

THE SOUTHWESTERN NATURALIST

An International Periodical Promoting Conservation and Biodiversity
Southwestern United States–Mexico–Central America

Una Revista Internacional para Fomentar la Conservación y Biodiversidad
El Suroeste de USA–México–Centroamérica

TAXONOMIC STATUS AND SPECIES LIMITS OF *PEROGNATHUS* (RODENTIA: HETEROMYIDAE) IN THE SOUTHERN GREAT PLAINS

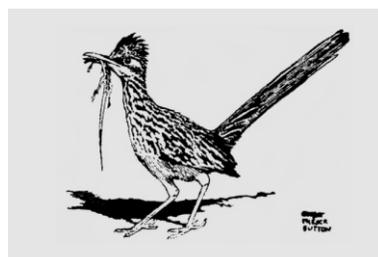
BRANDI S. COYNER,* THOMAS E. LEE, JR., DUKE S. ROGERS, AND RONALD A. VAN DEN BUSSCHE

Department of Zoology, Oklahoma State University, Stillwater, OK 74078 (BSC, RAVDB)

Department of Biology, Abilene Christian University, Abilene, TX 79699 (TEL)

Department of Biology and Monte L. Bean Life Sciences Museum, Brigham Young University, Provo, UT 84602 (DSR)

**Correspondent: Brandi.Coyner@okstate.edu*



TAXONOMIC STATUS AND SPECIES LIMITS OF *PEROGNATHUS*
(RODENTIA: HETEROMYIDAE) IN THE SOUTHERN GREAT PLAINS

BRANDI S. COYNER,* THOMAS E. LEE, JR., DUKE S. ROGERS, AND RONALD A. VAN DEN BUSSCHE

*Department of Zoology, Oklahoma State University, Stillwater, OK 74078 (BSC, RAVDB)**Department of Biology, Abilene Christian University, Abilene, TX 79699 (TEL)**Department of Biology and Monte L. Bean Life Sciences Museum, Brigham Young University, Provo, UT 84602 (DSR)***Correspondent: Brandi.Coyner@okstate.edu*

ABSTRACT—The cytochrome-*b* gene was sequenced and data were analyzed for 104 *Perognathus* from localities in Mexico, Arizona, California, New Mexico, Oklahoma, and Texas to evaluate the validity of *P. flavus* and *P. merriami* and to assess the occurrence of species of *Perognathus* in Oklahoma. Unweighted-parsimony, minimum-evolution, maximum-likelihood (TrN+I+G), and Bayesian analyses revealed seven well-supported terminal clades corresponding to currently recognized species of *Perognathus*. Results validate recognition of *P. flavus* and *P. merriami* as distinct species, document the occurrence of *P. merriami* within Oklahoma, and reveal a second potential contact zone between *P. flavus* and *P. merriami* in the Oklahoma Panhandle.

RESUMEN—El gen citocroma-*b* de 104 ejemplares de *Perognathus* de localidades en México, Arizona, California, Nuevo México, Oklahoma, y Texas fueron secuenciados y los datos analizados a fin de convalidar las especies *P. flavus* y *P. merriami* y para evaluar la presencia de las especies de *Perognathus* en Oklahoma. Los análisis filogenéticos (parsimonia sin peso, evolución mínima, probabilidad máxima (TrN+I+G), y el de Bayes) revelaron la existencia de siete clados terminales bien apoyados que corresponden a las especies de *Perognathus* actualmente conocidas. Los resultados confirman la validez de las especies *P. flavus* y *P. merriami*, documentan la presencia de *P. merriami* en el estado de Oklahoma, y revelan una segunda zona potencial de contacto entre *P. flavus* y *P. merriami* en el área noroeste del estado de Oklahoma conocido como el panhandle (mango de la sartén).

The North American rodent genus *Perognathus* occupies the western one-half of North America stretching from Canada to central Mexico (Schmidly et al., 1993) and currently consists of nine species (Patton, 2005) split into four species groups: the *fasciatus* group, the *flavus* group, the *longimembris* group, and the *parvus* group (Osgood, 1900; Williams, 1978). Informally, these species also can be divided into two overlapping geographic groups. A western desert group consisting of five species and a Great Plains group comprised of four. The southern Great Plains is home to three species of *Perognathus*: *P. flavescens*, *P. flavus*, and *P. merriami*.

