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Molecular phylogenetics of the *Handleyomys chapmani* complex in Mesoamerica

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Handleyomys chapmani (Chapman's Handley's mouse) is a Mexican endemic rodent inhabiting humid montane forest of the Sierra Madre Oriental (SMO), the Oaxacan Highlands (OH), and the Sierra Madre del Sur (SMS). The systematic status of populations currently classified as *H. chapmani* has been problematic and to date evolutionary relationships among populations remain unresolved. In this study we use sequences from the mitochondrial cytochrome-*b* gene (1,143 base pairs [bp]) and intron 7 of the beta fibrinogen gene (621 bp) to reconstruct a phylogeny, estimate divergence times, and assess patterns of sequence variation over geography among samples of *H. chapmani*. This species was recovered as 2 monophyletic clades corresponding to the SMO-OH and SMS mountain ranges. Moreover, *H. saturatior*, the purported sister taxon to *H. chapmani*, was consistently recovered as the sister lineage to the SMO-OH clade, rendering *H. chapmani* paraphyletic. The geographic distribution of the 2 *H. chapmani* clades and of *H. saturatior* strongly correlate with the geographic extent of the SMO-OH, SMS, and the Trans-Isthmian Highlands (highlands east of the Isthmus of Tehuantepec through Central America) mountain ranges. Divergence times associate their isolation to late Pleistocene climatic changes that likely were reinforced by barriers such as the Isthmus of Tehuantepec, the Tehuacán–Cuicatlán Valley, and the Central Valleys of Oaxaca. The fact that populations of *H. chapmani* represent 2 independent evolutionary lineages results in a substantial reduction in the distributional range for both entities. Therefore, the conservation status of *H. chapmani* should be re-evaluated.

Key words: conservation, cytochrome-*b*, *Fgb-I7*, *Handleyomys*, phylogeography, species delimitation, systematic

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Recent studies using molecular data have demonstrated that rodent populations from different mountain ranges in Mexico exhibit considerable levels of genetic differentiation (Sullivan et al. 1997; Harris et al. 2000; Arellano et al. 2005; León-Paniagua et al. 2007; Rogers et al. 2007; Rogers and González 2010; Vallejo and González-Cózatl 2012; Hardy et al. 2013). Within the *Handleyomys alfaroi* group, some forms are confined to medium- and high-elevation forests in different mountain ranges of Mesoamerica (Musser and Carleton 2005). Taxonomically, this species group had been included in *Oryzomys,* but a systematic evaluation conducted by Weksler et al. (2006) proposed that the genus be restricted to the "*palustris* group" and the remaining 10 clades were elevated to generic rank, including the "*alfaroi* group," which was provisionally assigned to *Handleyomys*. As a result, we refer to members of the *H. alfaroi* group as *Handleyomys* rather than *Oryzomys* throughout this paper.

The *H. alfaroi* group (Goldman 1918; Hall 1981), as currently defined (Weksler et al. 2006), is a complex of 6 species that includes *H. alfaroi* (Alfaro's Handley's mouse), *H. chapmani* (Chapman's Handley's mouse), *H. melanotis* (Black-eared Handley's mouse), *H. rostratus* (Long-nosed Handley's mouse), *H. rhabdops* (Highland Handley's mouse), and *H. saturatior* (Cloud Forest Handley's mouse). Although previous workers (Goldman 1915, 1918; Hall 1981; Musser and Carleton 1993, 2005) had retained *H. melanotis* and *H. rostratus* in the *melanotis* group, Weksler et al. (2006) included these 2 species in the *H. alfaroi* group. The systematics of this species group has been controversial and, over time, this complex has included from 5 (Goldman 1918) to 12 species (Allen 1891, 1913; Allen

and Chapman 1897; Merriam 1901; Goldman 1915). Within this complex, the taxonomy of the Mexican endemic *H. chapmani* also has been problematic. The 1st specimens referable to this taxon were collected near Jalapa, Veracruz, in the Sierra Madre Oriental (SMO), and were regarded as *H. melanotis* by Allen and Chapman (1897). Later, Thomas (1898) referred to these specimens as *H. chapmani*. In 1901, Merriam recognized *H. chapmani* (sensu Thomas 1898) and described specimens from northern Oaxaca (Oaxacan Highlands; OH) as *H. c. caudatus* and those from Puebla as *H. c. dilutior* (SMO). Goldman (1915) then described specimens from Guerrero and southern Oaxaca in the Sierra Madre Sur (SMS) as *H. guerrerensis*. In his revision of North American rice rats, Goldman (1918) retained *H. guerrerensis* as a full species, but relegated *H. chapmani* and the 2 subspecies contained therein (*caudatus* and *dilutior*) as subspecies of *H. alfaroi*. Specimens collected by Dalquest (1951) from Tamaulipas and San Luis Potosí (SMO) were described as a new subspecies of *H. alfaroi* (*H. a. huastecae*). Interestingly, Goodwin (1969) recognized 2 different forms of *Handleyomys* in the mountains of northeastern Oaxaca (OH); the larger specimens were described as *H. caudatus*, whereas the smaller form was viewed as *H. a. chapmani*. Additionally, specimens of *H. guerrerensis* from southern Oaxaca were relegated to a subspecies of *H. alfaroi* (*H. a. guerrerensis*—Goodwin 1969). Engstrom (1984) reported a unique karyotype for *H. caudatus* and recognized it as distinct from *H. alfaroi*. More recently, Musser and Carleton (1993, 2005) considered that all described forms restricted to cloud forests in the SMO, OH, and SMS (*H. a. chapmani, H. a. dilutior, H. a. guerrerensis*, *H. a. huastecae,* and *H. caudatus*) were conspecific and classified under the name *H. chapmani* with *H. saturatior* as the sister group.

Given that *H. chapmani* is distributed allopatrically across a series of mountain ranges in northern Mesoamerica and has a complicated taxonomic history, we used deoxyribonucleic acid (DNA) sequence data from the mitochondrial gene cytochrome-*b* (*Cytb*) and the nuclear intron 7 of the beta fibrinogen (*Fgb-I7*) as a first approach to estimate phylogenetic relationships among populations of *H. chapmani*. Other members of the *H. alfaroi* group for which tissue samples were available (*H. alfaroi*, *H. melanotis*, *H. rostratus*, and *H. saturatior*) also were included to estimate the phylogenetic affinities relative to *H. chapmani*. Specifically, we use our sequence data to test the proposal of Musser and Carleton (1993, 2005) that all forms of *Handleyomys* restricted to cloud forest elevations of the SMO, OH, and SMS and currently considered as conspecific forms of *H. chapmani* (*H. a. chapmani, H. a. dilutior, H. a. guerrerensis*, *H. a. huastecae,* and *H. caudatus*) represent a monophyletic assemblage. Also, we test the hypothesis that *H. chapmani* and *H. saturatior* represent sister species (Musser and Carleton 1993, 2005).

Materials and Methods

Specimens examined and genes sequenced.—Specimens used in this study were wild-caught following guidelines approved by the American Society of Mammalogists (Sikes et al. 2011) or obtained via tissue loans and represent localities sampled across the known distribution of *H. chapmani* and species representing other members of the *H. alfaroi* species group (*H. alfaroi*, *H. melanotis*, *H. rostratus,* and *H. saturatior*; Fig. 1). Taxonomy follows Musser and Carleton (2005) with nomenclatural updates from Weksler et al. (2006). A total of 79 individuals was used in this study, of which 72 and 39 individuals were sequenced for *Cytb* and intron *Fgb-I7*, respectively. In addition, 7 *Cytb* sequences were obtained from GenBank (Appendix I).

DNA extraction, amplification, and sequencing.—Total genomic DNA was extracted from liver tissue frozen or preserved in 95% ethanol either following Fetzner (1999) phenol–chloroform method, or using the QIAGEN DNeasy tissue kit (cat. no. 69504; Qiagen, Valencia, California). Amplification of *Cytb* and *Fgb-I7* was performed via polymerase chain reaction (PCR) with negative controls used for all amplifications. The complete *Cytb* gene was amplified with the primers MVZ-05 and MVZ-14-M (modified from Smith and Patton 1993 by Arellano et al. 2005) and internal primers MVZ-45, MVZ-16 (Smith and Patton 1993). *Fgb-I7* was amplified with primers B17 and Bfib (Wickliffe et al. 2003). For *Cytb*, the PCR master mix contained 1.0 µl of template DNA, 1.0 µl of deoxynucleotide triphosphates (dNTPs; 1.25 mM), 0.5 µl of each primer (100 µM), 3.0 µl of $MgCl_2$ (25 mM), 11.85 µl of distilled H_20, and 0.15 µl of Taq polymerase. For *Fgb-I7*, reactions included 3.0 µl of template DNA, 1.7 µl of dNTPs (1.25 mM), 2.5 µl of each primer (100 µM), 1.7 µl of $MgCl_2$ (25 mM), 0.8 µl of GeneAmp 10× PCR buffer, 13.7 µl of high-performance liquid chromatograpy-H_2O, and 0.125 µl of Platinum Taq polymerase (Promega Corp., Madison, Wisconsin). Thermal profiles for *Cytb* were: 3 min at 94°C, 39 cycles of 1 min at 94°C, 1 min at 50°C, 1 min at 72°C, and 5 min at 72°C followed by a soak at 4°C. For *Fgb-I7*, a hot start of 15 min at 85°C was used before the addition of dNTPs; this was followed by 10 min at 94°C, 32 cycles of 1 min at 94°C, 1 min at 65°C, and 1 min at 72°C, and a soak at 4°C. PCR products were purified either with a GeneClean PCR purification kit (Bio 101, La Jolla, California) or by using a Millipore (Billerica, Massachusetts) multiscreen PCR 96-well filtration system (cat. no. MANU03050). Sequencing reactions of purified PCR products were done with the Perkin–Elmer ABI PRISM dye terminator cycle sequencing ready reaction kit (Applied Biosystems, Foster City, California). Excess dye terminator was removed using a Sephadex 50G solution (3 g/50 ml H_2O) or with a Millipore multiscreen filter plate (cat. no. MAHVN4510). Light- and heavy-strand sequences were determined with an ABI 3100 automated sequencer (Applied Biosystems) housed in the DNA Sequencing Center at Brigham Young University or by Macrogen Inc., Seoul, Korea (http://www.macrogen.com). Sequences were edited manually using Sequencher version 4.1.1 and 4.1.2 (Gene Codes Corp., Ann Arbor, Michigan).

