

## PHYLOGENETICS OF SPINY POCKET MICE (GENUS *LIOMYS*): ANALYSIS OF CYTOCHROME *b* BASED ON MULTIPLE HEURISTIC APPROACHES

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We examined phylogenetic relationships among species of *Liomys*, including *L. adpersus* (Panamanian spiny pocket mouse), *L. irroratus* (Mexican spiny pocket mouse), *L. pictus* (painted spiny pocket mouse), *L. salvini* (Salvin's spiny pocket mouse), and *L. spectabilis* (Jaliscan spiny pocket mouse), several species of *Heteromys*, as well as representatives of other genera of heteromyids and 2 geomyids by using 1,140 base pairs of the mitochondrial cytochrome-*b* gene. Gene sequences analyzed under maximum-parsimony (MP), maximum-likelihood (ML), and Bayesian inference optimality criteria converged on essentially identical gene tree topologies. *Liomys* is paraphyletic relative to *Heteromys* and this relationship is well supported, with *L. adpersus* and *L. salvini* arranged as basal taxa relative to *Heteromys*. Our gene trees also recovered *L. pictus* as paraphyletic relative to *L. spectabilis* and these 2 taxa formed the sister group to *L. irroratus*. Constraint trees that held the genera *Heteromys* and *Liomys* as monophyletic (MP and ML criteria) were significantly longer or less likely ( $P < 0.009$  and  $0.046$ , respectively) than our optimal trees, whereas trees that arranged *L. pictus* as monophyletic relative to *L. spectabilis* were not significantly longer ( $P < 0.101$ ) under the MP criterion, but were significantly less likely under the ML criterion ( $P < 0.020$ ).

Key words: cytochrome *b*, *Heteromys*, *Liomys*, monophyly, paraphyly, spiny pocket mice

Spiny pocket mice of the genus *Liomys* occur from Sonora, Mexico, and southern Texas south to Panamá (Schmidly et al. 1993). The major, abiotic factor limiting their distribution most likely is rainfall. Members of the genus *Liomys* are not present in areas receiving <250 mm of annual precipitation nor do they inhabit areas that receive >500 mm annually (see Genoways 1973). In addition, spiny pocket mice rarely are sympatric with congeners. Areas of apparent sympatry usually are manifest as microallopatric distributions. As a result, presence of one species may serve to prevent the range expansion of another (Genoways 1973).

The 1st representatives of the genus *Liomys* were described by Gray (1868) as *Heteromys*, and the genus *Liomys* was not formally recognized until the beginning of the last century (Merriam 1902). Later, Goldman (1911) summarized the taxonomy of the subfamily Heteromyinae and recognized 10 species-level taxa in the genus *Liomys*. Over the next 6 decades the composition of the genus did not change substantially.

Goodwin (1932, 1956) described 2 species (*L. anthonyi* and *L. pinetorum*, respectively) and Hall (1981) subsumed Allen's (1908) *L. vulcani* under *L. salvini*. Based largely on morphological data, Genoways (1973) reduced the number of recognized species of *Liomys* from 11 to 4. He also described a 5th species (the Jaliscan spiny pocket mouse [*L. spectabilis*]). Genoways (1973) proposed that these 5 species, *L. adpersus* (Panamanian spiny pocket mouse), *L. irroratus* (Mexican spiny pocket mouse), *L. pictus* (painted spiny pocket mouse), *L. salvini* (Salvin's spiny pocket mouse), and *L. spectabilis* (Jaliscan spiny pocket mouse) were related as follows: *L. pictus* and *L. spectabilis* formed the *L. pictus* group, *L. salvini* and *L. adpersus* composed the *L. salvini* group (formerly *L. crispus*), and *L. irroratus* represented the *L. irroratus* group. Genoways (1973) also regarded *L. irroratus*, *L. pictus*, and *L. salvini* as polytypic, consisting of 7, 4, and 3 subspecies, respectively.

Based on allozyme data, Rogers (1990) generally supported the hypothesis of Genoways (1973) regarding relationships among members of the genus *Liomys*, but indicated that *L. pictus* was paraphyletic. Rogers and Engstrom (1992) examined allozymic variation among additional populations of *L. pictus* and concluded that genetic variation in or near zones of overlap along the west coast of Mexico for some populations regarded as subspecies of *L. pictus* (*L. p. plantinarenis* and

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*L. p. pictus* near Jalcoctán, Nayarit, and *L. p. hispidus* and *L. p. pictus* in Colima) were segregating as distinct species. In part, their conclusions supported those of Morales and Engstrom (1989), who used morphological characters to describe a zone of contact between *L. p. plantinarenensis* and *L. p. pictus* in Colima, Mexico. Morales and Engstrom (1989) suggested that *L. pictus*, as presently defined, is a composite taxon.

Although morphological variation among heteromyines is well documented (Best 1993; Genoways 1973; Rogers 1986) and *Heteromys* and *Liomys* traditionally have been viewed as sister taxa (Hafner 1993; Hafner and Hafner 1983; Ryan 1989; Wahlert 1985; Wood 1935), this hypothesis has not been tested explicitly. Indeed, examination of data from standard as well as differentially stained chromosomes (Genoways 1973; Mascarello and Rogers 1988; Patton and Rogers 1993) and allozymes (Rogers 1986, 1990) does not support recognition of 2 distinct genera.

The purpose of this study is 2-fold. First, we develop a phylogenetic hypothesis for the genus *Liomys* by using sequence data from the cytochrome-*b* (*Cytb*) gene. Specifically, we test the hypotheses developed by Genoways (1973) and Rogers (1990) of relationships among species within *Liomys*. Second, we include sequence data for multiple populations of *L. irroratus*, *L. pictus*, and *L. salvini*. This allows us to assess intraspecific *Cytb* differentiation and to test the suggestion by previous authors (Morales and Engstrom 1989; Rogers 1986, 1990; Rogers and Engstrom 1992) that *L. pictus* is paraphyletic.

## MATERIALS AND METHODS

**Sampling.**—The mitochondrial *Cytb* gene (1,140 base pairs) was sequenced for 87 mice representing all recognized species in the genus *Liomys*, 1 *Heteromys anomalus* (Trinidad spiny pocket mouse), 2 *H. desmarestianus* (Desmarest's forest spiny pocket mouse), 1 *Perognathus amplus* (Arizona pocket mouse), and 1 *P. flavus* (silky pocket mouse). In addition, we used *Cytb* sequences obtained from GenBank for *Chaetodipus penicillatus* (desert pocket mouse), *C. hispidus* (hispid pocket mouse), *Dipodomys merriami* (Merriam's kangaroo rat), *D. spectabilis* (banner-tailed kangaroo rat), *Heteromys gaumeri* (Gaumer's forest spiny pocket mouse), *Geomys bursarius* (plains pocket gopher), *Microdipodops megacephalus* (dark kangaroo mouse), and *Thomomys bottae* (Botta's pocket gopher). These sequences correspond to *Mus* positions 14139–15282 (GenBank Accession no. J01420—Bibb et al. 1981). Specimen collection in the field followed the guidelines for animal care and use established by the American Society of Mammalogists (Animal Care and Use Committee 1998).

