3	3
[5-e]	2	2	2	2	—	2	2	2	2	2	2	2	2	3	3	3	3	3	3	3	1	3
[6-f]	2	2	2	2	2	—	2	2	2	2	2	2	2	3	3	3	3	3	3	3	3	3
[7-g]	2	2	2	2	2	2	—	2	2	2	2	2	2	3	3	3	1	3	3	3	3	3
[8-h]	2	2	2	2	2	2	2	—	2	2	2	2	2	3	3	3	3	1	3	3	3	1
[9-i]	2	2	2	2	2	2	2	2	—	2	2	2	2	3	3	3	3	3	3	3	3	3
[A-j]	2	2	2	2	2	2	2	2	2	—	2	2	2	3	3	3	3	3	3	3	3	3
[B-k]	2	2	2	2	2	2	2	2	2	2	—	2	2	3	3	3	3	3	3	3	3	3
[C-l]	2	2	2	2	2	2	2	2	2	2	2	—	2	3	3	3	3	3	3	3	3	3
[D-m]	2	2	2	2	2	2	2	2	2	2	2	2	—	3	3	3	3	3	3	3	3	3
[E-ab]	1	1	3	3	3	3	3	3	3	3	3	3	3	—	2	2	2	2	4	2	4	4
[F-ac]	1	3	1	3	3	3	3	3	3	3	3	3	3	2	—	2	2	2	2	4	4	4
[G-ad]	1	3	3	1	3	3	3	3	3	3	3	3	3	2	2	—	2	2	4	2	4	4
[H-ag]	1	3	3	3	3	3	1	3	3	3	3	3	3	2	2	2	—	2	4	4	4	4
[I-ah]	1	3	3	3	3	3	3	1	3	3	3	3	3	2	2	2	2	—	4	4	4	2
[J-bc]	3	1	1	3	3	3	3	3	3	3	3	3	3	4	2	4	4	4	—	2	2	2
[K-bd]	3	1	3	1	3	3	3	3	3	3	3	3	3	2	4	2	4	4	2	—	2	2
[L-be]	3	1	3	3	1	3	3	3	3	3	3	3	3	4	4	4	4	4	2	2	—	2
[M-bh]	3	1	3	3	3	3	3	1	3	3	3	3	3	4	4	4	4	2	2	2	2	—
[N-cd]	3	3	1	1	3	3	3	3	3	3	3	3	3	4	2	2	4	4	2	2	4	4
[O-ce]	3	3	1	3	1	3	3	3	3	3	3	3	3	4	2	4	4	4	2	4	2	4
[P-cf]	3	3	1	3	3	1	3	3	3	3	3	3	3	4	2	4	4	4	2	4	4	4
[Q-cg]	3	3	1	3	3	3	1	3	3	3	3	3	3	4	2	4	2	4	2	4	4	4
[R-ci]	3	3	1	3	3	3	3	3	1	3	3	3	3	4	2	4	4	4	2	4	4	4
[S-de]	3	3	3	1	1	3	3	3	3	3	3	3	3	4	4	2	4	4	4	2	2	4
[T-dg]	3	3	3	1	3	3	1	3	3	3	3	3	3	4	4	2	2	4	4	2	4	4
[U-ef]	3	3	3	3	1	1	3	3	3	3	3	3	3	4	4	4	4	4	4	4	2	4
[V-efh]	4	4	4	4	2	2	4	2	4	4	4	4	4	5	5	5	5	3	5	5	3	3
[W-eg]	3	3	3	3	1	3	1	3	3	3	3	3	3	4	4	4	2	4	4	4	2	4
[X-eg]	3	3	3	3	1	3	3	1	3	3	3	3	3	4	4	4	4	2	4	4	2	2
[Y-fg]	3	3	3	3	3	1	1	3	3	3	3	3	3	4	4	4	2	4	4	4	4	4
[Z-fh]	3	3	3	3	3	1	3	1	3	3	3	3	3	4	4	4	4	2	4	4	4	2
[AA-fi]	3	3	3	3	3	1	3	3	1	3	3	3	3	4	4	4	4	4	4	4	4	4
[BB-gh]	3	3	3	3	3	3	1	1	3	3	3	3	3	4	4	4	2	2	4	4	4	2
[CC-gi]	3	3	3	3	3	3	1	3	1	3	3	3	3	4	4	4	2	4	4	4	4	4
[DD-gj]	3	3	3	3	3	3	1	3	3	1	3	3	3	4	4	4	2	4	4	4	4	4
[EE-gij]	4	4	4	4	4	4	2	4	2	2	4	4	4	5	5	5	3	5	5	5	5	5
[FF-gl]	3	3	3	3	3	3	1	3	3	3	3	1	3	4	4	4	2	4	4	4	4	4
[GG-hi]	3	3	3	3	3	3	3	1	1	3	3	3	3	4	4	4	4	2	4	4	4	2
[HH-hj]	3	3	3	3	3	3	3	1	3	1	3	3	3	4	4	4	4	2	4	4	4	2
[II-ij]	3	3	3	3	3	3	3	3	1	1	3	3	3	4	4	4	4	4	4	4	4	4

