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Phylogenetic relationships among spiny pocket mice (*Heteromys*) inferred from mitochondrial and nuclear sequence data

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In recent years molecular data have been used increasingly to estimate phylogenies and aid in species delimitation. We generated and analyzed sequence data for spiny pocket mice (*Heteromys*) for the mitochondrial gene cytochrome *b* (1,140 base pairs [bp]) and 2 nuclear gene segments, MYH6 (252 bp) and EN2 (189 bp). We used maximum-parsimony, maximum-likelihood, and Bayesian optimality criteria to estimate relationships among species and provide a framework for using a species-delimitation method to investigate the possibility of multiple species within the widespread *H. desmarestianus* group. We recovered several well-supported clades within this complex, including *H. goldmani*, *H. oresterus*, and *H. nubicolens*. Incorporating karyotype, allozyme, and morphological data from earlier studies, we found sufficient supporting evidence to justify maintaining *H. goldmani*, *H. oresterus*, and *H. nubicolens* as species and identifying 4 additional clades as candidate species. We present a revised taxonomic arrangement within the genus. The subgenus *Xylomys* should be retained and be composed of *H. nelsoni*. The subgenus *Heteromys* should be divided into 3 species groups: the *H. anomalus* group (*H. anomalus* and *H. australis*) together with *H. catopterius*, *H. oasicus*, and *H. teleus* (insertae sedis); the *H. gaumeri* group (*H. gaumeri*); and *H. desmarestianus* group (*H. desmarestianus*, *H. goldmani*, *H. oresterus*, *H. nubicolens*, and the 4 candidate species). DOI: 10.1644/09-MAMM-A-287.1.

Key words: cytochrome *b*, EN2, *Heteromys*, molecular phylogeny, MYH6, species boundaries, spiny pocket mice, *Xylomys*

Heteromys (spiny pocket mice in the family Heteromyidae) are exclusively neotropical in distribution, ranging from southern Mexico southward through Central America and into Colombia, Ecuador, and Venezuela. Species of *Heteromys* exhibit strong specificity to particular habitats, which include xeric to pluvial rain forests and montane cloud forests (Anderson 2003; Schmidly et al. 1993). The 1st species of the genus was described as *Mus anomalus* (=*H. anomalus* [Caribbean spiny pocket mouse]), from the island of Trinidad (Thompson 1815). By the mid-1800s 6 species of *Heteromys* were recognized (Gray 1868), but of these only *H. anomalus* and *H. desmarestianus* (Desmarest's spiny pocket mouse) currently are regarded as valid. In the early 1900s 3 new species were added to the genus, and the subgenus *Xylomys* was created to accommodate the morphologically divergent *H. nelsoni* (Nelson's spiny pocket mouse—Merriam 1902). In his revision of the subfamily Heteromyinae (*Heteromys* and *Liomys*) Goldman (1911) described *H. temporalis* and listed a total of 13 species of *Heteromys* divided into 2 subgenera (Table 1). Except for the descriptions of *H. oresterus* (mountain spiny pocket mouse) by Harris (1932) and *H. nigricaudatus* (Goodwin 1956), little systematic treatment was afforded this group for the next several decades. *H. oresterus* was placed with *H. nelsoni* in the subgenus *Xylomys* based on similar habitat preferences, skull and dental characters, and softness of pelage (Hall and Kelson 1959). Later, Hall (1981) summarized the state of affairs by remarking that current taxonomy and species identification keys for the genus *Heteromys* were inadequate. Rogers and Schmidly (1982) attempted to clarify relationships among Middle American members of the *desmarestianus* group (exclusive of *H. gaumeri* [Gaumer's spiny pocket mouse]) using morphology. Their multivariate study of external, cranial, and bacular morphology resulted in synonymy of *H. longicaudatus* and *H. lepturus* with *H. desmarestianus* and the reassignment of *H. temporalis* as a subspecies of *H. desmarestianus*. In addition, Rogers and Schmidly (1982) retained *H. goldmani* (Goldman's spiny pocket mouse) as a member of the *desmarestianus* group. Engstrom et al. (1987) examined karyotypic and morphological variation among populations of *H. gaumeri* and recommended that it be removed from the *H. desmarestianus* species group. More recently, Anderson and Jarrín-V. (2002),

Table 1.—Taxonomic summaries by Goldman (1911), Hall (1981), Williams et al. (1993), and Patton (2005). A dash (—) indicates that the taxon was not recognized by the author(s). This summary does not include *Heteromys nubicolens* (Anderson and Timm 2006) or *H. catopterius* (Anderson and Gutiérrez 2009).

Goldman	Hall	Williams et al.	Patton
Subgenus *Heteromys*	Subgenus *Heteromys*	Subgenus *Heteromys*	Subgenus *Heteromys*
H. anomalus group	*H. anomalus* group		
H. anomalus	*H. anomalus*	*H. anomalus*	*H. anomalus*
H. australis	*H. australis*	*H. australis*	*H. australis*
H. bicolor[a]	—	—	—
H. jesupi[b]	—	—	—
—	—	—	*H. oasicus*
—	—	—	*H. teleus*
H. desmarestianus group	*H. desmarestianus* group		
H. desmarestianus	*H. desmarestianus*	*H. desmarestianus*	*H. desmarestianus*
H. fuscatus	—	—	—
H. gaumeri	*H. gaumeri*	*H. gaumeri*	*H. gaumeri*
H. goldmani	*H. goldmani*	—	*H. goldmani*
H. lepturus	*H. lepturus*[c]	—	—
H. longicaudatus	*H. longicaudatus*[c]	—	—
H. repens	—	—	—
—	*H. nigricaudatus*[d]	—	—
H. temporalis	*H. temporalis*[c]	—	—
—	—	*H. oresterus*	*H. oresterus*
Subgenus *Xylomys*	Subgenus *Xylomys*	Subgenus *Xylomys*	Subgenus *Xylomys*
H. nelsoni	*H. nelsoni*	*H. nelsoni*	*H. nelsoni*
—	*H. oresterus*	—	—

[a] Considered a subspecies of *H. anomalus* (Osgood 1912).
[b] In synonymy with *H. a. anomalus* (Osgood 1912).
[c] In synonymy with *H. desmarestianus* (Rogers and Schmidly 1982).
[d] In synonymy with *H. lepturus* (Goodwin 1969).

Anderson (2003), Anderson and Timm (2006), and Anderson and Gutiérrez (2009) described *H. teleus* (Ecuadoran spiny pocket mouse), *H. oasicus* (Paraguaná spiny pocket mouse), *H. nubicolens* (cloud-dwelling spiny pocket mouse), and *H. catopterius* (overlook spiny pocket mouse), respectively, bringing the number of recognized species to 11 (Patton 2005; Table 1).

Based on his analysis of allozyme data, Rogers (1990) found that *H. desmarestianus* was divisible into 2 groups in southern Mexico but that *H. goldmani* was not strongly differentiated from geographically adjacent populations of *H. desmarestianus*. These data also supported the removal of *H. oresterus* from the subgenus *Xylomys*, prompting Rogers (1990) to suggest that *H. oresterus* be assigned to the *H. desmarestianus* species group. Removal of *H. gaumeri* from the *H. desmarestianus* group, as recommended by Engstrom et al. (1987), also was supported by allozyme data. Anderson et al. (2006) performed the 1st strictly phylogenetic evaluation of relationships among members of the subfamily Heteromyinae by coding the allozyme data from Rogers (1990) as a step matrix following the method of Mabee and Humphries (1993). In addition, Anderson et al. (2006) evaluated 21 morphological characters. Although their resulting phylogenetic trees were largely unresolved, they found weak support for monophyly of the *H. anomalus* group (*H. australis* [the southern spiny pocket mouse] and *H. anomalus*) as a basal clade relative to all other *Heteromys*. However, Anderson et al. (2006) found no support for the reciprocal monophyly of the *H. desmarestianus group* (*H. desmarestianus*, *H. goldmani*, *H. nubicolens*, and *H. oresterus*) relative to *H. gaumeri* and *H. nelsoni*. Recently, Hafner et al. (2007) evaluated basal clades in Heteromyidae using sequence data from 3 mitochondrial genes and including 1 individual each of 5 species of *Heteromys* (*H. anomalus*, *H. australis*, *H. desmarestianus*, *H. gaumeri*, and *H. nelsoni*) and 3 of *Liomys* (*L. irroratus* [Mexican spiny pocket mouse], *L. pictus* [painted spiny pocket mouse], and *L. salvini* [Salvin's spiny pocket mouse]). *H. nelsoni* was recovered as a basal clade relative to the other 4 *Heteromys* species, whereas *Liomys* was paraphyletic. In part, this prompted Hafner et al. (2007) to place *Liomys* in synonymy with *Heteromys*. However, we consider this change subject to evaluation pending a more thorough sampling of heteromyine taxa with multiple, independent data sets, and therefore we continue to use the name *Liomys* in this report.

Taken together, previous systematic studies using allozymes (Anderson et al. 2006; Rogers 1990), morphological characters (Anderson et al. 2006; Engstrom et al. 1987; Hafner and Hafner 1983; Homan and Genoways 1978; Rogers 1986; Rogers and Schmidly 1982; Wood 1935), and sequence data (Hafner et al. 2007) have resulted in conflicting phylogenetic hypotheses. Moreover, no comprehensive phylogenetic analysis based on DNA sequence data has been performed on the genus, nor has there been a clear resolution of kinship among species or potential species-level clades within the *H. desmarestianus* species group. To this end, the objectives of this study were 2-fold. First, we performed phylogenetic analyses based on mitochondrial DNA (mtDNA) and nuclear DNA sequences from a dense sampling of *Heteromys* taxa to

estimate relationships within the genus. Second, we use methods of species delimitation to test whether monophyletic groups within what currently is considered *H. desmarestianus* are sufficiently divergent to be considered species (de Queiroz 1998).

