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SYSTEMATICS OF A RARE SPECIES OF POCKET GOPHER, *PAPPOGEOMYS ALCORNI*

JAMES W. DEMASTES,* ANDREA L. BUTT, MARK S. HAFNER, AND JESSICA E. LIGHT

Department of Biology, University of Northern Iowa, Cedar Falls, IA 50614, USA (JWD, ALB)
Museum of Natural Science and Department of Biological Sciences, Louisiana State University, Baton Rouge, LA 70803, USA (MSH, JEL)

Pappogeomys alcorni is a peripherally isolated species that currently is designated as a rare species in Mexico. There are only 4 known museum specimens, all collected between the years 1950 and 1966, and recent attempts have failed to locate individuals in the wild. Although only 1 new specimen (skin only) has been collected since the original systematic work on *P. alcorni*, new analytical methods permit reexamination of existing specimens. DNA was isolated from a 48-year-old museum skin to investigate the phylogenetic relationship of this rare taxon to other species of *Pappogeomys* and *Cratogeomys*. Phylogenetic analysis based on 424 base pairs of the mitochondrial cytochrome-*b* gene and morphometric analysis of 101 individuals of *Pappogeomys* indicate that *P. alcorni* is a geographically disjunct subspecies of *P. bulleri* rather than a monotypic species.

Key words: ancient DNA, morphometrics, mtDNA, *Pappogeomys*, pocket gopher

Pocket gophers of the genus *Pappogeomys* are distributed across the Sierra Madre Occidental of Mexico in the states of Nayarit, Jalisco, Colima, and Michoacán (Fig. 1). Russell (1968) recognized 2 species in the genus *Pappogeomys* (*P. alcorni* and *P. bulleri*) based on morphological characters. *Pappogeomys bulleri* is a relatively widespread taxon with 8 described subspecies. The northernmost localities recorded for *P. bulleri* (near Tepic, Nayarit) are separated from the southernmost localities (south of Colima) by approximately 440 km. The intervening geography consists of mountains, volcanoes, and river basins. In contrast, *P. alcorni* is a peripherally isolated taxon known only from the immediate vicinity (within 9.6 km) of Mazamitla, Jalisco. Russell differentiated *P. alcorni* from its congener based on ''Outer (labial) three-fourths of posterior wall of M1 lacking enamel; bright buffy, instead of whitish, nasal patch; nasals broadly truncate instead of emarginate posteriorly; short, rounded (instead of long, narrow) incisive foramina'' (Russell 1968:616). These morphological differences, together with the geographic isolation of *P. alcorni*, led Russell to recognize *P. alcorni* as a distinct species.

Russell's (1968) work with *P. alcorni* was made difficult by the fact that only 3 specimens were available to be examined and one of these lacked cranial material. Since the time of Russell's (1957) original description of *P. alcorni*, only 1 additional specimen (skin only) has been collected. This specimen was collected in 1966 and is housed in the Colección Nacional de Mamíferos at Universidad Nacional Autónoma de México, Instituto de Biología (CNMA 26219). The remaining 3 specimens are housed in the University of Kansas, Museum of Natural History (KU 39805, 39806, and 61328).

The paucity of specimens of *P. alcorni* is likely the result of several factors, not the least of which is a low density of individ-

* *Correspondent: jim.demastes@uni.edu*

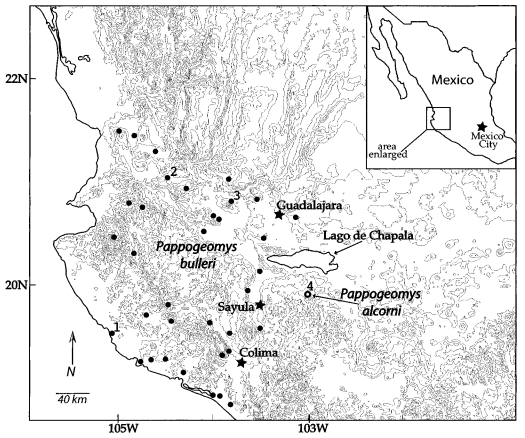

FIG. 1.—Distribution of the 2 species of *Pappogeomys* (*P. bulleri* and *P. alcorni*). Open circles represent *P. alcorni*, and filled circles represent historical records of *P. bulleri*; numbers (1–4) represent collecting localities for this study. Stars represent cities denoted for geographic reference.

