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CRYPTIC SPECIES IN THE MEXICAN POCKET GOPHER *CRATOGEOMYS MERRIAMI*

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A study of chromosomal variation in the Mexican pocket gopher *Cratogeomys merriami* revealed differences in diploid number that correspond to 3 major genetic and morphological clades within the species. Each of these 3 clades is diagnosable based on multiple characters, including chromosomal diploid number, quantitative and qualitative morphological characters, and mitochondrial DNA. Accordingly, we restrict the name *C. merriami* (Thomas) to include only pocket gophers of this genus from the states of México, México D.F., northern Morelos, and west-central Puebla. We resurrect the species name *C. fulvescens* Merriam to represent members of this genus from southern Tlaxcala, east-central Puebla, and parts of west-central Veracruz. Finally, we resurrect the species name *C. perotensis* Merriam to represent members of this genus from southern Hidalgo, northern Tlaxcala, north-central Puebla, and parts of west-central Veracruz. Based on the observation that differences in diploid number usually signal reproductive isolation between populations of pocket gophers, we hypothesize that *C. merriami*, *C. fulvescens*, and *C. perotensis* are reproductively incompatible. We provide synonymies and descriptions for these 3 species, along with a key to the *C. castanops* species group to which these species belong.

Key words: chromosomes, *Cratogeomys*, mitochondrial DNA, morphology, nuclear DNA, pocket gophers, systematics

During his distinguished career as curator of mammals at the British Museum, M. R. Oldfield Thomas (1858–1929) named and described *Geomys* (= *Cratogeomys*) *merriami* (Thomas 1893) in honor of his esteemed American colleague, C. Hart Merriam (1855–1942). Thomas's description was based on a single specimen (BM[NH] 1870.6.20.2, the holotype) labeled "southern México" and housed in the British Museum. Two years later, Merriam (1895), who was then Chief of the Division of Ornithology and Mammalogy for the United States Department of Agriculture, published his landmark monograph on the systematics of pocket gophers (exclusive of *Thomomys*). Merriam's (1895) detailed description and comparison of >1,000 specimens of pocket gophers in the various collections at his disposal resulted in a taxonomy of the Geomyidae that is generally valid to this day.

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In his monograph, Merriam (1895) determined the geographic range of *C. merriami* to include the southeastern rim of the Valley of Mexico, western Puebla, and parts of adjacent states (Fig. 1). Merriam also described 5 new species of *Cratogeomys* related to *C. merriami* (*estor*, *fulvescens*, *oreocetes*, *peregrinus*, and *perotensis*) from Puebla and surrounding states. In 1934, Nelson and Goldman described a new subspecies of *C. merriami* (*C. m. irolonis* from the vicinity of Irolo, Hidalgo), which was later elevated to full species status by Davis (1944). Unfortunately, Davis's study was based on 10 misidentified specimens, which were determined by Russell (1968) to be *C. tylorhinus* (recently synonymized under *C. fumosus* by Hafner et al. [2004]), not *C. merriami*.

Russell's (1968) revision of *Pappogeomys* (= *Cratogeomys*, exclusive of *P. bulleri*) is the most recent systematic treatment of *C. merriami*. Based on examination of cranial and dental features, body size, and pelage color and texture, Russell (1968) synonymized Merriam's (1895) species *estor*, *fulvescens*, *oreocetes*, *peregrinus*, and *perotensis* under *C. merriami* (Thomas). Russell recognized 7 subspecies of *C. merriami*, including *estor*, *fulvescens*, and *perotensis* (Merriam 1895),

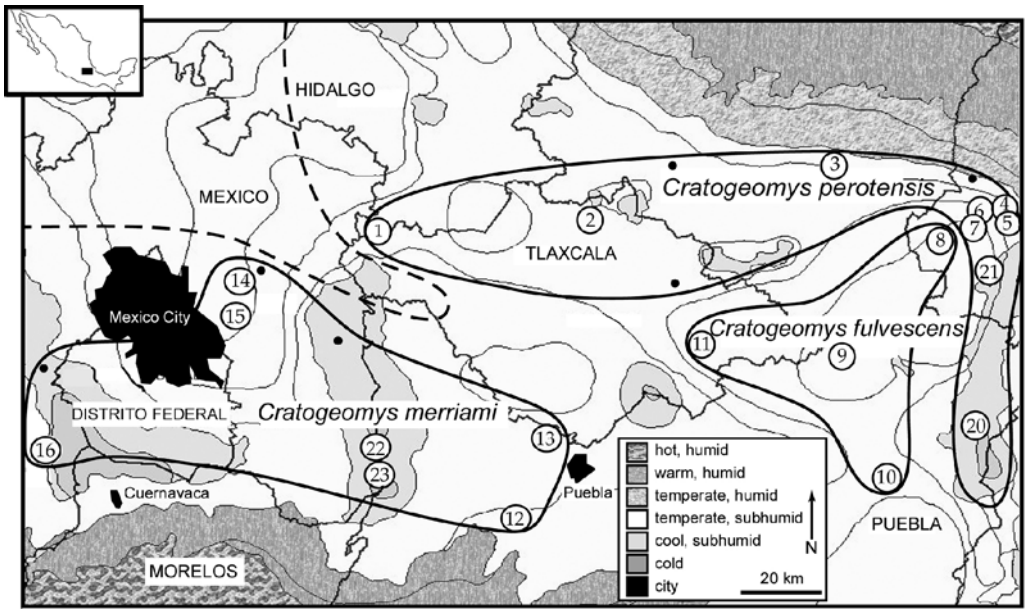

FIG. 1.—Geographic distribution of 3 species of *Cratogeomys* in central Mexico. These species, plus 3 others described by Merriam (1895) from this same region, were synonymized under *C. merriami* by Russell (1968). Results of the current study support retention of Merriam's (1895) original species *C. perotensis*, *C. fulvescens*, and *C. merriami*. Numbers refer to collecting localities listed in Appendix I. Black dots indicate marginal localities of specimens identified to species based on pelage markings (see diagnostic key), morphometrics (Appendix II), or both. Dashed line shows approximate eastern edge of geographic range of *Cratogeomys fumosus* (Hafner et al. 2004). The key indicates climatic regimes that correspond to elevational differences in this region of Mexico.

irolonis and *saccharalis* (Nelson and Goldman 1934), *peraltus* (Goldman 1937), and *merriami* (Thomas 1893). In his discussion of intraspecific variation in *C. merriami*, Russell (1968:697) commented, "Of the seven subspecies, *fulvescens* is most divergent."

Our recent studies of chromosomal variation in *C. merriami* revealed differences in diploid number that correspond geographically to Merriam's (1895) species *fulvescens* and *perotensis*. This discovery prompted additional investigation of *C. merriami* by using molecular and morphometric techniques, culminating in this systematic revision of *C. merriami* and related species.

MATERIALS AND METHODS

Specimens examined.—Twenty-nine specimens of *C. merriami* were used in the mitochondrial DNA (mtDNA), nuclear DNA, and chromosomal analyses (Appendix I), and 162 specimens were used in the morphometric analysis (Appendix II). Cytochrome-*b* (*Cytb*) sequence data for 5 specimens in Appendix I were taken from Demastes et al. (2002; GenBank accession numbers AF302157–AF302161). The remaining specimens are new to this study and were captured in the wild by using standard trapping methods. Animals collected for this project were treated in a humane manner as approved by the Louisiana State University Institutional Animal Care and Use Committee by following guidelines of the American Society of Mammalogists (Animal Care and Use Committee 1998). Either holotypes or topotypes of all of Merriam's (1895) named forms were examined for diagnostic morphological characters (see diagnostic key) and were karyotyped, and all named forms recognized by Russell (1968) were sequenced for mtDNA. Outgroup taxa consisted of 1 specimen each of *C. castanops*, *C. goldmani*, and *Pappogeomys bulleri* (Appendix I). *Cytb* sequence data for these species were obtained from Demastes et al. (2002; GenBank accession numbers AF302171, AF302176, and AF302177).

Mitochondrial DNA analysis.—Genomic DNA was isolated from liver or kidney tissue by using the DNeasy Tissue Kit (QIAGEN Inc., Valencia, California). Amplification by polymerase chain reaction (PCR) and sequencing of the mitochondrial *Cytb* gene (1,140 base pairs [bp]) were performed by using combinations of the following primers: L14724, L15513 (Irwin et al. 1991), L15049, H15579, H15906 (Spradling et al. 2001), and H15154 ("MVZ04" in Smith and Patton [1993]). PCR amplifications were performed in 50-µl reaction volumes, usually with primers L14724 with H15906. If this primer pair failed to yield acceptable internal sequences, reactions were performed by using primers L14724 with H15154, L15049 with H15579, and L15513 with H15906. Each reaction included 2.5 µl of each primer (10 µM), 4 µl of $MgCl_2$ (10 mM), 2 µl of deoxynucleotide triphosphate mixture (10 mM solution; deoxyadenosine triphosphate, deoxyguanosine triphosphate, deoxycytosine triphosphate, and deoxythymidine triphosphate, each 100 mM), 5 µl of 10X *Taq* buffer, and 0.2 µl of *Taq* DNA polymerase. The amplification protocol required an initial denaturation stage of 94°C for 2 min, followed by 38 PCR cycles of 94°C (1 min), 47°C (1 min; 4 cycles), then 56°C (1 min; 34 cycles), and 72°C (1 min), and a final extension of 72°C for 10 min.