**Data collection.**—Total genomic DNA was extracted from liver tissue either frozen or preserved in 95% ethanol by following the methods of Fetzner (1999), by the phenol–chloroform method, or by using the QIAGEN DNeasy Tissue Kit (catalog no. 69504, Valencia, California). Four microliters of DNA extraction product were electrophoresed on 1.75–2.0% agarose gels stained with ethidium bromide to estimate quality and amount of genomic DNA. If necessary, DNA samples were diluted before polymerase chain reaction (PCR) amplification. The primers L14724 with H15915 (Irwin et al. 1991) or MVZ-05-M and MVZ-14-M (Smith and Patton 1993) were used to amplify the entire mitochondrial *Cytb* gene. Light-strand internal primers used included 700L (Peppers and Bradley

2000), F1 (Whiting et al. 2003), MVZ 45 (Smith and Patton 1993), and MVZ 17 (Smith and Patton 1999), whereas heavy-strand internal primers used were CBH3 (Palumbi 1996), H1514 (Irwin et al. 1991), and MVZ 04 and MVZ 16 (Smith and Patton 1993).

The PCR amplification master mix contained 1.0  $\mu$ l of template DNA (approximate concentration estimated on a 2% agarose gel), 4  $\mu$ l of deoxynucleosidetriphosphates (1.25 mM), 2  $\mu$ l of 10 $\times$  *Taq* buffer, 0.5  $\mu$ l of each primer (100  $\mu$ M), 3  $\mu$ l of MgCl<sub>2</sub> (25 mM), 14  $\mu$ l of distilled water, and 0.25  $\mu$ l of *Taq* polymerase (5 U/ $\mu$ l; Promega Corp., Madison, Wisconsin). Thermal profiles for the majority of PCR reactions were 2–4 min at 94°C, 35–40 cycles (1 min at 94°C, 1 min at 45°C, and 1 min at 72°C), plus 5 min at 72°C. Some samples were more difficult to amplify. In these instances, annealing temperature was reduced to 42°C and cycles were increased to 40. Four microliters of double-stranded PCR-amplified product was assayed by electrophoresis on a 2% agarose gel. The remaining product (about 21  $\mu$ l) was purified either by the QIAquick PCR purification protocol (QIAGEN), the Gene-Clean purification method (Bio 101, La Jolla, California), or by using a Millipore Multiscreen PCR 96-Well Filtration System (catalog no. MANU03050, Millipore, Billerica, Massachusetts). Sequencing was performed by using the Perkin-Elmer ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems, Foster City, California). Excess dye terminator was removed by using a separation column with a solution of Sephadex 50G or by using Millipore Multiscreen Filter Plates for High Throughput Separations (catalog no. MAHVN4510). To verify the correctness of nucleotides sequenced, both strands of each DNA fragment were sequenced. *Cytb* sequences were determined by using either a Perkin-Elmer ABI Prism 377 automated sequencer or ABI 570 Genetic Analyzer (Applied Biosystems) housed in the DNA Sequencing Center at Brigham Young University. Resulting sequences were edited and aligned by using Sequencher versions 3.1 and 4.1.1 (Gene Codes Corporation 2000).

**Data analysis.**—Base frequencies, pairwise uncorrected sequence divergences, and statistical analyses were generated by using PAUP\* 4.0b10 (Swofford 2002). Gene phylogenies were estimated by using maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI). MP analyses were conducted with equal character weighting and 10,000 random addition sequences with tree-bisection-reconnection branch swapping. For MP trees, support for nodes was assessed by using nonparametric bootstrapping (Felsenstein 1985) with 10,000 bootstrap replicates of 100 random addition sequences. Bootstrap values  $\geq 70\%$  were considered well supported (Hillis and Bull 1993). Under the ML criterion, the model of evolution most appropriate for our data was selected by using Modeltest v3.6 (Posada and Crandall 1998). The general time reversible model with invariable sites and rate heterogeneity (GTR+ $\Gamma$ +I) was selected as the best-fit model of nucleotide substitution ( $\pi_A = 0.346$ ,  $\pi_C = 0.308$ ,  $\pi_G = 0.066$ , and  $\pi_T = 0.280$ ;  $r_{CT} = 6.891$ ,  $r_{CG} = 8.360$ ,  $r_{AT} = 0.821$ ,  $r_{AG} = 7.015$ , and  $r_{AC} = 0.455$ ; I = 0.450;  $\alpha = 0.863$ ).

Bayesian analyses were conducted by using MrBayes 3.0b4 software (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). In this methodology, a posterior probability of a phylogeny is estimated by sampling trees from the overall distribution of posterior probabilities. We did not define a priori a model of evolution. Instead, a likelihood-ratio test was performed to compare likelihood scores for each of 56 evolutionary models, partitioned among codon position and by protein domain (Ronquist and Huelsenbeck 2003) for *Cytb* by using MODELTEST v3.6 (Posada and Crandall 1998). Trees were derived by using the (HKY+ $\Gamma$ +I) model of evolution for the 2nd codon position and the (GTR+ $\Gamma$ +I) model for the 1st and 3rd codon positions. Separate series of BI analyses were run incorporating these

models of evolution. In 1 series, codon positions were treated as unlinked. In a 2nd, codon positions were designated as linked. Finally, *Cytb* data partitioned by domain were analyzed. All analyses were conducted 4 times, yielding a total of 12 BI analyses (Nylander et al. 2004). In each BI analysis, Markov chain Monte Carlo was used to sample phylogenies according to their posterior probabilities. This approach was initiated with a random tree and run for  $4 \times 10^6$  generations, with sampling every 1,000th generation. To ensure the Markov chain had become stable, log-likelihood values for sampling points were plotted against generation time. All sample points before stationarity ("burn-in") were omitted before posterior probabilities were calculated (Huelsenbeck and Bollback 2001). Independent analyses (derived from different starting trees) were considered to have converged if their log-likelihood scores approached similar mean values (Leaché and Reeder 2002). Four incrementally "heated" Markov chains were employed to search parameter space more thoroughly. A 50% majority-rule consensus tree was generated in PAUP\* and the percentage of samples recovered in a particular clade was assumed to be that clade's posterior probability (Huelsenbeck and Ronquist 2001). Congruence among independent analyses was determined by evaluating tree topologies and support for stable nodes across the 3 consensus trees. Bayesian posterior probabilities  $> 0.95$  were considered well-supported (Leaché and Reeder 2002).

**Outgroup choice and analysis.**—The *Cytb* sequences from *P. amplius* and *P. flavus*, together with sequences obtained from GenBank (representing all other genera of heteromyid rodents plus 2 genera of geomyids) were used as outgroups (Watrous and Wheeler 1981) in MP, ML, and BI analyses. For the MP analyses, we used a variety of outgroups singly and in various combinations. Initially we used 4 species of *Heteromys* in our analyses but later added additional *Heteromys* species to test whether or not resulting trees converged on the same topology.