Table 2. Extended.

	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	BB	CC	DD	EE	FF	GG	HH	II
[1-a]	3	3	3	3	3	3	3	3	4	3	3	3	3	3	3	3	3	4	3	3	3	3
[2-b]	3	3	3	3	3	3	3	3	4	3	3	3	3	3	3	3	3	4	3	3	3	3
[3-c]	1	1	1	1	1	3	3	3	4	3	3	3	3	3	3	3	3	4	3	3	3	3
[4-d]	1	3	3	3	3	1	1	3	4	3	3	3	3	3	3	3	3	4	3	3	3	3
[5-e]	3	1	3	3	3	1	3	1	2	1	1	3	3	3	3	3	3	4	3	3	3	3
[6-f]	3	3	1	3	3	3	3	1	2	3	3	1	1	1	3	3	3	4	3	3	3	3
[7-g]	3	3	3	1	3	3	1	3	2	1	3	1	3	3	1	1	1	2	1	3	3	3
[8-h]	3	3	3	3	3	3	3	3	4	3	1	3	1	3	1	3	3	4	3	1	1	3
[9-i]	3	3	3	3	1	3	3	3	4	3	3	3	3	1	3	1	3	2	3	1	3	1
[A-j]	3	3	3	3	3	3	3	3	4	3	3	3	3	3	3	3	1	2	3	3	1	1
[B-k]	3	3	3	3	3	3	3	3	4	3	3	3	3	3	3	3	3	4	3	3	3	3
[C-l]	3	3	3	3	3	3	3	3	4	3	3	3	3	3	3	3	3	4	1	3	3	3
[D-m]	3	3	3	3	3	3	3	3	4	3	3	3	3	3	3	3	3	4	3	3	3	3
[E-ab]	4	4	4	4	4	4	4	4	5	4	4	4	4	4	4	4	4	5	4	4	4	4
[F-ac]	2	2	2	2	2	4	4	4	5	4	4	4	4	4	4	4	4	5	4	4	4	4
[G-ad]	2	4	4	4	4	2	2	4	5	4	4	4	4	4	4	4	4	5	4	4	4	4
[H-ag]	4	4	4	2	4	4	2	4	3	2	4	2	4	4	2	2	2	3	2	4	4	4
[I-ah]	4	4	4	4	4	4	4	4	5	4	2	4	2	4	2	4	4	5	4	2	2	4
[J-bc]	2	2	2	2	2	4	4	4	5	4	4	4	4	4	4	4	4	5	4	4	4	4
[K-bd]	2	4	4	4	4	2	2	4	5	4	4	4	4	4	4	4	4	5	4	4	4	4
[L-be]	4	2	4	4	4	2	4	2	3	2	2	4	4	4	4	4	4	5	4	4	4	4