Phylogenetic analyses of the Cytb data set.—Alignment for *Cytb* was done by translating nucleotide sequences into amino

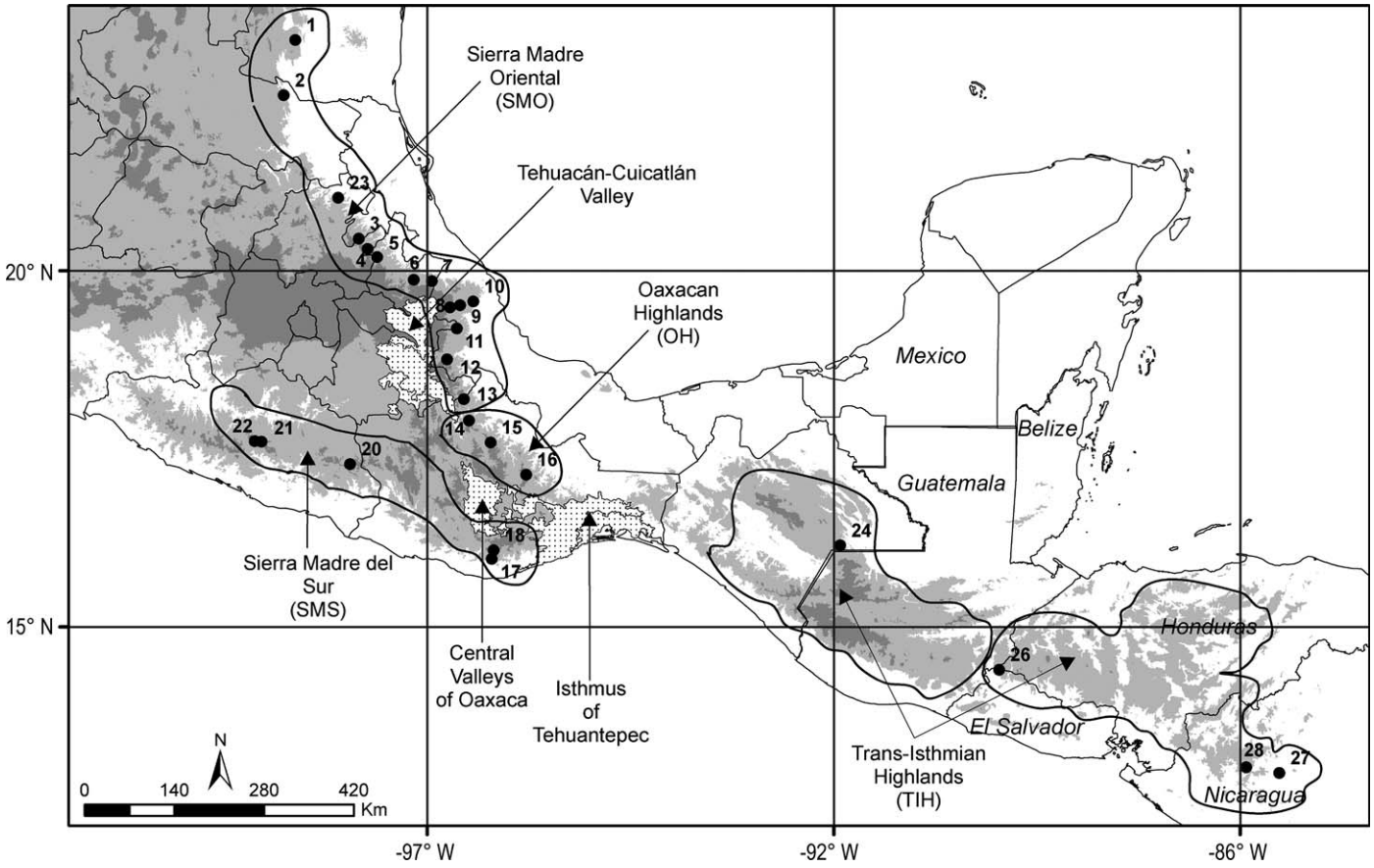

Fig. 1.—Map of Mexico and northern Central America showing collecting localities (numbered dots) for *Handleyomys chapmani* and *H. saturatior*. Main geological features in the region are delineated in black and hypothesized barriers for dispersion are shown as stippled areas. Numbers correspond to those shown in Figs. 2 and 3 and in Appendix I. An elevation gradient is represented with white < 800 m; light gray 800–2,200 m, and dark gray > 2,200 m.

acids with Codon Code Aligner v2.0.6 (Codon Code Corp., Dedham, Massachusetts). Unique haplotypes were identified with TCS v1.21 (Clement et al. 2000) and models of nucleotide substitution and genetic variation parameters that best fit our data were selected using jModelTest v1.1 (Posada 2008 using the Akaike information criterion). The model of evolution selected was TVM + Γ (Posada and Crandall 1998). Base frequencies were A = 0.3332, C = 0.3209, G = 0.0981, and T = 0.2480; transversion rates were (A-C) 0.2990, (A-G) 2.4623, (A-T) 0.3909, (C-G) 0.2127, (C-T) 2.4623, and the gamma shape parameter (Γ) was 0.2310. Maximum likelihood (ML—Felsenstein 1981) and Bayesian inference (BI—Yang and Rannala 1997) optimality criteria were used to estimate relationships among taxa using RAxML v7.4.8 (Stamatakis 2006) and MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003), respectively.

Handleyomys alfaroi was designated as the outgroup for our phylogenetic analyses following its current taxonomic position as the sister lineage to *H. chapmani* and *H. rostratus* (Weksler et al. 2006). We allowed *H. rostratus* and *H. melanotis* (its presumed sister lineage) to be part of the ingroup along with *H. saturatior*; this as an alternative test of monophyly for *H. chapmani* (Nixon and Carpenter 1993).

For BI, 2 analyses with 3 chains were run independently for 10 million Metropolis coupled Markov chain Monte Carlo (MCMC) generations using the default priors on model parameters starting from a random tree. For all analyses, a tree was sampled every 2,000 generations. Stationarity was determined by monitoring the fluctuating value of the likelihood parameters using Tracer v1.4 (Rambaut and Drummond 2007). All the trees before stationarity were discarded as "burn in." For ML, a heuristic search starting from a random tree was conducted with 1,000 replicates using RAxML v7.4.8 (Stamatakis 2006). Kimura 2-parameter (Kimura 1980) genetic distances were calculated to assess within- and among-species genetic divergence using PAUP v4.0b10 (Swofford 2002) as they are directly comparable with distance values reported in treatments dealing with phylogeny reconstruction or species definitions of mammals (Smith and Patton 1993; Baker and Bradley 2006; Tobe et al. 2010).

Phylogenetic analyses of the Fgb-I7 data set.—Alignment of *Fgb-I7* data was done using the software MUSCLE (Edgar 2004). The model of evolution selected by jModelTest v1.1 as most appropriate for *Fgb-I7* was HKY (Hasegawa et al. 1985). Base frequencies were A = 0.2949, C = 0.1725, G = 0.2035, and T = 0.3290; transition/transversion ratio = 1.2408.

Phylogeny estimation was done as for *Cytb* data set for ML and BI.

Phylogenetic analyses of the combined data set.—Before combining the data partitions, we assessed the level of disagreement between the *Cytb* and *Fgb-I7* data sets with the incongruence length difference test (ILD—Farris et al. 1995; see also Hipp et al. 2004) using simple taxon addition, nearest-neighbor interchange branch swapping, and a heuristic search using 1,000 replicates in PAUP v4.0b10 (Swofford 2002). Initially, the test was run by comparing 1,143 base pairs (bp) of *Cytb* with 621 bp of *Fgb-I7*, which resulted in rejecting the null hypothesis of data homogeneity ($P = 0.01$). Then, *Cytb* was reduced to its first 621 bp and to its last 621 bp to match the length of *Fgb-I7*. In both cases, these tests failed to reject the null hypothesis of data homogeneity ($P = 0.09$). This inconsistency highlights some of the criticisms of the ILD test (Yoder et al. 2001; Barker and Lutzoni 2002; Hipp et al. 2004). Alternatively, studies have demonstrated that total evidence may provide better resolution than separate analyses that are not fully resolved (Chippindale and Wiens 1994; Jackman et al. 1997), especially when the conflict is small and most regions of the tree are shared between partitions (Wiens 1998). Therefore, we followed Wiens (1998) methodology for data combinability and analyzed each partition separately to identify parts of the tree where there was incongruence; then combined the data sets and considered the conflicted branches with caution. All major haploclades recovered by *Cytb* were represented in the combined data set. For BI and ML combined analysis, the partition substitution models formerly selected were specified. Combined data analyses were run with the same settings as described for the *Cytb*.