Perognathus flavus and *P. merriami* are the two smallest representatives of the genus and are morphologically similar. Although these two species are allopatric over most of their range, an area of overlap occurs in southwestern Texas and northern Mexico where they are known to

hybridize (Wilson, 1973; Lee and Engstrom, 1991; Brant and Lee, 2006).

Baird (1855) described *P. flavus* from specimens collected near El Paso, Texas, whereas *P. merriami* was later described by Allen (1892) based on specimens collected near Brownsville, Texas, who noted differences in pelage coloration and the mastoid region between specimens he examined from Brownsville and those that Baird examined from El Paso. In his revision of the genus, Osgood (1900) recognized two subspecies of *P. merriami*: *P. m. merriami* occurring in central and southern Texas south to southern Tamaulipas, Mexico, and *P. m. gilvus* occurring in western Texas westward into eastern New Mexico. He noted that *P. m. gilvus* exhibited characteristics of both *P. flavus* and *P. merriami* and recognized the difficulty in distinguishing *P. flavus* and *P. merriami*. Despite their similarities, Osgood concluded that *P. flavus* and *P. merriami* were valid species.

Based on a multivariate morphological analysis, Wilson (1973) reported that *P. m. gilvus* was intermediate and likely represented hybrids between *P. flavus* and *P. merriami* and concluded that the two represented a single species, *P. flavus*. Martin (1977) agreed with conspecific recognition based upon similarities in behaviors exhibited by the two taxa, and several studies have documented that karyotypes of *P. flavus* and *P. merriami* are identical (Patton, 1967; Williams, 1978; Lee and Engstrom, 1991). Hall (1981) followed Wilson's (1973) recognition of a single species, but remarked that specimens from New Mexico and Chihuahua, Mexico, needed to be reexamined. However, allozymic data have since demonstrated that the two forms are genetically distinct (Lee and Engstrom, 1991). Recently, Brant and Lee (2006) noted significant differences in certain morphological characters separating *P. flavus* and *P. merriami*.

Perognathus flavus and *P. merriami* co-occur in New Mexico, Texas, and northern Mexico, with hybridization in areas of sympatry (Lee and Engstrom, 1991). Based upon range maps, the two species are also likely to co-occur in Oklahoma; however, northern areas of sympatry have not been examined (Schmidly, 2004; Brant and Lee, 2006). Species of *Perognathus* documented as occurring in Oklahoma are *P. flavus* and *P. flavescens* (Caire et al., 1989). However, no evaluation of the presence of *P. merriami* in Oklahoma has taken place, and no previous study included specimens from Oklahoma.

Therefore, the primary objective of this study was to evaluate distinctness of *P. flavus* and *P. merriami* as separate species using molecular-sequence data. Second, we hoped to determine how many species of *Perognathus* occur within Oklahoma, specifically if individuals representing *P. merriami* could be found within the state. Finally, we hoped to better define the northern range limits of each species and to determine if a second area of sympatry occurs in this region (Oklahoma or northern Texas).

MATERIALS AND METHODS—Specimens Examined—Specimens examined are listed in Appendix 1, including locality (Fig. 1) and identification information associated with museum vouchers and GenBank accession numbers. All individuals used in this study were collected following the American Society of Mammalogists Animal Care and Use Guidelines (Animal Care and Use Committee, 1998).