Materials and Methods

Specimens examined and genes sequenced.—A total of 116 specimens representing 7 *Heteromys anomalus*, 3 *H. australis*, 83 *H. desmarestianus*, 3 *H. gaumeri*, 4 *H. goldmani*, 4 *H. nelsoni*, 2 *H. nubicolens*, 6 *H. oresterus*, 2 *Liomys irroratus*, and 2 *L. salvini* was used in this study (Appendix I). Tissue samples were collected from natural populations, or obtained via tissue loans, in Belize, Costa Rica, Guatemala, El Salvador, Honduras, México, Nicaragua, Panama, and Venezuela (Fig. 1) following standard sampling methods approved by the American Society of Mammalogists (Gannon et al. 2007). For this study we used a rapidly evolving mitochondrial gene (cytochrome *b* [*Cytb*]) that provided phylogenetic signal for both intra- and interspecific relationships in previous studies of heteromyine rodents (Anderson and Jansa 2006; Rogers and Vance 2005). We also included 2 more slowly evolving nuclear gene segments; engrailed 2 (EN2) exon 3 and myosin heavy polypeptide 6 cardiac muscle alpha (MYH6) exon 35 and intron 35. These latter 2 loci were used to assess deeper nodes in an evaluation of phylogenetic relationships among species of *Neotoma* (Matocq et al. 2007). In addition to the sequence data generated in this study, *Cytb* sequences for 1 specimen of *H. anomalus* and 2 specimens of *H. desmarestianus* were taken from Rogers and Vance (2005; GenBank accession numbers DQ168468, DQ168466, and DQ168467, respectively), 1 specimen each of *H. desmarestianus* and *H. nubicolens* from Anderson and Jansa (2006; DQ450094 and DQ450090, respectively), and 1 specimen of *H. gaumeri* from Montgelard et al. (2002; AJ389536). *L. irroratus* and *L. salvini* were used as outgroup taxa (Hafner et al. 2007) in all analyses. Sequence data for these species were obtained from Rogers and Vance (2005; DQ168477, DQ168483, DQ168491, DQ168494, DQ168497, and DQ168501 for *L. irroratus* and DQ168540, DQ168542, DQ168545 for *L. salvini*). Tissue samples of *H. oasicus* and *H. teleus* were not available.

DNA extraction, amplification, and sequencing.—The entire mitochondrial *Cytb* gene (1,140 base pairs [bp]), EN2 (189 bp), and MYH6 (252 bp) were amplified and sequenced in both strand directions for this study. Total genomic DNA was extracted from liver, heart, or muscle tissue (either frozen or preserved in 95% ethanol) using the Qiagen DNeasy Tissue Kit (Qiagen Inc., Valencia, California). DNA amplification was accomplished using the polymerase chain reaction (Saiki et al. 1988). Four primers were used to amplify or sequence, or both, the *Cytb* gene in approximately 800-bp segments: L14724 and H15915 (Irwin et al. 1991), CB3H (Palumbi 1996), and F1 (Whiting et al. 2003). Primers MVZ-16 (Smith and Patton 1993), WDRAT 400F (Edwards et al. 2001), H15149 (Irwin et al. 1991), and Neo700L (Peppers and Bradley 2000) were used as necessary to amplify or sequence, or both, smaller segments. Primers for EN2 and MYH6 were those of Lyons et al. (1997). These authors designed primers for MYH2; however, according to Matocq et al. (2007), the sequences obtained using these primers should be regarded as MYH6.

Parameters for polymerase chain reactions were as follows. For *Cytb*, 1 cycle of 94°C (3 min) was followed by 36 cycles of 94°C (1 min) denaturing, 46°C annealing (1 min), and 72°C (1 min) extension; the polymerase chain reaction was concluded by 1 cycle of 72°C (3 min). For EN2, 1 cycle of 94°C (10 min) was followed by 32 cycles of 94°C (1 min) denaturing, 57°C annealing (1 min), and 72°C (1 min) extension. For MYH6, 1 cycle of 94°C (10 min) was followed by 32 cycles of 94°C (1 min) denaturing, 62°C annealing (1 min), and 72°C (1 min) extension. Negative (no DNA) controls were run with all amplifications to reveal instances of DNA contamination. Polymerase chain reaction products were visualized on an agarose gel with ethidium bromide. Successfully amplified products were purified with silica gel using the Gene Clean III Kit or by using a Multiscreen PCR 96-Well Filtration System (Millipore Corp., Billerica, Massachusetts). The purified polymerase chain reaction products were cycle-sequenced using the primers described above, and sequenced products purified using a Sephadex protocol or Multiscreen Filter Plates for High Throughput Separations (Millipore Corp.). Light and heavy strand sequences were collected on an ABI 377 automated sequencer (Applied Biosystems, Foster City, California) and then edited and compiled using Sequencher versions 3.1.1 and 4.1.2 (Gene Codes Corp., Ann Arbor, Michigan). Alignments for *Cytb* and EN2 were unambiguous (no insertions–deletions [indels]) and were performed using Se-Al version 2.0 (Rambaut 2002). MYH6 was aligned using Clustal_X (Thompson et al. 1997). Sequences were submitted to GenBank (GenBank accession numbers GU646919–GU647038 for *Cytb*, GU731466–GU731514 for EN2, and GU657039–GU647048 and GU731427–GU731465 for MYH6; Appendix I).

Phylogenetic analyses of the Cytb data set.—From the total *Cytb* data set of 116 individuals COLLAPSE version 1.2 (Posada 2004) identified 77 unique haplotypes. Phylogenies were estimated for these 77 haplotypes using maximum parsimony (MP) as implemented in PAUP* 4.0b10 (Swofford 2003), maximum likelihood (ML) as implemented in PhyML (Guindon and Gascuel 2003), and Bayesian inference (BI) with Markov chain Monte Carlo sampling for mixed models using MrBayes 3.0b4 (Ronquist and Huelsenbeck 2003). Model selection (both linked and unlinked) for BI was performed for each codon position using the Akaike information criterion (AIC—Akaike 1974) as implemented in MrModeltest version 3.7 (Nylander 2004) and as suggested by Posada and Buckley (2004). The best-fit model of evolution for the 1st and 3rd positions was TrN+I+Γ and HKY+Γ for the 2nd position. Bayesian posterior probabilities (p*P*) were determined by running 20 chains for 20 million Metropolis coupled Markov chain Monte Carlo generations

using the default priors on model parameters. For all analyses 1,000 trees were sampled from the posterior probability distribution (1 every 1,000 generations). A majority-rule consensus tree was produced after discarding the burn-in determined by Tracer version 1.4 (Rambaut and Drummond 2003; available from http://tree.bio.ed.ac.uk/software/tracer/). Each BI analysis was conducted twice, starting from a different, randomly chosen tree. Posterior probabilities for individual clades obtained from independent analyses were compared for congruence (Huelsenbeck and Imennov 2002; Huelsenbeck et al. 2002; Nylander 2004). Percent sequence divergence was estimated for this data set and the combined data set (see below), using both MP and ML as implemented in PAUP* 4.0b10 (Swofford 2003). MP analyses were conducted with equal character weighting with tree-bisection-reconnection branch swapping.

Under the ML criterion the model of evolution most appropriate for the *Cytb* gene was HKY+I+Γ (Hasegawa et al. 1985), as selected using AIC as implemented in Modeltest version 3.6 (Posada and Crandall 1998). The base frequencies were A = 0.3204, C = 0.2897, G = 0.0816, and T = 0.3083; transversion rates were (A–C) 1.0000, (A–G) 15.9820, (A–T) 1.6077, (C–G) 1.6077, and (C–T) 15.982. The proportion of invariable sites (I) was 0.5540, and the gamma distribution shape parameter (Γ) was 1.2844.

Phylogenetic analyses of the EN2 and MYH2 data sets.—From the MP *Cytb* haplotype tree a subset of 46 ingroup terminals was selected and sequenced for the EN2 and MYH6 gene segments following the hierarchical reduced sampling protocol of Morando et al. (2003). The evolutionary models selected by Modeltest and MrModeltest were HKY+I+Γ for EN2 and GTR+Γ for MYH6. These models were used in both the ML and BI analyses.

Phylogenetic analysis of combined data set.—In all but 4 instances the same specimen was sequenced for all 3 genes for the 46 ingroup terminals. In these 4 instances another individual from the same *Cytb* haploclade was used as a substitute. With the exception of a single individual from Panama (*H. desmarestianus* complex clade VII, locality 45; see Appendix I), for which we could not obtain either EN2 or MYH6 sequence, all *Cytb* haploclades were represented in all combined data analyses.

Following Wiens (1998) and Liu and Miyamoto (1999), we analyzed each gene individually and then with all sequences concatenated into a single analysis to explore the extent of congruence and conflict among of data sets more fully. Because of low numbers of parsimony-informative characters in the EN2 and MYH6 gene segments (González 2005) and the computational time required, MP analyses for these gene segments were not performed. For the MP analysis of combined data, parameters were the same as for the *Cytb* haplotype data set. We found 2 gaps (each a single base pair in length) resulting from 2 indel events in MYH6. These 2 gaps each were treated as a 5th state. For the BI analysis *Cytb* partitions or models, or both, and models of evolution for EN2 and MYH6 remained the same.

Under the ML criterion the model of evolution most appropriate for the combined data was (HKY+I+Γ). The base frequencies were A = 0.2883, C = 0.2807, G = 0.1731, and T = 0.2579; transversion rates were (A–C) 2.3510, (A–G) 10.0432, (A–T) 3.8820, (C–G) 0.9356, and (C–T) 32.5061. The proportion of invariable sites (I) was 0.6124, and the gamma distribution shape parameter (Γ) was 1.2170.

Nodal support.—For MP trees branch support (BS) for nodes was estimated using nonparametric bootstrapping (Felsenstein 1985) with 10,000 pseudoreplicates of heuristic searches using tree-bisection-reconnection branch swapping and 100 random sequence additions. Additionally, partitioned Bremer supports (Baker and DeSalle 1997; Baker et al. 1998; Bremer 1988) were calculated using TreeRot, version 2c (Sorenson 1999) to evaluate support for nodes for each gene or gene segment. Negative Bremer support values indicate conflict among gene trees at that particular node. For Bayesian analyses trees not discarded as burn-in were used to construct a 50% majority rule consensus tree, with values representing posterior probabilities. Posterior probabilities (pP) $\geq$0.95 were considered evidence of significant support for a clade (Huelsenbeck and Ronquist 2001). BS support values for ML, reported as bootstrap proportions (BP), were estimated using nonparametric bootstrapping with 1,000 pseudoreplicates in PhyML (Guindon and Gascuel 2003).

Hypothesis testing.—Alternative phylogenetic hypotheses were evaluated for statistical significance using the 1-tailed Shimodaira–Hasegawa test (Shimodaira and Hasegawa 1999) with restricted ML, a method of estimating components of variance as implemented in PAUP* 4.0 (Swofford 2003). Ten thousand bootstrap replicates were performed using the Shimodaira–Hasegawa topology test by resampling the partial likelihoods for each site (RELL model).