Polymerase chain reaction amplifications of the entire mitochondrial gene cytochrome oxidase subunit I (*CoI*; 1,551 bp) were performed in 50-µl reaction volumes by using primers *CoI*-5285f and *CoI*-6929r (Spradling et al. 2004) and the *Cytb* protocol listed above. The primers *CoI*-5285f, *CoI*-6929r, GcoIF1, GcoIR1, and *CoI*-570F (Spradling et al. 2004) were used to perform sequencing reactions.

Before sequencing, amplified *Cytb* and *CoI* products were purified by using the QIAquick PCR Purification Kit protocol (QIAGEN, Inc.). Amplified products were sequenced in both directions at the Museum of Natural Science, Louisiana State University. Each 10-µl reaction

included 2 μl of BigDye (Applied Biosystems, Inc. [ABI], Perkin-Elmer Corporation, Boston, Massachusetts), 3.2 μl of 0.5 μM primer, 1.8 μl of double-distilled H_2O, and 3 μl of amplification product. An alternative 10-μl reaction included 1.6 μl of BigDye (ABI, Perkin-Elmer Corporation), 0.32 μl of 10 μM primer, 4.08 μl of double-distilled H_2O, 2 μl of 5× ABI sequencing buffer, and 2 μl of amplification product. Samples were sequenced for 30 cycles at 96°C (10 s), 50°C (5 s), and 60°C (4 min). Templates were purified with Centri-Sep spin columns (Princeton Separations, Inc., Adelphia, New Jersey) and were electrophoresed by using an ABI Prism 377 Genetic Analyzer (Perkin Elmer, Foster City, California). Sequences were edited by using Sequencher Version 4.1 software (Gene Codes Corporation, Ann Arbor, Michigan) and then aligned by using Se-Al v2.0a11 (http://evolve.zps.ox.ac.uk/Se-Al/Se-Al.html) and submitted to GenBank (GenBank accession numbers AF302158, AF302171, AF302176, AF302177, and AY649447–AY649468 for *Cytb*; and AY331076–AY331078, AY331084, and AY649469–AY649490 for *CoI*).

Three mtDNA data sets were analyzed: *Cytb, CoI*, and *Cytb* + *CoI*. Phylogenetic analyses were conducted by using maximum-parsimony, maximum-likelihood, and Bayesian approaches. Equally weighted maximum-parsimony searches were performed with 100 random taxon addition replicates and tree bisection-reconnection branch swapping (PAUP* 4.0b10—Swofford 2002). Nonparametic bootstrap (1,000 pseudoreplicates and 10 random sequence additions) analyses were performed to assess nodal support (Felsenstein 1985).

To generate the best maximum-likelihood tree, Modeltest (Version 3.6—Posada and Crandall 1998) was used to examine the fit of 56 models of nucleotide substitution to the sequence data. Models of evolution providing the best approximation of the data using the fewest parameters were chosen for subsequent analyses according to hierarchical likelihood-ratio tests and the Akaike information criterion (AIC—Huelsenbeck and Rannala 1997; Posada and Buckley 2004). Only the results of the hierarchical likelihood-ratio tests are presented here because both approaches selected similar models and phylogenetic analysis using these models of evolution yielded the same topology. The Tamura–Nei model (TrN—Tamura and Nei 1993), including among-site rate variation (TrN+Γ—Gu et al. 1995), was chosen as the best model of evolution according to hierarchical likelihood-ratio tests for both the *Cytb* and *Cytb* + *CoI* data sets. Similarly, the Hasegawa–Kishino–Yano model (HKY—Hasegawa et al. 1985), again including among-site rate variation (HKY+Γ—Gu et al. 1995), was chosen for the *CoI* data set. A full heuristic maximum-likelihood search was conducted by using the successive-approximations approach with the preferred model in PAUP* 4.0b10 (Swofford 2002). A full heuristic bootstrap (200 pseudoreplicates) also was performed on both genes by using the preferred model on a Beowolf cluster (8 alpha-processor nodes).

Bayesian phylogenetic analyses were performed by using MrBayes 3.0B4 (Huelsenbeck and Ronquist 2001). The GTR+Γ model was used in all analyses and model parameters were treated as unknown variables with uniform priors and were estimated as part of the analysis. Bayesian analyses were initiated with random starting trees, performed for 2×10^6 generations with 4 incrementally heated chains (Metropolis-coupled Markov chain Monte Carlo—Huelsenbeck and Ronquist 2001), and sampled at intervals of 100 generations. Two independent Bayesian analyses were performed to avoid entrapment on local optima and log-likelihood scores were compared for convergence (Huelsenbeck and Bollback 2001; Leaché and Reeder 2002). Stationarity was assessed by plotting the log-likelihood scores of sample points against generation time. All burn-in points (the first 2,500 trees) were discarded. The retained equilibrium samples were used to generate a 50% majority-rule consensus tree with the percentage of samples recovering any particular clade representing that clade's posterior probability (Huelsenbeck and Ronquist 2001).

Phylogenetic congruence of the gopher mitochondrial genes *Cytb* and *CoI* data sets was evaluated by using the partition homogeneity test (Farris et al. 1994) in PAUP* 4.0b10 (Swofford 2002). One thousand partition replicates were analyzed by maximum parsimony (heuristic search option and random addition replicates of tree bisection-reconnection branch swapping). Alternative phylogenetic hypotheses were compared statistically by using the Kishino–Hasegawa and Shimodaira–Hasegawa tests as implemented in PAUP* 4.0b10 (maximum-parsimony and maximum-likelihood analyses using resampling estimated log-likelihood optimization and 1,000 bootstrap replicates—Goldman et al. 2000; Shimodaira and Hasegawa 1999). Sequence divergence between taxa was calculated by using uncorrected *p* distances in PAUP* 4.0b10. Average divergence within and between clades was calculated by hand.

Ancient mtDNA analysis.—Extraction of DNA from a 3-mm^2 section of dried skin from specimen FMNH 14061 (collected in 1904) was accomplished by using the DNeasy Tissue Kit (QIAGEN, Inc.) following the manufacturer's protocol with the following modifications: The dried skin was initially soaked in phosphate-buffered saline for 5 h (with 3 solution changes), addition of 3 μg of a homopolymer (15-deoxythymidine) to act as carrier DNA, and pH adjustment of the sample to 6.5–7.0 before adding the sample to the column to ensure DNA binding (Iudica et al. 2001).

Because of the age of the specimen (>100 years old) a necessarily short section (88 bp) of the *Cytb* gene was identified that allowed discrimination among the 3 *Cratogeomys* species in question. Two primers were designed to amplify this 88-bp region: H109 5′-GTAGGATTAAGCATAATCCTA and L21 5′-AACTAATGACAATTATACGAAAATCT. Primer names indicate the DNA strand (H = heavy or L = light) and the position of the 3′ end of the oligonucleotide sequence relative to the 1st nucleotide position of *Cytb* (Anderson et al. 1981).

Double-stranded PCR amplifications were performed in 20-μl reaction volumes. Buffer conditions were optimized for difficult templates (100 mM Tris-HCl, pH 8.3; 500 mM KCl; 17.5 mM $MgCl_2$; 5% [by volume] dimethylsulfoxide; 0.5% Triton-X; 10% [weight by volume] bovine serum albumin). After an initial denaturation stage of 95°C for 1 min, 55 PCR cycles were performed with the following thermal-cycling parameters: 1 min denaturation at 95°C, 45 s annealing at 38°C, and 1 min extension at 72°C.

Several precautions were taken to avoid contamination. Before each PCR reaction was set up, laboratory benches and pipettors were washed with DNase (DNA Away, Molecular Bio-Products, San Diego, California) to remove any DNA, and all PCR reactions were run with negative controls. After sequences were obtained and proofed, searches were performed by using BLAST software (Basic Local Alignment Search Tool, National Center for Biotechnology Information, Bethesda, Maryland) to ensure that the sequences were the result of amplification of the target template. In addition to BLAST searches, sequences also were compared to all sequences that were the result of unrelated PCR activity in the laboratory. Before sequencing, amplified products were purified by using the QIAquick PCR Purification Kit protocol (QIAGEN, Inc.). Sequencing was performed at the Iowa State University Sequencing and Synthesis Facility, Ames, Iowa. Both DNA strands were obtained. The resulting sequence was deposited in GenBank (GenBank accession number AY943306).

Nuclear DNA analysis.—Amplification of a portion of the nuclear recombination activating gene 1 (*Rag1*) was performed by using primers *Rag1*-S70 and *Rag1*-S115 (Steppan et al. 2004) as outlined by Spradling et al. (2004). Amplification of a portion of the 7th intron of

the β-fibrinogen gene (β-*fib*) was accomplished by using the FIB-B17U and FIB-B17L primers of Prychitko and Moore (1997) as outlined by Spradling et al. (2004). *Rag1* and β-*fib* PCR products were prepared for sequencing by using the QIAquick PCR Purification Kit (QIAGEN, Inc.). Sequencing reactions were performed by using the CEQ 2000 Dye Terminator Cycle Sequencing with Quick Start Kit and were assessed using the CEQ 8000 Genetic Analysis System (Beckman Coulter, Inc., Fullerton, California). Sequences were aligned and heterozygosity (defined as peaks of equal height) was evaluated by eye by using Sequencher 4.1.2 software (Gene Codes Corporation). Sequences were submitted to GenBank (GenBank accession numbers AY331222 and AY581194–AY581196 for *Rag1*; and AY880889–880911 for β-*fib*).