uals in the population, which is reflected in its designation as a rare species (SEDESOL 1994). Interestingly, *P. alcorni* historically was found in sympatry with *Cratogeomys tylorhinus*, with the smaller *P. alcorni* occupying burrow systems above the much larger burrows of *C. tylorhinus* (Russell 1968). Recent attempts by experienced field researchers have failed to locate and collect additional specimens of *P. alcorni* (Demastes et al. 2002). Despite repeated efforts, this failure to locate individuals at published localities (and all nearby areas) raises concern for the present status of *P. alcorni*.

Hall (1981) recognized that the only dental character used by Russell (1968) to distinguish *P. alcorni* from *P. bulleri* was quite variable in *P. bulleri*, and he questioned its use as a diagnostic character. Accordingly, Hall stated, ''If more specimens of *P. alcorni* are obtained, a study of them would be warranted to learn if the taxon differs specifically or only subspecifically from *P. bulleri*'' (Hall 1981:517). Although additional specimens of *P. alcorni* are not available, additional data can be generated from existing museum specimens, and modern analytical techniques can be used to examine morphological variation. Here, we use DNA sequences obtained from a dried museum skin to determine the phylogenetic affinity of *P. alcorni*. In addition, we conducted a morphometric analysis to determine if *P. alcorni* is distinguishable from its congener based on cranial measurements.

MATERIALS AND METHODS

Analysis of DNA.—Two small (5 mm^2) sections of dried skin were removed from a specimen of *P. alcorni* housed in the University of Kansas, Museum of Natural History (KU 61328). This skin-only specimen was collected in 1954 by A. A. Alcorn. The sample, which included some hair and subcutaneous material, was removed from the edge of an existing opening in the skin caused by the original trapping of the specimen.

Because of the rarity of *P. alcorni* specimens, care was taken to use the most efficient method of DNA extraction for subsequent analysis. Three techniques were tested using a teaching specimen (*Geomys*) that was >10 years old. These techniques included standard chelex extraction (Walsh et al. 1991) and procedures associated with 2 commercially available kits, including an Aquapure Genomic DNA Isolation Kit (Bio Rad, Hercules, California) and a DNeasy Tissue Kit (Qiagen, Valencia, California). Extraction methods for the commercial kits followed those supplied by the manufacturers, with 1 exception: the Qiagen protocol was modified to include pH adjustment of the sample to 6.5–7.0 before adding the sample to the column (Iudica et al. 2001). Quality of the resulting samples was determined by amplification (via polymerase chain reaction [PCR]) of 402 base pairs (bp) of the mitochondrial DNA (mtDNA) cytochrome-*b* (*Cytb*) gene using the primers L14724 (Irwin et al. 1991) and H15154 (''MVZ04''—Smith and Patton 1993). Primer names indicate the DNA strand (H = heavy, L = light) and the position of the 3′ end of the oligonucleotide sequence relative to the human sequence (Anderson et al. 1981). Although all 3 methods of extraction yielded DNA of sufficient length and quality to allow the amplification of the 402-bp target fragment for the *Geomys* specimen, no method did so for the *P. alcorni* material. Al-

though the Qiagen method yielded workable DNA from the *P. alcorni* material, this DNA was sufficiently degraded to warrant new primer design to target sequences shorter than 400 bp.