Molecular Methods—Whole genomic DNA was extracted from frozen skeletal muscle or organ tissue

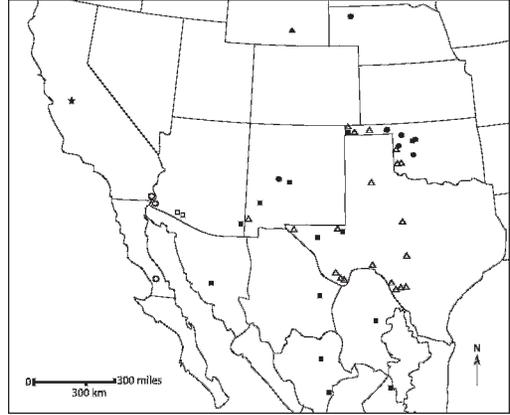


FIG. 1.—Localities in the southwestern United States and northern Mexico where specimens were collected for *Perognathus amphus* (open squares), *P. fasciatus* (closed triangles), *P. flavescens* (closed circles), *P. flavus* (closed squares), *P. inornatus* (stars), *P. longimembris* (open circles), and *P. merriami* (open triangles). Specific localities are listed in Appendix 1.

following standard lysis-phenol methods (Longmire et al., 1997), following Fetzner (1999), or using the DNEasy Kit (Qiagen Inc., Foster City, California). To estimate amount of genomic DNA, extractions were electrophoresed on 1.75–2.0% agarose gels stained with ethidium bromide.

The complete cytochrome-*b* gene was amplified and sequenced using combinations of a series of external and internal primers (Table 1). Polymerase chain reactions (PCR) were carried out in 25–30 μ L reactions containing 0.167–0.2 μ M of each primer, 2–3 mM of $MgCl_2$, 6 μ L of 5 \times buffer or 2 μ L of 10 \times buffer, 0.14–0.20 mM of each deoxynucleoside triphosphate, and 0.6–1.25 units of Taq DNA polymerase (Promega Corp., Madison, Wisconsin). For some reactions, 0.8 mg/mL of bovine serum albumen (BSA) was added. Two thermal profiles were used to amplify samples: one consisted of an initial denaturation of 95 $^{\circ}$ C for 3 min, followed by 35 cycles of 95 $^{\circ}$ C for 30 s, 45 $^{\circ}$ C for 50 s, and 72 $^{\circ}$ C for 1 min, and followed by a final elongation of 72 $^{\circ}$ C for 10 min, whereas the other consisted of an initial denaturation of 94 $^{\circ}$ C for 2–4 min, followed by 35–40 cycles of 94 $^{\circ}$ C for 1 min, 50 $^{\circ}$ C for 1 min, 72 $^{\circ}$ C for 1 min, and followed by a final elongation of 72 $^{\circ}$ C for 5 min.

Double-stranded amplifications were purified using the Wizard PCR prep DNA purification system (Promega Corp., Madison, Wisconsin), the QIAquick PCR purification protocol (QIAGEN, Chatsworth, California), or the Gene-Clean purification method (Bio 101, La Jolla, California). Purified samples were sequenced in both directions using BigDye version 3.1 chain terminators and electrophoresed on a 3130 Avant Genetic Analyzer (Applied Biosystems, Inc., Foster City, California) or using Perkin Elmer ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems, Foster City, California) and se-

TABLE 1—Primers used for amplification and sequencing of the cytochrome-*b* gene for *Perognathus*. Sources are listed for primer sequences previously published and sequences are given for primers developed by the authors.

Primer	Source or sequence (5' to 3')
External Light	
L14724	Irwin et al., 1991
MVZ05	Smith and Patton, 1993
MVZ-05-M	CTTGATATGAAAAACCATCGTTG modified from Smith and Patton, 1993
External Heavy	
H15915	Irwin et al., 1991
MVZ14	Smith and Patton, 1993
MVZ-14-M	TCTTCATCTYHGGYTTACAAGAC modified from Smith and Patton, 1993
Internal Light	
700L	Peppers and Bradley, 2000
F1	Whiting et al., 2003
flavus-cytb-A	ACCATCGTTGTCTATTCAACTATA
flavus-cytb-C	ATYATTGCAGCMATAGCYATAGT
MVZ17	Smith and Patton, 1999
MVZ35	Smith and Patton, 1993
MVZ45	Smith and Patton, 1993
Pero565F	ATTGCAGCMATAGCCATAGT
Internal Heavy	
CBH3	Palumbi, 1996
flavus-cytb-D	TAGTARGGRTGRAATGGAATT
H15149	Irwin et al., 1991
MVZ04	Smith and Patton, 1993
MVZ16	Smith and Patton, 1993
Pero700R	CTTTATAYGAGTAGTAGGGGTG

quenced on a Perkin-Elmer ABI Prism 377 automated sequencer.