[M-bh]	4	4	4	4	4	4	4	4	5	4	2	4	2	4	2	4	4	5	4	2	2	4
[N-cd]	—	2	2	2	2	2	2	4	5	4	4	4	4	4	4	4	4	5	4	4	4	4
[O-ce]	2	—	2	2	2	2	4	2	3	2	2	4	4	4	4	4	4	5	4	4	4	4
[P-cf]	2	2	—	4	2	4	4	2	3	4	4	2	2	2	4	4	4	5	4	4	4	4
[Q-cg]	2	2	2	—	2	4	2	4	5	2	4	2	4	4	2	2	2	3	2	4	4	4
[R-ci]	2	2	2	2	—	4	4	4	5	4	4	4	4	2	4	2	4	5	4	2	4	2
[S-de]	2	2	4	4	4	—	2	2	3	2	2	4	4	4	4	4	4	5	4	4	4	4
[T-dg]	2	4	4	2	4	2	—	4	3	2	4	2	4	4	2	2	2	3	2	4	4	4
[U-ef]	4	2	2	4	4	2	4	—	1	2	2	4	2	2	4	4	4	5	4	4	4	4
[V-efh]	5	3	3	5	5	3	5	1	—	1	3	1	3	3	3	3	3	4	3	5	5	5
[W-eg]	4	2	4	2	4	2	2	2	1	—	2	2	4	4	2	2	2	3	2	4	4	4
[X-eg]	4	2	4	4	4	2	4	2	3	2	—	4	2	4	2	4	4	5	4	2	2	4
[Y-fg]	4	4	2	2	4	4	2	4	1	2	4	—	2	2	2	2	2	3	2	4	4	4
[Z-fh]	4	4	2	4	4	4	4	2	3	4	2	2	—	2	2	4	4	5	4	2	2	4
[AA-fi]	4	4	2	4	2	4	4	2	3	4	4	2	2	—	4	4	4	5	4	2	4	2
[BB-gh]	4	4	4	2	4	4	2	4	3	2	2	2	2	4	—	2	2	3	2	2	2	4
[CC-gi]	4	4	4	2	2	4	2	4	3	2	4	2	4	4	2	—	2	1	2	2	4	2
[DD-gj]	4	4	4	2	4	4	2	4	3	2	4	2	4	4	2	2	—	1	2	4	2	2
[EE-gij]	5	5	5	3	5	5	3	5	4	3	5	3	5	5	3	1	1	—	3	3	3	1
[FF-gl]	4	4	4	2	4	4	2	4	3	2	4	2	4	4	2	2	2	3	—	4	4	4
[GG-hi]	4	4	4	4	2	4	4	4	5	4	2	4	2	2	2	2	4	3	4	—	2	2
[HH-hj]	4	4	4	4	4	4	4	4	5	4	2	4	2	4	2	4	2	3	4	2	—	2
[II-ij]	4	4	4	4	2	4	4	4	5	4	4	4	4	2	4	2	2	1	4	2	2	—