Nodal support.—ML branch support was determined with 1,000 nonparametric bootstrap pseudoreplicates (Felsenstein 1985). Bootstrapping was synchronized with phylogeny reconstruction in RAxML v7.4.8 (Stamatakis 2006). Clades with bootstrap proportions (BP) above 70% were considered relatively well supported (Hillis and Bull 1993). For Bayesian analyses, the posterior probabilities (*pP*) for individual clades were obtained by constructing a majority-rule consensus of the trees not discarded as burn-in using MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003).

Topology tests.—Statistical support for the tree topologies was estimated as the *pP* for the subset of possible trees in agreement with the topology we recovered (Huelsenbeck and Rannala 2004). Specific ML topology tests were performed with the approximately unbiased (AU) test (Shimodaira 2002) with 10 sets of 10,000 bootstrap replicates. Both tests were performed on the combined data set and run in Consel (Shimodaira and Hasegawa 2001) using site log-likelihoods calculated with PAUP v4.0b10 (Swofford 2002). The model of nucleotide substitution for the AU test was GTR with optimized parameters using RAxML.

Divergence times estimates.—Phylogeny dating was assessed with the coalescent Bayesian approach for multilocus data implemented in BEAST v1.7.4 (Drummond and Rambaut 2007). For each partition, parameters for the model of nucleotide substitution were the same used for the phylogenetic analyses (unlinked substitution models). Two MCMC analyses were run for 10,000,000 generations with trees sampled every 1,000 generations. Stationary, appropriate effective sample size (ESS) and convergence of independent MCMC was visualized with Tracer v1.5. The first 1,000 trees of each run (10% respectively) were discarded as burn-in and the remaining trees were then combined to build a maximum credibility tree in TreeAnnotator v1.6. Analyses were done under the assumption of a relaxed molecular clock to account for heterogeneity of substitution rates among lineages (Arbogast et al. 2002). Using a Yule tree prior on the net rate of speciation, rates among lineages were assumed to be uncorrelated, and the rate for each branch was independently drawn from a lognormal distribution (uncorrelated lognormal model—Drummond et al. 2006). As calibration points we used fossil records for *H. alfaroi* and *H. rostratus* (= *H. melanotis*) from the Rancholabrean 0.3 million years ago (mya—Ferrusquía-Villafranca et al. 2010) and *H. alfaroi* from the late Quaternary (0.5–1.0 mya—Arroyo-Cabrales et al. 2002). This information was incorporated in the *H. rostratus* and *H. alfaroi* nodes to set hard lower bounds of 0.3 mya and 0.5 mya, respectively for the time of most recent common ancestor (tMRCA).

Delimiting species boundaries.—Although species delimitation is an inherent practice in phylogenetics, until recently, implementation of species boundaries had lacked a theoretical framework on which such limits could be tested explicitly (Sites and Marshall 2003, 2004; Wiens 2007; Rogers and Gonzalez 2010). This is attributable, at least in part, to the natural subjectivity of species concepts and incompatibilities among them (de Queiroz 2007). Nevertheless, this topic is receiving more attention and hypothesis-testing methods for delimitation of species boundaries have been developed (see Wiens 2007; Camargo et al. 2012; Fujita et al. 2012).

The amount and direction of gene flow was estimated using MIGRATE-N v.3.3 (Beerli and Felsenstein 2001) as the mutation scaled effective migration rate (M) to account for the autosomal inheritance of *Fgb-I7*. M in turn was multiplied by the estimated effective population size (theta = Θ) to obtain the effective number of migrants per generation (Nm). F_{ST} estimates were used as starting values to run 3 replicate chains with 100,000 genealogies. If migration between lineages was not perceived, the degree of exclusive ancestry was quantified with the genealogical sorting index (GSI—Cummings et al. 2008). GSI ranges from 0 to 1, where values < 1 basically reflect additional coalescent events from the minimum required to unite all members of the group through a most recent common ancestor. Statistical significance for the GSI values (probability of finding that degree of exclusive ancestry in our groupings by chance) is estimated with a permutation test (Cummings et al. 2008). For BI trees (*Cytb*, *Fgb-I7*, and concatenated), GSI values were calculated for the last 100 trees of the MCMC search. For ML trees (*Cytb*, *Fgb-I7*, and concatenated), GSI values were calculated on the best tree found during the heuristic search (with RAxML v7.4.8; see

"Materials and Methods"). We also calculated the GSI for the *Cytb* and *Fgb-I7* gene topologies ensemble (GSI_T).

Geographic association of the recovered lineages was assessed with the nested clade phylogeographical analysis (NCPA—Templeton 1998) and GeoDis (Posada et al. 2000) run in their automated form as implemented in ANeCA (Panchal 2007). Although the NCPA has been criticized, most of these arguments are based on the lack of statistical assessment of uncertainty and related to inconsistencies of the inferences of complex phylogeographic histories, particularly involving high migration rates (Beaumont and Panchal 2008; see Beaumont et al. 2010 for a detailed review). However, the performance of this method has been defended (Templeton 2008, 2010a, 2010b). For the purpose of this paper, migration rates were explicitly estimated previous to this test, and under those circumstances the test provides a concrete way to describe the distribution of genetic variation over geography.

Finally, using the sequence data from both markers, we estimated the posterior probability (BpP) for a model of speciation using those clades that were characterized by a lack of migration and suggested as significantly exclusive on the basis of results of the GSI tests. These analyses were implemented in the software Bayesian phylogenetics and phylogeography (BPP v.2.0—Yang and Rannala 2010). The coalescent species delimitation method used in BPP relies on a reversible-jump Markov chain Monte Carlo (rjMCMC) for taking into account uncertainty due to unknown gene trees and ancestral coalescent processes. An equal prior probability was assigned to all species delimitation models (1–4 species, 5 species, and 6 species), and to ensure convergence of the estimates, rjMCMC was run with algorithm 0 and 1 (with fine-tune parameter $\varepsilon = 15$) starting from 3 different trees (fully resolved; 6 species, 5 species, and 1 species). The rjMCMC was run for 500,000 generations with a sampling frequency of 5 after a burn-in period of 10,000. The mean value for the ancestral population size (Θ) was estimated with MIGRATE-N v.3.3 to set a gamma prior (α, β) of $\Theta = (5.0, 100)$ and root age (tau = τ) $\tau 0 = (2, 1{,}000)$. The Kimura 2-parameter (Kimura 1980) genetic distances for *Cytb* were then used for sequence divergence comparisons within and among the lineages identified.

Results

Phylogenetic analysis of individual genes.—Of the 1,143 *Cytb* nucleotides, 329 were variable. Both ML and BI phylogenetic analyses converged on basically the same tree topology (Fig. 2). Nodal support was high for all species-level taxa and geographically exclusive clades. A total of 65 haplotypes in the *Cytb* data set were identified, of which 49 represented samples of *H. chapmani* and 16 belonged to other species of *Handleyomys*. With only 3 exceptions, *H. chapmani* haplotypes also were exclusive by locality. These exceptions included 1 haplotype found at localities 11 (Colección de Mamíferos del Centro de Investigación en Biodiversidad y Conservación, Universidad Autónoma del Estado de Morelos [CMC] 779, CMC 782) and 7 (CMC 1052; Fig. 1), and 1 haplotype present in localities 9 (CMC 1450), 6 (Monte L. Bean Life Science Museum, Brigham Young University [BYU] 15803), and 7 (CMC 1049, CMC 1054); all within SMO. Similarly, within the OH a haplotype from locality 16 (CMC 114) was found at locality 14 (CMC 1347). Haplotypes representing *H. alfaroi*, *H. melanotis*, and *H. rostratus* also were exclusive by locality, except for a *H. melanotis* haplotype that was present at locality 33 (ASNHC 3418) and locality 34 (ASNHC 3419). Topologies generated under both ML and BI optimality criteria showed that *H. chapmani* is not a monophyletic group. Samples of this species were recovered in 2 divergent clades (SMO-OH and SMS; Fig. 2) with high nodal support ($pP = 1.0$, BP = 100 for both). Furthermore, *H. saturatior* was placed as the sister group to the *H. chapmani* SMO-OH clade ($pP = 0.92$, BP = 91). *H. melanotis* and *H. rostratus* were recovered as sister taxa ($pP = 1.00$, BP = 100), and samples of each species constituted strongly supported monophyletic assemblages ($pP = 1.00$, BP = 100). *H. rostratus* was recovered in 2 well-supported clades ($pP = 1.0$, BP = 91–94) corresponding to samples from east and west of the Isthmus of Tehuantepec (Fig. 2).