After DNA extraction from *P. alcorni* using the Qiagen method, 3 nonoverlapping sections of the *Cytb* gene were amplified using PCR with *Taq* DNA polymerase (Promega, Madison, Wisconsin; Saiki et al. 1986, 1988). Double-stranded amplifications and sequencing reactions were performed using combinations of the following primers: L14724, L15513, H15579 (Irwin et al. 1991), H15906 (Spradling et al. 2001), H15154 (''MVZ04''—Smith and Patton 1993), and 2 primers designed specifically for this study, viz., L14945 5′-CCTCACAGCCTTCTCATCAGT-3′ and H14926 5′-CTGATGAGAAGGCTGTGAG-3′.

Double-stranded PCR amplifications were performed in 50-μl reaction volumes, usually using primers L14724 with H14926, L14945 with H15154, and L15513 with H15579. Each reaction included 3 μl of each primer (10 μM) and was performed using EasyStart PCR tubes (Molecular Bioproducts, Inc., San Diego, California). After an initial denaturation stage of 95°C for 1 min, 36 PCR cycles were performed with the following thermal-cycling parameters: 1 min denaturation at 95°C, 1 min annealing at 46°C, and 1 min extension at 72°C.

Several precautions were taken to avoid potential problems of contamination. Before each PCR reaction was set up, laboratory benches and pipettors were washed with DNase (DNA Away, Molecular BioProducts) to remove any DNA, and all PCR reactions were run with negative controls. After sequences were obtained and proofed, searches were performed 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 with all sequences that were the result of unrelated PCR activity in the laboratory. The 3 fragments of the *Cytb* gene reported herein were obtained using 3 different sets of PCR primers. Diagenetic modification (i.e., postmortem changes) of DNA is a potential problem in some studies, but in phylogenetic studies these changes should result in nothing more than uninformative autapomorphies (Villablanca 1994). Because only 1 DNA sequence is being compared with several sequences obtained from properly preserved tissues, there is no opportunity for systematic diagenetic modification caused by damaged DNA in multiple taxa.

Before sequencing, the double-stranded PCR product was purified using the Quickstep™ PCR Purification Kit (Edge Biosystems, Gaithersburg, Maryland). Sequencing was performed at the Iowa State University Sequencing and Synthesis Facility, Ames, Iowa. Both DNA strands were obtained for all gene segments. The resulting sequences were deposited in GenBank (accession numbers AF454094–454096). Sequences for *P. bulleri* representatives of individuals throughout the range of this species ($n = 3$; see Fig. 1) were obtained from GenBank (accession numbers AF392168, AF302177, L11900), as were the sequences for 2 out-group taxa, *C. castanops* ($n = 1$; L11902) and *C. fumosus* ($n = 1$; L11903). These outgroups were chosen to represent the 2 major lineages within *Cratogeomys*, the sister taxon of *Pappogeomys* (Demastes et al. 2002; Russell 1968).

Genetic distances were calculated using the Kimura 2-parameter model of substitution (Kimura 1980), assuming minimum evolution. For comparison, genetic distances were recalculated for the *Cratogeomys* data of Demastes et al. (2002), using the same 3 gene fragments and substitution model as for *P. alcorni*.

To generate the best maximum-likelihood tree for these data, the software program MODELTEST Version 3.0 (Posada and Crandall 1998) was used to examine the fit of several ($n = 56$) models of nucleotide substitution to the sequence data. Of the 56 models tested, a submodel of the general time-reversible model (TrN+G [alpha = 0.1985]—Yang 1994; Gu et al. 1995) was chosen for subsequent analyses because it provided the best approximation of the *Pappogeomys Cytb* data using the fewest parameters, as assessed by likelihood ratio tests (Huelsenbeck and Rannala 1997). A full heuristic maximum likelihood search was conducted using the TrN+G model and the Jumble option (10 replicates) in PAUP* software (Swofford 1998). A full heuristic bootstrap (1,000 replicates) also was performed using the TrN+G model.