Data Analyses—Sequences were visualized using Sequencher versions 3.1.1 and 4.1.1 (Gene Codes Co., Ann Arbor, Michigan) or ChromasLite (Technelysium Pty Ltd., Tewantin, Australia) and AssemblyLign 1.0.9 (Oxford Molecular Group PLC, 1998) was used to assemble overlapping fragments for each sample. Nine sequences of *Perognathus* (Appendix I) were obtained from GenBank (*P. amplus*—AY926403; *P. fasciatus*—AY926410; *P. flavescens*—AY926411, AY926412; *P. flavus*—DQ168551, AY926405; *P. inornatus*—AY926404; *P. longimembris*—AY926408; *P. merriami*—AY926406) along with three sequences of *Chaetodipus* to serve as outgroup taxa (*C. baileyi*—AY926393; *C. hispidus*—AY926391; *C. intermedius*—AY926389). All 117 sequences were aligned using ClustalX (Thompson et al., 1997) with default gap parameters. Alignments were visualized, as translated amino acids, and edited in MacClade (version 4.0; Maddison and Maddison, 2000) to ensure that no insertion, deletion, or stop codon was present in the dataset. Completed sequences were submitted to GenBank (FJ514829–FJ514931).

Nucleotides were coded as unordered, discrete characters, and character-state changes were polarized by designating individuals of *Chaetodipus* as outgroups. PAUP (Swofford, 2000) was used to estimate phylogenetic relationships among samples of *Perognathus* under the criteria of unweighted-parsimony, minimum-evolution (Kimura two-parameter corrected distances), and maximum-likelihood, whereas MRBAYES (Huelsenbeck and Ronquist, 2001) was used to estimate Bayesian phylogenetics. MODELTEST (Posada and Crandall, 1998) was used prior to maximum-likelihood analysis to determine the model of evolution that best fit the data (TrN+I+G, base frequencies = 0.3130, 0.2950, 0.0782, 0.3138; nst = 6; rmat = 1.0000, 15.2443, 1.0000, 1.0000, 7.8073; rates = gamma with shape parameter (α) = 1.3845 and proportion of invariant sites = 0.5167). Stability of clades obtained from the maximum-likelihood analysis was evaluated using 100 bootstrap replicates and Nearest-Neighbor Interchange branch-swapping.

For unweighted-parsimony analysis, a heuristic search with 25 random additions of input taxa and tree-bisection-reconnection branch-swapping was performed to estimate phylogenetic relationships. Kimura two-parameter (K2P) correction was used to infer phylogenetic relationships for minimum evolution. For both unweighted-parsimony and minimum-evolution analyses, stabilities of clades were estimated by performing 1,000 heuristic bootstrap replicates and TBR branch-swapping.

The GTR+ Γ model of DNA sequence evolution was used, along with site-specific rates of variation calculated for each of the three positions of the codon via the ssgamma option in MRBAYES to estimate phylogenetic relationships under Bayesian criterion. Four simultaneous Markov chains were run for 2,000,000 generations, starting with random, unconstrained trees. Temperature was set at 0.02 to facilitate greater movement between the four Markov chains, and trees were sampled every 10 generations. Resulting burn-in values (the point at which the model parameters and tree scores became stationary) were determined empirically by evaluating likelihood scores. Three independent runs of MRBAYES were performed using a different outgroup taxon (*C. baileyi*, *C. hispidus*, or *C. intermedius*) to ensure that final trees converged upon the same topology. Pairwise comparisons were performed between clades to determine percentage sequence divergences based upon Kimura two-parameter corrected distances.

RESULTS—Complete cytochrome-*b* sequences were obtained for 103 individuals and a partial cytochrome-*b* sequence was obtained for one sample (ACUNHC 504). Of the 1,140 sequenced bases, 617 sites were constant and 523 sites were variable with 125 at the first codon position, 33 at the second codon position, and 365 at the third codon position. Maximum-likelihood analysis produced a single optimal tree of length -10422.48133 and bootstrap analysis revealed 45 clades supported in $\geq 70\%$ of iterations.