Delimiting species boundaries.—The discovery of species is generally recognized as 1 of the 2 stated empirical goals of systematic biology (Wiley and Mayden 2000), and the number of species definitions or concepts is numerous (Baker and Bradley 2006; de Queiroz 1998; Wheeler 1999; Wiley and Mayden 2000). Although these definitions typically allude to the same general idea of what a species is, in many instances workers propose different properties as being the essential determinant of a species. Unfortunately, the majority of species definitions provide no framework with which species boundaries actually could be tested. Recently, this area of systematics has received much needed attention (Sites and Marshall 2003, 2004; Wiens 2007). Although the methods may not agree, progress continues toward viewing species limits as testable hypotheses rather than vague assemblages of characters or de facto thresholds of genetic distance (Bradley and Baker 2001). The DNA tree-based approach of species delimitation of Wiens and Penkrot (Wiens and Penkrot 2002) was selected a priori to delimit species in a hypothesis-testing framework. This method uses a dichotomous key approach in making decisions at the species level and takes advantage of the more rapid coalescing time of mtDNA. A focal species is selected and examined for exclusivity (=monophyly), mean-

ing that haplotypes representing the focal species do not interdigitate with haplotypes representing other entities. If other clusters of haplotypes are recovered as strongly supported basal clades, which are exclusive by locality, those terminals also may be considered a species-level clade. This method can identify cryptic species, particularly when well-supported clades are concordant with geography and may have been overlooked based on morphological comparisons (Wiens and Penkrot 2002).

We selected *H. desmarestianus* as our focal species, because examination of karyotypic (Rogers 1989) and allozyme (Anderson et al. 2006; Rogers 1990) data suggested a strong possibility of multiple species. The method of Wiens and Penkrot (Wiens and Penkrot 2002) was used to delimit species at the deepest level of divergence in the tree, and we followed the protocol of Morando et al. (2003) in defining basal clades as those represented by at least 2 haplotypes and separated beyond the TCS (Clement et al. 2000) network connections (of 95% confidence; 14 steps for this data set—González 2005) among *Cytb* haplotypes. After using the approach of Wiens and Penkrot (Wiens and Penkrot 2002), we incorporated data from previous studies of *Heteromys* (e.g., morphology and karyology) in our determination of species limits. Uncorrected ''p'' distances (*p*-distance) for *Cytb* were generated using PAUP* to allow for comparison of relative sequence divergence among clades but were not used in assessing species-level status.

Results

Phylogenetic analysis of individual genes.—Analyses of the *Cytb* haplotypes data set (1,140 bp) based on MP, ML, and BI optimality criteria converged on essentially the same tree topology, but with less resolution using the MP optimality criterion (results available on request). MP analysis of the *Cytb* haplotypes data set resulted in a single most-parsimonious tree (length = 1,944; consistency index = 0.342; retention index = 0.818, tree not shown). The data matrix contained 461 variable characters, of which 426 were parsimony informative (González 2005). For the ML tree topology, except for clade II, nodal support was high for all species-level taxa and haploclades (Fig. 2). Of the 77 *Cytb* haplotypes evaluated in these analyses, all were recovered as exclusive by locality with a few exceptions. A single individual of *H. anomalus* from Miranda, Venezuela (locality 2 in Fig. 1), grouped with individuals from Sucre (locality 1). Several haplotypes representing *H. desmarestianus* also were found in more than 1 locality; these instances were confined to several locations in the Guatemalan Peten and the Yucatan Peninsula of Mexico (samples 19 and 21; samples 13–15 and 20).

Heteromys anomalus, *H. australis*, *H. gaumeri*, and *H. nelsoni* were recovered as highly supported monophyletic clades ($pP \geq 0.95$, BP = 100, BS $\geq$ 70%). The remaining specimens grouped together in what we refer to as the *H. desmarestianus* complex, composed of 11 clades (Fig. 2), 3 of which currently are recognized as *H. goldmani*, *H. nubicolens*, and *H. oresterus*. Within this complex both ML and BI analyses recovered relationships among clades I, VII, and VIII as (VII–VIII) (I); however, this grouping is not well supported and was not present in the MP tree. Relationships among the remaining clades and substructure within clades were largely concordant across optimality criteria.

Bayesian and ML analyses of EN2 and MYH6 resulted in less-resolved trees in comparison with our analysis of *Cytb* (González 2005). Analyses of EN2 sequences yielded the least-resolved trees, which was not surprising because of few informative characters (13 of 15 variable characters). However, BI and ML analyses of the EN2 data set recovered a clade consisting of a *H. desmarestianus* complex (clades II, III, IV, and V in Fig. 2) together with *H. goldmani*, *H. nubicolens*, and *H. oresterus*. Additionally, clade I was recovered as monophyletic, as was *H. gaumeri*. EN2 analyses did not recover the genus *Heteromys* as monophyletic relative to *Liomys*, although support for this nonmonophyly was not significant. Bayesian analysis of the MYH6 gene segment resulted in a more-resolved consensus tree than did EN2 (González 2005). The MYH6 gene segment yielded 44 variable characters of which 30 were parsimony informative. In addition, 2 single base pair indels were identified within the MYH6 data set. The 1st defined a clade consisting of *Liomys* + *Heteromys*, and the 2nd indel was a synapomorphy for the *H. desmarestianus* species group sensu Goldman (1911) and Hall (1981), which includes the *H. desmarestianus* complex (including *H. goldmani*, *H. nubicolens*, and *H. oresterus*). In both BI and ML analyses of the MYH6 gene *Heteromys* was recovered as monophyletic, as were *H. nelsoni* and the *H. desmarestianus* complex clades IV, VII, and *H. oresterus* (González 2005).

Phylogenetic analysis of combined data set.—Using the qualitative method of evaluating congruence among data sets as described by Wiens (1998), *Cytb*, MYH6, and EN2 tree topologies were compared and instances of incongruence were noted. MYH6 and EN2 topologies were not well resolved, and the majority of nodes in the trees were comprised of polytomies that did not disagree with nodes resolved in the *Cytb* topology. The only incongruence was in the EN2 topology in which a clade composed of *H. australis*, *H. anomalus*, and *Liomys irroratus* was recovered ($pP = 0.78$—data available on request).

Trees estimated from concatenating *Cytb*, MYH6, and EN2 sequence data converged on essentially the same tree topology regardless of the optimality criterion used; differences were the result of less resolution. The ML tree (lnL = −9,765.58; Fig. 3) recovered the same major relationships as the *Cytb* tree (Fig. 2), with the exception that groups I and VII were not associated with each other. This was due, at least in part, to ''clade'' VIII not being represented in the combined analysis. Bayesian p*P* were significant, and ML bootstrap values were high (BP $\geq$ 86) for all species-level taxa and haploclades. Partitioned Bremer support indicated that most of the support for the topology was from the *Cytb* data set; however, 9 of 15 interior nodes were supported by 2 or more genes as indicated by positive Bremer

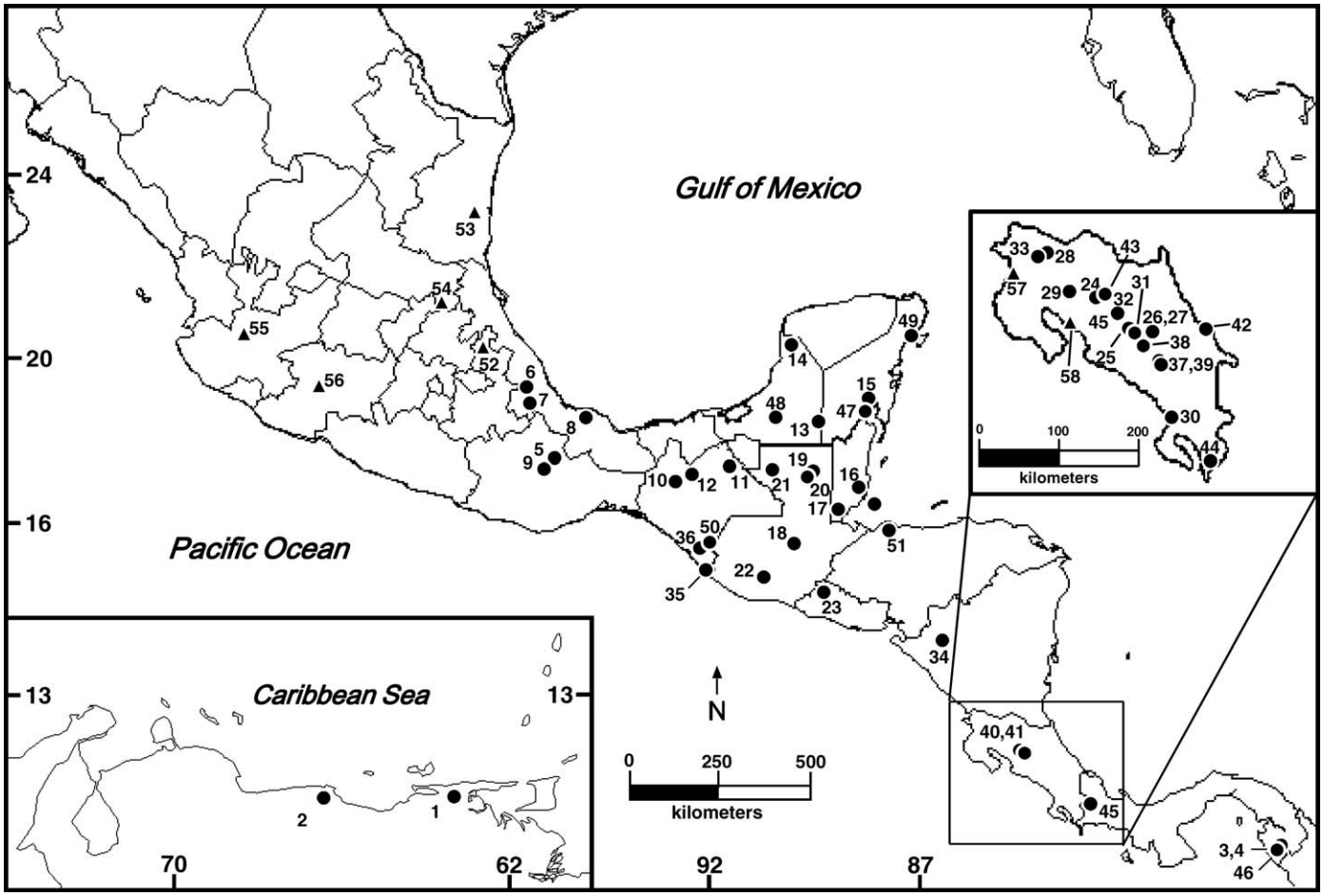

Fig. 1.—Map of Mexico and Central America with an inset of northern South America illustrating the geographic location of samples included in this study. Numbers for localities are the same as those used in Figs. 2 and 3 and Appendix I. Samples of *Heteromys* are represented by ● and *Liomys* by ▲.

support values, including monophyly of the genus *Heteromys* and monophyly of the *H. desmarestianus* group. *H. anomalus*, *H. australis*, and *H. gaumeri* each were recovered as monophyletic groups with high support in the combined analysis ($pP \geq 0.95$, BP = $\geq$ 99). BI and ML analyses supported a clade composed of *H. anomalus* and *H. australis*, but Bremer support for this association was not strong. *H. nelsoni* consistently was recovered as a basal clade relative to the other species in the genus. The remaining clades in the topology comprise the *H. desmarestianus* complex, which includes *H. goldmani*, *H. nubicolens*, and *H. oresterus*. This latter clade also was supported by a single base pair deletion in MYH2. We view the ML topology with Bayesian analysis of the combined data set (Fig. 3) to be our best working hypothesis and use this topology as our tree of reference.