The *Fgb-I7* data set consisted of 621 characters, of which 52 were variable. Three indels were inferred for our *Fgb-I7* sequences based on *H. alfaroi* as the outgroup. One was assigned at position 283 (single bp deletion for *H. chapmani* and *H. saturatior*), a 2nd gap was identified at positions 382–383 (an insertion inferred for all ingroup taxa), and a 3rd indel was set at positions 407–417 (a deletion inferred for *H. rostratus*). There were 14 *Fgb-I7* haplotypes identified by TCS (Phylogenetic network estimation using statistical parsimony; Clement et al. 2000), 8 of which were present only in *H. chapmani*. Of these 8, 3 were unique haplotypes and 5 were shared but exclusive by regions (SMS, SMO-OH). *H. saturatior* was represented by 2 haplotypes corresponding to different localities, *H. alfaroi* by the same haplotype found at 2 localities, *H. melanotis* by 1 haplotype present in all 5 localities, and *H. rostratus* by 2 haplotypes from a single locality.

Phylogenetic analyses of *Fgb-I7* based on ML and BI optimality criteria estimated genealogies that were highly concordant, albeit less resolved than those recovered with *Cytb* (Fig. 3a). Samples of *H. chapmani* and *H. saturatior* grouped together as a well-supported monophyletic assemblage ($pP = 1.00$, BP = 100), although this clade resulted in an unresolved polytomy. Nonetheless, within this clade samples were arranged following a geographic pattern by mountain range. Samples of *H. chapmani* from the SMS formed 2 clades, 1 comprising CMC 1655 and CMC 1657 from El Tejocote, Guerrero (locality 20; $pP = 1.00$, BP = 89) and the other comprising the remaining samples. Similarly, all *H. saturatior* samples except ECOSCM 1231 (locality 24) also were recovered in a well-supported clade ($pP = 1.00$, BP = 98). *H. melanotis* and *H. rostratus* were recovered as monophyletic clades ($pP = 1.00$, BP = 100, for both clades). However, *H.*

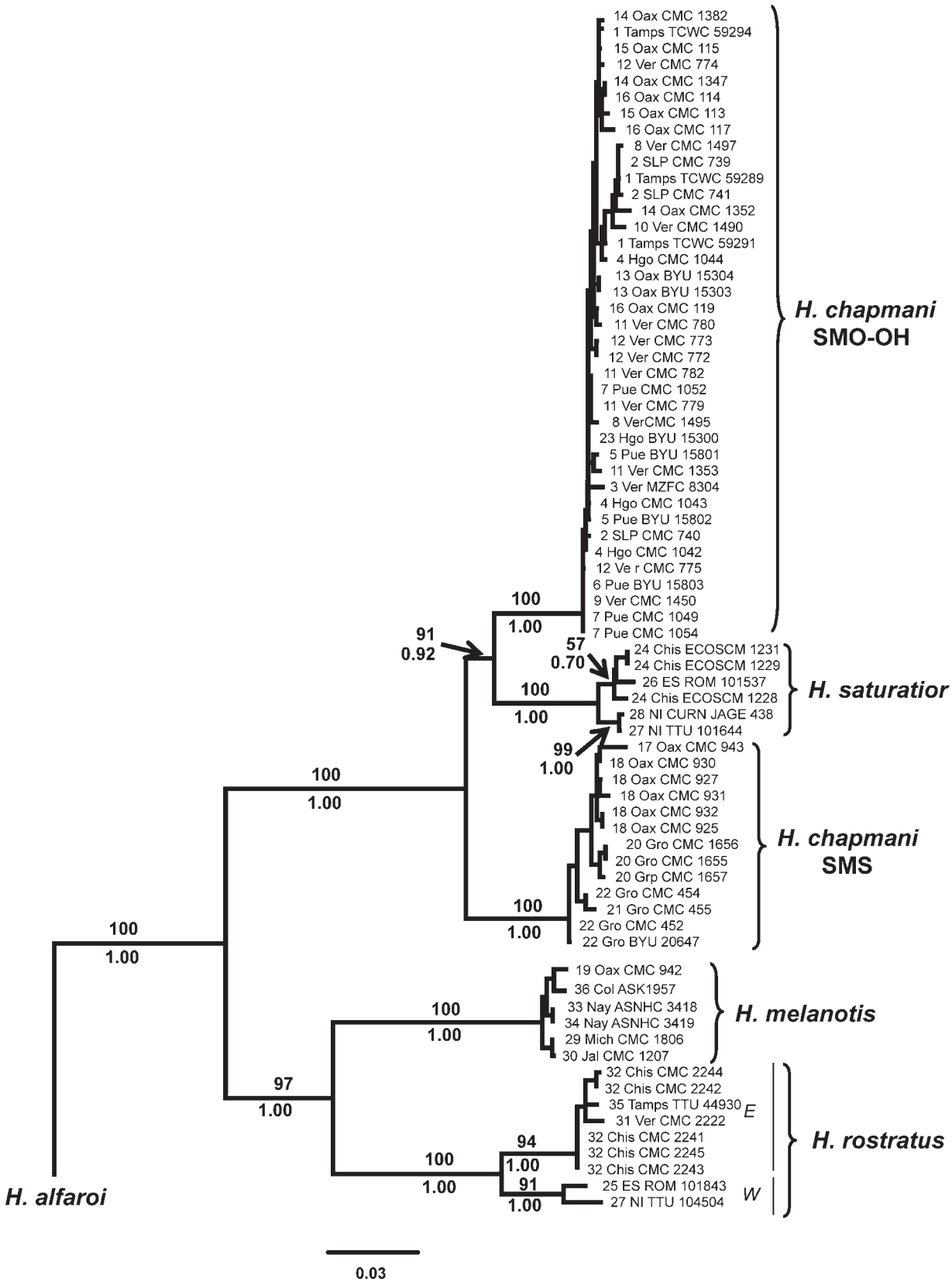

Fig. 2.—Maximum likelihood (ML) phylogram based on *Cytb* sequence data. ML bootstrap values are shown above nodes and Bayesian inference (BI) posterior probabilities are shown below. Terminal labels indicate locality number; abbreviation for country (ES = El Salvador and NI = Nicaragua) or the Mexican states as listed in Appendix I, museum acronym, and museum voucher number.

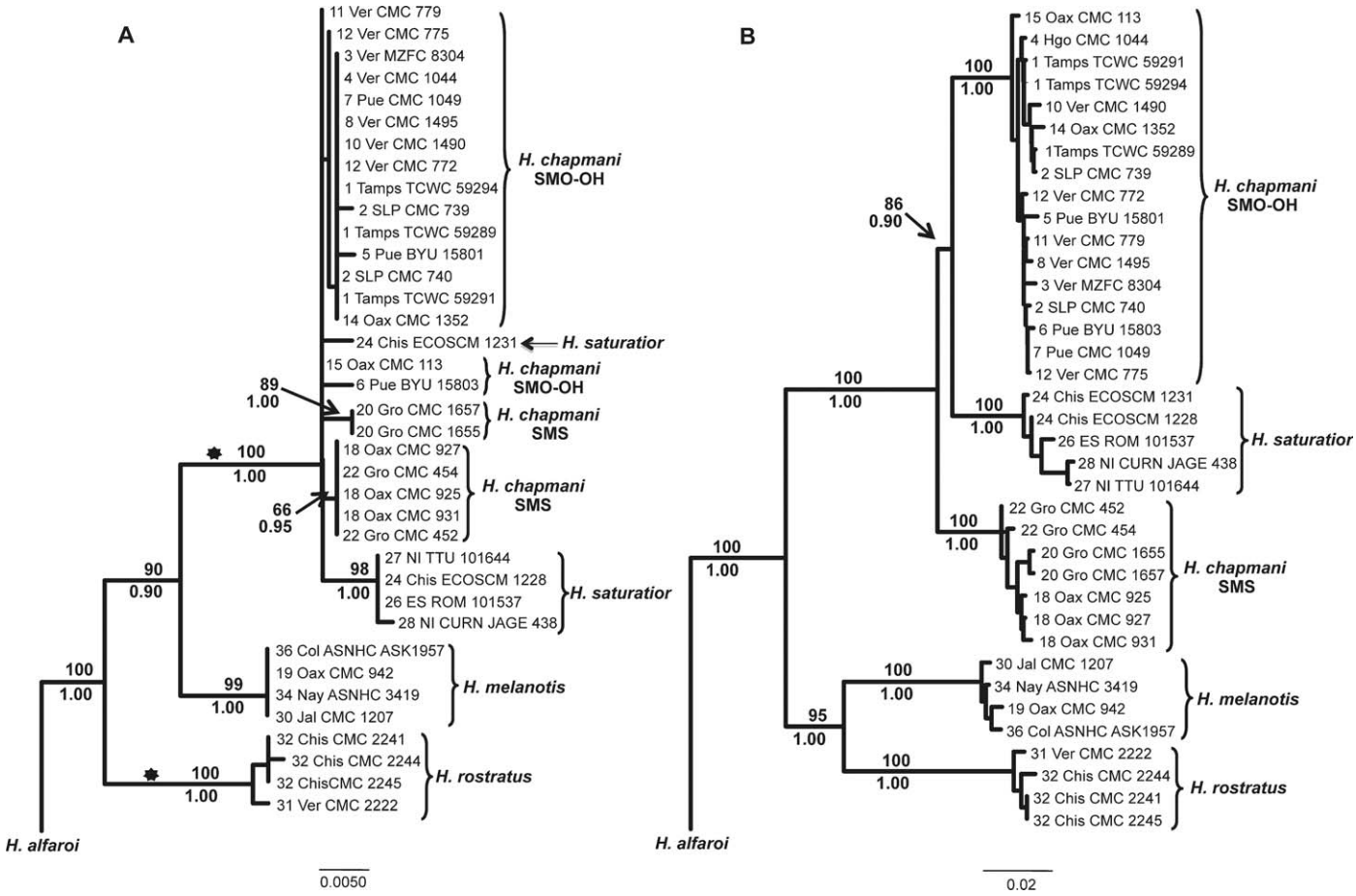

Fig. 3.—Maximum likelihood (ML) phylograms based on a) *Fgb-I7* sequence data and b) the combined (*Cytb* and *Fgb-I7*) data set. For both trees, ML bootstrap values are shown above nodes and Bayesian inference (BI) posterior probabilities are shown below. Stars represent inferred gaps in the *Fgb-I7* sequence data mapped onto the tree. Terminal labels are as in Fig. 2.

melanotis was placed as sister group to the *H. chapmani* and *H. saturatior* clade ($pP = 0.90$, BP = 90).