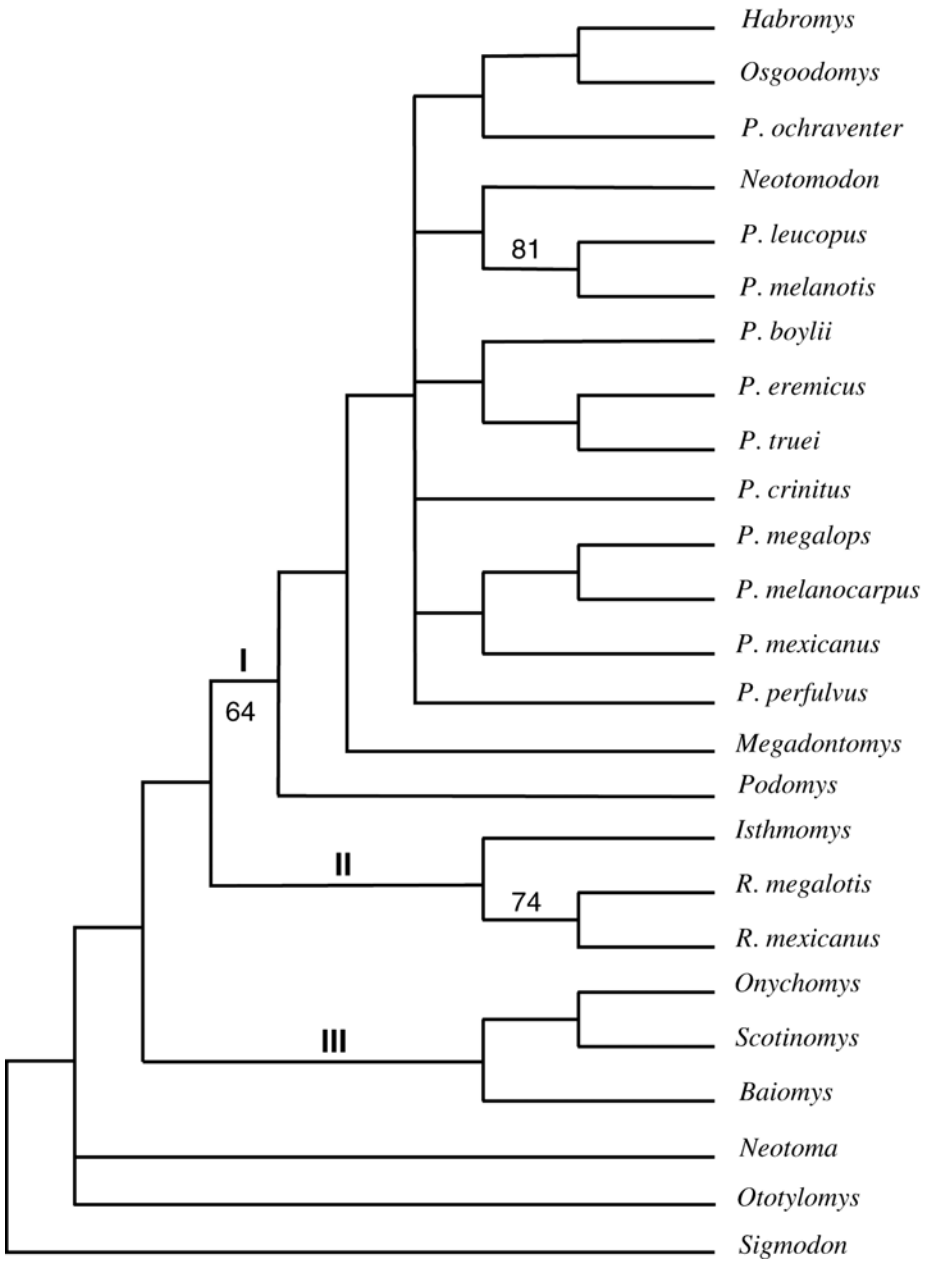

Fig. 1. Maximum-parsimony consensus cladogram (50% majority rule) derived from 917 equally parsimonious trees (length = 224 steps) estimating phylogenetic relationships among selected neotomine-peromyscine rodents using *Sigmodon hispidus* as the out-group. Clade designations are explained in text. Numbers on branches are bootstrap percentages based on 1000 iterations.

resolution. Clade II was formed by *Reithrodontomys* and *Isthmomys*, whereas Clade III comprised the genera *Onychomys*, *Baiomys*, and *Scotinomys.*

To further investigate relationships among the peromyscines, we performed a series of phylogenetic analyses using single and multiple taxa (various combinations of the genera *Baiomys*, *Neotoma*, *Onychomys*, *Ototylomys*, and *Scotinomys)* as outgroups. All analyses resulted in recovery of Clades I and II, but provided no additional resolution (trees not shown). Inasmuch as the composition of Clades I and II was stable regardless of the combination of outgroup taxa used, we performed an analysis in which *Isthmomys* and *Reithrodontomys* (Clade II

taxa) were used as multiple outgroup taxa to further resolve relationships within Clade I. This analysis resulted in 720 equally parsimonious trees (Fig. 2) and provided additional resolution. Within Clade I, three clades were recognized. Clade A (composed of all respresentatives of the genus *Peromyscus* [sensu stricto] plus *Habromys,* Neotomodon, and *Osgoodomys)*, Clade B (*Megadontomys*), and Clade C (*Podomys*).