Inferred species boundaries.—Heteromys anomalus, *H. australis*, *H. gaumeri*, and *H. nelsoni* were recovered as strongly supported clades in all analyses, thus supporting their species-level status. Following the dichotomous key presented in Wiens and Penkrot (2002), we determined that our focal species, *H. desmarestianus*, was nonexclusive (i.e., paraphyletic) with respect to 1 or more distinct, exclusive species (*H. goldmani*, *H. nubicolens*, and *H. oresterus*). Based on criteria of the method of Wiens and Penkrot (Wiens and Penkrot 2002), the *H. desmarestianus* complex has 11 distinct haplotype groups exclusive by locality (clades I–VIII and *H. goldmani*, *H. oresterus*, and *H. nubicolens*; Fig. 2).

Hypothesis testing.—Using the Shimodaira–Hasegawa test (Shimodaira and Hasegawa 1999), we tested 8 a priori hypotheses derived from the literature (Table 2) for the *Cytb* and the combined data sets. All constraint tests resulted in significantly less-likely tree topologies with the exception of monophyly of the *H. anomalus* species group for both *Cytb* and the combined data.

Discussion

Relationships among species of Heteromys.—We tested the relationships developed by Rogers (1990) and Anderson et al. (2006) against the tree topologies depicted in Figs. 2 and 3. The relationships among taxa recovered from *Cytb* or the combined gene analyses were significantly more likely than the topology based on allozymes (Rogers 1990) or on the alternative topology based on a combination of allozymes and morphology (Anderson et al. 2006). Aside from the topological differences between our phylogenetic hypothesis and

FIG. 2.—Maximum-likelihood phylogenetic hypothesis (log-likelihood score −1,070.58) for *Heteromys* based on *Cytb* sequence data using the HKY+I+Γ model of evolution and rooted with *Liomys salvini* and *L. irroratus*. Numbers to the right of terminals indicate sampling localities (Appendix I), followed by specimen voucher numbers and abbreviations designating countries in which the samples were collected as follows: BL = Belize; CR = Costa Rica; ES = El Salvador; GU = Guatemala; HN = Honduras; MX = Mexico; NI = Nicaragua; PN = Panama; VZ = Venezuela. Nodal support is represented by maximum-likelihood (ML) bootstrap replicates (1,000 iterations) and bootstrap proportions (BP) above nodes, maximum-parsimony (MP) nonparametric bootstrap pseudoreplicates and values (branch support [BS] ≥70% shown with an asterisk and derived from 10,000 pseudoreplicates) below nodes, and solid circles at nodes indicating Bayesian posterior probabilities (p*P*) ≥0.95%. Bootstrap support for terminal nodes (samples from a single locality) is not shown. Roman numerals refer to clades currently regarded as *H. desmarestianus* and as detailed in text. Letters indicate candidate species as discussed in text.

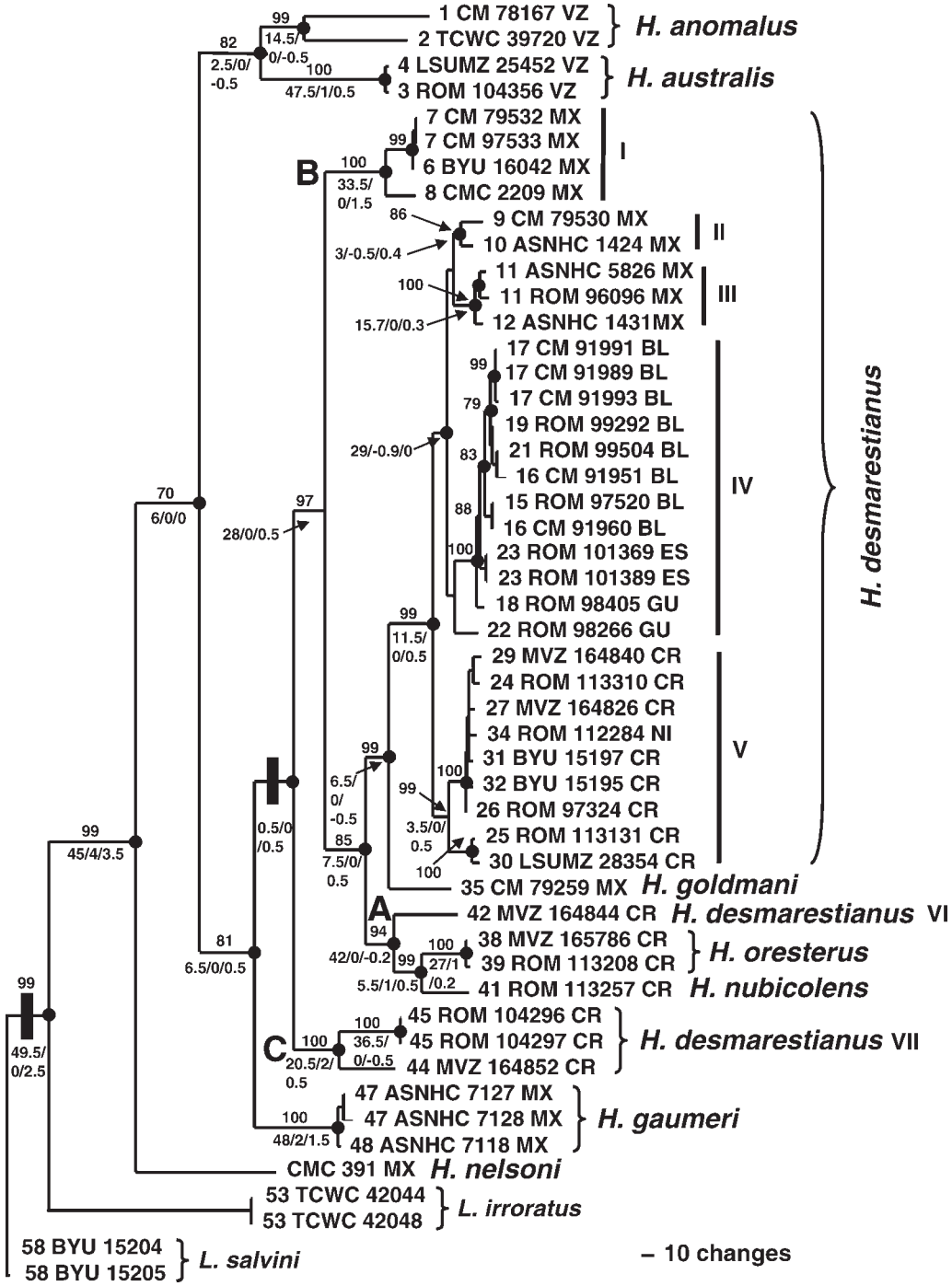

Fig. 3.—Maximum-likelihood phylogenetic hypothesis (log-likelihood score −9,765.58) for the genus *Heteromys* based on combined *Cytb*, MYH6, and EN2 sequence data using the HKY+I+Γ model of evolution and rooted with *Liomys salvini* and *L. irroratus* as outgroup taxa. Numbers to the right of terminals indicate sampling localities (Appendix I), followed by specimen voucher numbers and country abbreviations as in Fig. 2. Closed bars at nodes indicate unique MYH6 indels. Nodal support from maximum-likelihood and Bayesian posterior probabilities is as in Fig. 2. Partitioned Bremer support values (*Cytb*/MYH6/EN2) are provided below nodes. Bootstrap support for terminal nodes is not shown. Roman numerals and letters are as defined in Fig. 2.

those developed earlier, the differences may be due, at least in part, to earlier trees being less resolved because both the allozyme and morphological data sets were composed of fewer characters.

Heteromys nelsoni consistently was recovered as a basal clade relative to the remaining taxa in the genus (Figs. 2 and 3). In his phenetic analysis of morphological and allozyme characters, Rogers (1986, 1990) also recovered *H. nelsoni* as distinct and basal to all other *Heteromys*. No support was found for the subgenus *Xylomys* consisting of *H. oresterus* and *H. nelsoni* (Anderson et al. 2006; Hall 1981). Moreover, forcing a sister-group relationship between *H. nelsoni* and *H.*

Table 2.—Topological tests of a priori hypotheses using Shimodaira–Hasegawa tests (Shimodaira and Hasegawa 1999). Log-likelihood scores for the cytochrome-*b* and combined data trees were −1,070.58 and −9,765.58, respectively. Probabilities ≤0.05 indicate statistical rejection of the respective hypothesis.

Hypothesis	ML cytochrome-*b*		ML combined	
	Log score difference	Probability	Log score difference	Probability
1. Monophyly of *Xylomys* (Anderson et al. 2006:figure3; Hall 1981)	106.92	<0.0001	120.75	<0.0001
2. Phylogeny of *Heteromys* (Rogers 1990:figure 3)	68.65	<0.0001	128.17	<0.0001
3. Phylogeny of *Heteromys* (Anderson et al. 2006:figure 3)	51.04	<0.0001	66.72	<0.0001
4. Nonmonophyly of the *H. anomalus* group (Anderson et al. 2006; Goldman 1911; Hall 1981)	7.73	0.1000	7.86	0.0820
5. Nonmonophyly of the *H. desmarestianus* group (Goldman 1911; Hall 1981)	10.15	0.0350	9.37	0.0410
6. Nonmonophyly of *H. goldmani* (Rogers and Schmidly 1982) relative *H. desmarestianus*	9.12	0.0470	13.60	0.0200
7. Nonmonophyly of *H. nubicolens* (Anderson and Timm 2006) relative to *H. oresterus*	39.18	0.0010	25.69	0.0030
8. Monophyly of *H. oresterus* (Harris 1932) relative to *H. nubicolens*	39.18	0.0010	25.69	0.0030

oresterus resulted in a significantly less-likely tree (Table 2). Based on the results of Rogers (1986, 1990), the present study and the unique karyotype possessed by *H. nelsoni* (2n = 42—Rogers 1989), maintaining the subgenus *Xylomys*, composed only of *H. nelsoni*, is supported (test 1; Table 2).