Phylogenetic analysis of combined data set.—Trees estimated from the combined data set (*Cytb* and *Fgb-I7*) converged on basically the same tree topology for both ML and BI optimality criteria, as recovered by the *Cytb* tree (Fig. 2). Fig. 3b depicts the ML tree (lnL = –5754.025). *H. chapmani* was recovered as 2 polyphyletic clades (SMO-OH and SMS). Each of the clades recovered was strongly supported by Bayesian *pP* and ML bootstrap values ($pP = 1.00$, BP = 100). Additionally, the *H. chapmani* SMO-OH clade was placed as the sister group to *H. saturatior* ($pP = 0.90$, BP = 83).

Topology tests.—The *pP* value for a topology that constrained clades representing *H. chapmani* to be monophyletic was $pP = 0.142$, whereas the probability of a topology with *H. chapmani* paraphyletic (as recovered in this study) was $pP = 0.858$. With the AU test, the hypothesis of *H. chapmani* monophyly was rejected (AU = 0.0451; $P < 0.05$). Log-likelihood of the constrained topology (*H. chapmani* monophyletic) was lnL = –5,762.05701, whereas the unconstrained topology (*H. chapmani* paraphyletic) was lnL = –5,754.025.

Divergence times estimates.—The MCMC combined runs reached ESS above 450 for all parameters. The standard deviation of the uncorrelated lognormal relaxed clock for *Cytb* had a mean of 0.883 and 1.982 for *Fgb-I7*, indicating that both were not behaving in a clock-like manner. The mean substitution rate (per site per million years) was 0.027 for *Cytb* and 0.009 for *Fgb-I7*. On a timescale, *H. alfaroi* was not used to root the tree because the root is implicit as the most recent common ancestor (MRCA). The root was placed with a mean age of 2.51 mya (highest posterior density interval [95% HPD] = 1.30, 3.76). A mean divergence time of 1.45 mya was estimated for the *H. chapmani* SMS clade (95% HPD = 0.65, 2.40), whereas the *H. chapmani* SMO-OH and *H. saturatior* split was estimated at 1.08 mya (95% HPD = 0.54, 1.86). The divergence time estimate for *H. melanotis* and *H. rostratus* was placed at 1.53 mya (95% HPD = 0.68, 2.50). When it was not constrained as the root, *H. alfaroi* was positioned as sister to the *H. melanotis* and *H. rostratus* clade; the tMRCA for this clade was estimated at 2.07 mya (95% HPD = 1.08, 2.97).

Inferred species boundaries.—There was no evidence of gene flow between *H. chapmani* (SMO-OH) and *H. saturatior* ($Nm = 0.09$ [95% HDP = 0.00–0.18]), between *H. chapmani* (SMO-OH) and *H. chapmani* (SMS; $Nm = 0.07$ [95% HDP =

Table 1.—Genealogical sorting index (GSI) and Bayesian phylogenetics and phylogeography (BPP) posterior probability (BpP) for *Handleyomys chapmani* as currently recognized (Sierra Madre Oriental–Oaxacan Highlands [SMO-OH] and Sierra Madre del Sur [SMS] clades labeled as *H. chapmani*), and for the SMO-OH and SMS clades labeled as different groups. Values for *H. saturatior* also are shown as a reference for a recognized and diagnosable species in the group. GSI values correspond to maximum likelihood (ML) and Bayesian inference (BI) topologies from individual genes (GSI_{Cytb} and $GSI_{Fgb\text{-}I7}$), the concatenated data set ($GSI_{Concatenated}$), and an ensemble from the *Cytb* and *Fgb-I7* trees (GSI_T). All GSI values had highly significant *P*-values (< 0.0004).

	ML				BI				BpP
	GSI_{Cytb}	$GSI_{Fgb\text{-}I7}$	$GSI_{Concatenated}$	GSI_T	GSI_{Cytb}	$GSI_{Fgb\text{-}I7}$	$GSI_{Concatenated}$	GSI_T	
SMO-OH	1.000	0.808	1.000	0.827	1.000	0.655	1.000	0.827	1.000
SMS	1.000	0.604	1.000	0.703	1.000	0.830	1.000	0.762	1.000
H. saturatior	1.000	0.379	1.000	0.664	1.000	0.379	1.000	0.813	1.000
H. chapmani SMO-OH/SMS	0.817	0.552	0.766	0.870	0.817	0.797	0.894	0.845	0.004

0.00 – 0.14]), or between *H. chapmani* (SMS) and *H. saturatior* (*Nm* = 0.09 [95% HDP = 0.00 – 0.18]). The opposite migration estimates were equivalent and are not shown.

The GSI values for *H. chapmani* as currently defined (SMO-OH and SMS clades labeled as *H. chapmani*) averaged 0.794 (min. = 0.552, max. = 0.894; Table 1). When *H. chapmani* was labeled according to the 2 clades recovered in this study, the GSI values were higher for each lineage (SMO-OH averaged 0.889 [min. = 0.655, max. = 1] and SMS averaged 0.862 [min. = 0.604, max. = 1]). The GSI values for *H. saturatior* were smaller (min. = 0.379, max. = 1, average = 0.779) than those calculated for each *H. chapmani* clade. For each basal lineage recovered in our study, *Cytb* and concatenated data topologies consistently recovered values of 1 (achieved monophyly); and weighted GSI analyses (*Cytb* and *Fgb-I7* topologies combined; GSI_T) ranged from 0.664 to 0.827. All GSI statistics had significant *P*-values (< 0.0004). *Fgb-I7* trees yielded lower GSI values for all the groupings (Table 1).

The species delimitation analyses (BPP) strongly supported a model of 5 speciation events (6 species; Fig. 4), corresponding to *H. alfaroi*, *H. melanotis*, *H. rostratus*, *H. chapmani* SMS, *H. saturatior*, and *H. chapmani* SMO-OH (BpP = 0.99620). Under this model, *H. chapmani* SMO-OH, *H. chapmani* SMS, and *H. saturatior* have a BpP = 1.00000; *H. alfaroi* a BpP = 0.99999, and *H. rostratus* and *H. melanotis* a BpP = 0.99666. In contrast, a model of 5 species had a BpP = 0.00374, and a model of < 5 species had a BpP = 0.00005. To examine the effect of excessive a priori subdivision, we further split *H. chapmani* SMO-OH to create a model with 7 species (all of the above species plus *H. chapmani* OH populations as the 7th). This model had a much lower probability BpP = 0.09518 than our 6-species speciation model (BpP = 0.99620).

The *Cytb* haplotype networks we identified corresponded to the clades recovered by ML and BI analyses. Samples of *H. chapmani* were represented in 2 separate networks (SMS and SMO-OH). The NCPA indicated that haplotypes of *H. chapmani* corresponding to the SMS and SMO-OH clades