For comparison with the maximum likelihood tree, parsimony trees were constructed (PAUP*—Swofford 1998) using all characters

TABLE 1.—Genetic distances (Kimura 2-parameter) for *Pappogeomys* and *Cratogeomys*. Numbers in parentheses refer to locality numbers in Figs. 1 and 2.

		1	2	3	4	5	6
1	*C. castanops*	—					
2	*C. fumosus*	0.134	—				
3	*P. bulleri* (1)	0.133	0.151	—			
4	*P. bulleri* (2)	0.140	0.124	0.070	—		
5	*P. buller* (3)	0.145	0.136	0.064	0.060	—	
6	*P. alcorni* (4)	0.165	0.154	0.092	0.084	0.098	—

and using transversions only. A full heuristic bootstrap (1,000 replicates) was performed using parsimony criteria.

Morphological analysis.—For morphometric analyses, 99 adult females and 60 adult males of *P. bulleri* (Appendix I) and 2 adult females of *P. alcorni* (the only 2 skulls known) were analyzed 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 molars 1 and 2, and occlusal length of molar 3. These characters have proven useful in previous morphometric analyses of pocket gophers (Patton and Smith 1990; Smith and Patton 1988). All statistical analyses were performed using Systat 8.0 software (SPSS 1998).

Mensural data for *P. bulleri* were examined for sexual dimorphism 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 ($P < 0.01$ for all characters except occlusal length of molars 1 and 2 and occlusal length of molar 3). Because both the existing *P. alcorni* skulls are from females, only data from female specimens were analyzed in the following statistical tests. Statistical significance between the raw cranial measurements of *P. bulleri* and *P. alcorni* was examined using analysis of variance (ANOVA) with a Bonferroni adjustment. To decrease the effect of individual size variation, all characters were transformed logarithmically (Burbrink 2001; Corruccini 1975; Gould 1966). Principal components analysis (PCA) was performed on the $\log_{10}$-transformed characters to determine whether the 2 species could be separated without an a priori hypothesis of group membership. The small sample size for *P. alcorni* precludes use of a discriminant function analysis.

RESULTS

DNA isolation and amplification.—Although the Qiagen column did yield DNA that was suitable for PCR, this DNA was severely degraded. When electrophoresed on a 1% agarose gel and visualized with ethidium bromide, the DNA from the *P. alcorni* museum skin appeared as a distinct smear beginning at approximately 400 bp and stretching to well below 100 bp. The greatest concentration of intact DNA appeared in the 200-bp range. Not surprisingly, the longest fragment successfully amplified from *P. alcorni* was only 204 bp. Three nonoverlapping fragments were successfully amplified for a total of 424 bp, representing segments throughout the *Cytb* gene.

Phylogenetic analyses.—Of the 424 bp amplified and sequenced for *P. alcorni*, 327 were invariant and 45 were parsimony informative. For the in-group, genetic distances (Kimura 2-parameter) ranged from 5.9% to 9.8% for all positions (Table 1). A phylogenetic tree constructed using maximum likelihood criteria (Fig. 2) revealed that *P. bulleri* was not a monophyletic group. However, bootstrap values are relatively low, and a log-likelihood ratio test indicated that this tree ($\ln = -1{,}148.8299$) was not significantly different from a tree in which *P. bulleri* was forced to remain monophyletic ($\ln = -1{,}147.84$; $\chi^2 = 0.92$, $P = 0.98$). Maximum parsimony (weighting all character state changes equally) yielded

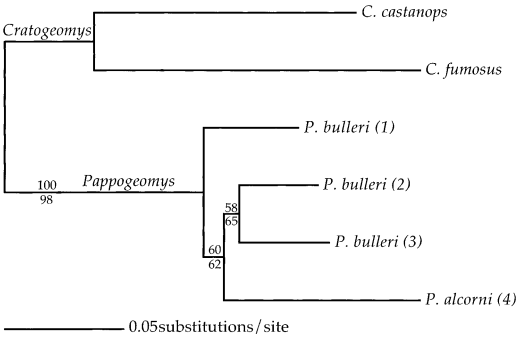

FIG. 2.—Maximum likelihood tree based on 424 base pairs of the mitochondrial cytochrome-*b* gene. Bootstrap values are for 1,000 replications using the full maximum likelihood model (TrN+G; above branches) and for 1,000 replications using transversion parsimony (below branches).