**Hypothesis testing.**—Alternative phylogenetic hypotheses were tested with both MP- and ML-based approaches. Tree searches were conducted with constraints designed to match tree topologies for each hypothesis. Differences in tree scores between all equally optimal trees from constrained searches were compared to optimal trees overall by using the Kishino and Hasegawa test (Kishino and Hasegawa 1989) for trees generated by using the MP criterion and the Shimodaira and Hasegawa test (Shimodaira and Hasegawa 1999) with restricted ML as implemented in PAUP\* 4.0b10 (Swofford 2002). Goldman et al. (2000), Buckley (2002), and Strimmer and Rambaut (2002) noted that the Shimodaira–Hasegawa test may be biased in that the number of trees included in the confidence set increases as the number of trees to be compared increases, which results in a conservative test.

## RESULTS

**Sequence divergence.**—Nucleotide composition was similar to those reported for the majority of mammals (Irwin et al. 1991) and no internal stop codons were detected, indicating that the sequences we generated were mitochondrial in origin. There were 568 variable characters in the data matrix, of which 494 were potentially parsimony informative. Mean uncorrected pairwise genetic distances (*P*-values) among species of *Liomys* ranged from 7.75% between samples of *L. adspersus* and *L. salvini* to 19.50% between samples of *L. pictus* and *L. salvini* (Table 1). Among members of the genus *Heteromys*, uncorrected *P* values ranged from 5.30% between 2 populations of *H. desmarestianus* to 15.65% between the

**TABLE 1.**—Pairwise uncorrected *P* values (in percentage) for 8 species in the subfamily Heteromyinae (*Liomys* and *Heteromys*). Numbers in parentheses are *P* values within species; dashes (—) indicate that only a single specimen was examined.

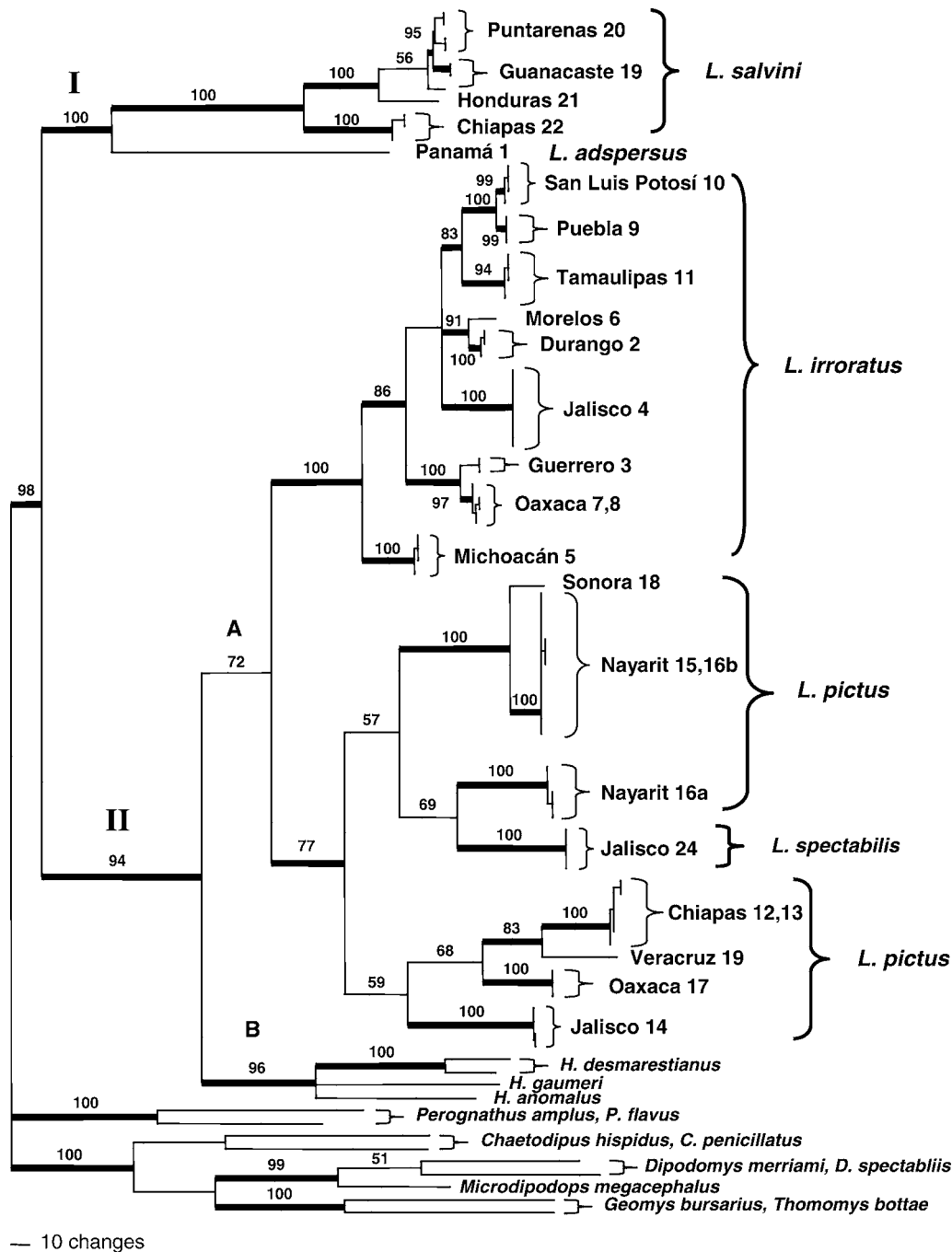
Taxon	Taxon							
	1	2	3	4	5	6	7	8
1 <i>L. adspersus</i>	—							
2 <i>L. irroratus</i>	18.78	(5.17)						
3 <i>L. pictus</i>	19.40	14.73	(10.61)					
4 <i>L. salvini</i>	7.75	19.64	17.10	(2.29)				
5 <i>L. spectabilis</i>	19.50	19.07	12.76	19.07	(0.04)			
6 <i>H. desmarestianus</i>	19.64	19.15	16.45	19.15	16.87	(5.34)		
7 <i>H. anomalus</i>	19.21	19.71	16.44	19.71	16.54	15.65	—	
8 <i>H. gaumeri</i>	20.70	16.45	17.72	20.30	17.20	14.65	15.26	—

sample of *H. desmarestianus* from Mexico and *H. anomalus* from Venezuela (Table 1).