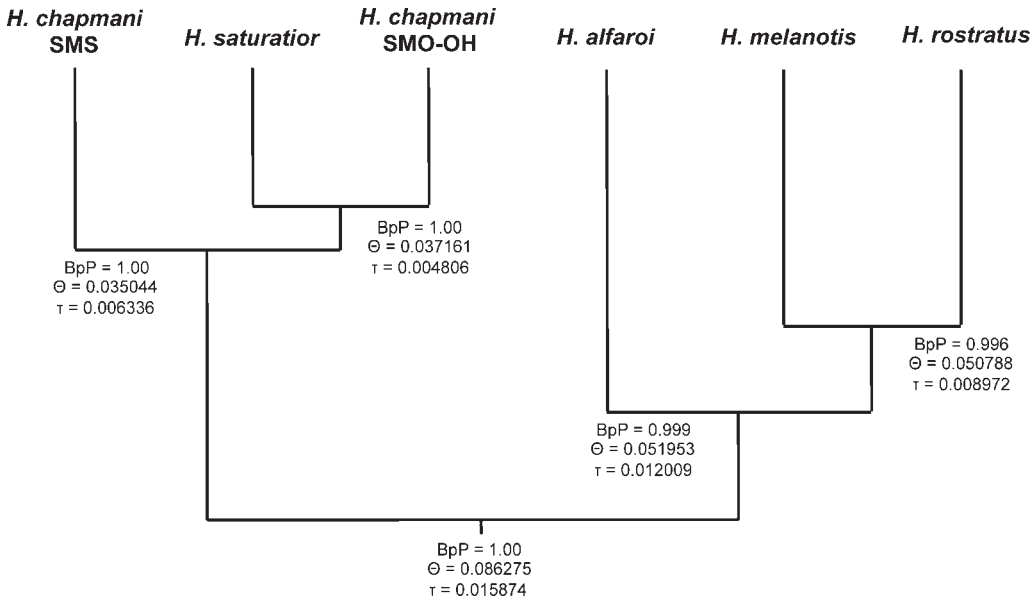

Fig. 4.—Bayesian species delimitation (6 species) BpP = 0.99620. A model with 5 species had a BpP = 0.00374, a model with 7 species a BpP = 0.09518; and a model of < 5 species a BpP = 0.00005. Bayesian posterior probability (BpP) for a lineage split and mean posterior estimates for Θ and τ are shown below nodes.

are geographically isolated genetic clusters ($P = 0.0277$ and $P = 0.0411$ respectively). Within SMS (Geodis Dc), the southern Oaxaca and western Guerrero haplotypes are the most geographically restricted ($P = 0.0121$). Within SMO, haplotypes found in San Luis Potosí connected to those in Hidalgo but with a significantly large nested clade distance (Dn; $P = 0.0249$). In contrast, haplotypes from northern Oaxaca (OH) were recovered as a separate genetic unit from the rest of *H. chapmani* SMO ($P = 0.0010$; Figs. 1 and 2).

Discussion

Our molecular phylogeny demonstrates that *H. chapmani* is comprised of 2 nonsister lineages that are restricted to different mountain systems (SMO-OH and SMS). Moreover, the SMO-OH clade is the sister group to *H. saturatior*, which occurs to the east of the Isthmus of Tehuantepec in the highlands of Chiapas and Central America (TIH). Together, these 3 lineages constitute a well-supported monophyletic assemblage. By extension, our phylogenetic analyses do not support the proposal of Musser and Carleton (1993, 2005) that all forms of *H. chapmani* (*H. a. chapmani, H. a. dilutior, H. a. guerrerensis*, *H. a. huastecae,* and *H. caudatus*) restricted to cloud forest habitat in the SMO, OH, and SMS are conspecific.

The mean *Cytb* genetic distance among clades of *H. chapmani* from the SMO-OH and SMS was 6.5%, whereas values between *H. chapmani* SMO-OH and *H. saturatior* and between *H. chapmani* SMS and *H. saturatior* were 6.0% and 6.9%, respectively. These values are comparable with those among many cryptic species of mammals (Baker and Bradley 2006). Also, the degree of genetic differentiation is in agreement with our divergence time estimates, which suggests that the 2 lineages of *H. chapmani* (SMO-OH and SMS) and *H. saturatior* are the most recently derived lineages within the *H. alfaroi* group (1.08–1.45 mya). According to Arroyo-Cabrales et al. (2002), Ceballos et al. (2010), and Ferrusquía-Villafranca et al. (2010), *H. rostratus* and *H. alfaroi* were well-differentiated forms in the Pleistocene fauna of Mexico. This proposal is consistent with the estimated MRCA for *H. alfaroi–H. rostratus* and *H. melanotis* (2.07 mya) and the relatively large percent *Cytb* sequence divergence between them (13.2%).

Although *Fgb-I7* has been useful in resolving intrageneric relationships in other rodent groups (Matocq et al. 2007; Hanson and Bradley 2009), it typically has a slower substitution rate than *Cytb* in mammals (Wickliffe et al. 2003). We interpret the lack of resolution in the *Fgb-I7* topology as a case of incomplete lineage sorting. This is supported by the lack of evidence for gene flow and the *Fgb-I7* GSI values showing a substantial amount of exclusivity for each *H. chapmani* lineage (0.65 to 0.83) despite the partially resolved phylogeny.

Lack of detectable gene flow between *H. chapmani* clades SMO-OH and SMS ($Nm = 0.11$) and between any of these 2 lineages and *H. saturatior* (average $Nm = 0.13$) also support the notion that these groups represent separate biological species. Similarly, the geographic distributions of these 3 lineages are allopatric as supported by the NCPA analysis. The GSI values for *H. chapmani* clades SMO-OH and SMS showed considerable amounts of exclusive ancestry (mean GSI values of 0.889 and 0.862, respectively) and achieved monophyly (GSI = 1) with *Cytb* and concatenated data topologies. Moreover, the GSI values for the 2 *H. chapmani* clades were consistently larger than for *H. saturatior*, whose average GSI value was 0.779. Accordingly, BPP assigned the highest probability to a speciation model in which *H. chapmani* SMO-OH and *H. chapmani* SMS constitute 2 separate species (BpP = 1.0 for each lineage). This interpretation is also consistent with the phylogenetic species concept (Cracraft 1989). Therefore, we regard the SMS evolutionary lineage of *H. chapmani* as an unrecognized species on the basis of our molecular genealogy and morphological differences (Goldman 1915, 1918) from the SMO-OH clade.

Goldman (1915) described individuals from Omiltemi, Guerrero (SMS; locality 21; CMC 455) as *H. guerrerensis*. Later, he incorporated individuals from southern Oaxaca (SMS; ~10 km E locality 17; CMC 943) and extended the geographic distribution of this taxon to the "forested Pacific slopes of the Sierra Madre del Sur in Guerrero and Oaxaca" (Goldman 1918:69). In comparison with *H. chapmani* from the SMO-OH, Goldman (1918:70) described skulls representing the SMS form as "smaller and flatter; zygomata tending to curve evenly outward, the sides less nearly parallel; sides of rostrum more tapering anteriorly; ascending branches of premaxillae usually broader posteriorly; maxillary arms of zygoma more slender; incisors smaller." Goodwin (1956) acknowledged the morphological uniqueness of the SMS form described by Goldman (1918) and retained it as species. In a review of the mammals of Oaxaca, Goodwin (1969) included individuals from the SMS localities 17 (CMC 943), 18 (CMC 925, CMC 930, CMC 931, CMC 932, CMC 927), and ~80 km NE (by road) locality 20 (CMC 1655, CMC 1656, CMC 1657) and compared them with *H. chapmani* from the SMO-OH including locality15 (CMC 113, CMC 115) and locality 16 (SMO-OH; CMC 114, CMC 117, CMC 119). Although Goodwin (1969) acknowledged the morphological features underlined by Goldman (1918), he relegated *H. guerrerensis* to a subspecies of *H. alfaroi*.

Because the name *chapmani* first was assigned to voucher specimens of *Handleyomys* from Xalapa, Veracruz (Thomas 1898—but originally described as *H. melanotis* by Allen and Chapman [1897]), and our sampling included 1 specimen from this locality (CMC1450; locality 9) plus 4 more from a nearby location (CMC 1495, CMC 1497; locality 8; CMC 1353, CMC 1490; locality 10), we propose that the SMO-OH clade should retain the name *chapmani*. Determining a valid name for the SMS clade would require sequence data from specimens collected at or near type localities of names currently in synonymy. Goldman (1915) first used the term *guerrerensis* and Goodwin (1956, 1969) preserved the name *guerrerensis*. Therefore, taking into account that our sampling of the SMS included specimens from the type locality of *guerrerensis* (CMC 455; locality 21), we propose that *guerrerensis* is the

name with priority and should be applied to the *H. chapmani* SMS clade. *H. saturatior* originally was described as a subspecies of *H. chapmani* (Merriam 1901) and later was retained as a subspecies of *H. alfaroi* (Goldman 1918). Our results support recognition by Musser and Carleton (1993, 2005) of *H. saturatior* as a species-level taxon.

There is general agreement that the highlands of the SMO, OH, SMS, and the different mountain ranges in the TIH represent different biogeographic provinces (Halffter 1987; Liebherr 1994; Marshall and Liebherr 2000; Contreras-Medina et al. 2007; Morrone 2010). Overall, the distributional patterns observed in these studies are supported by a variety of taxa, including plants and various animal groups (Luna-Vega et al. 2001; García-Moreno et al. 2004, 2006; Contreras-Medina et al. 2007; Puebla-Olivares et al. 2008; Bryson et al. 2011). The SMS and SMO provinces are thought to have been separated by intense volcanism in the Miocene (~15 mya) during the formation and migration of the Mexican Transvolcanic Belt, with continuing volcanism until ~3.5 mya (Ferrari et al. 1999). Similarly, the highlands south of the Isthmus of Tehuantepec were repeatedly isolated from mountain ranges to the north and east, with the most recent marine incursion thought to have occurred in the late Pliocene ~3.6 mya (Maldonado-Koerdell 1964; Beard et al. 1982; Coates and Obando 1996). Climatic changes during the Pleistocene (~2.5 mya) could have reinforced the isolating effects of a low-lying isthmus (Toledo 1982), as supported by our divergence time estimates.