Clade A is divisible into two groups. One consists of *Neotomodon*, together with the sister taxa *P. leucopus* and *P. melanotis*. The second is formed by 8 species groups in the genus *Peromyscus (boylii, crinitus, megalops, melanocarpus, mexicanus, ochraventer, perfulvus, and truei)* together with *Haplomylomys* (*P. eremicus*), *Habromys*, and Osgoodomys. Within this latter group, five nodes are consistently resolved, including (Carleton's [1989] classification follows species names): 1) *P. perfulvus (P. melanophrys* species group), 2) (*P. mexicanus* (*P. megalops, P. melanocarpus*)) (*P. mexicanus* species group (*P. megalops* species group))): 3) (*P. boylii* (*P. truei, P.*

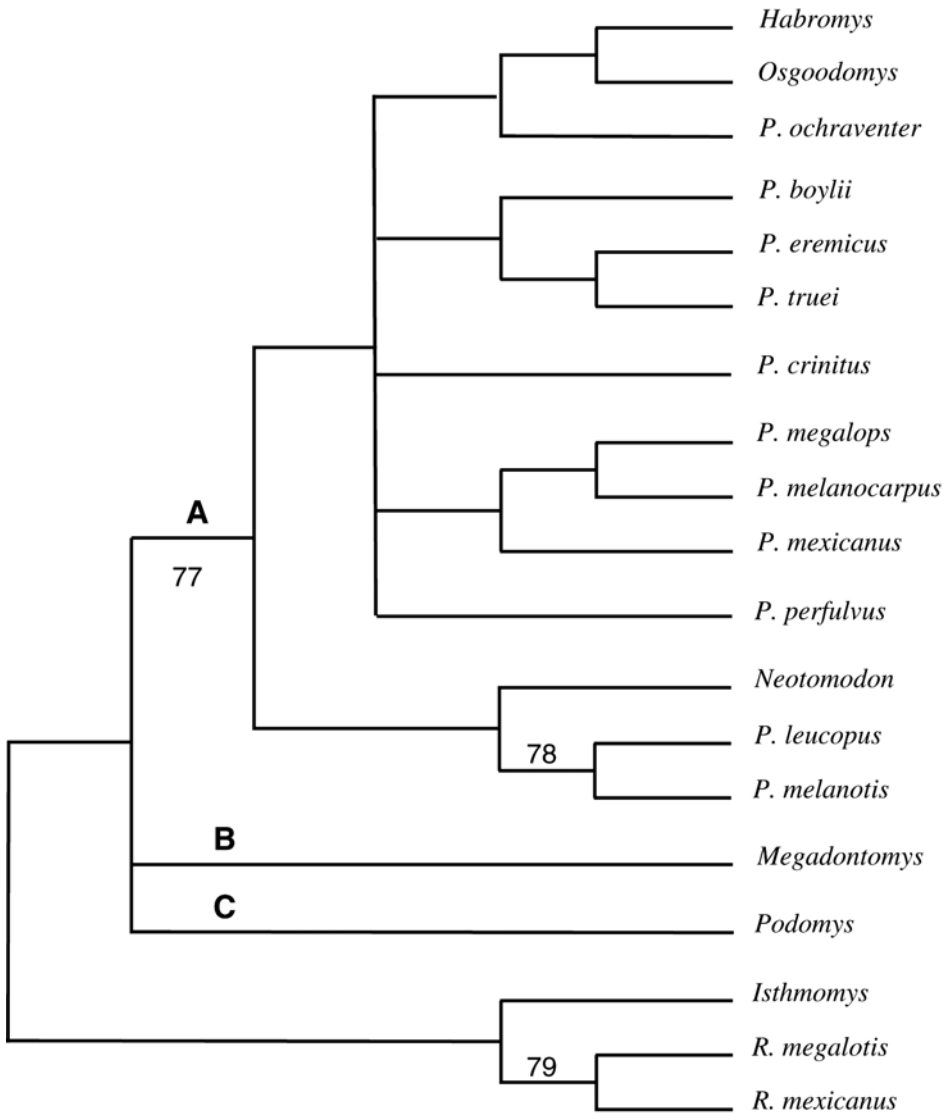

Fig. 2. Maximum-parsimony consensus cladogram (50% majority rule) derived from 720 equally parsimonious trees (length = 136 steps) estimating phylogenetic among *Peromyscus* (*sensu lato*) using *Isthmomys* and *Reithrodontomys* as out-group taxa. Clade designations are explained in text. Numbers on branches are bootstrap percentages based on 1000 iterations.

eremicus)) (*P. boylii* species group (*P. truei* species group, *Haplomylomys*)); 4) *P. crinitus* (*P. crinitus* species group); 5) (*P. ochraventer* (*O. banderanus, H. lepturus* = *ixtlani*)) (*P. furvus* species group (*Osgoodomys, Habromys*)). Phylogentic relationships among the nodes were not consistently resolved, however, relationships within clades that contain multiple taxa were consistent regardless of the outgroup taxa.