**Interspecific phylogenetics.**—Maximum parsimony analyses with equal character weighting and using outgroup taxa in various combinations all recovered identical consensus tree topologies for the ingroup taxa (*Heteromys* and *Liomys*). In addition, inclusion of *Heteromys australis* (southern forest spiny pocket mouse), *H. desmarestianus*, *H. goldmani* (Goldman's forest spiny pocket mouse), *H. nelsoni* (Nelson's forest spiny pocket mouse), and *H. oresterus* (mountain forest spiny pocket mouse) did not alter tree topologies with respect to placement of *Liomys* species (M. W. González and D. S. Rogers, pers. comm.). Likewise, ML and all BI analyses (4 replicate analyses each partitioned by protein domain [linked versus unlinked] and codon position [linked versus unlinked]) resulted in nearly identical gene trees, regardless of outgroup taxa or taxon sampling of *Heteromys*. Figs. 1 and 2 depict relationships among heteromyine taxa based on MP and BI analyses, respectively. BI replicate analyses generated slightly different posterior probabilities (values available upon request from DSR) than depicted in Fig. 2. Therefore, the posterior probabilities given in Fig. 2 were based on our 1st BI analysis using the unlinked codon position model.

All MP, ML, and BI analyses indicated that members of subfamily Heteromyinae comprised a monophyletic group relative to other heteromyids and this lineage has strong nodal support. Within the heteromyines, 2 strongly supported clades are recovered. Clade I is represented by *L. salvini* and *L. adspersus*, whereas clade II included all other heteromyine taxa (Figs. 1 and 2). In turn, clade II consisted of 2 lineages. Group B represented species of *Heteromys*, with *H. desmarestianus* and *H. gaumeri* arranged as sister taxa relative to *H. anomalus*. Taxa comprising group A included *L. irroratus*, *L. pictus*, and *L. spectabilis*. Samples referable to *L. irroratus* formed a monophyletic group with strong nodal support; however, *L. pictus* was recovered as paraphyletic relative to *L. spectabilis*.

**Intraspecific relationships.**—Genetic divergence (uncorrected *P*-values) among samples of *L. salvini* averaged 2.29% (Table 1). Populations of *L. salvini* from Costa Rica (localities 19 and 20; Fig. 3) and Honduras (locality 21) formed a lineage with strong nodal support (Figs. 1 and 2) compared to *L. salvini* from Chiapas, Mexico (locality 22).



**FIG. 1.**—Maximum-parsimony phylogenetic hypothesis (50% majority rule) for the genus *Liomys* and 3 species of *Heteromys*, rooted with *Chaetodipus hispidus*, *C. penicillatus*, *Dipodomys merriami*, *D. spectabilis*, *Geomys bursarius*, *Microdipodops megacephalus*, *Perognathus amplus*, *P. flavus*, and *Thomomys bottae* as outgroup taxa. Numbers to the right of Mexican states (or Latin American countries) indicate sampling localities (Appendix I). Nodal support is represented by maximum-parsimony bootstrap indices derived from 10,000 pseudoreplicates (above nodes). Nodes for which no values are given were recovered with a frequency > 50%. Nodes in bold are strongly supported by bootstrap values > 70%. Roman numerals I and II and letters A and B refer to groupings as detailed in text.

Among localities of *L. irroratus*, mice from Michoacán, Mexico (locality 5), were recovered as a separate clade relative to all other populations and this result was consistent for all analyses and optimality criteria (Figs. 1 and 2). Kinship among mice representing localities from northeastern Mexico (Puebla, San Luis Potosí, and Tamaulipas) and the Mexican plateau (Durango and Morelos) was strongly supported in all analyses.

However, affinities among mice from Jalisco relative to populations 2, 6, and 9–11 varied among analyses and nodal support for subdivision among these samples was weak.

The mean uncorrected *P* value for pairwise comparisons between populations of *L. pictus* was 10.61% (0–15.31%). Samples of *L. pictus* formed 2 strongly supported clades in ML and BI analyses (Fig. 2), but were less well supported based