The aligned β-*fib* sequences were pruned to include only sites that showed variation in one or more individuals ($n = 7$ sites). The genotype of each individual was determined, and relationships among all β-*fib* alleles ($n = 9$ alleles) were inferred by parsimony analysis.

Chromosomal analysis.—After the initial discovery of major chromosomal differences within *C. merriami* (sensu lato), chromosomal variation was examined in specimens from localities selected to represent the 3 major groups identified in the mtDNA analysis. Non–preferentially stained chromosome preparations were made from 20 individuals (Appendix I) by following the postmortem field protocol described by Hafner and Sandquist (1989). Diploid number (2n) and fundamental number (FN) were determined for each individual by examination of at least 10 metaphase spreads.

Morphometric analysis.—One hundred twenty-two specimens of *C. merriami* (62 females and 60 males; Appendix II) were measured for 12 mensural characters. These variables included occipital–nasal length, occipital–incisor length, nasal length, rostral width, width of interorbital constriction, zygomatic breadth, cranial width, mastoid breadth, diastema length, length of maxillary toothrow, occlusal length of upper molars 1 and 2, and occlusal length of upper molar 3. This set of characters has proven useful in previous morphometric analyses of pocket gophers (Patton and Smith 1990; Smith and Patton 1988). Specimens with missing data and specimens that were clearly not adults based on skull size (occipital–nasal length < 50 mm) were excluded from the analysis. Univariate and multivariate statistical analyses were performed by using SAS software (SAS Institute Inc. 2000) and Systat 8.0 (SPSS, Inc. 1998). The measured cranial characters for specimens of *C. merriami* were examined for sexual dimorphism by using an unpaired *t*-test. Past work has shown strong sexual dimorphism in pocket gophers (Patton and Smith 1990; Smith and Patton 1988), and our results support this finding ($P < 0.05$ for all characters except maxillary toothrow, occlusal length of upper molars 1 and 2, and occlusal length of upper molar 3). Accordingly, males and females were analyzed separately.

To decrease the effect of individual size variation, all characters were transformed logarithmically and standardized by using the methods of Burbrink (2001), Corruccini (1975), and Gould (1966). Discriminant function analysis and size-free canonical discriminant analysis (dos Reis et al. 1990) were performed on both the raw and size-adjusted characters to determine if gophers could be separated with an a priori hypothesis of group membership to the 3 mitochondrial clades identified in this study (hereafter referred to as the *perotensis*, *fulvescens*, and *merriami* clades). Sample sizes for each clade were as follows: *perotensis* = 38, *fulvescens* = 7, and *merriami* = 17 for females; *perotensis* = 20, *fulvescens* = 15, and *merriami* = 25 for males. Before discriminant function analysis, stepwise discriminant function analyses (forward and backward) were performed on the size-adjusted cranial characters to identify those characters most useful in discriminating among groups. Discriminant function analyses were performed by using all cranial characters as well as those selected by stepwise discriminant analysis. Analyses also were performed by using equal and estimated prior probabilities of group membership. The analyses generated classification matrices (jackknifed and unjackknifed) that showed the percentage of specimens correctly assigned to their a priori groupings. Qualitative morphological characters used in the diagnostic key were obtained from Merriam (1895).

Results

Mitochondrial DNA analysis.—Of the 1,140 bp of the *Cytb* gene examined, 180 bp were potentially parsimony informative. Parsimony analysis of the *Cytb* gene produced 1 most-parsimonious tree (not shown; length = 502; consistency index [CI] = 0.755; retention index [RI] = 0.834; rescaled CI [RC] = 0.629). Of the 1,551 bp of the *CoI* gene examined, 223 bp were potentially parsimony informative. Parsimony analysis of the *CoI* gene produced 16 equally parsimonious trees (not shown; length = 607; CI = 0.738; RI = 0.825; RC = 0.609). Phylogenetic analyses using maximum-parsimony, maximum-likelihood, and Bayesian methods for the 23 ingroup and 3 outgroup taxa for both *Cytb* and *CoI* yielded trees that differed only in minor rearrangements or polytomies of terminal branches. The partition homogeneity test did not detect significant heterogeneity between the 2 mitochondrial genes ($P = 0.321$). Given this result and the strong topological similarity observed in independent analyses of the *Cytb* and *CoI* data sets, these data were combined in subsequent analyses, resulting in a total of 2,691 bp of which 403 bp were potentially parsimony informative. Parsimony analysis of the combined data set yielded 18 equally parsimonious trees (length = 1,108; CI = 0.768; RI = 0.829; RC = 0.619). Phylogenetic analyses using maximum-parsimony, maximum-likelihood, and Bayesian methods for the 23 ingroup and 3 outgroup taxa for the combined data set yielded trees with the same clade structure, although they differed in the position of terminals within clades. Likewise, trees generated in the combined analysis had the same clade structure as those from the independent analyses, although they differed occasionally in the position of terminals within clades.

Maximum-parsimony and maximum-likelihood bootstrap support values were broadly similar across all 3 analyses. Posterior probabilities from the Bayesian analysis were similar to, but generally higher than, the maximum-likelihood bootstrap support values (Cummings et al. 2003; Erixon et al. 2003—posterior probability values available upon request). All analyses resolved 3 well-supported clades: *perotensis, fulvescens*, and *merriami* (Fig. 2). Although the mitochondrial data unite *C. perotensis* with *C. merriami*, bootstrap support for this association is not particularly strong. The specimen from 1 km southeast of San Miguel Xoxtla, Puebla (CNMA 41826; locality 13 in Figs. 1 and 2) groups with *C. perotensis*, despite having chromosomes and pelage characteristic of *C. merriami* (see diagnostic key).

Percentage sequence divergence values (uncorrected pairwise distance; Table 1) were similar within and between clades

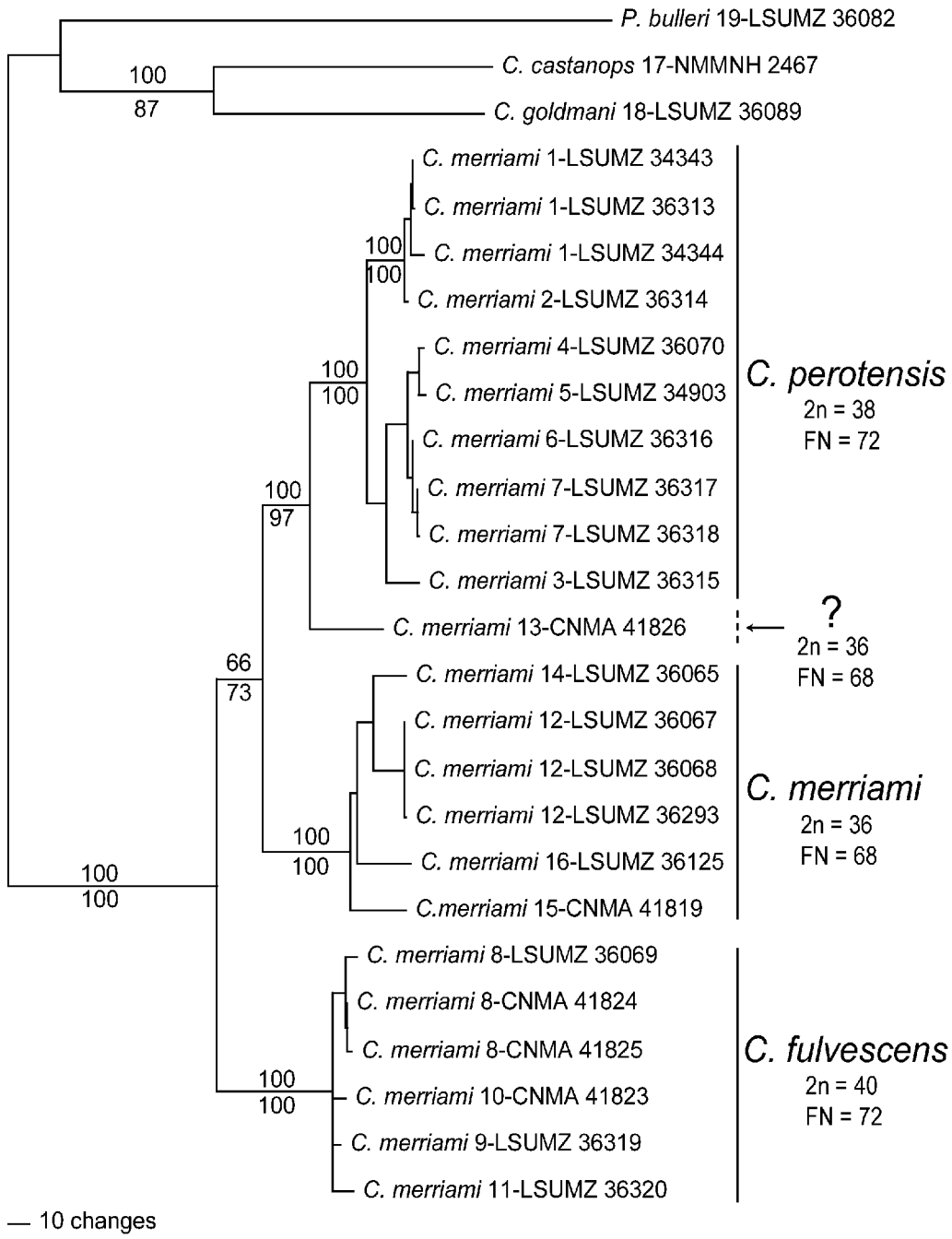

FIG. 2.—Parsimony phylogram based on the combined mitochondrial DNA (mtDNA) analysis (*Cytb* plus *CoI*) for 23 ingroup specimens (*Cratogeomys merriami*, sensu lato) and 3 outgroup specimens (*C. castanops*, *C. goldmani*, and *Pappogeomys bulleri*). The species names applied to the 3 major gopher clades in this study are indicated at the right. Data also were analyzed by using maximum-likelihood and Bayesian analyses, yielding trees with very similar branching structure (see text). Bootstrap support values are indicated only for the deeper splits (maximum-likelihood support values above the nodes, and maximum-parsimony support values below the nodes; other support values are available upon request). Diploid (2n) and fundamental numbers (FN) are indicated for the 3 major clades. The specimen from locality 13 (San Miguel Xoxtla, Puebla; CNMA 41826) groups with *C. perotensis* based on mtDNA data, but groups with *C. merriami* based on karyotype, morphology, and parasitological data.