The *H. anomalus* species group is represented here by *H. anomalus* and *H. australis* and represents a 2nd, basal clade. This arrangement is supported by combined cladistic analysis of morphological and allozyme data by Anderson et al. (2006) but is discordant with allozyme results of Rogers (1990), which revealed no affinity or similarity between *H. anomalus* and *H. australis*. Forcing nonmonophyly of the *H. anomalus* species group did not result in significantly less-likely trees (test 4; Table 2; $P = 0.082$). *H. australis* is restricted in its distribution, known only from humid evergreen forests in eastern Panama, western and Andean Colombia, northwestern Venezuela, and northwestern Ecuador (Anderson 1999, 2003), whereas *H. anomalus* occurs in deciduous and evergreen tropical forests in Colombia, Venezuela, Trinidad, and Tobago (Anderson 2003). Although the test for nonmonophyly was not

Table 3.—Summary of taxonomic recommendations for members of the genus *Heteromys* included in this study and evidence for species-level status. Karyotypic data are summarized by Anderson et al. (2006), Patton and Rogers (1993), and Rogers (1989); allozyme data are from Rogers (1986, 1990); and morphological data are summarized by Rogers and Schmidly (1982), Anderson and Jarrín-V. (2002), Anderson and Timm (2006), and Anderson et al. (2006). Habitat preference is derived from museum collection records and Anderson (1999, 2003).

	Morphologically diagnosable	Habitat preference	Karyotype	Other evidence
Subgenus *Heteromys*				
H. anomalus group				
H. anomalus	Yes	Deciduous humid forests	2n = 60, FN = 68	Allozyme fixed differences
H. australis	Yes	Low- to midelevation humid forests	Unknown	Allozyme fixed differences
H. desmarestianus group				
H. desmarestianus	No	Low- to midelevation humid forests	2n = 60, FN = 67–86	
H. goldmani	Yes	Low- to midelevation humid forests	2n = 60, FN = 78	Allozyme fixed differences
H. oresterus	Yes	Cloud forests	2n = 60, FN = 86	Allozyme fixed differences, chromosome banding data
H. nubicolens	Yes	Cloud forests	2n = 60, FN = 86	Allozyme fixed difference
Candidate species A (clade VI)	Unknown	Low- to midelevation humid forests	2n = 60, FN = 86	Chromosome banding data
Candidate species B (clade I)	Yes	Low-elevation humid forests	2n = 60, FN = 82 or 86	Allozyme fixed differences
Candidate species C (clade VII)	Unknown	Low-elevation humid forests	2n = 60, FN = 90	Allozyme fixed differences, chromosome banding data
Candidate species D (clade VIII)	Unknown	Cloud forests	Unknown	Allozyme fixed differences
H. gaumeri group				
H. gaumeri	Yes	Xeric forests	2n = 56, FN = 76	Allozyme fixed differences
Subgenus *Xylomys*				
H. nelsoni	Yes	Cloud forests	2n = 42, FN = 72	Allozyme fixed differences

significant, evidence from this study does not refute the sister-group relationship between *H. anomalus* and *H. australis*. Moreover, other South American taxa (*H. oasicus*, *H. teleus*, and *H.* sp.) were not included in our analysis. According to Anderson et al. (2006), *H. oasicus* and *H. teleus* share morphological features with *H. anomalus* and *H. australis*. Therefore, we follow Anderson et al. (2006) by placing both taxa in the *H. anomalus* species group (Table 3). In addition, Anderson and Gutiérrez (2009) described *H. catopterius* from Venezuela and indicated that this taxon corresponded to part of the samples considered *H. anomalus* by Anderson et al. (2006). Therefore, we tentatively place this new species in the *H. anomalus* species group.

Individuals of *H. anomalus* included in our analyses did not sort by locality. Specifically, a specimen from Miranda, Venezuela (CM 78170), formed a clade with specimens from Sucre. These results were concordant with those of Rogers (1986), who found 3 fixed allozyme differences between mice from Sucre and most from Miranda; however, CM 78170 from Miranda shared the same alleles with individuals from Sucre. The *p*-distances for *Cytb* between these 2 clades range from 7.8% to 8.0%. This *p*-distance is comparable to the 7.7% sequence divergence between the sister taxa *Liomys adspersus* (Panamanian spiny pocket mouse) and *L. salvini* (Rogers and Vance 2005). Additional sampling within Venezuela is needed to determine if the 2nd clade we recovered represents *H. catopterius* or another species-level entity within *H. anomalus* proper.

Heteromys gaumeri was recovered as the sister clade to the *H. desmarestianus* complex in all analyses, with strong nodal support. However, *H. gaumeri* showed no well-supported kinship with *H. desmarestianus* in allozyme or morphological analyses (Anderson et al. 2006; Rogers 1986, 1990) and has a unique karyotype (2n = 56, FN = 76). Based on allozyme, morphological, and chromosomal differences together with results of our study, we support the recommendation of Engstrom et al. (1987) that *H. gaumeri* be removed from the *desmarestianus* species group and placed in a group of its own.

Species-level phylogenetics in the H. desmarestianus group.—As currently recognized, *H. desmarestianus* is not monophyletic. Nodal support for this complex (8 clades previously referable to *H. desmarestianus* together with *H. goldmani*, *H. nubicolens*, and *H. oresterus*) is significant in terms of Bayesian posterior probabilities but is not particularly strong based on MP or ML bootstrap values. This complex also is defined by a single base pair deletion in MYH6. Forcing nonmonophyly of the *H. desmarestianus* group resulted in significantly less-likely trees for both *Cytb* and the combined data sets (test 5; Table 2).

The tree topology of the *H. desmarestianus* species group has a high level of geographic structure (Fig. 4). Clades I, II, III, and *H. goldmani* are located in southern Mexico (states of Veracruz, Oaxaca, and Chiapas, respectively). Clade IV is composed of specimens restricted to Central America (Mexican states of Campeche and Quintana Roo, Belize, Guatemala, El Salvador, and Honduras); clade V is found in Costa Rica and Nicaragua; *H. oresterus*, *H. nubicolens*, and clade VI are located in Costa Rica; clade VII occurs in eastern Costa Rica and western Panama; and clade VIII is made up exclusively of specimens from the Panamanian Darien.

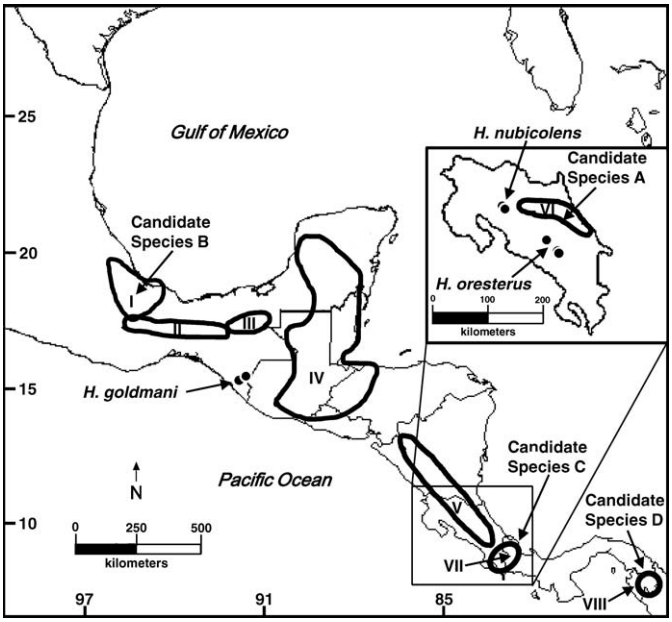

FIG. 4.—Map of Mexico and Central America illustrating the geographic location of species, candidate species, and major clades within the *Heteromys desmarestianus* complex. Roman numerals and letters are those used in Figs. 2 and 3 and the text.

Key to sorting out the nonexclusivity of *H. desmarestianus* is the question of whether *H. goldmani*, *H. nubicolens*, and *H. oresterus* merit species-level status. Otherwise, the *H. desmarestianus* complex could be viewed as a widespread, monophyletic entity with high levels of genetic and karyotypic diversity. Earlier studies all have documented subdivisions within *H. desmarestianus* (Anderson et al. 2006; Rogers 1986, 1989, 1990), and with the range of *H. desmarestianus* spanning nearly 2,000 km from Mexico to eastern Panama and northern South America (Rogers 1986; Williams et al. 1993), geographic extent alone would argue for the possibility of cryptic species. Inasmuch as *H. goldmani* is nested more internally than either *H. nubicolens* or *H. oresterus* (Figs. 2 and 3), we address this taxon first.

Rogers and Schmidly (1982) noted that compared to *H. d. desmarestianus*, *H. goldmani* was darker and lacked a pronounced sprinkling of ochraceous hairs, with several larger cranial features and a smaller, more rounded baculum. Rogers (1986) included additional samples of *H. desmarestianus* and found that although *H. goldmani* averaged larger than *H. desmarestianus* in many cranial features, it fell within the range of morphological variation present in *H. desmarestianus* from southern Mexico. Rogers (1990) determined that *H. goldmani* was not significantly different morphologically from the adjacent populations of *H. desmarestianus*, but he also documented 2 fixed allozyme differences relative to *H. desmarestianus*. In all of our analyses *H. goldmani* was recovered as a strongly supported, monophyletic clade (Figs. 2

and 3) separate from nearby *H. desmarestianus* in Mexico (Fig. 4). *H. goldmani* is genetically divergent from *H. desmarestianus* (*p*-distances ranging from 8.0% to 9.2%) and possesses a unique karyotype (2n = 60, FN = 78—Rogers 1989). Constraint tests for the monophyly of *H. goldmani* relative to adjacent Mexican populations of *H. desmarestianus* result in significantly less likely trees for a nonmonophyletic *H. goldmani* (test 6; Table 2). According to the flow chart for species delimitation (Wiens and Penkrot 2002), Goldman's spiny pocket mouse fits case ''1c.'' Therefore, we regard *H. goldmani* as a species-level taxon and recommend that it remains in the *H. desmarestianus* species group within the subgenus *Heteromys*.