Overall, levels of genetic differentiation within the *H. chapmani–H. saturatior* complex are in agreement with 3 main clades that occur in isolated mountain ranges (SMO-OH, SMS, and TIH). Therefore, it is reasonable to assume that these lineages have been subjected to similar historical genetic isolation and diversification, as have other montane rodent taxa such as *Peromyscus* (Sullivan et al. 1997; Harris et al. 2000), *Reithrodontomys* (Arellano et al. 2005; Hardy et al. 2013), *Habromys* (León-Paniagua et al. 2007; Rogers et al. 2007), and *Glaucomys* (Kerhoulas and Arbogast 2010), as well as a variety of other vertebrate taxa (see Almendra and Rogers 2012 for a recent summary). However, the degree of divergence of the splits among the 3 main lineages of the *H. chapmani–H. saturatior* complex is not completely consistent with the general patterns observed in other taxonomic groups inhabiting montane systems. Although the lowlands of the Tehuacán–Cuicatlán Valley and the Central Valleys of Oaxaca separate the SMO-OH and SMS, biogeographically, it would be more plausible to expect a closer relationship between the 2 lineages of *H. chapmani* (SMO-OH and SMS). This is because their distributional ranges are closer to each other than either is to *H. saturatior* (TIH).

It has been suggested that the Isthmus of Tehuantepec represents the deepest biogeographic break for closely related taxa of rodents with a geographic distribution along the highlands of México and Central America (Sullivan et al. 2000). Likewise, genetic differentiation recovered herein has been replicated for other rodent clades whose distributions span the Isthmus of Tehuantepec: *Peromyscus* (Sullivan et al. 1997); *Reithrodontomys* (Arellano et al. 2005; Hardy et al. 2013), *Habromys* (León-Paniagua et al. 2007), and *Neotoma* (Edwards and Bradley 2002) as well as other highland taxa (birds—Weir et al. 2008; Barber and Klicka 2010; and reptiles—Castoe et al. 2009). Nevertheless, our data show that within the *H. chapmani–H. saturatior* complex, the deepest split corresponds to the Tehuacán–Cuicatlán Valley and the Central Valleys of Oaxaca, rather than the Isthmus of Tehuantepec. The isthmus has played an important role in the evolutionary diversification of *H. chapmani* (SMO-OH)–*H. saturatior*, as noted by Musser and Carleton (1993, 2005).

It is interesting to note that even though the distribution area of the *H. chapmani* SMO-OH clade includes 2 mountain ranges that are split by the Rio Santo Domingo valley in Oaxaca, samples from each mountain system were not separated in our phylogenetic analyses. This pattern is consistent with that of other rodent species that are continuously distributed along highlands of the SMO and OH, but with no apparent genetic differentiation between samples occurring on each mountain system (i.e., the Mexican harvest mouse, *Reithrodontomys mexicanus*—Arellano et al. 2005). There are, however, examples of other groups of rodents in which the Rio Santo Domingo has played an important role in the diversification of populations on either side of this geological barrier (Jico deermouse, *Habromys simulatus* [SMO]; Chinanteco deermouse, *H. chinanteco* [OH]—Carleton et al. 2002; Rogers et al. 2007; Nelson's big-toothed deermouse, *Megadontomys nelsoni* [SMO]; Oaxacan big-toothed deermouse, *M. cryophilus* [OH]—Vallejo and González-Cózatl 2012). The only evidence of differentiation between samples of *Handleyomys chapmani* from SMO and OH was generated by our NCPA analysis, which found that haplotypes from northern Oaxaca (OH) constitute an allopatric genetic unit from the rest of *H. chapmani* SMO ($P = 0.0010$).

Mexico is considered a biodiversity hot spot for mammals, both in terms of species richness and endemism (Ceballos et al. 1998; Ceballos and Ehrlich 2006; Ceballos 2007; Giam et al. 2011). Tropical montane cloud forest is the most diverse vegetation type in Mexico, but comprises only 1% of the land surface of the country (Pedraza and Williams-Linera 2003). Unfortunately, cloud forest habitat has had a loss of 41% of its original land area, and of what remains, more than 52% is degraded (Mas et al. 2009; Sánchez Colón et al. 2009). As a result of habitat loss, *H. saturatior* is currently listed as near threatened (Reid et al. 2008) and *H. rhabdops* is listed as vulnerable (Reid and Vázquez 2008). Despite the high rates of cloud forest deforestation, *H. chapmani* is listed as least concern mainly because of its relatively large distribution (Castro-Arellano and Vázquez 2008). However, the fact that we recovered 2 evolutionary units within *H. chapmani* results in a substantial reduction in range for both lineages. As a result, the conservation status of *H. chapmani* should be re-evaluated.

Resumen

Handleyomys chapmani (ratón de Handley de Chapman) es un roedor endémico de México con distribución en la Sierra

Madre Oriental (SMO), Sierra Norte de Oaxaca (OH) y Sierra Madre del Sur (SMS). El estatus taxonómico de las poblaciones actualmente clasificadas como *H. chapmani* ha sido problemático y hasta la fecha, las relaciones evolutivas entre dichas poblaciones continúan sin resolverse. En este estudio, usamos secuencias del gen mitocondrial citocromo *b* (1143pb) y del intron 7 del gen beta fibrina (621pb) para estimar una filogenia del grupo, tiempos de divergencia y analizar los patrones de variación genética entre poblaciones de *H. chapmani* en un sentido geográfico. *H. chapmani* fue recuperado en 2 clados monofiléticos correspondientes a los sistemas montañosos de la SMO-OH y SMS. Además, *H. saturatior* (ratón de Handley de bosque nublado), reconocido como el grupo hermano de *H. chapmani*, fue consistentemente recuperado como el linaje hermano al clado de las SMO-OH; revelando a *H. chapmani* como un taxón parafilético. La distribución geográfica de los 2 clados en *H. chapmani* y *H. saturatior* muestra una fuerte correlación con la extensión geográfica de la SMO-OH, la SMS y las Tierras Altas Trans-Istmicas (TIH; tierras altas al este del Istmo de Tehuantepec en Chiapas y América Central). Los tiempos de divergencia asocian el aislamiento de éstas entidades con cambios climáticos del Pleistoceno superior, que posiblemente fue reforzado por barreras geográficas como el Istmo de Tehuantepec, el Valle Tehuacán-Cuicatlán y los Valles Centrales de Oaxaca. El hecho de que las poblaciones de *H. chapmani* constituyan 2 entidades evolutivas, tiene como consecuencia la reducción significativa del rango de distribución de estos 2 linajes. Por lo tanto, el estatus de conservación de *H. chapmani* debe ser reevaluado.

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APPENDIX I

Specimens examined.—For each voucher specimen of *Handleyomys* we list the museum acronym and catalog number as follows: ASNHC = Angelo State Natural History Collections; BYU = Monte L. Bean Life Science Museum, Brigham Young University; CMC = Colección de Mamíferos del Centro de Investigación en Biodiversidad y Conservación, Universidad Autónoma del Estado de Morelos; CURN = Centro Universitario Regional del Norte de la Universidad Autónoma de Nicaragua; ECOSCM = El Colegio de la Frontera Sur; MZFC = Museo de Zoología, Facultad de Ciencias, Universidad Nacional Autónoma de México; ROM = Royal Ontario Museum; and TCWC = Texas Cooperative Wildlife Collection, Texas A&M University. For sequences from Genbank we list the accession ID. Specimens are listed by taxon, country, collecting location, locality number, museum voucher number, and specimen field number. Abbreviations *Cytb,* and *Fgb-I7* indicate which gene or gene segment was sequenced for each individual.

Handleyomys alfaroi.—ECUADOR: Esmeralda, Comuna San Francisco de Bogotá (**37**; *Cytb* = EU579488); MEXICO: Veracruz (Ver), Catemaco, 13.0 km NW (by road) Sontecomapan, Estación Los Tuxtlas-IBUNAM, 150 m (**31**; CMC 2246 = DSR 8543 [*Cytb* = KF658401, *Fgb-I7* = KF658443], CMC 2247 = DSR 8544 [*Cytb* = KF658400, *Fgb-I7* = KF658444]); NICARAGUA: Matagalpa, Selva Negra, 250 m (**27**; *Cytb* = EU579489).