Relatively few bootstrap values were large for the trees depicted in Figs. 1 and 2. However, Arellano *et al.* (2003) demonstrated that for relatively small data sets, boostrap values increase dramatically when data sets are increased artificially (*e.g.* increasing number of characters by duplicating the original data matrix two, three or four-fold).

Discussion

Taxonomic history of peromsycine rodents. In the first significant departure from Osgood's (1909) traditional systematic arrangement of peromyscines, Hooper and Musser (1964) formally described several aberrant species of *Peromyscus* as subgenera *(Habromys, Isthmomys,* and *Osgoodomys*), retained the rank of some existing subgenera (*Megadontomys, Podomys,* and *Haplomylomys*), and supported the previous removal of other supraspecific taxa from the genus *Peromyscus* (*Ochrotomys* and *Baiomys*) based mainly on the analysis of phallic morphology. *Peromyscus* and its included subgenera were then depicted as monophyletic relative to other peromyscines (*i.e.*, *Reithrodontomys, Neotomodon, Ochrotomys, Onychomys, Baiomys, and Scotinomys*, in relative order of phylogenetic distance from *Peromyscus*). According to Hooper and Musser (1964:9), the genus *Peromyscus* "becomes a morphologically coherent and probably a close phylogenetic unit".

This arrangement was modified substantially by Carleton (1980), who, based on analysis of a large suite of skeletal and soft anatomical traits, placed *Baiomys* and *Scotinomys* in a separate tribe (Baiomyines), and elevated all the subgenera of *Peromyscus* recognized by Hooper and Musser (1964) with the exception of *Haplomylomys* to genera. This decision was based, in part, on the apparent sister group relationship of the subgenera *Haplomylomys* and *Peromyscus* to *Reithrodontomys*, exclusive of other genera of peromyscines including the other former subgenera (Carleton 1980). The close phylogebetic relationship between *Reithrodontomys* and *Peromyscus* (sensu stricto), however, was not supported by subsequent biochemical (Brownell 1983; Patton *et al.* 1980; Rogers and Engstrom 1992) or chromosomal data sets (Rogers 1983; Rogers *et al.* 1984; Stangl and Baker 1984). Based in part on this new information, Carleton (1989) modified his hypothesis regarding the phylogenetic placement of *Reithrodontomys* relative to the genus *Peromyscus sensu stricto*. However, Carleton (1989) retained the aberrant subgenera (*Habromys, Isthmomys, Megadontomys, Osgoodomys,* and *Podomys*) of Hooper and Musser (1964) as genera, and further restricted the scope of the informal tribe peromycini to those taxa (together with *Neotomodon* and *Onychomys)*, while excluding *Reithrodontomys* and *Ochrotomys*. This latter hypothesis and restricted definition was the starting point for our initial phylogenetic analysis and was the basis for our regarding *Baiomys* and *Scotinomys* (Baiomyini), *Neotoma* (Neotomini), *Ototylomys* (Tylomyini), and *Sigmodon* as outgroups to peromyscines (Musser and Carleton 1993).

Monophyly of Peromyscus. Continued recognition of the genus *Peromyscus* (*sensu stricto*) as a taxonomic entity requires that various data sets support monophyly of the subgenera *Peromyscus* and *Haplomylomys* relative to other peromyscine taxa, including those genera that formerly were regarded as subgenera within *Peromyscus* (sensu lato). This has not been the case. Previous investigations that have included one or more of the subgenera (sensu Hooper and Musser, 1964) together with various representatives of the subgenera *Haplomylomys* and *Peromyscus* have failed to support monophyly of *Peromyscus*. These studies include both phenetically and phylogenetic assessments using allozymes (Avise *et al.* 1979, Patton *et al.* 1980; Schmidly *et al.* 1985), banded chromosomes (Rogers, 1983; Rogers *et al.* 1984; Stangl and Baker, 1984; Yates *et al.* 1979), micro-complement fixation (Fuller *et al.* 1984), as well as mtDNA sequences (Engel *et al.* 1998). Admittedly, taxon sampling for the majority of these studies, including our own, was not robust and failure to sample sufficient taxa can reduce phylogenetic accuracy (Hillis, 1998; Poe, 1998). Nevertheless, our study and that of Stangl and Baker (1984) included all the subgenera of *Peromyscus* (*sensu* Hooper and Musser, 1964) and both fail to support monophyly of *Peromyscus* (*sensu* Carleton,