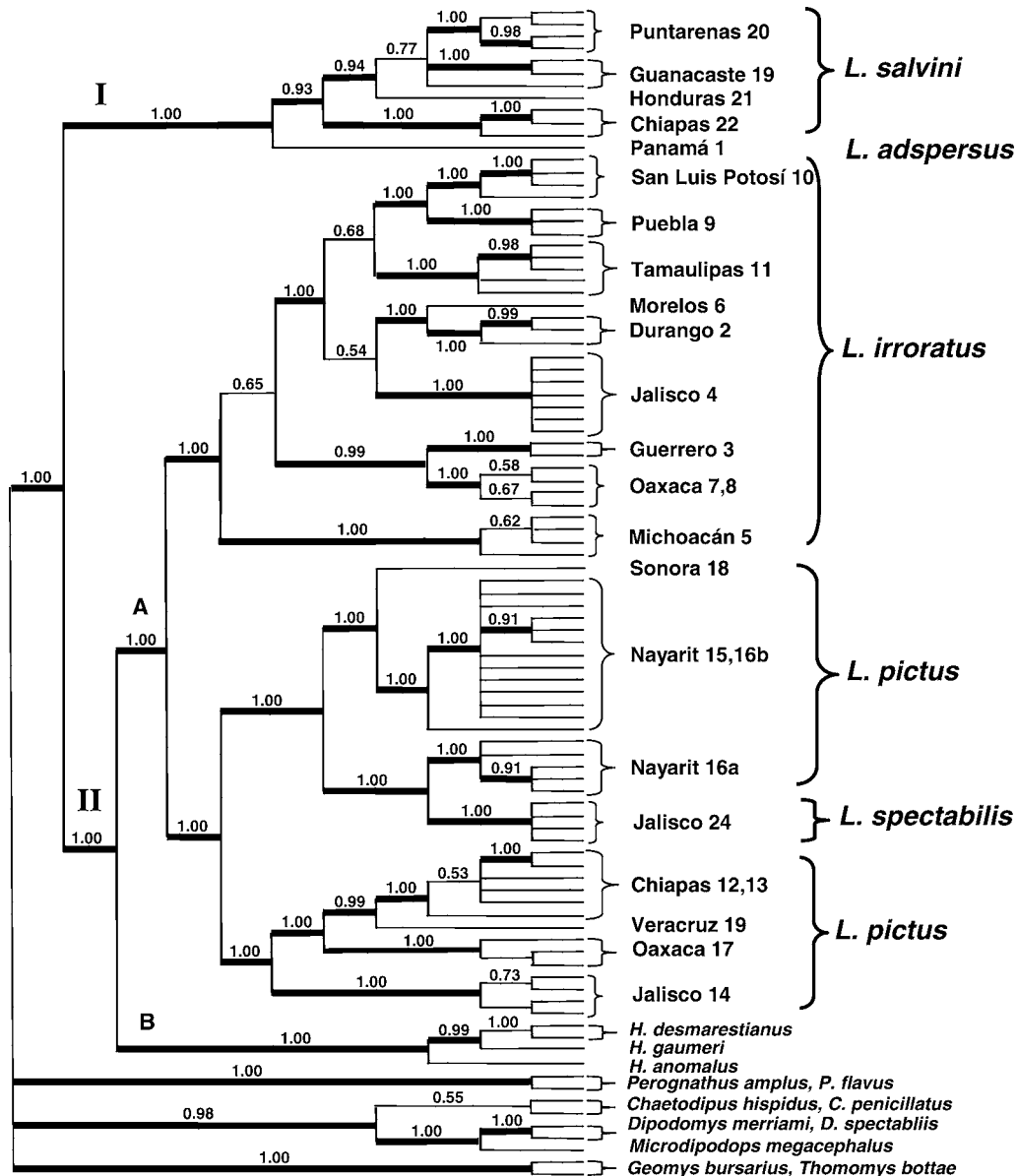


FIG. 2.—Bayesian phylogenetic hypothesis (50% majority rule) for the taxa as in Fig. 1. This tree is derived from analysis of cytochrome-*b* sequence data partitioned by codon position by using the (HKY+Γ+I) model of evolution for the 2nd codon position and the (GTR+Γ+I) model for the 1st and 3rd codon positions. Values above nodes are Bayesian posterior probabilities. Nodes for which no values are given were recovered with a frequency >50%. Nodes in bold are strongly supported (>0.95%). Numbers, roman numerals, and letters are as defined in Fig. 1.

on MP (Fig. 1). Within 1 subclade, samples from locality 16 (Jalcocotán, Nayarit) segregated into 2 lineages. One form (sample 16a) was genetically more similar to *L. spectabilis* (uncorrected *P* value = 9.36%), whereas the other lineage showed kinship with mice from Nayarit and Sonora (localities 15 and 16b; Fig. 3). The 2nd subclade within *L. pictus* was composed of localities 12, 13, 17, and 19 from southern Mexico (Figs. 1 and 2) together with sample 14 to the north and west (Jalisco; Fig. 3). Within this subclade the mean uncorrected *P* value = 10.61% (6.65–13.03%).

**Constraint analyses.**—By using MP and ML optimality criteria, we tested for monophyly of the genus *Liomys* relative to *Heteromys*. Resulting MP consensus trees were significantly longer (15 steps, *P* < 0.009) or had a significantly larger log-

likelihood score (+8.006, *P* < 0.046). Likewise, we tested for monophyly of *L. pictus* compared to *L. spectabilis*. In this case, MP length difference was not significant (11 steps, *P* < 0.101), but the constrained ML tree had a significantly larger score (+20.022, *P* < 0.020).

### DISCUSSION

**Monophyly of the genus *Liomys* and intrageneric relationships.**—Rogers (1986) conducted a cladistic analysis of morphological characters traditionally used to separate *Liomys* from *Heteromys* by using *Peridomys*, the most likely progenitor (Wood 1931, 1935), as the outgroup taxon. He determined that there were no synapomorphic characters available to distinguish

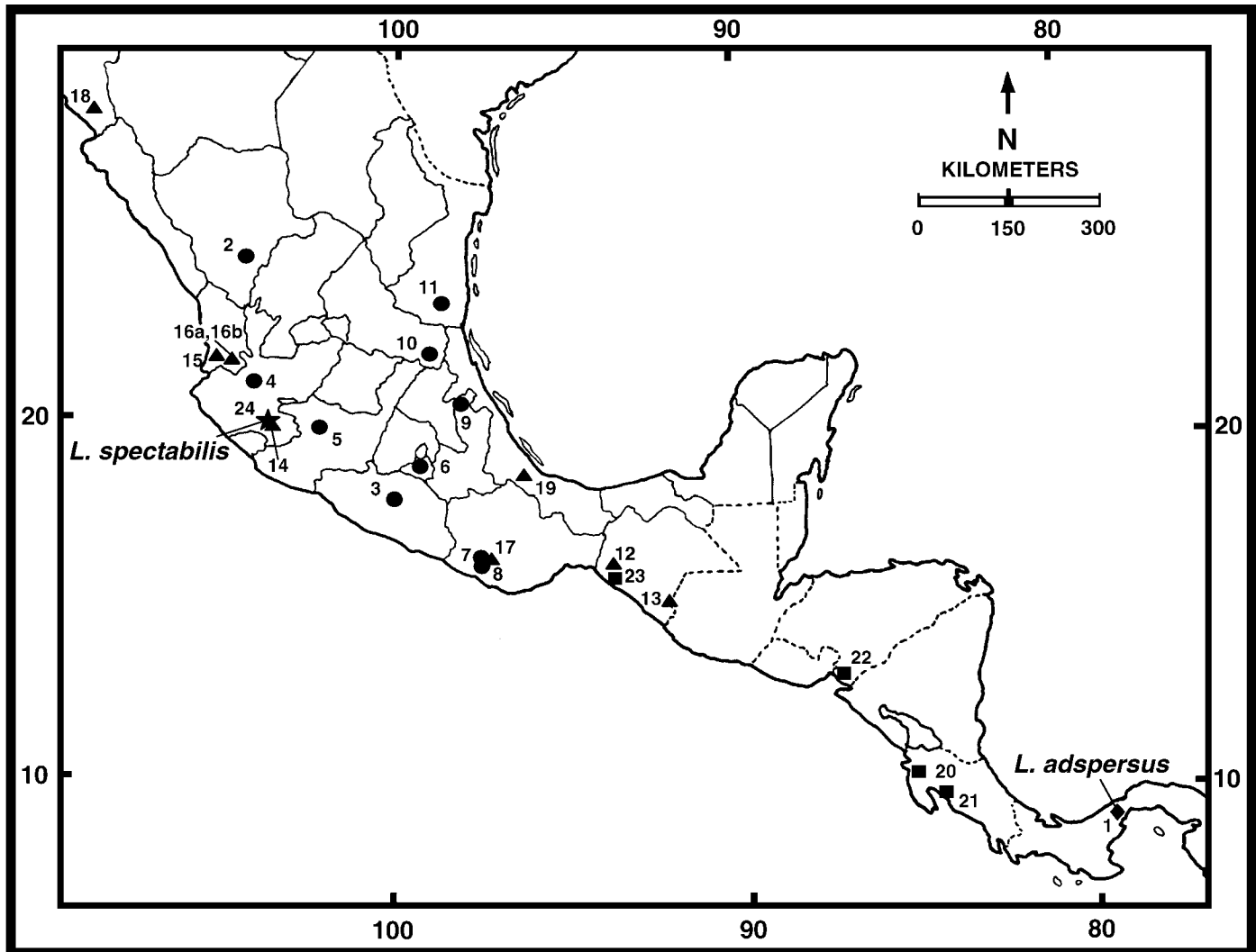


FIG. 3.—Map of Mexico and Central America illustrating the geographic location for samples of *Liomys* included in this study. Numbers are the same as those used in Figs. 1 and 2 and Appendix I. Samples of *L. irroratus* are represented by the symbol ●, *L. pictus* by ▲, and *L. salvini* by ■. Locality 1 is referable to *L. adspersus*, whereas locality 24 represents *L. spectabilis*.

one genus from the other. Rogers (1990) also used allozymes to infer relationships among all species-level taxa within the subfamily Heteromyine. Based on distance–Wagner analyses (Swofford and Selander 1981) of genetic distance values of Rogers (1972), Rogers (1990) resolved members of the genus *Heteromys* as paraphyletic relative to *Liomys*. However, the consensus distance–Wagner tree depicted 8 unresolved lineages (3 representing the genus *Liomys* and 5 of *Heteromys*), leading Rogers (1990:681) to conclude that “genetic data do not support the interpretation that *Heteromys* and *Liomys* represent independent, monophyletic lineages.”