5 trees (tree length = 138, consistency index [CI] = 0.7754), each depicting a different arrangement within *Pappogeomys*. A consensus tree of these 5 parsimony trees depicted *Pappogeomys* as an unresolved polychotomy. Transversion-only parsimony yielded a tree whose topology was identical to the maximum likelihood tree with similar bootstrap values (tree length = 29, CI = 0.9310). The topology of the *Pappogeomys* clade involving individuals 1–3 (Fig. 2) was congruent with maximum likelihood and parsimony trees produced using the entire *Cytb* gene (1,140 bp—Demastes et al. 2002).

Although terminal branches were long (especially in the case of *P. alcorni*), internodal branch lengths (Fig. 2) were very short, suggesting a rapid radiation of these lineages (Kraus and Miyamoto 1991; Sudman and Hafner 1992). A rapid radiation also would explain why bootstrap values were low for the clades within *Pappogeomys*, yet monophyly of the genus was strongly supported (Fig. 2; Lara et al. 1996). If these lineages did, in fact, undergo a rapid radiation, a well-supported phylogeny would be difficult to produce regardless of the completeness of taxon sampling or the amount of data generated (Lanyon 1988).

Morphological analysis.—There were no significant differences between *P. bulleri* and *P. alcorni* specimens for each of the 12 characters examined (ANOVA, *P*-values ranged from 0.13 to 0.73). PCA indicated that the 2 species cannot be differentiated based on cranial morphology (Fig. 3). As expected, all factors loaded positively on principal component (PC) 1 (range 0.5–0.97). PC 1 accounted for 64.06% of the total variation, and PC2 and PC3 accounted for 11.62% and 6.54%, respectively.

DISCUSSION

The data presented herein indicate that Hall (1981) was justified in suggesting the need for further study concerning the validity of species status for *P. alcorni*. Russell's (1968) morphological analysis likely was confounded by the extreme plasticity of geomyid morphology; skull morphology, body size, and coloration of pocket gophers vary in response to soil types and abundance of available nutrients (Patton and Brylski 1987; Smith and Patton 1988; Wilkins and Swearingen 1990). Russell points out that one of the presumed diagnostic characters for *P. alcorni* (loss of enamel on M1) "... occurs on adjacent populations of *P. bulleri*" (Russell 1968:600) and is thus not diagnostic. In the original description of *P. alcorni*, Russell was duly cautious in stating, "The features which distinguish *Pappogeomys alcorni seem to be* [emphasis added] beyond the range of variation in *Pappogeomys bulleri*" (Russell 1957:360). Furthermore, in a recent study of the sister genus, *Cratogeomys*, Demastes et al. (2002) concluded that the morphological characters used by Russell to delimit *Cratogeomys* species were of limited taxonomic value. Clearly, the morphometric analysis (Fig. 3) suggests that *alcorni* is indistinguishable from *bulleri* based on cranial characters.

Russell's decision to recognize *P. alcorni* also was influenced by the degree of its