for all analyses, although the *Cytb* gene appeared to be evolving slightly more rapidly than the *CoI* gene (average percentage sequence divergence within clades was 1.097 for *Cytb* and 1.024 for *CoI*). Percentage sequence divergence among clades averaged 5.220 for *Cytb* and 4.596 for *CoI*. Percentage sequence divergence calculated from the combined data set averaged 0.986 within clades and 4.835 between clades.

Ancient mtDNA analysis.—Specimen FMNH 14061 from the original type series of *C. merriami peraltus* (Goldman) had

TABLE 1.—Average percentage sequence divergence (and uncorrected *P* value) within and between clades identified by phylogenetic analyses of the 3 mitochondrial DNA (mtDNA) data sets. Values in parentheses are average percentage sequence divergence when the specimen from San Miguel Xoxtla, Puebla (CNMA 41826) is constrained to group with the *Cratogeomys merriami* clade (see text).

	mtDNA data set		
	Cytb	*CoI*	*Cytb* + *CoI*
Within *perotensis*	1.711 (1.009)	1.549 (0.838)	1.562 (0.911)
Within *fulvescens*	0.395 (0.395)	0.454 (0.454)	0.428 (0.428)
Within *merriami*	1.184 (2.370)	1.069 (2.355)	0.969 (2.254)
perotensis versus *fulvescens*	5.395 (5.482)	4.463 (4.489)	4.944 (4.980)
perotensis versus *merriami*	4.518 (4.079)	4.546 (3.805)	4.503 (3.890)

an mtDNA haplotype identical to that of *C. perotensis* for the 88 bp sequenced.

Nuclear DNA analysis.—The *Rag1* sequences (520 bp) provided no cladistic resolution. The single specimen representing each of the 3 clades identified in the mtDNA analysis (Fig. 2) plus the specimen from near San Miguel Xoxtla (CNMA 41826; locality 13 in Figs. 1 and 2) were identical, except for 1 autapomorphic base substitution in the specimen representing the *C. fulvescens* clade (CNMA 41823; locality 10 in Figs. 1 and 2).

Of the 529 bp of β-*fib* examined, only 7 nucleotide positions were variable (Fig. 3). These sites comprised 9 alleles in the gophers examined, with allele 2 presumed primitive for the entire clade because it differs by only 1 step (GACCTTA) from the allele present in all outgroup taxa (GACCGTA). Four equally parsimonious networks of the remaining alleles are possible (not shown), with differences among the networks involving only alleles 3, 6, and 8. Allele 1 is widespread in the *merriami* clade and is present in only 1 individual of the *perotensis* clade. Conversely, alleles 3 and 6 are widespread in the *perotensis* clade and present in only 1 individual of the *merriami* clade. These alleles (1, 3, and 6) can be derived directly from the presumed primitive allele (1 step each; Fig. 3), and because these alleles are present only in individuals of the *perotensis* and *merriami* clades, they are presumed synapomorphies for the *perotensis* + *merriami* lineage. However, it is possible that one or more of these alleles is the result of introgression between *perotensis* and *merriami* (see ''Discussion''). The remaining 5 alleles support the 3 clades identified in the mtDNA analysis (Fig. 2). Specifically, alleles 4, 7, and 9 are restricted to *C. fulvescens* with the following genotype proportions: 4,4 (3 individuals); 4,9 (1 individual); 7,7 (1 individual). Allele 5 was found only in *C. merriami* (homozygous in 1 individual), and allele 8 was restricted to *C. perotensis* (heterozygous in 1 individual).

Morphometric analysis.—Multivariate analysis (canonical discriminant analysis or discriminant function analysis) of both female and male pocket gophers was able to discriminate among the 3 clades (*perotensis*, *fulvescens*, and *merriami*) by using the cranial characters selected for analysis (Fig. 4). Results for the different data treatments (standardized and log-transformed) were similar, with the standardized data presented below. For female pocket gophers, a posteriori rates of correct classification into the 3 mitochondrial clades were 82% (*perotensis*), 100% (*fulvescens*), and 100% (*merriami*). Seven gophers were misclassified; however, only 3 of these were misclassified with high confidence (>80%). For male pocket gophers, rates of correct classification into mitochondrial groups were 90% (*perotensis*), 100% (*fulvescens*), and 92% (*merriami*). Four male gophers were misclassified, and 2 of these were misclassified with high confidence (>80%).

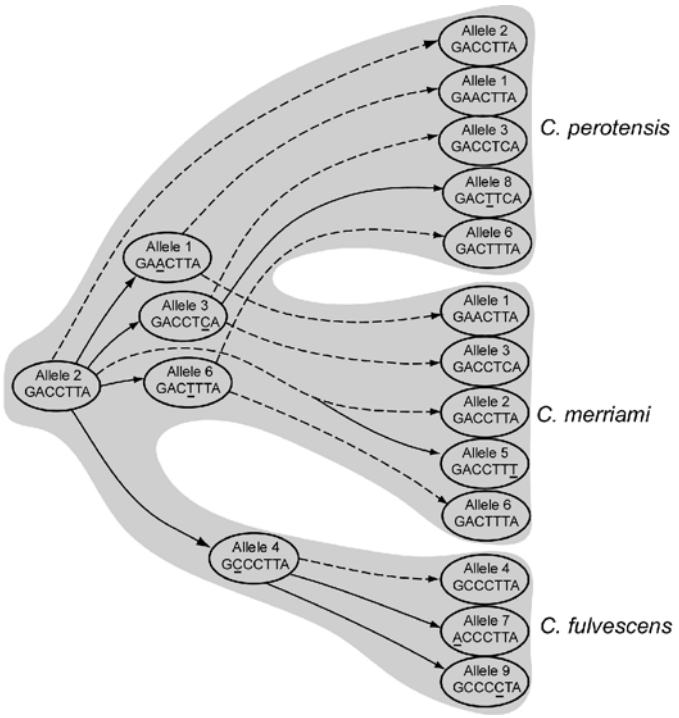

FIG. 3.—One of 4 most-parsimonious networks of 9 alleles identified in the analysis of 529 base pairs of the β-fibrinogen gene. Each solid line represents 1 base substitution (underlined); dashed lines indicate no base change (retention of ancestral state). The other 3 networks show different arrangements of alleles 3, 6, and 8, but the arrangement shown (with alleles 3 and 6 primitive) is preferred because both alleles are present in both the *merriami* and *perotensis* lineages.

Regardless of how the data were treated (log-transformed or standardized), similar characters for both males and females showed consistently high loading on the 1st discriminant axis, which explained approximately 70% of the total variation in the data set. These characters included mastoid breadth, rostal width, and nasal length for females, and mastoid breadth, zygomatic breadth, and nasal length for males.

Chromosomal analysis.—Each of the 3 gopher clades possessed different chromosomal complements. Specimens karyotyped from the *merriami* clade had 2n = 36 and FN = 68, those from the *perotensis* clade had 2n = 38 and FN = 72, and specimens from the *fulvescens* clade had 2n = 40 and FN = 72 (Fig. 5). If *fulvescens* is the outgroup to *merriami* + *perotensis* (as indicated in Fig. 2), then it would require only a single fusion event to yield the *perotensis* karyotype from the *fulvescens* karyotype, followed by another fusion (plus loss,