1980; 1989). Instead, our results indicate that although *Megadontomys* and *Podomys* are outliers, their affinities lie with Clade I (Fig. 1). The only taxon formerly included within *Peromyscus* (sensu Hooper and Musser, 1964) that is not aligned phylogenetically with *Peromyscus* is *Isthmomys*. This taxon, together with two representatives of *Reithrodontomys*, form a monophyletic clade that is the sister group to *Peromyscus* (*sensu lato*). This relationship also was suggested by Patton *et al.* (1980). However, this relationship rests primarily on one fixed, shared derived allele at the highly conservative MD1 locus and we regard the apparent sister-group relationship between *Isthmomys* and *Reithrodontomys* as tantalizing but tentative.

Our data also suggest that *Onychomys, Scotinomys* and *Baiomys* form a clade that represents the sister group to the Peromyscines, and that *Onychomys* is not closely allied to *Peromyscus,* in contrast to relationships hypothesized by previous workers (Brownell, 1983; Dickerman 1992; Engel *et al.*, 1998; Patton *et al.*, 1980). Carleton (1980:122) tentatively placed *Onychomys* in his peromysine group, but noted "An equally plausible hypothesis of relationship is to consider *Onychomys* arising from the stem leading to *Baiomys* and *Scotinomys*." This latter hypothesis is consistent with our data. Likewise Hamilton *et al.* (1992) found that 3 or 4 satellite DNA probes isolated from *P. leucopus* hybridized with chromosomes of 9 species of *Peromyscus* (sensu lato and including *Haplomylomuys* and *Peromyscus (Megadontomys) thomasi*, but these DNA probes did not hybridize with the chromosomes of other "non-peromyscine" taxa including *Baiomys, Ochrotomys, Onychomys or Riethrodontomys.* Their data suggest a peripheral relationship between *Onychomys* and *Peromyscus.* Likewise, *Baiomys, Ochrotomys, and Onychomys* display a copulatory lock (a derived feature – see Voss, 1979). Clearly the phylogenetic position of *Onychomys* relative to *Peromyscus* merits further investigation.

Taxonomic Considerations – Strict interpretation of our data set is inconsistent with the current phylogenetic concept of *Peromyscus* (*sensu stricto*- Carleton, 1980; 1989). As noted by Carleton (1989), systematic estimates dictate two options with regard to defining generic boundaries among peromyscines. One tack would be to circumscribe a broadly defined genus *Peromyscus* to include at least the current genera and subgenera *Habromys, Haplomylomys, Neotomodon, Osgoodomys,* and *Peromyscus. Megadontomys and Podomys* are outliers but still form a monophyletic lineage with *Peromyscus,* and therefore their inclusion in the genus or recognition as separate genera is subjective. Alternatively, the genus *Peromyscus* could be further restricted to a number of monophyletic generic entities. Given the equivocal nature of allozymic data in phylogenetic analysis, including inherent problems of homoplasy in electrophoretic mobilities, polymorphic nature of the data, shallow taxonomic and geographic sampling, and limited number of characters, we do not recommend that current taxonomy be revised based on our data. Nevertheless, our results strongly suggest that the current scope and context of the genus *Peromyscus* is flawed. A more accurate estimate of phyletic relationships will be attained only after additional molecular markers (such as DNA sequences data from multiple, nuclear genes-Graybeal, 1998) are considered together with morphological and perhaps other (behavior, ecological) data sets.

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Appendix

Specimens examined. The 143 specimens examined are listed below by taxa, localities, and museum acronym (Hafner *et al.* 1997). Abbreviations for voucher numbers are as follows: ASNHC = Angelo State Natural History Collections; MVZ = University of California, Berkeley, Museum of Vertebrate Zoology; TCWC = Texas A&M University, Texas Cooperative Wildlife Collections; TTU = Texas Tech University, Museum of Texas Tech University.