Our data are consistent with this view that *Liomys* is not monophyletic. Specifically, we recovered strong evidence that *Liomys* is paraphyletic relative to *Heteromys* and that the clade comprised of *L. adspersus* and *L. salvini* is basal to all other heteromyine lineages.

*Interspecific differentiation.*—Although the number of species-level taxa recognized within the genus *Liomys* has varied, 3 primary lineages have been recognized: the *L. irroratus* group,

the *L. salvini* (formerly *L. crispus*) group, and the *L. pictus* group. These lineages were delimited primarily on the basis of the number of plantar tubercles on the hind feet (the *L. irroratus* and *L. pictus* groups had 5, or 5 or 6, respectively, and the *L. salvini* group possessed 6). Goldman (1911) initially suggested that the *L. irroratus* and *L. pictus* groups shared a closer affinity with one another relative to the *L. salvini* group; this judgment was based on Merriam’s (1902) view that the *L. salvini* group possessed slightly more complex dentition. However, Genoways (1973) depicted relationships among these 3 groups as unresolved. The consensus distance–Wagner tree depicted by Rogers (1990) also identified these 3 groups within *Liomys* but did not recover the genus as monophyletic. Rogers (1990:681) stated that “. . . relationships among these [3] lineages or their relationship to species of *Heteromys* could not be resolved with genetic [allozyme] data.” Our sequence data provide additional resolution. Although the genus *Liomys* is paraphyletic, we recover strong support for a clade consisting of *L. adspersus*–*L. salvini* (clade I; Figs. 1 and 2) and another comprised of

*L. irroratus*, *L. pictus*, and *L. spectabilis* together with representatives of the genus *Heteromys* (clade II; Figs. 1 and 2).

Paraphyly among populations of *L. pictus* with respect to *L. spectabilis* was first described by Rogers (1986, 1990) based on a limited number of samples and by using allozymes. This finding was confirmed by Rogers and Engstrom (1992), who surveyed allozymic variation among 12 populations of *L. pictus*. *L. spectabilis* is sympatric with *L. pictus* near Contla, Jalisco, and can be distinguished from it morphologically and karyotypically (Genoways 1973). Moreover, *L. spectabilis* differs from the sympatric *L. pictus* in that each is fixed for different alleles at 7 allozyme loci (Rogers and Engstrom 1992). Our results are concordant with these previous studies. *L. spectabilis* differs from the sympatric *L. pictus* from Contla, Jalisco, by about 13.60% (uncorrected p-distance). This population of *L. pictus* is most similar, phylogenetically, to populations of *L. pictus* further south in the Mexican states of Oaxaca, Veracruz, and Chiapas (Fig. 1). In turn, *L. spectabilis* shows closest kinship with the "large" form (locality 16a) of *L. pictus* collected near Jalcocotán in Nayarit. The other entity taken near Jalcocotán (locality 16b) is morphologically smaller and genetically is most similar to *L. pictus* to the north in Sonora, Mexico.

*Species-level phylogenetics.*—We made decisions regarding species boundaries by employing the phylogenetic species concept (Cracraft 1983; Nixon and Wheeler 1990), which defines a species as the smallest group of organisms delimited by a unique combination of character states, within which there is a pattern of ancestry and descent. Because strict application of this species concept can result in recognition of temporarily isolated demes as species, we also applied the tree-based species delimitation method as outlined by Wiens and Penkrot (2002). Accordingly, delimiting species requires concordance among 2 or more independent data sets and can involve both non-tree- and tree-based methods (Marshall and Sites 2003).

The sample of *L. irroratus* from the vicinity of the type locality, Omiltemi, Guerrero, is referable to *L. irroratus guerrerensis*. This taxon formerly was treated as a species-level entity by Goldman (1911). Genoways (1973) recognized that *L. guerrerensis* differed morphologically and occupied a habitat type unique for *Liomys* (cloud forest). However, Genoways (1973) also found that several individuals of *L. irroratus* from the locality nearest to Omiltemi (60 km to the south near Chilpancingo, Guerrero) were intermediate morphologically and thought it best to recognize *L. guerrerensis* as a morphologically well-defined subspecies of *L. irroratus*. Examination of our *Cytb* sequence data demonstrates that samples of *L. irroratus* from western Oaxaca (both from relatively high-elevation sites) form a well-supported clade with examples *L. i. guerrerensis*. Because these mice occupy different habitats and are distinct morphologically and genetically, we hypothesize that they represent a species-level taxon (candidate species A). However, we recommend that formal application of the name *L. guerrerensis* to these populations be deferred until data for the morphologically intermediate samples from near Chilpancingo, Guerrero, are available.

A similar situation exists with respect to our sample of *L. irroratus* from near Pátzcuaro, Michoacán, Mexico. This

population represents mice from the type locality for *L. i. acutus*, originally described by Hall and Villa-R. (1948). Genoways (1973) noted that *L. i. acutus* differed from other populations of *L. irroratus* in that the frequency of divided interparietal bones and posteriorially truncated nasals was much lower. However, he also determined that morphological differences attributable to this taxon either were "non-existent, clinal in nature, or restricted to local situations" (Genoways 1973:100). Therefore, Genoways (1973) subsumed *L. i. acutus*, together with *L. i. canus* and *L. i. pullus*, under the name *L. i. alleni*. Examination of our data demonstrates that *L. irroratus* from Pátzcuaro is genetically distinct and we refer to these populations as candidate species B. However, we believe that additional sampling of *L. irroratus* in this part of Mexico is necessary and should include topotypic material for *L. i. canus*, *L. i. pullus*, and *L. i. alleni*.

Previous investigations (Morales and Engstrom 1989; Rogers 1990; Rogers and Engstrom 1992) documented that *L. pictus* is a composite taxon. For example, 2 forms of *L. pictus* are microallopatric near Jalcocotán, Nayarit. These 2 entities differed ecologically and morphologically, could be distinguished by 7 fixed allozyme differences, and differed reproductively in that only the smaller form (sample 16b) was breeding at the time both were collected. Examination of our *Cytb* data also demonstrates that these 2 forms are divergent (p-distance value between these 2 entities is about 12.2%). However, our level of sampling does not allow us to demarcate the distribution of these 2 forms, nor can we propose a name for either without samples from the type localities of names in synonymy or from currently recognized subspecies of *L. pictus*.