Unweighted-parsimony analysis resulted in 300 equally parsimonious trees of 2,145 steps (consistency index, excluding uninformative characters = 0.4037; retention index = 0.9029). The large number of equally parsimonious trees primarily was due to lack of phylogenetic resolution among individuals within the larger, species-level clades. Bootstrap analysis revealed 48 clades supported in $\geq 70\%$ of iterations. Minimum evolution with Kimura two-parameter corrected distances revealed a single shortest tree with a minimum-evolution score of 1.66991 and 48 clades receiving bootstrap support $\geq 70\%$.

Bayesian analysis reached stationarity with *C. baileyi* at 7,100 generations, with *C. hispidus* at 10,560 generations, and with *C. intermedius* at 10,100 generations. Topology, posterior probabilities, and model parameters were in agreement for all runs and the topology resulting from Bayesian analysis with the gamma option resulted in the same topology as that from the other three analyses with 43 clades receiving posterior probabilities of ≥ 0.95 . Clades receiving bootstrap support $\geq 70\%$, Bayesian probabilities of ≥ 0.95 in ≥ 3 of the 4 analyses, or both, were considered strongly supported. When results from maximum-likelihood, unweighted-parsimony, minimum-evolution, and Bayesian analyses were considered together, the topologies are similar with six strongly supported species-level clades (Fig. 2).

Major clades correspond to *P. amplus*, *P. flavus*, *P. inornatus*, *P. longimembris*, and *P. merriami*, and a clade containing individuals identified as *P. fasciatus* and *P. flavescens*. Clades that correspond to *P. flavus* and *P. merriami* are monophyletic, supporting recognition of the *flavus* species group. Sister to the *flavus* species group is the *longimembris* species group consisting of *P. longimembris*, *P. inornatus*, and *P. amplus* with a strongly supported sister relationship between *P. longimembris* and *P. inornatus*. Within the *fasciatus* group, only the clade of Oklahoma *P. flavescens* is strongly supported. Denser sampling of the *fasciatus* group is required to provide better resolution for making conclusions about the group including the monophyly of *P. flavescens*. No sample of *P. parvus* or *P. alticolus* was included in this study, so we are unable to address the validity of the *parvus* group.

Within clades, percentage sequence-divergence values (Table 2) ranged from 1.125% in *P. amplus* to 6.633% in the clade consisting of *P. fasciatus*

TABLE 2.—Genetic distances (percentage sequence divergence) corrected by Kimura two-parameter model for comparisons within and between clades of *Perognathus* recovered in phylogenetic analyses (Fig. 2). Number of pairwise comparisons is given in parentheses.

Taxon	Between clades				
	1	2	3	4	5
1. <i>P. fasciatus</i> + <i>P. flavescens</i>	6.633 (136)				
2. <i>P. flavescens</i> —Oklahoma	1.437 (91)				
3. <i>P. amplus</i>	1.125 (3)				
4. <i>P. inornatus</i>	—	16.135 (3)			
5. <i>P. longimembris</i>	5.125 (3)	16.008 (9)	7.304 (3)		
6. <i>P. flavus</i>	4.256 (406)	19.847 (87)	19.123 (29)	19.996 (87)	
7. <i>P. merriami</i>	5.920 (1,770)	19.948 (180)	18.548 (60)	19.369 (180)	17.407 (1,740)