Baiomys musculus: Mexico, Veracruz, 2 km S (by road) Cuautlapan, Cerro Chicohuaxtla, 1500 m (MVZ 163058-163060).

Habromys ixtlani: Mexico, Oaxaca, Distrito Ixtlan, 5.2 mi NNE (by road) El Machin, ca. 2600 m (MVZ 159722-159724, 161263, 161265, 161268, 161270-161273, 182897, 182898, 182900).

Isthmomys pirrensis: Panama, Darien Province, 6 km E Cana, E Slope Cerro Pirri (1 TTU).

Megadontomys nelson: Mexico, Veracruz, 3.1 km S (by road) Puerto del Aires, ca. 2,300 m (MVZ 163048-163050).

Neotoma albigula: Mexico, Sonora, 1 mi N Guasimas (MVZ 147647-147650).

Neotomodon alstoni: Mexico, Distrito Federal, 3 mi N Parres (3 ASNHC).

Onychomys torridus: California, San Bernardino Co., 7.5 mi E Boron (1 MVZ), 11.8 mi E Boron, 2,500 ft. (1 MVZ), 2 mi E Searles Station, 9 mi NNE Johannesburg (1 MVZ).

Osgoodomys banderanus: Mexico, Jalisco, 7 mi S El Tuito (TCWC 42814, 42817), 9.5 mi NW Melaque (TCWC 42818-42823).

Ototylomys sp.: Mexico, Chiapas, Pozo de Petroleo, 7 mi N (by road) Berriozabal, 950 m (MVZ 161245, 161246).

Peromyscus boylii: Mexico, Chiapas, 4 mi W (by road) San Cristobal, ca. 2300 m (MVZ 159580-159584).

P. crinitus: California, Inyo Co., Darwin Falls, 4 mi W Paramint Springs, ca. 3000 ft. (MVZ 157081-157084).

P. eremicus: California, Inyo Co., Suprise Canyon, Chris Wick Camp, 3 mi N, 2 mi E Ballarat, ca. 2000 ft. (MVZ 157092-157096).

P. leucopus: Mexico, Quintana Roo, 18.5 km E San Miguel, Isla Cozumel (1 ASNHC), 30 km SE (by road) San Miguel, Isla Cozumel (2 ASNHC), 1 km S, 1 km E San Miguel, Isla Cozumel (1 ASNHC), 20.3 km SE (by road) San Miguel, Isla Cozumel (1 ASNHC).

P. megalops: Mexico, Guerrero, 7 mi SW Filo de Caballo, 8,200 ft (TCWC 43019-43025, 43027-43029, 43031).

P. melanocarpus: Mexico, Oaxaca, Distrito Ixtlán, 16 mi WSW La Esperanza (TCWC 43062-43066).

P. melanotis: Mexico, Distrito Federal, 3 mi N Parres (2 ASNHC).

P. mexicanus: Mexico, Oaxaca, Vista Hermosa, 1,000 m (MVZ 159719-159721, 161282, 161283, 161285, 161286).

P. ochraventer: Mexico, San Luís Potosí, 26 mi W Cuidad Valles (TCWC 43092, 43093).

P. perfulvus: Mexico, Jalisco, 9.5 mi NW Melaque (TCWC 43224, 43217-43220).

P. truei: California, Alameda Co., Grizzly Peak Blvd. at Grizzly Peak (MVZ 157316-157319).

Podomys floridanus: Florida, Archbold Field Station (MVZ 165787-165790).

Reithrodontomys megalotis: California, Mendocino Co., 4.25 mi W, 2.5 mi S Leggett, 1,150 ft. (MVZ 148511-148515).

R. mexicanus: Mexico, Chiapas, 3.9 mi NE (by road) Bochil, ca. 1,200 m (MVZ 159516), 5.1 mi SE Rayon, ca. 1,050 m, (MVZ 159520, 159521).

Scotinomys xerampilinus: Costa Rica, San José Province, 2.2 km E (by rd.) La Trinidad de Dota, 2,600 m (MVZ 164958, 164959, DSR 2237).

Sigmodon hispidus: Mexico, Veracruz, 8.5 mi ENE (by road) Sontecomapan, 25 m (MVZ 182907).