Heteromys oresterus (San José and Cartago provinces, Cordillera Central, Costa Rica) consistently forms a sister-group relationship with individuals of *H. nubicolens* from the nearby provinces of Guanacaste and Puntarenas (Cordillera de Guanacaste and Tilarán; Figs. 2 and 3, localities 40 and 41). This relationship was not recovered by Rogers (1986, 1990), who included a sample of *H. nubicolens* from Monte Verde, Guanacaste Province (then regarded as *H. desmarestianus*), in his allozyme analysis. Likewise, a cladistic analysis of allozyme and morphological data for members of the subfamily Heteromyinae by Anderson et al. (2006) did not recover *H. nubicolens* and *H. oresterus* as sister taxa. However, in contrast to support values in those analyses, nodal support for the sister-group relationship between these 2 taxa is strong across all of our data sets and analyses. *H. oresterus* (FN = 78) differs from *H. nubicolens* (FN = 86) karyotypically and allozymically (7 fixed differences—Rogers 1990), and *Cytb p*-distances between these 2 taxa range from 6.6% to 6.9%. Qualitative morphological differences also exist (Anderson and Timm 2006). Finally, forcing nonmonophyly of samples regarded as either *H. oresterus* or *H. nubicolens* results in significantly less likely trees (tests 7 and 8; Table 2). Both taxa conform to case ''1e'' of the method of Wiens and Penkrot (Wiens and Penkrot 2002). Therefore, we recognize these 2 taxa as valid sister species belonging to the *H. desmarestianus* species group (Table 3).

The clade composed of *H. oresterus* and *H. nubicolens* is divergent genetically from *H. desmarestianus*, clade VI, which is comprised of spiny pocket mice from the Caribbean coastal province of Limón (Fig. 3) and 1 individual from the inland province of Alajuela (Fig. 2). Both *H. oresterus* and *H. nubicolens* occur in cloud forests, whereas individuals representing clade VI inhabit low- or midelevation forests (Table 3). Although the nondifferentially stained karyotypes of both *Heteromys* from coastal Limón and *H. nubicolens* are FN = 86 (cytotype F in Rogers [1989]), the *Cytb p*-distances between the *H. oresterus–H. nubicolens* clade and clade VI range from 10.0% to 10.3%. Likewise, spiny pocket mice from Limón differ by 3 and 7 allozyme fixed differences from individuals representing *H. nubicolens* and *H. oresterus*, respectively (Rogers 1990). These results fit case ''1e'' of the approach of Wiens and Penkrot (Wiens and Penkrot 2002). Therefore, we consider clade VI to represent candidate species A (Figs. 2 and 3).

Clade I forms the sister group to clades II–VI together with *H. goldmani*, *H. nubicolens*, and *H. oresterus* (Fig. 3). Spiny pocket mice representing this clade are known from low-elevation localities in Oaxaca and Veracruz, Mexico (Fig. 4), and possess an FN = 82 or FN = 86 karyotype (Rogers 1989). This clade was recognized by Rogers (1990), who documented 2 allozyme fixed differences distinguishing these lowland populations from other samples of *H. desmarestianus* in Mexico and Honduras. Clade I also was recovered by Anderson et al. (2006) in their analysis of allozyme and morphological data for the subfamily Heteromyinae. Clade I is highly divergent genetically (*Cytb p*-distances range from 11.6% to 15.8%) from all other *Heteromys*, including other samples of *H. desmarestianus*. This pattern is consistent with case ''1c'' of the method of Wiens and Penkrot (Wiens and Penkrot 2002). We refer to this entity as candidate species B (Figs. 2 and 3).

Based on the combined data analyses, clade VII is basal to the rest of the *H. desmarestianus* complex. The Costa Rican and Panamanian samples forming this clade separated into 2 haplotype networks (González 2005), and the *Cytb p*-distance between these 2 subclades was 8.7%. This clade is well supported in all analyses (p*P* = 1.0, BP = 100). Compared with other *Heteromys* in our analyses, *Cytb p*-distances for clade VII range from 12.8% to 17.0%. The Costa Rican individuals included in this study were identified as *Heteromys* sp. by Mascarello and Rogers (1988) and *H. desmarestianus planifrons* by Anderson et al. (2006). Mascarello and Rogers (1988) documented standard and differentially stained karyotypic differences (Table 3) between these samples and both *H. desmarestianus*, from near Tilarán, Province Guanacaste, Costa Rica (clade V), and *H. oresterus*. Compared with other *Heteromys* examined by Rogers (1990), the Costa Rican samples of clade VII were distinguished by 2 fixed allozyme differences. This taxon fits case ''1e'' of the method of Wiens and Penkrot (Wiens and Penkrot 2002). We consider this clade to be candidate species C (Figs. 2 and 3).

Clade VIII is composed of a single individual from Cana, Panama (Fig. 4). Although collected at a locality proximate to *H. australis*, this individual is highly divergent from all other *Heteromys* (*Cytb p*-distances ranged from 10.9% to 15.8%), was identified by Anderson et al. (2006) as *H. d. crassirostris*, and differs from *H. australis* morphologically (Anderson 1999). Unfortunately, this animal was not included in the combined analyses because of our inability to obtain nuclear sequence data. However, this individual also possesses 2 fixed allozymic differences (Rogers 1990) compared with samples of *H. anomalus*, *H. australis*, or *H. desmarestianus*. Although the method of Wiens and Penkrot (Wiens and Penkrot 2002) could not be applied to this entity because it is represented by a single individual, we consider this clade to be candidate species D (Fig. 2).

Within the ''interior'' of the *H. desmarestianus* complex (see Figs. 2–4), there are 2 reciprocally monophyletic groups

that each exhibit substructure. The 1st (clades II, III, and IV) is found in Mexico, Belize, El Salvador, and Guatemala with low FN values (67, 68, or 72). Members of this clade inhabit low- to moderate-elevation tropical forests. The 2nd group (clade V) is mostly Costa Rican and includes 2 localities with an FN = 86 karyotype together with a single individual from Nicaragua. This second group also occupies both low- and moderate-elevation habitats. These low and high FN groups formed independent haplotype networks at the 95% confidence level (González 2005), but the average *Cytb p*-distance between clades II–IV and clade V is moderate (4.9–6.3%). Because our study included only 2 samples of *H. desmarestianus* from the region between these 2 groups (Honduras, clade IV and Nicaragua, clade V), it is possible that more sampling within Central America could provide the haplotypes necessary to connect these 2 clades. However, given the level of karyotypic variation, moderate genetic differentiation, and diversity in habitat preference, we predict that multiple species-level entities exist within these groups.

Recognizing clades for candidate species status has been aided greatly by the advance of DNA sequence data, because it is now possible to find divisions within species that have been overlooked based on gross morphological comparisons. However, this information must be used with caution because DNA sequences sometimes can make it easy to distinguish populations so that some workers feel justified in declaring unique haplotypes a species (Agapow et al. 2004). In addition, incomplete lineage sorting and reliance on a single marker (or linked markers) can lead to spurious phylogenetic reconstructions (Moore 1995). Therefore, we have taken a conservative approach in defining candidate species by following a protocol of tree-based species delimitation and seeking concordance with evidence provided by earlier studies of *Heteromys*. Although use of genetic data is a powerful tool, it is essential to ensure that species designations retain both meaningful and practical value, and this requires incorporating as much supporting data as possible. Unfortunately, the majority of characters helpful in delimiting some species of *Heteromys* involve genetic features rather than discrete and easily discernable external morphological characters that are most useful to field workers.

Our recommendations for *Heteromys* taxonomy are summarized below and in Table 3. We maintain the subgenera *Heteromys* and *Xylomys*, with the latter remaining monotypic (*H. nelsoni*). We divide the subgenus *Heteromys* into 3 species groups: the *H. anomalus* group (*H. anomalus* and *H. australis*), *H. desmarestianus* group (*H. desmarestianus*, *H. goldmani*, *H. nubicolens*, *H. oresterus*, and candidate species A, B, C, and D), and the *H. gaumeri* group (*H. gaumeri*). We tentatively assign *H. teleus* and *H. oasicus* to the *H. anomalus* species group following Anderson et al. (2006) and suggest that a new species of *Heteromys* described by Anderson and Gutiérrez (2009) also should tentatively be placed in the *H. anomalus* group.

Valid names for candidate species cannot be suggested at this time, inasmuch as many names are available either in synonymy or as subspecies of *H. desmarestianus*, and determining the valid name (or proposing a new one) will require additional investigation. Based on their geographic proximity to the localities representing candidate species A, several subspecific names might be applied including *H. d. planifrons* (Goldman 1937), *H. d. underwoodi* (Goodwin 1943), and *H. d. zonalis* (Goldman 1912). Specimens representing candidate species B include individuals collected from the type locality (locality 7 in this study) of *H. temporalis* (Goldman 1911), a name in synonymy with *H. desmarestianus*. However, other names with priority might properly be applied to this taxon, including *H. longicaudatus*, with a type locality of ''Mexico'' (Gray 1868) and defined by Goldman (1911) to match most nearly specimens of *Heteromys* from Montecristo [Emiliano Zapata], Tabasco, and *H. lepturus* (Merriam 1902) described from near Guichicovi, Oaxaca. Genetic data from topotypes would shed light on which, if any, of the available names for this candidate species should be applied. Candidate species C is known from the Pacific lowlands in southern Costa Rica and northern Panama (Fig. 4). Here, the 2 most likely available names are *H. d. chiriquensis* (Enders 1938) and *H. d. repens* (Bangs 1902). Candidate species D is represented by only a single specimen from the Panamanian Darien. The most probable name to be applied to this entity would be *H. d. crassirostris* (Goldman 1912). Clearly, the relatively dense geographic sampling of museum specimens in Mexico and Central America compared with South America will facilitate the morphological revisionary studies necessary to assign names to these candidate taxa.

Resumen

En los años recientes, los datos moleculares han sido utilizados cada vez con mayor frecuencia para estimar relaciones filogenéticas y ayudar en la delimitación de especies. En el presente estudio generamos y analizamos secuencias del gen mitocondrial citocromo *b* (1,140 pb) y segmentos de los genes nucleares MYH6 (252 pb) y EN2 (189 pb) del ratón mochilero (*Heteromys*). Para ello, empleamos los métodos de máxima parsimonia, máxima verosimilitud e inferencia bayesiana como criterios de optimalidad para establecer relaciones interespecíficas y proveer un marco de referencia para la delimitación de posibles especies dentro del grupo *H. desmarestianus*. Obtuvimos varios clados con buen soporte dentro de este complejo, incluyendo a *H. goldmani*, *H. oresterus*, y *H. nubicolens*. Al incorporar datos cariológicos, aloenzimáticos y morfológicos de estudios previos, encontramos suficiente evidencia para mantener a *H. goldmani*, *H. oresterus*, y *H. nubicolens* como aloespecies así como también para identificar 4 clados adicionales como candidatos a especies plenas. En virtud de la evidencia aquí mostrada, presentamos una nueva propuesta para la taxonomía del género. Consideramos que el subgénero *Xylomys* debe mantenerse e incluir a *Heteromys nelsoni*, el subgénero *Heteromys* debe ser dividido en 3 grupos

de especies: *H. anomalus* (*H. anomalus* y *H. australis*) con *H. catopterius*, *H. oasicus*, y *H. teleus* (insertae sedis), *H. gaumeri* (*H. gaumeri*), y *H. desmarestianus* (*H. desmarestianus*, *H. goldmani*, *H. oresterus*, *H. nubicolens*, y las 4 especies candidatas).