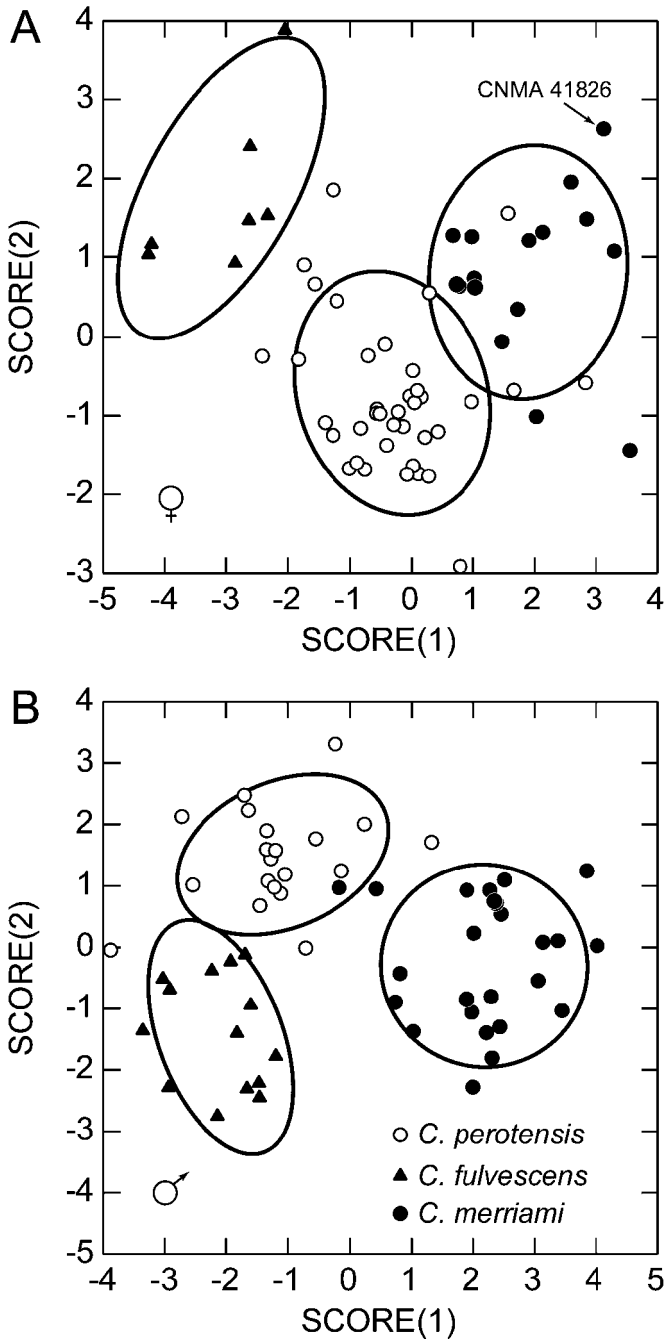

FIG. 4.—Discriminant function plot of standardized cranial measurements for A) female and B) male individuals of *Cratogeomys perotensis*, *C. fulvescens*, and *C. merriami*. The ovals surrounding each clade represent 95% confidence intervals. The specimen (female) from 1 km southeast of San Miguel Xoxtla, Puebla (CNMA 41826) is indicated in the plot (see text for discussion).

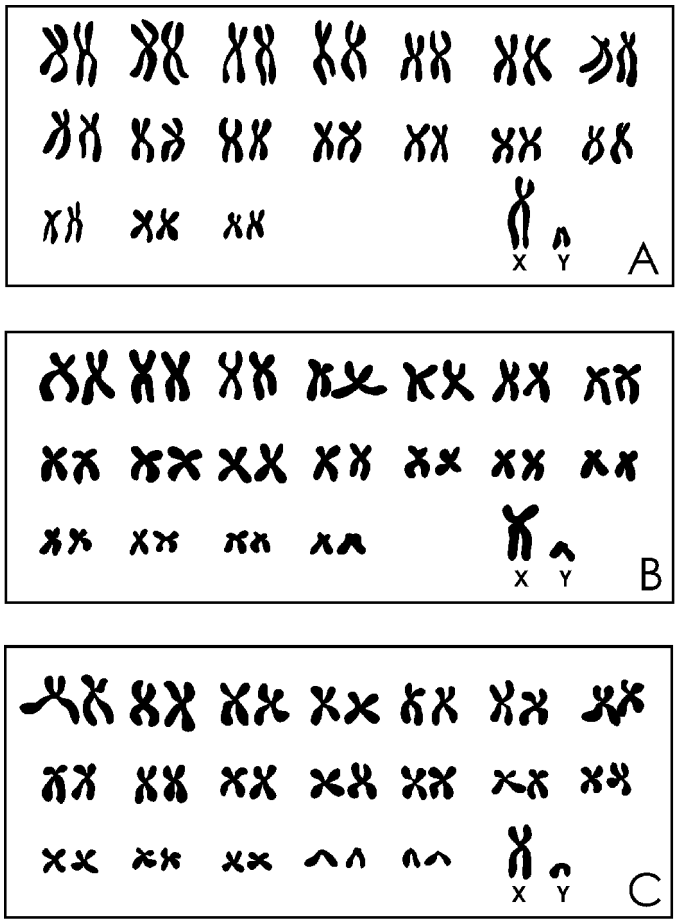

FIG. 5.—Non–differentially stained karyotypes representing A) *Cratogeomys merriami*, 2n = 36, FN = 68 (LSUMZ 36293); B) *C. perotensis*, 2n = 38, FN = 72 (LSUMZ 34903); and C) *C. fulvescens*, 2n = 40, FN = 72 (CNMA 41825).

transversion, or translocation of arms) to yield the *merriami* karyotype.

DISCUSSION

Species concepts.—Where possible, we employ the biological species concept in this study, although we realize that the small population size, patchy distribution, and genetic structure of pocket gopher populations often makes this, or any other, species concept operationally difficult to apply (Steinberg and Patton 2000). In such cases, our species concept is based on monophyly and diagnosability of lineages. In pocket gophers, reproductive barriers between species usually, but not always, are signaled by chromosomal differences and, in most cases, differences in diploid number (Hafner et al. 1987; Patton 1985; but see Patton et al. [1984] and Thaeler [1968] for contrary examples). Thus, differences in diploid number, if present, between otherwise monophyletic and diagnosable populations of pocket gophers usually lend additional support to species designations.

Relationships within Cratogeomys merriami (sensu lato).—Molecular and morphological analyses of *C. merriami* identify 3 strongly supported clades (Figs. 1–4). Each of these clades is characterized by a different karyotype (Fig. 5), and each has pelage characteristics that distinguish it from the other 2 clades (see diagnostic key). The monophyly and diagnosability of each of these clades, plus the fact that differences in diploid number between gopher clades often signal reproductive incompatibility, suggest that these clades should be regarded as separate species.

Only 1 specimen in this analysis showed conflicting mitochondrial and nuclear genetic data. This specimen, from 1 km southeast of San Miguel Xoxtla, Puebla (specimen CNMA 41826; locality 13 in Figs. 1 and 2), has a nuclear genotype (based on karyotype and morphology) characteristic of *C. merriami*, but a mitochondrial haplotype characteristic of *C. perotensis* (the sequence lacked dual peaks, insertions–deletions, and stop codons, indicating that the gene sequenced in specimen CNMA 41826 is not a nuclear copy of the mitochondrial gene—Smith et al. 1992). This specimen also possesses β-*fib* allele 3, which is otherwise restricted to *C. perotensis*. When this specimen was constrained to group with *C. merriami* in maximum-parsimony and maximum-likelihood phylogenetic analyses of the combined mitochondrial data set (*Cytb* plus *CoI*), the resulting trees were significantly different from the tree in Fig. 2 (Kishino–Hasegawa test [maximum parsimony] $P = 0.0005$; Kishino–Hasegawa test [maximum likelihood] and Shimodaira–Hasegawa test [maximum likelihood] $P = 0.015$). These results reinforce the fact that specimen CNMA 41826, despite having a nuclear background that is predominantly *C. merriami*, has a mitochondrial haplotype characteristic of *C. perotensis* (Fig. 2).

Presence of the mitochondrial haplotype of *C. perotensis* in a specimen with a nuclear genome that is predominantly *C. merriami* may result from past mitochondrial introgression from *C. perotensis* into the *C. merriami* clade. Presently, the closest point of contact between these taxa is approximately 25 km, with possible intervening populations of *C. fumosus* (Fig. 1). The possibility of introgression from *C. perotensis* into *C. merriami* is consistent with presence of β-*fib* allele 3 (otherwise restricted to *C. perotensis*) in the specimen from San Miguel Xoxtla (CNMA 41826). However, if this specimen received its mitochondrial haplotype and β-*fib* allele 3 from *C. perotensis*, one would expect this specimen to cluster more closely with the particular *perotensis* lineage from which it inherited its mtDNA (or, if the source lineage was not sampled, the closest relative thereof), rather than appear as the outgroup to all populations of *perotensis* sampled (Fig. 2). Given the basal position of CNMA 41826 relative to the populations of *C. perotensis* sampled (Fig. 2), it is more likely that this incongruity in nuclear and mitochondrial relationships is due to incomplete lineage sorting. Both morphological and chromosomal data, as well as ectoparasite associations observed by one of us (JEL), suggest that the specimen from 1 km southeast of San Miguel Xoxtla is a member of the *C. merriami* clade.

Taxonomic Conclusions

Based on the foregoing analyses, the name *merriami* (Thomas) is restricted to include only pocket gophers of this genus from the state of México and the Distrito Federal, as well as northern Morelos and west-central Puebla (Fig. 1). Examination of the holotype of *C. merriami* in the British Museum (BM[NH] 1870.6.20.2 from ''southern México'') confirmed its identity when using the diagostic key (below). The name *fulvescens* Merriam is resurrected to represent members of this genus from southern Tlaxcala, east-central Puebla, and parts of west-central Veracruz. Finally, the name *perotensis* Merriam is resurrected to represent members of this genus from southern Hidalgo, northern Tlaxcala, north-central Puebla, and parts of west-central Veracruz (Fig. 1).