*Liomys pictus annectens* originally was described by Merriam (1902) as *Heteromys annectens*, from Pluma Hidalgo, Oaxaca, Mexico, and later was placed in the genus *Liomys* by Goldman (1911). Genoways (1973) relegated this taxon to a subspecies of *L. pictus* and characterized its distribution as high elevation (more than 750 m) in the Sierra Madre del Sur from Guerrero to Oaxaca, Mexico. Our samples from El Polvorin, Oaxaca, are genetically distinct from other populations of *L. pictus* and match, morphologically, the descriptions of *L. p. annectens* as summarized by Genoways (1973). However, Genoways (1973) demonstrated that some mice from localities between the type locality for *L. p. annectens* and representatives of the smaller, coastal form of *L. pictus* were morphologically intermediate. Therefore, the systematic position of *L. p. annectens* cannot be resolved until samples from coastal and intermediate localities are evaluated.

*Conclusions and prospectus.*—Allozyme data were used to address relationships among heteromyines (Rogers 1990) and presumably these markers indirectly track multiple, independent nuclear genes. However, resolution when using allozymes was relatively poor with regard to the issue of monophyly for *Heteromys* and *Liomys*, whereas examination of our *Cytb* data supports the hypothesis that *Liomys* is paraphyletic relative to *Heteromys*. Examination of karyotypic data (summarized by Patton and Rogers 1993) sheds no light on this issue. Members of the genus *Heteromys* possess a series of derived dental characters, but no synapomorphic morphological characters

unite members of the genus *Liomys* (Rogers 1986) relative to *Heteromys*. Thus, morphological data are not inconsistent with our hypothesis that the genus *Liomys* is paraphyletic. Given the strong nodal support for paraphyly of the genus *Liomys* that we recovered (Figs. 1 and 2), and the fact that forcing monophyly of *Liomys* results in significantly longer (or less likely) trees, we hypothesize that other molecular markers will support this arrangement.

We have clear evidence from both the *Cytb* data presented herein and allozymes (Rogers 1990; Rogers and Engstrom 1992) that *L. pictus* is paraphyletic and likely consists of several species-level taxa. Although not free of difficulties (gene versus species trees), the mitochondrial *Cytb* gene offers the advantage of relatively rapid lineage sorting and therefore can be a superior marker for inferring relationships among closely related taxa (Morondo et al. 2003; Wiens and Penkrot 2002) in the absence of introgression, retention of ancestral polymorphisms, or both (Funk and Omland 2003; Harrison 1991). We are not certain which of the available junior synonyms should be applied to the multiple species-level entities we recovered within *L. pictus* and *L. irroratus*. Therefore, we argue that a formal taxonomic revision of the genus *Liomys* must await additional data from nuclear markers as well as directed sampling of type localities.

## RESUMEN

Nosotros estudiamos las relaciones filogenéticas entre las especies de *Liomys*, incluyendo *L. adpersus* (ratón espinoso de Panamá), *L. irroratus* (ratón espinoso de México), *L. pictus* (ratón espinoso manchado), *L. salvini* (ratón espinoso de Salvin) y *L. spectabilis* (ratón espinoso de Jalisco), varias especies de *Heteromys*, junto a representantes de otros géneros de heterómidos y de geómidos, mediante el uso de 1,140 pares de bases del gen mitocondrial citocromo *b*. Las secuencias analizadas mediante criterios de optimización por parsimonia (MP), máxima verosimilitud (ML) e inferencia Bayesiana convergieron sobre topologías de árboles prácticamente idénticas. *Liomys* es parafiletico respecto a *Heteromys* cuya relación es bien soportada, y con *L. adpersus* y *L. salvini* ubicados como taxa basales en relación a *Heteromys*. Nuestros árboles de genes también recuperaron a *L. pictus* como parafiletico en relación a *L. spectabilis* y estos 2 taxa formaron el grupo hermano de *L. irroratus*. Los árboles constreñimiento que sostienen al género *Heteromys* y *Liomys* como monofiléticos (criterios MP y ML), fueron significativamente más largos o menos probables ( $P < 0.009$  y  $0.046$ , respectivamente) que nuestros árboles óptimos, mientras que los árboles que muestran a *L. pictus* como monofilético en relación a *L. spectabilis* no fueron significativamente más largos bajo el criterio MP ( $P < 0.101$ ), pero fueron significativamente menos probables bajo el criterio de ML ( $P < 0.020$ ).

## ACKNOWLEDGMENTS

F. A. Cervantes (Universidad Nacional Autónoma de México), T. E. Lee, Jr. (Abilene Christian University), J. W. Bickham and R. L. Honeycutt (Texas A&M University), and R. J. Baker (Texas Tech University) kindly supplied tissue samples. Permits for fieldwork

in Mexico were issued by the Secretaria de Medio Ambiente, Recursos Naturales y Pesca (SEMARNAP) to E. Arellano, F. A. Cervantes, and F. X. González-Cozátl. Financial support was provided by National Science Foundation grant DBI-0139501, Professional Development grants, the Department of Integrative Biology and the M. L. Bean Life Science Museum, Brigham Young University (to DSR), and the Office of Research and Creative Works and the Honors Program, Brigham Young University (to VLV). We thank E. Arellano, R. C. Dowler, M. D. Engstrom, F. X. González-Cozátl, N. Lewis-Rogers, R. Mercado, E. Rios, and Q. R. Shurtliff for assistance in the field and M. Pérez-Losada and N. Lewis-Rogers for help with data analysis. Finally, we thank 2 anonymous reviewers for comments that greatly improved our paper.

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Submitted 22 December 2004. Accepted 20 April 2005.

Associate Editor was Eric A. Rickart.

## APPENDIX I

Specimens examined in the molecular analysis listed by taxon, locality number (only members of the genus *Liomys* have numeric designations), collecting locality, museum acronym and voucher specimen number, and GenBank accession number. Locality numbers for *Liomys* specimens are indicated in Figs. 1 and 2 and are mapped in Fig. 3. Museum acronyms are as follows: ACUNHC = Abilene Christian University Natural History Collection; BYU = Monte L. Bean Life Science Museum, Brigham Young University; CM = Carnegie Museum of Natural History; CMC = Colección de Mamíferos, del CEAMISH (Centro de Educación Ambiental e Investigación Sierra de Huautla), Universidad Autónoma del Estado de Morelos; CNMA = Colección Nacional de Mamíferos, Universidad Nacional Autónoma de México; MVZ = Museum of Vertebrate Zoology, University of California, Berkeley; TCWC-AK = Texas Cooperative Wildlife Collection, Texas A&M University; TTU = Natural Science Research Laboratory, Texas Tech University. Sequence data for specimens listed after *Perognathus flavus* were obtained from GenBank (voucher specimen numbers and locality data, if available, are provided).

*Heteromys anomalus*.—VENEZUELA: Miranda, 25 km N Altigracia de Oricuto (TCWC 31844, DQ168468).