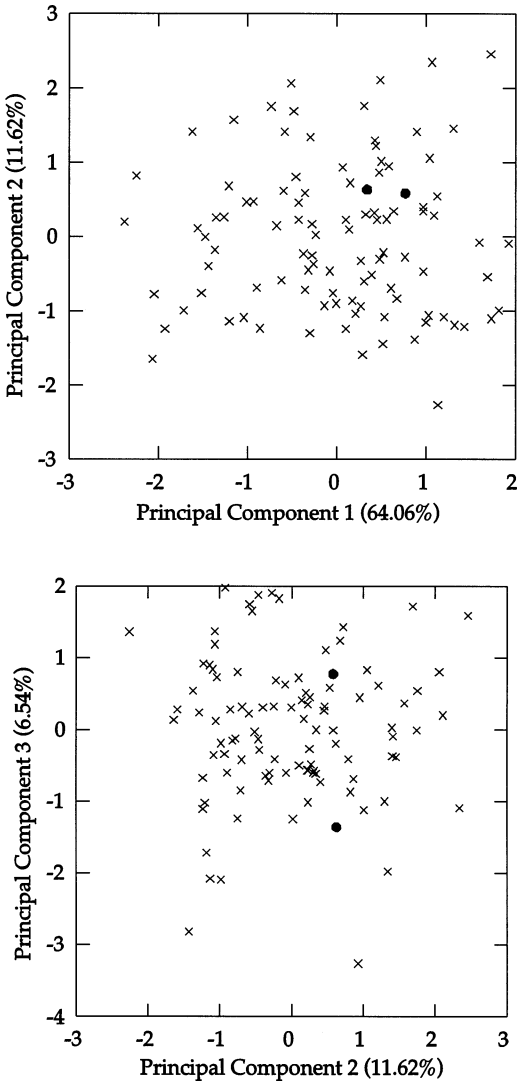

FIG. 3.—Principal component plots of cranial measurements for all female individuals of *Pappogeomys alcorni* (indicated by ●; $n = 2$) and *P. bulleri* (indicated by ×; $n = 99$) on axes 1 and 2 (above) and 2 and 3 (below). The proportion of total variation explained by each component is indicated in parentheses.

geographic isolation from populations of *P. bulleri* to the west: ''Because of the qualitative nature of the distinguishing characters of *alcorni* and its separation geographically from *bulleri, alcorni* is arranged as a species instead of as a subspecies of *P. bulleri*'' (Russell 1968:616). Because Russell believed that *P. alcorni* more closely resembled populations of *P. bulleri* to the west, he assumed the isolating barrier to be the dry lowlands to the west of *P. alcorni* (the area surrounding Sayula, Jalisco; Fig. 1). However, the mtDNA data suggest that *P. alcorni* is more closely allied with populations of *P. bulleri* to the north, near Guadalajara (Fig. 1). Unlike the dry, low-lying basin to the west of *P. alcorni*, which Russell recognized as a formidable barrier, there is no such geographic barrier present to the north, assuming that gophers dispersed around the eastern edge of Lago de Chapala.

Under other circumstances, the phylogeny based on mtDNA *Cytb* data (Fig. 2) would not be sufficient evidence to warrant a taxonomic revision of *Pappogeomys*. The bootstrap values for the tree presented in Fig. 2 are relatively low, and the likelihood ratio test revealed that a tree constrained to uphold monophyly of *P. bulleri* is not significantly different from the tree shown in Fig. 2. However, in the absence of evidence supporting recognition of *P. alcorni* at the species level, it is appropriate to revert to the null hypothesis of conspecificity, which is supported by both mtDNA and morphometric evidence.

It is possible that the mtDNA phylogeny (Fig. 2) is the result of incomplete lineage sorting in the *Pappogeomys* clade (Avise et al. 1984). However, the *Pappogeomys* lineages are relatively old (divergences approaching 10%; Table 1), and the relatively rapid extinction of mtDNA lineages (Avise et al. 1984) lessens the likelihood of incomplete lineage sorting. It also is important to point out that there is no reliable evidence (morphological or otherwise) to suggest that the phylogeny presented herein is incorrect. Furthermore, genetic distances among *Pappogeomys* specimens (6.0–9.8%; Table 1) are within the range of genetic distances for *Cytb* demonstrated by Bradley and Baker (2001) to be indicative of potentially conspecific lineages of mammals (≤11%—Bradley and Baker 2001).