Cratogeomys fulvescens appears to be the most divergent of the 3 gopher clades analyzed in this study, a relationship 1st suggested by Russell (1968). Although the outgroup status of *C. fulvescens* is only weakly supported by the mitochondrial data (Fig. 2), *fulvescens* possesses an autapomorphic *Rag1* allele that distinguishes it from both *perotensis* and *merriami*. *C. fulvescens* also is distinguishable from *C. perotensis* and *C. merriami* based on qualitative morphological features (see diagnostic key). Ectoparasite associations observed by one of us (JEL) further support the basal position of *C. fulvescens* relative to *C. perotensis* and *C. merriami*.

Results of this study clarify the taxonomic status of other previously named forms of *C. merriami* (sensu lato). Merriam's (1895) species *Cratogeomys oreocetes* and *C. peregrinus* (known only from the boreal slopes of Mt. Popocatépetl and Mt. Iztaccíhuatl, respectively) were synonymized under *C. merriami* by Davis (1944). The chromosomes of topotypes of *C. oreocetes* and *C. peregrinus* (LSUMZ 36366 and 36369, respectively; Appendix I) and the pelage of the holotypes clearly fit the description of *C. merriami* (see diagnostic key). Merriam's (1895) species *C. estor* from Las Vigas, Veracruz, was synonymized under *C. merriami* by Russell (1968). Topotypes and near-topotypes of *C. estor* (LSUMZ 34903 and 34903) possess the *perotensis* karyotype (Fig. 5), and all specimens of this taxon examined, including the holotype, possess pelage markings characteristic of *C. perotensis* (see diagnostic key). Similarly, *Cratogeomys irolonis* (from Irolo, Hidalgo), originally described by Nelson and Goldman (1934) as a subspecies of *C. merriami* and elevated to species rank by Davis (1944), is synonymized herein under *C. perotensis*. Topotypes of this taxon (LSUMZ 34343, 34344, and CNMA 41904) possess the *C. perotensis* karyotype (Fig. 5) and mtDNA haplotype (Fig. 2), and all specimens examined possess the pelage markings characteristic of *C. perotensis* (see diagnostic key). The holotype and 5 topotypes examined of *C. merriami peraltus* (Goldman 1937) from Mt. Orizaba, Puebla, all have the pelage markings characteristic of *C. perotensis*. In addition, 2 topotypes of *C. merriami peraltus* (LSUMZ 36371 and CNMA 42510) have karyotypes typical of *C. perotensis*, and analysis of ancient mtDNA from topotype specimen FMNH 14061 showed it to have the *perotensis* haplotype. Finally, *C. saccharalis* (Nelson and Goldman 1934) is synonymized herein under *C. merriami* (sensu stricto). Again, topotypes of this taxon (LSUMZ 36067, 36068, and 36293) possess the *C. merriami* karyotype (Fig. 5) and mtDNA haplotype (Fig. 2), and all specimens examined possess pelage markings characteristic of *C. merriami* (see diagnostic key).

Cratogeomys Merriam, 1895

Cratogeomys Merriam, 1895:150. Type species *Geomys merriami* Thomas. *Cratogeomys* was regarded as a subgenus of *Pappogeomys* by Russell (1968:592), but was returned to generic status by Honeycutt and Williams (1982:212).

Platygeomys Merriam, 1895:162. Type species *Geomys gymnurus* Merriam. Regarded as inseparable from *Cratogeomys* by Hooper (1946:397).

Before this study, the genus *Cratogeomys* Merriam included 7 species divided into 2 species groups, the *castanops* and *gymnurus* species groups. The *gymnurus* species group, which originally contained 5 species (Russell 1968), was renamed "*fumosus* species group" by Hafner et al. (2004) and revised to include only 2 species, *C. fumosus* and *C. planiceps*. The *castanops* species group, which formerly included 2, possibly 3, species (*castanops*, *merriami*, and possibly *goldmani*—Berry and Baker 1972; Lee and Baker 1987; Russell 1968), is revised herein to include 2 additional species related to *C. merriami*, namely *C. fulvescens* and *C. perotensis*.

Cratogeomys fulvescens Merriam, 1895
Oriental Basin Pocket Gopher

Cratogeomys fulvescens Merriam, 1895:161. Type locality "Chalchicomula [= Ciudad Serdán], State of Puebla, Mexico." Type specimen adult male, skin and skull, United States National Museum number 58168, collected 15 January 1894 by E. W. Nelson and E. A. Goldman, original number 5651.

Cratogeomys fulvescens subluteus Nelson and Goldman, 1934:152. Type locality "Perote, 7800 ft., Veracruz, Republic of México."

Geographic range.—Patchily distributed around the Oriental Basin of eastern Puebla from Esperanza, Puebla, north to Perote, Veracruz, and west to the base of Volcán La Malinche in Tlaxcala. Elevational range approximately 2,300–2,700 m.

Description.—Size small for genus (cranial width usually <26 mm in adults). Fur color grizzled yellowish brown dorsally, with strong mixture of black-tipped hairs imparting a "salt-and-pepper" appearance; underparts paler. See key for additional characters.

Cratogeomys merriami (Thomas, 1893)
Merriam's Pocket Gopher

Geomys merriami Thomas, 1893:271. Type locality "southern México" (restricted to "probably the Valley of Mexico" by Merriam 1895:152). Type specimen age and sex unknown, skin and skull, British Museum number BM(NH) 1870.6.20.2. Collected by A. Bouchard, date of collection and original number, if any, unknown.

Cratogeomys merriami: Merriam, 1895:152. First use of current name combination.

Cratogeomys oreocetes Merriam, 1895:156. Type locality "Mount Popocatapetl [= Popocatépetl], Mexico (altitude, 11,000 ft)."

Cratogeomys peregrinus Merriam, 1895:158. Type locality "Mount Iztaccihuatl [= Iztaccíhuatl], Mexico (altitude 11,500 ft)."

Cratogeomys saccharalis Nelson and Goldman, 1934:149. Type locality "Atlixco, 5400 ft., Puebla, Mexico."

Geographic range.—Patchily distributed in the southern part of the Valley of Mexico, Sierra de Las Cruces, Sierra de Ajusco, Mt. Popocatépetl, Mt. Iztaccíhutl, and from Lerma at the eastern end of the Valley of Toluca eastward into western Puebla. Elevational range approximately 1,800–4,000 m.

Description.—Generally large for genus (cranial width usually >26 mm in adults). Fur color highly variable, from dull chestnut brown to slate black dorsally; underparts similar, but paler. See key for additional characters.

Cratogeomys perotensis Merriam, 1895
Cofre de Perote Pocket Gopher

Cratogeomys perotensis Merriam, 1895:154. Type locality "Cofre de Perote, Vera Cruz [= Veracruz] (altitude 9,500 ft)." Type specimen adult female, skin and skull, United States National Museum number 54299, collected 28 May 1893 by E. W. Nelson, original number 4889.

Cratogeomys estor Merriam, 1895:155. Type locality "Las Vigas, Vera Cruz [= Veracruz] (altitude 8,000 ft)."

Cratogeomys merriami irolonis Nelson and Goldman, 1934:150. Type locality "Irolo, 7600 ft., Hidalgo."

Cratogeomys irolonis: Davis, 1944:387. Elevation to species rank.

Cratogeomys merriami peraltus Goldman, 1937:403. Type locality "Mount Orizaba, about 12,500 ft., Puebla."

Geographic range.—Patchily distributed in the Plain of Apan in southern Hidalgo eastward through the mountains of northern Puebla and the pine forest zone of west-central Veracruz, southward to Cofre de Perote and Mt. Orizaba. Elevational range approximately 2,400–4,000 m.

Description.—Size medium for genus (cranial width usually <26 mm in adults), except in western portions of range, where size approaches that of *C. merriami*. Fur color varying from light to dark brown dorsally; underparts similar, but paler. Most, perhaps all, specimens of *C. perotensis* have 1 or more small patches of white fur near the base of the tail (area of patch varies from approximately 1 cm^2 to more than 2 cm^2). In study skins, the skin at the base of the tail is often folded under skin in the rump region, thereby obscuring the white patch at the base of the tail. See key for additional characters.

KEY TO THE *CRATOGEOMYS CASTANOPS* SPECIES GROUP

This key is based on examination of 42 adult specimens in the *C. castanops* species group and 27 in the *C. fumosus* species group. All of these specimens were correctly assigned to species group by using the combination of morphological characters in step 1 of this key. Similarly, all 42 specimens in the *C. castanops* species group were assigned to the correct species (as determined genetically) by using only the morphological characters in steps 2–4 of this key. Reliability of each morphological character (i.e., percentage of specimens correctly identified by using this character alone) is indicated after the character description. Chromosomal characters are 100% reliable based on individuals sampled to date. This key is for adult specimens only, with adulthood determined by fusion of the basioccipital–basisphenoid suture.