Handleyomys chapmani.—MEXICO: Tamaulipas (Tamps), El Cielo, San José, 1,329 m (**1**; TCWC 59291 = ICA 36 [*Cytb* = KF658365, *Fgb-I7* = KF658451], TCWC 59294 = ICA 69 [*Cytb* = KF658373, *Fgb-I7* = KF658452], TCWC 59289 = ICA 75 [*Cytb* = KF658375, *Fgb-I7* = KF658450]; San Luis Potosí (SLP), El Naranjo, 3.5 km N 3 km W, Maguey de Oriente (**2**; CMC 739 = FXG 527 [*Cytb* = KF658376, *Fgb-I7* = KF658422], CMC 740 = FXG 528 [*Cytb* = KF658356, *Fgb-I7* = KF658423], CMC 741 = FXG 529 [*Cytb* = KF658377); Veracruz (Ver), Zacualpan (**3**; MZFC 8304 = HBR 069 [*Cytb* = KF658379, *Fgb-I7* = KF658448]); Hidalgo (Hgo), 26.5 km NE (by road) Metepec, 2,210 m (**4**; CMC 1042 = FXG 804 [*Cytb* = KF658348], CMC 1043 = FXG 823 [*Cytb* = KF658353], CMC 1044 = FXG 827 [*Cytb* = KF658361, *Fgb-I7* = KF658431]); Puebla (Pue), Huauchinango, Rancho El Paraíso, 6 km SW Huahuchinango, 2000 m (**5**; BYU 15801 = EAA 643 [*Cytb* = KF658362, *Fgb-I7* = KF658417], BYU 15802 = EAA 644 [*Cytb* = KF658354); Puebla (Pue), La Gloria Falls, Apulco River, 10 km N Zacapoaxtla, 1,500 m (**6**; BYU 15803 = EAA 642 [*Cytb* = KF658344, *Fgb-I7* = KF658418]); Puebla (Pue), 4.7 km NE (by road) Teziutlán, 1,750 m (**7**; CMC 1049 = FXG834 [*Cytb* = KF658345, *Fgb-I7* = KF658432], CMC 1052 = FXG 837 [*Cytb* = KF658349], CMC 1054 = FXG 839 [*Cytb* = KF658346]); Veracruz (Ver), Xico, Matlalapa, 2,070 m (**8**; CMC 1497 = RMV 50 [*Cytb* = KF658378], CMC 1495 = RMV48 [*Cytb* = KF658355, *Fgb-I7* = KF658436]); Veracruz (Ver)**,** Xalapa, El Haya, Old road to Coatepec km 25 (Botanic Garden Francisco Javier Clavijero), 1,235 m (**9**; CMC 1450 = RMV 01[*Cytb* = KF658343]; Veracruz (Ver), Acajete, Mesa de la Yerba, 3.4 km intersection to Mazatepec (Xalapa-Perote by road), 2,004 m (**10**; CMC 1353 = FXG 873 [*Cytb* = KF658366], CMC 1490 = RMV 84 [*Cytb* = KF658380, *Fgb-I7* =

KF658435]); Veracruz (Ver), Huatusco, Las Cañadas, 1,340 m (**11**; CMC 779 = FXG 618 [*Cytb* = KF658350, *Fgb-I7* = KF658426], CMC 780 = FXG 619 [*Cytb* = KF658360], CMC 782 = FXG 621 [*Cytb* = KF658351]); Veracruz (Ver), Texhuacán, 1.2 km SE Xochititla, 1,670 m (**12**; CMC 772 = FXG 578 [*Cytb* = KF658358, *Fgb-I7* = KF658424], CMC 773 = FXG 579 [*Cytb* = KF658357], CMC 774 = FXG 580 [*Cytb* = KF658372], CMC 775 = FXG 581 [*Cytb* = KF658347, *Fgb-I7* = KF658425]); Oaxaca (Oax), Puerto de la Soledad, 2,600 m (**13**; BYU 15303 = EAA 310 [*Cytb* = KF658364], BYU 15304 = EAA 311 [*Cytb* = KF658363]); Oaxaca (Oax), Concepción Pápalo, 14.4 km NE (by road) Santa Flor, 2,600 m (**14**; CMC 1382 = FXG 943 [*Cytb* = KF658370], CMC 1347 = FXG 944 [*Cytb* = KF658367], CMC 1352 = FXG 949 [*Cytb* = KF658381, *Fgb-I7* = KF658434], CMC 1389 = FXG 950 [*Cytb* = KF658369]); Oaxaca (Oax), Ixtlán, 11 km SW (by road) La Esperanza, 2,400 m (**15**; CMC 113 = DSR 5800 [*Cytb* = KF658374, *Fgb-I7* = KF658419], CMC 115 = DSR 5827 [*Cytb* = KF658371]); Oaxaca (Oax), Santa María Tlahuitoltepec, Santa María Yacochi, 2,400 m (**16**; CMC 114 = DSR 5701 [*Cytb* = KF658368], CMC 117 = DSR 5763 [*Cytb* = KF658382], CMC 119 = DSR 5765 [*Cytb* = KF658359]); Oaxaca (Oax), Candelaria Loxicha, 0.7 km E (by road) La Soledad, 1,025 m (**17**; CMC 943 = FXG 682 [*Cytb* = KF658395]); Oaxaca (Oax), Miahuatlán, San Miguel Suchixtepec, Río Molino, 2,353 m (**18**; CMC 925 = FXG 691 [*Cytb* = KF658388, *Fgb-I7* = KF658427], CMC 930 = FXG 737 [*Cytb* = KF658389], CMC 931 = FXG 738 [*Cytb* = KF658391, *Fgb-I7* = KF658429], CMC 932 = FXG 739 [*Cytb* = KF658387], CMC 927 = FXG 734 [*Cytb* = KF658390, *Fgb-I7* = KF658428]); Guerrero (Gro), Malinaltepec, 3 km E El Tejocote, 2,620 m (**20**; CMC 1656 = FXG 1043 [*Cytb* = KF658392], CMC 1657 = FXG 1044 [*Cytb* = KF658394, *Fgb-I7* = KF658438], CMC 1655 = FXG 1041 [*Cytb* = KF658393, *Fgb-I7* = KF658437]); Guerrero (Gro), Chilpancingo de los Bravos, 6.1 km SW (by road) Omiltemi, 2,480 m (**21**; CMC 455 = FXG 412 [*Cytb* = KF658399]); Guerrero (Gro), Leonardo Bravo, 3.4 km (by road) Carrizal, 2,480 m (**22**; CMC 452 = FXG 462 [*Cytb* = KF658397, *Fgb-I7* = KF658420], BYU 20647 = FXG 463 [*Cytb* = KF658396], CMC 454 = FXG 464 [*Cytb* = KF658398, *Fgb-I7* = KF658421]); Hidalgo (Hgo), Tlanchinol, 3 km E (by road) Tlanchinol, 1,451 m (**23**; BYU 15300 = EAA 272 [*Cytb* = KF658352]).

Handleyomys melanotis.—MEXICO: Oaxaca (Oax), Putla Villa de Guerrero, 5.5 km S (by road) Concepción de Guerrero, 936 m (**19**; CMC 942 = FXG 789 [*Cytb* = KF658412, *Fgb-I7* = KF658430], CMC 939 = FXG 793 [*Cytb* = KF658413]; Nayarit (Nay), Peñita de Jaltemba, 1.8 km N of La Peñita de Jaltemba (ASNHC 3418 = ASK1601 [**33**; *Cytb* = KF658408]); Michoacán (Mich), Coalcomán, 10.9 km NW (by road) Coalcomán (**29**; CMC 1806 = DSR 7715 [*Cytb* = KF658410]); Jalisco (Jal), San Sebastián, 3.4 km W (by road) San Sebastián del Oeste, 1,450 m (**30**; CMC 1207 = DSR 7414 [*Cytb* = KF658411, *Fgb-I7* = KF658433]); Nayarit (Nay), 8 KM E of San Blas (**34**; ASNHC 3419 = ASK 1538 [*Cytb* = KF658409, *Fgb-I7* = KF658415]); Colima (Col), Comala, Hacienda San Antonio (**36**; ASNHC = ASK1957 [*Cytb* = KF658414, *Fgb-I7* = KF658416]).

Handleyomys rostratus.—MEXICO: Veracruz (Ver), Catemaco, 13 km NW (by road) Sontecomapan, Estación Los Tuxtlas, IBUNAM, 150 m (**31**; CMC 2222 = DSR 8560 [*Cytb* = KF658407, *Fgb-I7* = KF658439]); Chiapas (Chis), Berriozabal, 12 km N (by road) Berriozabal, 1,060 m (**32**; CMC 2241 = DSR 8464 [*Cytb* = KF658403, *Fgb-I7* = KF658440], CMC 2242 = DSR 8465 [*Cytb* = KF658406], CMC 2243 = DSR 8466 [*Cytb* = KF658402], CMC 2244 = DSR 8467 [*Cytb* = KF658405, *Fgb-I7* = KF658441], CMC 2245 = DSR 8468 [*Cytb* = KF658404, *Fgb-I7* = KF658442]); Tamaulipas (Tamps), Rancho Calabazas (near Ciudad Victoria), 3.2 km W Calabazas (**35**; *Cytb* = EU579492). EL SALVADOR: Ahuachapán, Ahuachapán, El Imposible (**25**; *Cytb* = EU579493). NICARAGUA: Matagalpa, Matagalpa, El Tigre (**27**; *Cytb* = EU579491).

Handleyomys saturatior.—MEXICO: Chiapas (Chis), La Trinitaria, Lagos de Montebello (**24**; ECOSCM 1228 [*Cytb* = KF658384, *Fgb-I7* = KF658446], ECOSCM 1229 [*Cytb* = KF658385], ECOSCM 1231 [*Cytb* = KF658383, *Fgb-I7* = KF658447]). NICARAGUA: Matagalpa, Selva Negra-Atajo Trail (**27**; TTU 101644 [*Cytb* = DQ224410, *Fgb-I7* = KF658453]); (**28**; CURN = JAGE 438 [*Cytb* = KF658386, *Fgb-I7* = KF658445]). EL SALVADOR: Santa Ana, Montecristo National Park (**26**; ROM 101537 [*Cytb* = EU579494, *Fgb-I7* = KF658449]).