Likewise, the genetic distances among *Pappogeomys* specimens also are within the range of genetic distances (recalculated using identical criteria) for conspecific clades within the closely related pocket gopher genus *Cratogeomys* (average interclade distances = 2.8–9.9%—Demastes et al. 2002). We conclude that the combined genetic and morphological evidence warrants recognition of *P. alcorni* as a subspecies of *P. bulleri* (*P. bulleri alcorni*; see ''Subspecies Account''). Therefore, *P. bulleri* will now comprise 9 described subspecies.

Subspecies Account

Pappogeomys bulleri alcorni Russell, 1957

Pappogeomys alcorni Russell, University of Kansas Publications, Museum of Natural History, 9:359, 25 January.

Holotype.—Adult female, skull and skin; KU 39806, University of Kansas Museum of Natural History; MEXICO: Jalisco, 4 mi (6 km) W Mazamitla, 6,600 ft (2,013 m).

Diagnosis.—A subspecies of *bulleri* characterized by relatively small body size; incisive foramina relatively short and broad, enamel plate absent on outer (labial) three-fourths of posterior wall of M1, nasal patch relatively large and buffy.

Distribution.—Known only from vicinity of type locality.

Comparisons.—*Pappogeomys bulleri alcorni* is relatively small compared with other subspecies of *P. bulleri*, lacks enamel on outer (labial) three-fourths of posterior wall of M1 (enamel usually present in other subspecies of *P. bulleri*); nasal patch buffy rather than whitish (as in most other subspecies), nasals broadly truncate instead of emarginate posteriorly, incisive foramina short and rounded instead of long and narrow.

Remarks.—The characters originally used by Russell (1957) to diagnose *P. b. alcorni* are present in the holotype (KU 39806) and one of the 2 paratypes (KU 39805; the other paratype lacks cranial material). However, Russell's subsequent examination of additional specimens of *P. bulleri* (Russell 1968) revealed presence of supposedly diagnostic *alcorni* characters in *P. bulleri* specimens. Based on this evidence, combined with new evidence from mtDNA and morphometric analyses (this study), we conclude that *alcorni* is a geographically isolated subspecies of *P. bulleri*.

Specimens examined.—MEXICO: Jalisco, 4 mi (6 km) W Mazamitla (KU 39805, 39806 holotype); 3 mi (5 km) WSW Mazamitla (Sierra del Tigre; KU 61328; skin only); 6 mi (10 km) S Mazamitla, 6,200 ft (1,891 m; CNMA 26219; skin only).

Resumen

Pappogeomys alcorni es una especie periférica aislada que actualmente está designada como especie rara en México. Existen solamente 4 especímenes de museo, todos colectados entre los años 1950–1966, y esfuerzos recientes no lograron localizar especímenes en el medio silvestre. Aunque solo un espécimen ha sido colectado (piel únicamente) desde el trabajo sistemático original sobre *P. alcorni*, nuevos métodos analíticos han permitido re-examinar los especímenes existentes. Se aisló ADN proveniente de una piel de un espécimen de museo de 48 años para investigar la relación filogenética de este raro taxón con otras otras especies de *Pappogeomys* y *Cratogeomys*. Un análisis filogenético basado en 424 pares de bases del gen del citocromo *b* mitocondrial y un análisis morfométrico de 101 individuos de *Pappogeomys* indican que *P. alcorni* es una subespecie de *P. bulleri*, más que una especie monotípica.

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APPENDIX I

Specimens examined and their geographic localities. Of these 162 specimens of *P. bulleri*, a total of 161 specimens were examined for morphometric analysis of crania, and 1 skin-only specimen was sampled for DNA analysis. All localities are in Mexico. Museum acronyms are as follows: the University of Kansas Natural History Museum (KU); Louisiana State University Museum of Natural Science (LSUMZ); the University of Michigan Museum of Zoology (UMMZ).