*Heteromys desmarestianus*.—HONDURAS: Atlantida (TCWC 51233, DQ168466); MEXICO: Oaxaca, Vista Hermosa, 1,000 m (MVZ 161229, DQ168467).

*Liomys adspersus*.—PANAMA: locality 1: Province Panamá, 1.8 km (by road) N Fort Clayton (MVZ 165784, DQ168469).

*Liomys irroratus*.—MEXICO: locality 2: Durango 25 km E, 22 km S Vicente Guerrero, Municipio Vicente Guerrero, 1,950 m (BYU 15767, DQ168472), 21 km E, 5.8 km N Vicente Guerrero, Municipio Vicente Guerrero, 1,937 m (BYU 15765, DQ168470; BYU 15766, DQ168471); locality 3: Guerrero, 6.1 km SW (by road) Omiltemi, 17°32.950'N, 99°43.260'W, 2,490 m (BYU 20646, DQ168473; CMC 399, DQ168474); locality 4: Jalisco, 24 km W (by road) Ameca, 1,470 m (BYU 16045, DQ168475; BYU 16046, DQ168476; BYU 16047, DQ168477; BYU 16048, DQ168478; BYU 16049, DQ168479; BYU 16050, DQ168480; BYU 16044, DQ168481); locality 5: Michoacán, 10 km S (by road) Pátzcuaro 10°27'35"N, 101°36'27.3"W, 2,200 m (BYU 16051, DQ168482; BYU 16052, DQ168483; BYU 16053, DQ168484; BYU 16055, DQ168485); locality 6: Morelos, Cuernavaca, 18°59.142'N, 99°14.130'W, 2,210 m (CMC 404, DQ168486); locality 7: Oaxaca, La Cumbre, 18.5 km S (by road) Sola de Vega, 16°27.174'N, 97°00.141'W, 2,175 m (BYU 20652, DQ168487; CMC 419, DQ168531); locality 8: Oaxaca, El Polvorín, 5.3 km turn off Lachao Viejo (by road), 16°11.996'N, 97°08.038'W, 1,735 m (CMC 408, DQ168488; CMC 410, DQ168489); locality 9: Puebla, 3.5 miles SW Xicotepec de Juárez (TCWC 41724, DQ168490), 4 miles

SW Xicotepec de Juárez (CM 79450, DQ168491; CM 70451, DQ168492); locality 10: San Luis Potosí, Rancho Plan de la Laja, Xilitilla, 6 km W Xilitla, Municipio Xilitla, 785 m (BYU 15265, DQ168493; BYU 15266, DQ168494; BYU 15267, DQ168495; BYU 15268, DQ168496); locality 11: Tamaulipas, 2.2 miles N Soto la Marina (TCWC 42044, DQ168497; TCWC 42045, DQ168498; TCWC 42046, DQ168499; TCWC 42047, DQ168500; TCWC 42048, DQ168501).

*Liomys pictus*.—MEXICO: locality 12: Chiapas, 7.5 miles SW Ixtápa (TCWC 37055, DQ168504; TCWC 37056, DQ168505; TCWC 37057, DQ168506; AK 4200, DQ168507); locality 13: Chiapas, 5 km NE (by road) Mazapa de Madero, 15°23.558'N, 92°09.857'W, 1,100 m (BYU 20653, DQ168502; CMC 421, DQ168503); locality 14: Jalisco, 3 miles NE Contla (TCWC 42402, DQ168508; TCWC 42403, DQ168509), Rancho Laurel, 1.5 km S Contla, Municipio Tamazula de Gordiano, 1,200 m (CNMA 28194, DQ168511; CNMA 28198, DQ168510); locality 15: Nayarit, 2 km N Chapalilla, Municipio Santa Maria del Oro, 1,020 m (BYU 15768, DQ168521; BYU 15770, DQ168522; BYU 15771, DQ168523; BYU 15773, DQ168524; BYU 15775, DQ168525; BYU 15776, DQ168526; BYU 15777, DQ168527; BYU 15778, DQ168528; BYU 15779, DQ168529; BYU 15781, DQ168530); locality 16a: Nayarit, 2.1 miles E Jalcocotán (TCWC 42226, DQ168513; TCWC 42236, DQ168514; TCWC 42237, DQ168515; TCWC 42240, DQ168516; TCWC 42249, DQ168517); locality 16b: Nayarit, 2.1 miles E Jalcocotán (TCWC 42251, DQ168518; TCWC 42252, DQ168519; TCWC 42253, DQ168520); locality 17: Oaxaca, El Polvorín, 5.3 km turn off Lachao Viejo (by road), 16°11.996'N, 97°08.038'W, 1,735 m (CMC 406 FXG 303, DQ168532; BYU 20654, DQ168533; BYU 20656, DQ168534); locality 18: Sonora (AK 11725, DQ168535); locality 19: Veracruz, Estación Biológica "La Mancha" 30 km N, 3 km E Cardel, Municipio Actopan, 8 m (CNMA 34324, DQ168512).

*Liomys salvini*.—COSTA RICA: locality 20: Guanacaste Province, 3.9 km SE (by road) Playas del Coco (MVZ 164808, DQ168544; MVZ 164809, DQ168545; MVZ 164812, DQ168546); locality 21: Puntarenas Province, Chomes, Finca Mamos, 60 m (BYU 15202, DQ168539; BYU 15203, DQ168541; BYU 15204, DQ168540; BYU 15205, DQ168542); HONDURAS: locality 22: Valle Province, 2.6 miles S, 2.6 miles W Jicaro Galen (TCWC 52263, DQ168543); MEXICO: locality 23: Chiapas, 1.1 miles SE Cabeza de Toro (CM 79513, DQ168536; CM 79514, DQ168537; CM 79515, DQ168538).

*Liomys spectabilis*.—MEXICO: locality 24: Jalisco, 3 miles NE Contla (TCWC 42412, DQ168547; TCWC 42413, DQ168548; TCWC 42405, DQ168549; TCWC 42414, DQ168550).

*Perognathus amplus*.—ARIZONA: Maricopa Co., 14.5 miles S, 80 miles E Yuma (ACUNHC 22, DQ168552).

*Perognathus flavus*.—MEXICO: Coahuila, 66 miles SW Cuatro Ciénegas (TTU 35363, DQ168551).

*Chaetodipus penicillatus*.—AF155868.

*Chaetodipus hispidus*.—TEXAS: Willacy Co., Las Palomas Wildlife Management Area, UTM 14-648509N-2912530E (TTU 77344, AF172832).

*Dipodomys merriami*.—ARIZONA: Maricopa Co., 11.2 km N Gila Bend (TTU 41781, AF173502).

*Dipodomys spectabilis*.—NEW MEXICO: Socorro Co., 51.2 km S, 35.2 km W Socorro (TTU 37019, AF173503).

*Geomys bursarius*.—AF158695.

*Heteromys gaumeri*.—AJ389536.

*Thomomys bottae*.—U65278.