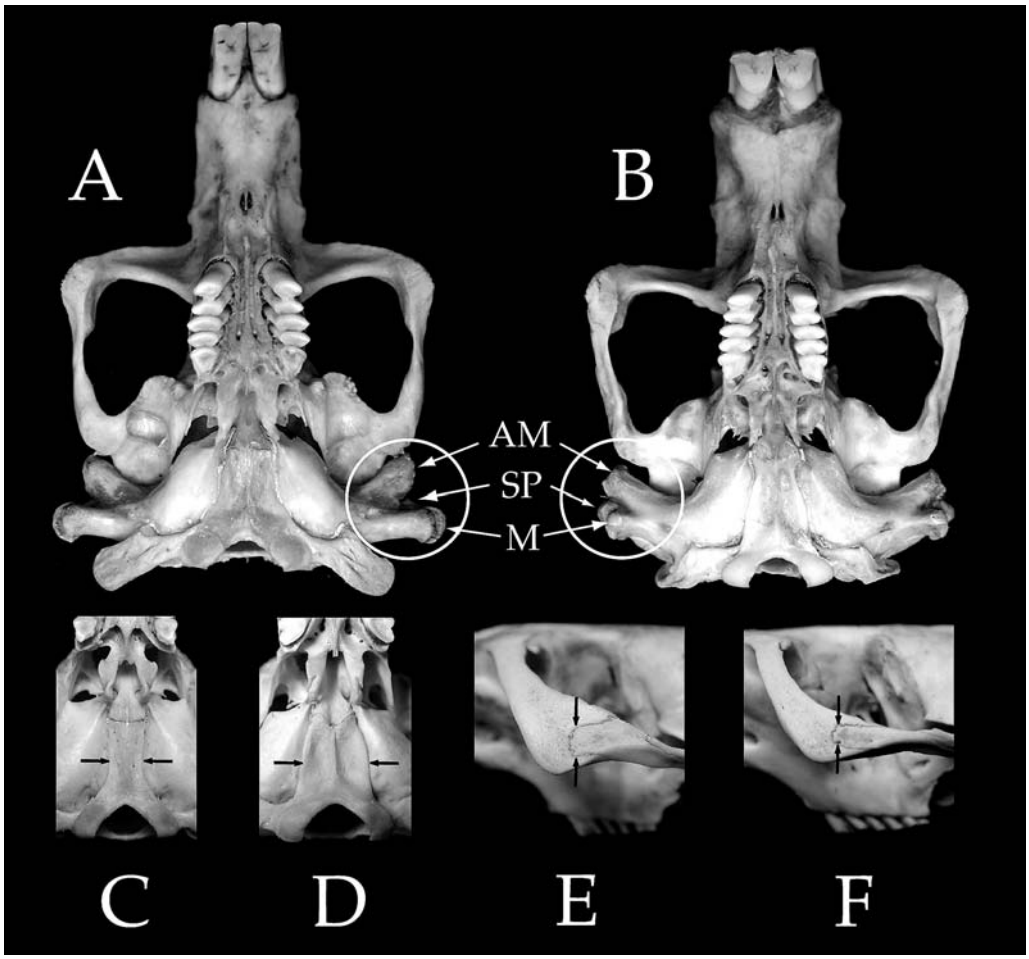

Fig. 6.—A) Ventral view of cranium of *Cratogeomys fumosus* (LSUMZ 36081), a member of the *C. fumosus* species group (Hafner et al. 2004). In members of this species group, the mastoid process (M) extends laterally well beyond the external auditory meatus (AM) and obscures most of the supraoccipital process (SP) when viewed from the ventral perspective. B) Ventral view of cranium of *C. merriami* (LSUMZ 36068), a member of the *C. castanops* species group. In members of this species group, the mastoid process does not extend laterally beyond the external auditory meatus (note that the supraoccipital process [SP] is visible from the ventral perspective). C) Basioccipital region of *C. castanops* (LSUMZ 31454) showing the narrow (usually <4 mm at midpoint [indicated by arrows]) and parallel-sided (occasionally hour-glass–shaped) basioccipital bone characteristic of *C. castanops* and *C. goldmani*. D) Basioccipital region of *C. merriami* (LSUMZ 36065) showing the broad, wedge-shaped basioccipital bone (usually >4 mm at midpoint) characteristic of *C. merriami*, *C. fulvescens*, and *C. perotensis*. E) Lateral view of left zygomatic arch of *C. fulvescens* (USNM 58169); note the broad anterior edge of the jugal bone (usually >2 mm [at arrows] in *C. fulvescens* and >2.5 mm in *C. merriami*). F) Lateral view of left zygomatic arch of *C. perotensis* (USNM 54294); note the narrow (usually <2 mm) anterior edge of the jugal bone.

1 Mastoid process extending laterally beyond auditory meatus (Fig. 6A; 96% reliable in the 69 specimens examined); greatest breadth across angular processes of mandible greater than greatest length of mandible, including incisors (the mandibular symphysis in *Cratogeomys* is fused in adults [87.5% reliable]) . . . . . *C. fumosus* species group

Possession of at least 1 of the 2 following characters: Mastoid process not extending laterally beyond auditory meatus (Fig. 6B; 96% reliable); greatest breadth across angular processes of mandible less than greatest length of mandible, including incisors (87.5% reliable) . . . . . *C. castanops* species group 2

2 Basioccipital narrow (usually <4 mm at midpoint) and parallel sided or hour-glass shaped (Fig. 6C; 97% reliable); chromosomes 2n = 42 or 46 3

Basioccipital broad (usually >4 mm at midpoint) and wedge shaped (converging anteriorly; Fig. 6D; 97% reliable); chromosomes 2n = 36, 38, or 40 4

3 Condylobasal length < 47 mm (100% reliable) and length of palate < 31.5 mm (96% reliable); chromosomes 2n = 42 . . . . . *C. goldmani*

Condylobasal length > 47 mm (100% reliable) and length of palate > 31.5 mm (96% reliable); chromosomes 2n = 46 . . . . . *C. castanops*

4 Dorsal pelage grizzled yellowish brown, giving a decidedly yellow or golden hue to the fur (100% reli-

able); anterior edge of jugal broad (usually >2 mm; Fig. 6E; 89% reliable); chromosomes 2n = 40, FN = 72 . . . . . *C. fulvescens*

Dorsal pelage not grizzled yellowish brown. Small patch of white (not light brown) fur near base of the tail; patch may be very small (<1 cm^2) and may be located dorsally, ventrally, or both (93% reliable; 4 of 57 study skins examined appear to lack the white patch, which may have been present but obscured because of the method of preparation—see *C. perotensis* species account); anterior edge of jugal narrow (usually <2 mm; Fig. 6F; 89% reliable); chromosomes 2n = 38, FN = 72 . . . . . *C. perotensis*

Pelage not grizzled yellowish brown (100% reliable); white patch near base of tail absent (93% reliable); anterior edge of jugal broad (usually >2.5 mm; Fig. 6E; 92% reliable); chromosomes 2n = 36, FN = 68 . . . . . *C. merriami*

Geomyid diversity in the Trans-Mexican Volcanic Belt (TMVB).—This revision of *C. merriami* (sensu lato) of the *C. castanops* species group, coupled with the recent revision of the *C. fumosus* (formerly *C. gymnurus*) species group by Hafner et al. (2004) and the study of *Pappogeomys alcorni* by Demastes et al. (2003), brings the total number of geomyid species in the TMVB region (latitude 18°–22°N) to 12 species representing 5 of the 6 extant geomyid genera. Six of these 12 species are in the genus *Cratogeomys*, 3 in *Orthogeomys*, and 1 each in *Pappogeomys*, *Thomomys*, and *Zygogeomys*; only the genus *Geomys*, which is predominately temperate in distribution, is unrepresented in the TMVB. As such, the latitudinal belt containing the TMVB is the pinnacle of pocket gopher diversity at both the specific and generic levels. Russell (1968:558) suggested that modern geomyine genera may have originated in southern Mexico. If true, then the high pocket gopher diversity seen in this area may be, at least in part, simply a product of time. However, in the absence of a good fossil record of pocket gophers in Mexico, it is premature to conclude that the TMVB represents the center of origin of the Geomyidae (Wahlert and Souza 1988). Instead, the high diversity of pocket gophers in this area, which is shared with many other plant and animal groups (Ramamoorthy et al. 1993; Strattersfield et al. 1998) may be a response to the varied ecological and physiographic features of the TMVB, including the confluence of boreal forest, high desert, and tropical forest habitats augmented by high elevational relief.

Resumen

Un estudio referente a la variación cromosómica en la tuza Mexicana *Cratogeomys merriami* reveló diferencias en el nùmero diploide correspondiente a 3 principales clados genéticos y morfológicos dentro de la especie. Cada uno de los 3 clados es diagnosticable basado en mùltiples caracteres, incluyendo cromosomas, nùmero diploide, caracteres morfológicos cuantitativos y cualitativos, y ADN mitocondrial. Por lo tanto, restringimos el nombre *C. merriami* (Thomas) para incluir ùnicamente tuzas de este género de los estados de México, México D.F., norte de Morelos, y la porción central oeste de Puebla. Resucitamos el nombre de especie de *C. fulvescens* Merriam para representar los miembros de este género para el sur de Tlaxcala, la porción central este de Puebla, y partes del oeste central de Veracruz. Finalmente, resucitamos el nombre de especie de *C. perotensis* Merriam para representar miembros de este género para el sur de Hidalgo, norte de Tlaxcala, región central norte de Puebla, y partes de la región central oeste de Veracruz. Basados en la observación de que diferencias en el nùmero diploide son reflejo de aislamiento reproductivo entre poblaciones de tuzas, hipotetizamos que *C. merriami*, *C. fulvescens*, y *C. perotensis* son reproductivamente incompatibles. Proveemos sinonimias y descripciones para estas 3 especies junto con una clave para el grupo de especies *C. castanops*, grupo al cual pertenecen estas especies.