COLIMA: Armeria, ca. 3 km S (LSUMZ 11111–11112), 1 km SE, Manzanillo-Cuyutlan Hwy (LSUMZ 11113–11115); Santiago, 4 mi (6 km) W, 1 mi (2 km) S, 10 ft (3 m; KU 36675–36676); Santiago, 6 mi (10 km) N (KU 87422).

JALISCO: Ameca, 5 mi (8 km) NNW, Cerro Ameca, 5,500 ft (1,676 m; KU 33452); Ameca, 13 mi (21 km) WSW, 5,100 ft (1,554 m; KU 97150–97151); Atemajac de Brizuela, 4 mi (6 km) E, 8,000 ft (2,438 m; KU 109204–109205); Autlan, 20 mi (32 km) SE, 7,700 ft (2,347 m; KU 111727–111733); Barra de Navidad, 5 mi (8 km) NE, 200 ft (61 m; KU 39817); Barra de Navidad, 10 mi (16 km) NNW (KU 100443); Ciudad Granja, 5,100 ft (1,554 m; KU 95959–95961); Cuautla, 14 mi (23 km) NW, 7,500 ft (2,286 m; KU 100435–100437); Cuautla, 5 mi (8 km) NW, 6,550 ft (1,996 m; KU 100438–100442); Guadalajara, 13 mi (21 km) W, 4 mi (6 km) N, west side La Venta (KU 30995–31006, KU 31878); Guadalajara, 2 mi (3 km) N, 0.5 mi (1 km) W (KU 31007–31019, KU 31021–31026); Guadalajara, 4 mi (6 km) W, 5,100 ft (1,554 m; KU 31027–31036, KU 31877); Guadalajara, 10 mi (16 km) S, 8 mi (13 km) W (KU 31037–31043, KU 31045–31050); Jazmin, 2.5 mi (4 km) ENE, 6,800 ft (2,073 m; KU 109213–109218); Jazmin, 4 mi (6 km) ENE, 7,700 ft (2,347 m; KU 111734–111738); La Cuesta, 2 mi (3 km) S, 1,500 ft (457 m; KU 111718–111721); La Huerta, 10 mi (16 km) WSW, 1,400 ft (427 m; KU 107585–107591); Mascota, 14 mi (23 km) NW, 6,500 ft (1,981 m; KU 111712–111717); Nevado de Colima, northern slope Volcano de Nieve, ca. 8,500 ft (2,591 m; LSUMZ 10462); Mazamitla, 4 mi (6 km) W (KU 39805–39806); Mazamitla, 3 mi (5 km) WSW (Sierra del Tigre; KU 61328; skin only—used for DNA analysis); Purificacion, 5 mi (8 km) S (KU 33453); Sierra de Autlan, 7,600 ft (2,316 m; UMMZ 94624); Sierra de Cuale, 7,300 ft (2,225 m; KU 92986–92987, KU 92984 holotype *P. b. lutulensis*); Talpa de Allende, 15 mi (24 km) S, 9 mi (14 km) E, 6,900 ft (2,103 m; KU 97152–97154, KU 98759–98760); Tapalpa, 7 mi (11 km) S, 6,800 ft (2,073 m; KU 109206–109211); Tecomates, 7.5 mi (12 km) SE, 1,500 ft (457 m; KU 109212, KU 111723–111726, KU 111722 holotype *P. b. melanurus*); Tequila, 7 mi (11 km) SSW, 9,000 ft (2,743 m; KU 105654–105655); Tequila, 7 mi (11 km) S, 2 mi (3 km) W, Cerro Tequila, 10,000 ft (3,048 m; LSUMZ 36082, KU 33444–33450, KU 33451 holotype *P. b. infuscus*).

NAYARIT: Ahuacatlan, 8.4 km W, 1,100 ft (335 m; LSUMZ 34338); Ixtlan de Rio, 6 mi (10 km) S, 6,800 ft (2,073 m; KU 39801–39802); Jalcocotan, 2 mi (3 km) WNW, 3,000 ft (914 m; KU 36670–36674).