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APPENDIX I

Specimens examined.—Heteromys and *Liomys* included in this study. For each voucher specimen we list the museum catalog number (including museum acronyms) as follows: AMNH = American Museum of Natural History; ASNHC = Angelo State Natural History Collections; BYU = Monte L. Bean Life Science Museum, Brigham Young University; CM = Carnegie Museum of Natural History; CMC = Collecion de Mamiferos CIBC (Centro de Investigación en Biodiversidad y Conservación), Universidad Autónoma del Estado de Morelos; KU = University of Kansas Biodiversity Institute; LACM = Los Angeles County Museum of Natural History; LSUMZ = Louisiana State University Museum of Natural Science; MNHN = Muséum National d'Histoire Naturelle; MVZ = Museum of Vertebrate Zoology, University of California, Berkeley; ROM = Royal Ontario Museum; TCWC = Texas Cooperative Wildlife Collection, Texas A&M University. Field collector number, tissue number, or both are also given for each specimen. Specimens are listed by taxon, country, locality number (as in Figs. 1–3), collecting location, and specimen identification numbers. Abbreviations *Cytb*, EN2, or MYH6 indicate which gene or gene segment was sequenced for each individual.

Heteromys anomalus.—VENEZUELA: **locality 2a**: Miranda, 25 km N Altagracia de Orituco, 500 m (CM 78170 = MDE 2087 = AK 3468 [*Cytb* = GU646919], CM 78172 = MDE 2130 = AK 3483 [*Cytb* = GU646920]; TCWC 37494 = MDE 2091 = AK 3472 [*Cytb* = GU646921]); **locality 2b**: Miranda, 40 km N Altagracia (TCWC 39720 = MDE 2129 = AK 3482 [*Cytb* = DQ168468, EN2 = GU731466, MYH6 = GU647039]; **locality 1**: Sucre, 40 km NW Caripito, 250 m (CM 78166 = MDE 1999 = AK 3411 [*Cytb* = GU646922]; CM 78167 = MDE 2033 = AK 3436 [*Cytb* = GU646923, EN2 = GU731467, MYH6 = GU647040], CM 78168 = MDE 2034 = AK 3437 [*Cytb* = GU646924]; CM 78169 = MDE 2062 = AK 3449 [*Cytb* = GU646925]).

Heteromys australis.—PANAMA: **locality 4**: Darién, approximately 6 km NW Cana, E. slope Cerro Pirre, 1,200 m (LSUMZ 25452 = MSH 1187 = TK 22565 [*Cytb* = GU646926, EN2 = GU731468, MYH6 = GU647041]); **1ocality 3**: Darién, Cerro Pirre, Parque Nacional Darien (ROM 104356 = F38215 [*Cytb* = GU646927, EN2 = GU731469, MYH6 = GU647042], ROM 104357 = F38216 [*Cytb* = GU646928]).

Heteromys desmarestianus.—BELIZE: **locality 16a**: Stann Creek District, 3.4 km WNW Quam Bank, Cockscomb Basin (CM 91988 = AK7663 [*Cytb* = GU646929]); **locality 16b**: Stann Creek District, 6.8 km WNW Quam Bank, Cockscomb Basin (CM 91980 = AK7688 [*Cytb* = GU646930]); **locality 16c**: Stann Creek District, 7.7 km WNW Quam Bank, Cockscomb Basin (CM 91960 = AK7664 [*Cytb* = GU646931, EN2 = GU731470, MYH6 = GU647043], CM 91951 = AK 7665 [*Cytb* = GU646932]); **locality 17a**: Toledo District, 1.0 km NNE Salamanca, Forestry Camp, Columbia Forest Reserve

(CM 91991 = AK7540 [*Cytb* = GU646933, EN2 = GU731471, MYH6 = GU647044]); **locality 17b**: Toledo District, 2.4 km NNW Salamanca, Forestry Camp, Columbia Forest Reserve (CM 91989 = AK7555 [*Cytb* = GU646934, EN2 = GU731472, MYH6 = GU647045]); **locality 17c**: Toledo District; 2.1 km NNE Salamanca, Forestry Camp, Columbia Forest Reserve (CM 91993 = AK7588 [*Cytb* = GU646935, EN2 = GU731473, MYH6 = GU647046], CM 91994 = AK7586 [*Cytb* = GU646936], CM 91995 = AK7589 [*Cytb* = GU646937]). COSTA RICA: **locality 24**: Alajuela; 10 km E of Sucre, Parque Nacional, Juan Costro Blanco (ROM 113310 = F48617 [*Cytb* = GU646938, EN2 = GU731474, MYH6 = GU647047], ROM 113311 = F48618 [*Cytb* = GU646939]); **locality 25**: Cartago, Iztaru, Cerros de la Carpintera (ROM 113130 = F48436 [*Cytb* = GU646940], ROM 113131 = F48437 [*Cytb* = GU646941, EN2 = GU731475, MYH6 = GU647048]); **locality 27**: Cartago, Rio Reventazón, 5.6 km SE (by road) Turrialba, 450 m (MVZ 164823 = DSR 2153 [*Cytb* = GU646942], MVZ 164824 = DSR 2154 [*Cytb* = GU646943], MVZ 164825 = DSR 2166 [*Cytb* = GU646944], MVZ 164826 = DSR 2167 [*Cytb* = GU646945, EN2 = GU731476, MYH6 = GU731427], MVZ 164827 = DSR 2246 [*Cytb* = GU646946]); **locality 26**: Cartago, 4 km SE of Turrialba by road, Catie, 600 m (ROM 97324 = FAR 111 [*Cytb* = GU646947, EN2 = GU731477, MYH6 = GU731428], ROM 97325 = FAR 112 [*Cytb* = GU646948]); **locality 33**: Guanacaste, Area de Conservación Guanacaste, approximately 20 km NNE Liberia, Pailas, Sendero Pailas, near Rio Colorado, 800 m (KU 158615 = MK 00-112 [*Cytb* = DQ450094]); **locality 29a**: Guanacaste, 4.1 km NE (by road) Tilarán, 650 m (MVZ 164828 = DSR 2123 [*Cytb* = GU646949], MVZ 164829 = DSR 2124 [*Cytb* = GU646950], MVZ 164830 = DSR 2125 [*Cytb* = GU646951], MVZ 164831 = DSR 2134 [*Cytb* = GU646952], MVZ 164833 = DSR 2141 [*Cytb* = GU646953], MVZ 164835 = DSR 2143 [*Cytb* = GU646954]); **locality 29b**: Guanacaste: 5.0 km NE (by road) Tilarán, 675 m (MVZ 164839 = DSR 2121 [*Cytb* = GU646955], MVZ 164840 = DSR 2122 [*Cytb* = GU646956, EN2 = GU731478, MYH6 = GU731429]); **locality 28**: Guanacaste: Volcán Santa Maria (ROM 113244 = F48551 [*Cytb* = GU646957], ROM 113245 = F48552 [*Cytb* = GU646958]); **locality 30**: Puntarenas, 1 km N, 5 km W Palmar Norte, 33 m (LSUMZ 28354 = MSH 1260 = M1833 [*Cytb* = GU646959, EN2 = GU731479, MYH6 = GU731430]); **locality 31**: San José, Bajo de Iglesia, SW Volcán Irazu, Cascajal de Coronado (BYU 15197 = EA 21 [*Cytb* = GU646960, EN2 = GU731480, MYH6 = GU731431], BYU 15198 = EA 22 [*Cytb* = GU646961]); **locality 32**: San José, Parque Nacional Braulio Carillo, Moravia, Cerro Honduras (BYU 15195 = EA 78 [*Cytb* = GU646962, EN2 = GU731481, MYH6 = GU731432], BYU 15196 = EA 79 [*Cytb* = GU646963]). EL SALVADOR: **locality 23**: Santa Ana, Parque Nacional Montecristi, Bosque Nebuloso, 2,200 m (ROM 101369 = F35547 [*Cytb* = GU646964, EN2 = GU731482, MYH6 = GU731433], ROM 101389 = F35567 [*Cytb* = GU646965, EN2 = GU731483, MYH6 = GU731434]). GUATEMALA: **locality 18**: Baja Verapaz: 5 km E of Puruhla, 1,550 m (ROM 98405 = FN31394 [*Cytb* = GU646966, EN2 = GU731484, MYH6 = GU731435], ROM 98406 = FN31395 [*Cytb* = GU646967], FN31402 [*Cytb* = GU646968]); **locality 19**: El Peten: Biotope, Cerro Cahui, El Remate (ROM 99603 = FN32272 [*Cytb* = GU646969], ROM 99604 = FN32273 [*Cytb* = GU646970]); **locality 20**: El Peten: Campo los Guacamayos, Biotopo Laguna del Tigre, 40 km N El Naranjo (ROM 99469 = FN32318 [*Cytb* = GU646971], ROM 99504 = FN32353 [*Cytb* = GU646972, EN2 = GU731485, MYH6 = GU731436]); **locality 21**: El Peten, Tikal (ROM 99292 = FN31842 [*Cytb* = GU646973, EN2 = GU731486, MYH6 = GU731437], ROM 99293 = FN31843 [*Cytb* = GU646974]); **locality 22**: Sacatepequez, 5 km W San Miguel Duanas, 1,765 m (ROM 98266 = FN31252 [*Cytb* = GU646975, EN2 = GU731487, MYH6 = GU731438], ROM 98265 = FN31254 [*Cytb* = GU646976]). HONDURAS: **locality 51**: Atlántida, Lancetilla (TCWC 52259 = BEL 865 = AK 9696 [*Cytb* = DQ168466]). MEXICO: **locality 14**: Campeche, 10 km N El Refugio (ROM 97050 = FN 30853 [*Cytb* = GU646977], ROM 97051 = FN 30854 [*Cytb* = GU646978]); **locality 13**: Campeche: 25 km N Xpujil (ROM 96089 = FN 29880 [*Cytb* =GU646979]); **locality 10**: Chiapas, 12 km N (by road) Berriozábal (ASNHC 3515 = LAF1689 = MDE 5003 [*Cytb* = GU646980], ASNHC 1424 = ASK 660 [*Cytb* = GU646981, EN2 = GU731488, MYH6 = GU731439], ASNHC 1425 = ASK 689 [*Cytb* = GU646982]); **locality 11a**: Chiapas, 6.6 km S Palenque (ASNHC 1426 = ASK 51 [*Cytb* = GU646983]); **locality 11b**: Chiapas, 9.0 km S Palenque (ASNHC 5826 = ASK 49 [*Cytb* = GU646984, EN2 = GU731489, MYH6 = GU731440]); **locality 11c**: Chiapas, 12.5 km S Palenque (ROM 96096 = FN29887 [*Cytb* = GU646985, EN2 = GU731490, MYH6 = GU731441], ROM 96105 = FN29896 [*Cytb* = GU646986]); **locality 11d**: Chiapas, 1.2 km E Ruinas de Palenque (ASNHC 1440 = ASK 29 [*Cytb* = GU646987], ASNHC 1441 = ASK 31[*Cytb* = GU646988]); **locality 12a**: Chiapas, 6 km E of Rayon, 1,560 m (ROM 97542 = FN 33018 [*Cytb* = GU646989]); **locality 12b**: Chiapas, 9 km SE Rayon (ASNHC 1431 = ASK 589 [*Cytb* = GU646990, EN2 = GU731491, MYH6 = GU731442], ASNHC 1432 = ASK 591 [*Cytb* = GU646991]); **locality 15**: Quintana Roo, 1 km N Noh-Bec (ROM 97520 = FN 30995 [*Cytb* = GU646992, EN2 = GU731492, MYH6 = GU731443], ROM 97521 = FN 30996 [*Cytb* = GU646993]); **locality 9a**: Oaxaca, Vista Hermosa, 1,000 m (CM 79530 = DSR 934 = AK 3108 [*Cytb* = GU646994, EN2 = GU731493, MYH6 = GU731444]); **locality 9b**: Oaxaca; Distrito Ixtlán, Vista Hermosa, 1,000 m (MVZ 161229 = DSR 1685 [*Cytb* = DQ168467], MVZ 161230 = DSR 1686 [*Cytb* = GU646995]). NICARAGUA: **locality 34**: Esteli, Esteli (ROM 112284 = F48170 [*Cytb* = GU646996, EN2 = GU731494, MYH6 = GU731445]).