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APPENDIX I

List of the 23 localities from which 29 ingroup and 3 outgroup specimens of *Cratogeomys* and *Pappogeomys* were examined in the molecular and chromosomal analyses (all localities are in Mexico). Specimens are grouped into the 3 clades identified in the molecular analysis (Fig. 2). Twenty-four of the 32 specimens listed were used in the mitochondrial DNA (mtDNA) analysis (indicated by [mt]). Specimens also used in the chromosomal analysis are indicated by [k], and those also used in the nuclear DNA analysis are indicated by [n]. Collection acronyms are as follows: Colección Nacional de Mamíferos, Universidad Nacional Autónoma de México (CNMA); Field Museum of Natural History (FMNH); Louisiana State University Museum of Natural Science (LSUMZ); New Mexico Museum of Natural History (NMMNH). Locality numbers are mapped in Fig. 1.

Cratogeomys perotensis.—1. HIDALGO: Irolo, 2,500 m, LSUMZ 34343[mt,n], CNMA 37206[mt,n], CNMA 41904[mt,k,n; all 3 specimens are topotypes of *C. merriami irolonis* Nelson and Goldman]; 2. TLAXCALA: Llanos de Teopa, 8 km (by rd.) N Tlaxco, 2,887 m, CNMA 41905[mt,k,n]; 20. PUEBLA: 5.5 km N, 3 km W Pico de Orizaba, 3,820 m, CNMA 42510[k], LSUMZ 36371[k; both specimens are topotypes of *C. perotensis peraltus* Goldman]; 3. PUEBLA: 2.5 km N Zaragosa, 2,250 m, CNMA 41906[mt,k,n]; 21. VERACRUZ: Cofre de Perote, 1 km SSW El Conejo, 3,442 m, CNMA 42508[k; topotype of *C. perotensis* Merriam]; 4. VERACRUZ: Las Vigas, 7,900 feet, LSUMZ 36070[mt,n; topotype of *C. estor* Merriam]; 5. VERACRUZ: 2 km S Las Vigas, 2,568 feet; LSUMZ 34903[mt,k,n]; 6. VERACRUZ: Cruz Blanca, 2,450 m, CNMA 41909[mt,k,n]; 7. VERACRUZ: 9 km NE Perote, 2,440 m, CNMA 41910[mt,k,n], CNMA 41911[mt,k,n].

Cratogeomys fulvescens.—8. VERACRUZ: 2 km NE Perote, 7,900 ft, LSUMZ 36069[mt,n], CNMA 41824[mt,k], CNMA 41825[mt,k,n; all 3 specimens are topotypes of *C. fulvescens subluteus* Nelson and Goldman]; 9. PUEBLA: 1 km NW Zacatepec, 2,380 m, CNMA 41907[mt,k,n]; 10. PUEBLA: 1 km SE Ciudad Serdán, 2,700 m, CNMA 41823[mt,k,n; topotype of *C. fulvescens* Merriam]; 11. TLAXCALA: Huamantla, 2,380 m, CNMA 41908[mt,k,n].

Cratogeomys merriami.—12. PUEBLA: 1 km S Atlixco, 6,300 feet, LSUMZ 36067[mt,n], LSUMZ 36068[mt,n], LSUMZ 36293[mt,k,n; all 3 specimens are topotypes of *C. merriami saccharalis* Nelson and Goldman]; 13. PUEBLA: 1 km SE San Miguel Xoxtla, 2430 m, CNMA 41826[mt,k,n]; 22. MÉXICO: Volcan Iztaccíhuatl, 4 km N Paso de Cortéz, 3,842 m, LSUMZ 36369[k; topotype of *C. peregrinus* Merriam]; 23. MÉXICO: Volcan Popocatépetl, 1 km NW Tlamacas, 3,884 m, LSUMZ 36366[k; topotype of *C. oreocetes* Merriam]; 14. MÉXICO: 5 km SSW Texcoco, 7,000 feet, LSUMZ 36065[mt,n]; 15. MÉXICO: 15 km SSW Texcoco, 2,253 m, CNMA 41819[mt,k,n]; 16. MÉXICO: 2 km SE Coatepec, 8,600 feet, LSUMZ 36125[mt,n].

Outgroups.—17. DURANGO: 7 miles NNW La Zarca, 5,700 feet, *Cratogeomys castanops*, NMMNH 2467[mt]; 18. SAN LUIS POTOSÍ: 6 km E Río Verde, 3,350 ft, *Cratogeomys goldmani*, LSUMZ 36089[mt]; 19. JALISCO: Cerro Tequila, 7 miles S, 2 miles W Tequila, 10,000 ft, *Pappogeomys bulleri*, LSUMZ 36082[mt].

Ancient mtDNA.—20. PUEBLA: Mt. Orizaba (near timberline); FMNH 14061[mt, topotype of *C. perotensis peraltus* Goldman].

APPENDIX II

Specimens examined in the morphometric analysis (all localities are in Mexico). Collection acronyms are listed in Appendix I, with the following additions: University of Kansas Natural History Museum (KU); University of California Museum of Vertebrate Zoology (MVZ); The Museum, Texas Tech University (TTU); University of Michigan Museum of Zoology (UMMZ); and United States National Museum of Natural History (USNM).

Cratogeomys perotensis (n = 58).—HIDALGO: 10 miles NW Apam, [= Apan], 7,750 feet (KU 48539); Irolo, 2,500 m (CNMA 37206, 41904; LSUMZ 34343; USNM 53495). PUEBLA: Cruz Alta, S. Aquixtla, 9,000 feet (UMMZ 112607, 112609, 112610); Rancho Ocotal Colorado, S. Aquixtla, 8,800 feet (UMMZ 112611–112617); 2.5 km N Zaragosa, 2,250 m (CNMA 41906). TLAXCALA: Llanos de Teopa, 8 km (by road) N Tlaxco, 2,887 m (CNMA 41905). VERACRUZ: 6 km SSE Altotonga, 9,000 feet (KU 19329); Cofre de Perote, 9,500 feet (USNM 54288–54291, 54293–54298); N slope Cofre de Perote, 10,300 feet (UMMZ 94625, 94626); Cruz Blanca, 2,450 m (CNMA 41909); 7 km SE Jalacingo, 8,000 feet (KU 19328); Las Vigas, 8,500 feet (LSUMZ 36070; KU 30010, 30011, 30013, 30016–30021, 30023, 30026; USNM 54306, 54307); 2 km S Las Vigas, 2,568 m (LSUMZ 34903); 2 km E Las Vigas, 8,000 feet (KU 30008, 30009); 3 km E Las Vigas, 8,000 feet (KU 19337); 9 km NE Perote, 2,440 m (CNMA 41910, 41911); 1 km NW Pescados, 10,500 feet (KU 19331–19335); 2 km S Sierra de Agua, 8,500 feet (KU 19350).

Cratogeomys fulvescens (n = 22).—PUEBLA: Chalchicomula [= Ciudad Serdán], 8,200 feet (USNM 53497, 53498, 58166, 58167, 58169, 58170); 1 km SE Ciudad Serdán (CNMA 41823); 1 km NW Zacatepec, 2,380 m (CNMA 41907). TLAXCALA: Huamantla, 2,380 m (CNMA 41908). VERACRUZ: 3 km W Limón, 7,500 feet (KU 19351, 19353); 2 km E Perote, 8,300 feet (KU 19340, 19342–19347); 2 km N Perote, 8,000 feet (KU 30029, 30030); 2 km NE Perote, 2,430 m (CNMA 41825; LSUMZ 36069).

Cratogeomys merriami (n = 42).—DISTRITO FEDERAL: 1.8 miles E San Gregoria Altapulco, 2,270 m (KU 28043); 0.5 miles S Rancho del Llano, 4 miles S, 0.8 miles E Churubusco (KU 28035, 28036); Desierto de Leones (UMMZ 104635, 104637); Falda SW Cerro Zacatepec, 3.9 miles SW Monumento a Obergón (KU 28044); Ixtapalapa [= Iztapalapa] (CNMA 498; UMMZ 104638); 1 km N Morelea [= Highway 95, 1 km N Morelos–D.F. border] (TTU 38111, 38113); Santa Cruz Acalpixca, 2,270 m (KU 28040); Rancho la Noria, 4 km W Xochimilco, 2,270 m (KU 28037, 28038). MÉXICO: Amecameca (USNM 57962); 5 miles E Amecameca, 9,600 feet (MVZ 91252); 2 km SE Coatepec, 8,600 feet (LSUMZ 36125); Lerma, 8,650 feet (USNM 50110); Monte Río Frío, 47 km ESE México City (UMMZ 86513); 1 mile SSW Río Frío (KU 61661); 5 km W Río Frío, 10,000 ft (KU 19326, 19327); Salazar, 11,000 feet (USNM 50109); Piramides de San Juan Teotchuacan [= Teotihuacán] (MVZ 91251); 5 km SSW Texcoco, 7,000 feet (LSUMZ 36065); 6 miles S, 1 mi W Texcoco, 7,350 feet (KU 48540); 15 km SSW Texcoco, 2,253 m (CNMA 41819). PUEBLA: Atlixco (USNM 55346, 55349); 1 km S Atlixco, 1,812 m (LSUMZ 36068, 36293); 2 miles S Atlixco, 5,800 feet (KU 62513–62517); Cholula Barro San Pablo [= Cholula, Barrio San Pedro?] (USNM 540920); Hacienda San Pedro Coxtocan, km 96.5 on Puebla–Mexico Highway (USNM 540934, 540935); 1 km SE San Miguel Xoxtla, 2,430 m (CNMA 41826); Texmelucan (KU 66164–66166).