Heteromys gaumeri.—MEXICO: **locality 48**: Campeche, 7 km N Escarcega (ASNHC 7118 = FN 32736 [*Cytb* = GU646997, EN2 = GU731495, MYH6 = GU731446]); **locality 47**: Quintana Roo, 7 km NE Xul-Ha (ASNHC 7127 = FN32575 [*Cytb* = GU646998, EN2 = GU731496, MYH6 = GU731447], ASNHC 7128 = FN32576 [*Cytb* = GU646999, EN2 = GU731497, MYH6 = GU731448]); **locality 49**: Quintana Roo, Puerto Morelos (MNHN 2000-234 [*Cytb* = AJ389536]).

Heteromys goldmani.—MEXICO: **locality 36**: Chiapas El Triunfo, 10 km SSE Finca Prusia (LACM 74200 = LJB 3108 = LAF1773 [*Cytb* = GU647000], LACM 74201 = LJB 3109 = LAF1774 [*Cytb* = GU647001], ASNHC 3523 = MDE 5049 = LAF1790 [*Cytb* = GU647002]); **locality 35**: Chiapas: 15.5 miles SE (by road) Mapastepec, 150 feet (CM 79529 = MDE 1224 = AK 3150 [*Cytb* = GU647003, EN2 = GU731498, MYH6 = GU731449]).

Heteromys nelsoni.—MEXICO: **locality 50**: Chiapas, Cerro Mozotal, 15°25.866′N, 92°20.274′W, 2,930 m (CMC 391 = DSR 7181 [*Cytb* = GU647012, EN2 = GU731502, MYH6 = GU731453], CMC 398 = DSR 7212 [*Cytb* = GU647015], BYU 20643 = DSR 7187 [*Cytb* = GU647013], BYU 20644 = DSR 7189 [*Cytb* = GU647014]).

Heteromys nubicolens.—COSTA RICA: **locality 40**: Guanacaste: Monteverde, Campbell's Woods (MVZ 161225 = DSR 1745 [*Cytb* = GU647010]); **locality 41a**: Puntarenas, Monte Verde Biological Station (ROM 113257 = F48564 [*Cytb* = GU647011, EN2 = GU731501, MYH6 = GU731452]); **locality 41b**: Puntarenas,

Monteverde, Monteverde Cloud Forest Reserve, Cerro Amigos, 1,800–1,840 m (KU 159101 = CMM 222 [*Cytb* = DQ450090]).

Heteromys oresterus.—COSTA RICA: **locality 37**: Cartago, Villa Mills, 4 km by road Pan American Highway, Catie Project (ROM 102753 [*Cytb* = GU647004]); **locality 39**: San José: Cerro la Muerte, San Gerardo de Dota (ROM 113208 = F48514 [*Cytb* = GU647005, EN2 = GU731499, MYH6 = GU731450], ROM 113229 = F48535 [*Cytb* = GU647006]); **locality 38**: San José, 2.2 km E (by road) La Trinidad de Dota, 2,600 m (MVZ 164861 = DSR 2092 [*Cytb* = GU647007], MVZ 164863 = DSR 2107 [*Cytb* = GU647008], MVZ 165786 = DSR 2244 [*Cytb* = GU647009, EN2 = GU731500, MYH6 = GU731451]).

Heteromys candidate species A.—COSTA RICA: **locality 43**: Alajuela: 7 road km NE Quesada, 2,297 m (LSUMZ 26357 = DJH 2469 = M607 [*Cytb* = GU647016]); **locality 42**: Limón: 4.6 km W (by road) Limón, 25 m (MVZ 164844 = DSR 2150 [*Cytb* = GU647017, EN2 = GU731503, MYH6 = GU731454], MVZ 164845 = DSR 2151 [*Cytb* = GU647018], MVZ 164846 = DSR 2155 [*Cytb* = GU647019], MVZ 164850 = DSR 2173 [*Cytb* = GU647020], MVZ 164851 = DSR 2245 [*Cytb* = GU647021]).

Heteromys candidate species B.—MEXICO: **locality 5**: Oaxaca, 23 miles SSW (by road) Tuxtepec, 250 feet (AMNH 254697 = DSR 936 = AK 3110 [*Cytb* = GU647022]); **locality 7**: Veracruz, 1 mile NW Motzorongo, 700 feet (CM 79532 = DSR 922 = AK 3099 [*Cytb* = GU647023, EN2 = GU731504, MYH6 = GU731455], CM 79533 = DSR 923 = AK 3100 [*Cytb* = GU647024, EN2 = GU731505, MYH6 = GU731456]); **locality 6a**: Veracruz, Ojo de Agua (AMNH 254694 = MDE 1010 [*Cytb* = GU647025]); **locality 6b**: Veracruz: Ojo de Agua, 1400 ft (AMNH 254693 = DSR 921 = AK 3098 [*Cytb* = GU647026]); **locality 6c**: Veracruz; Ojo de Agua, 18°55′35.6″N, 96°52′58.8″W, 600 m (BYU 16042 = EA 823 [*Cytb* = GU647027, EN2 = GU731506, MYH6 = GU731457], BYU 16043 = EA 836 [*Cytb* = GU647028]); **locality 8**: Veracruz, 13.0 km NW (by road) Sontecomapán, 18°35.080′N, 95°04.525′W, 40 m (CMC 2209 = DSR 8551 [*Cytb* = GU647029, EN2 = GU731507, MYH6 = GU731458]).

Heteormys candidate species C.—COSTA RICA: **locality 44**: Puntarenas, 1.1 km SE (by road) Ciudad Nielly, 25 m (MVZ 164852 = DSR 2193 [*Cytb* = GU647030, EN2 = GU731508, MYH6 = GU731459], MVZ 164854 = DSR 2195[*Cytb* = GU647031], MVZ 164865 = DSR 2222 [*Cytb* = GU647032]). PANAMA: **locality 45**: Chiriqui, Ojo de Agua, 2 km N Santa Clara (ROM 104296 = F38147 [*Cytb* = GU647033, EN2 = GU731509, MYH6 = GU731460], ROM 104297 = F38148 [*Cytb* = GU647034, EN2 = GU731510, MYH6 = GU731461]).

Heteromys candidate species D.—PANAMA: **locality 46**: Darién, approximately 7 km NW Cana, E slope Cerro Pirre, 1,500 m (LSUMZ 25451 = DJH 2427 [*Cytb* = GU647035]).

Liomys irroratus.—MEXICO: **locality 55**; Jalisco, Ameca (BYU 16047 = EA 816 [*Cytb* = DQ168477]); **locality 56**: Michoacán, 10 km S (by road) Pátzcuaro 10°27′35″N, 101°36′27.3″W, 2,200 m (BYU 16052 = DSR 5215 [*Cytb* = DQ168483]); **locality 52**: Puebla, 4 miles SW Xicotepec de Juarez (CM 79450 = AK 3083 [*Cytb* = GU647036]); **locality 54**: San Luis Potosí, Rancho Plan de la Laja, Xilitlilla, 6 km W Xilitla, Município Xilitla, 785 m (BYU 15266 = EA 223 [*Cytb* = DQ168494]); **locality 53**: Tamaulipas, 2.2 miles N Soto la Marina (TCWC 42044 = AK 4335 [*Cytb* = GU647037, EN2 = GU731511, MYH6 = GU731462], TCWC 42048 = AK 4339 [*Cytb* = GU647038, EN2 = GU731512, MYH6 = GU731463]).

Liomys salvini.—COSTA RICA: **locality 57**: Guanacaste, 3.9 km SE (by road) Playas del Coco (MVZ 164809 = DSR 2128 [*Cytb* = DQ168545]); **locality 58**: Puntarenas, Finca Mamos, Chomes, 60 m (BYU 15204 = EA 63 [*Cytb* = DQ168540, EN2 = GU731513, MYH6 = GU731464], BYU 15205 = EA 64 [*Cytb* = DQ168542, EN2 = GU731514, MYH6 = GU731465]).