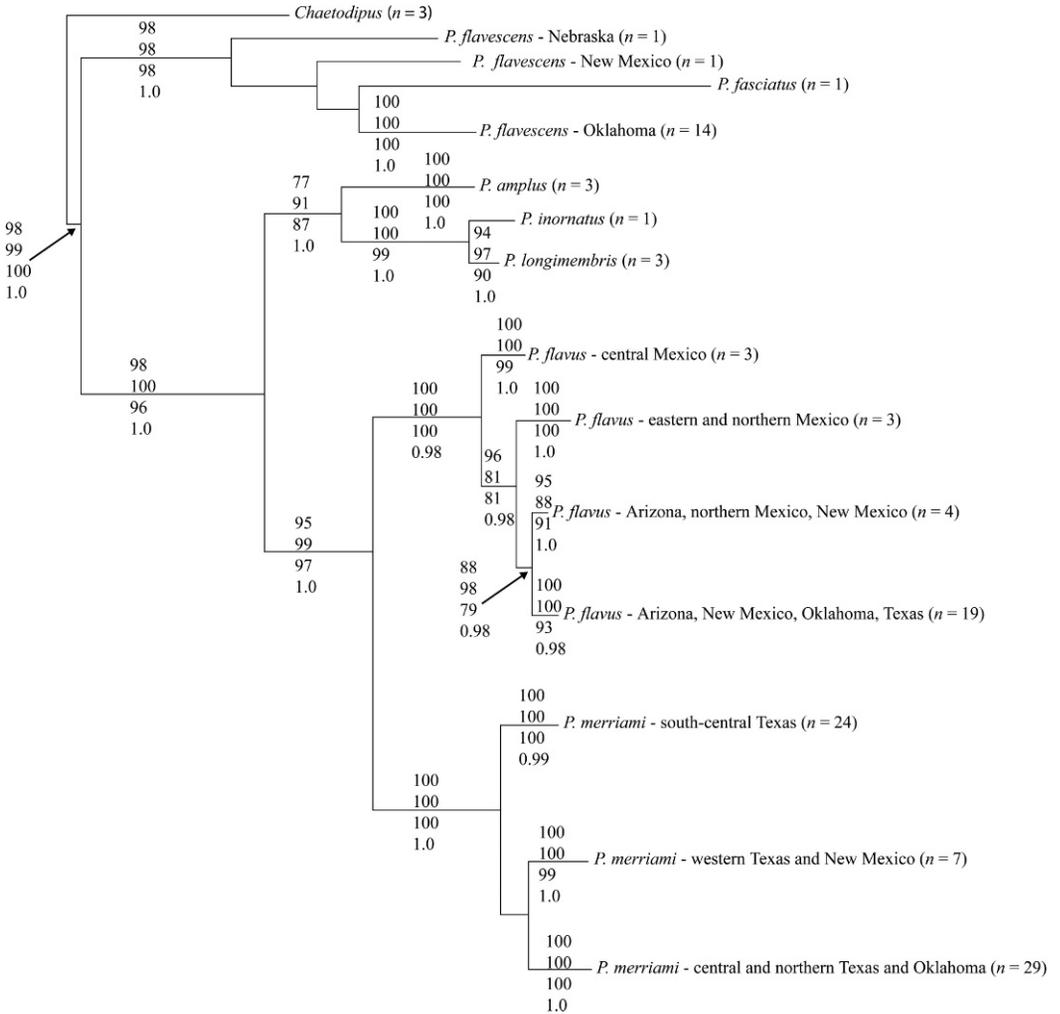


FIG. 2—Maximum-likelihood tree depicting relationships of cytochrome *b* within *Perognathus*. Bootstrap values and posterior probabilities are given at each branch from top to bottom as follows: maximum parsimony, minimum evolution, maximum likelihood, and Bayesian posterior probabilities, respectively. Only nodes receiving bootstrap values of $\geq 70\%$ or Bayesian posterior probabilities ≥ 0.95 in three of the four analyses are shown.

and *P. flavescens*. Percentage sequence-divergence values between clades was low only in *P. longimembris* at 7.304%. Values for all other between-clade comparisons were 16.008–27.484%, with average sequence-divergence between *P. flavus* and *P. merriami* = 17.407%. Within the *flavus* subclades, percentage sequence-divergence values (Table 3) ranged from 0.234% for the central Mexico subclade to 2.745% in the Arizona, northern Mexico, and New Mexico subclade. Percentage sequence-divergence values between the *flavus* subclades were 5.574–8.784%. Within the *merriami* subclades, divergence values (Table 4) ranged from 0.613% in the south-central

Texas subclade to 0.698% in the western Texas and New Mexico subclade, whereas between-subclade values were 8.755–9.684%.

DISCUSSION—Previous studies based on morphology, allozymes, karyology, and sequence data of the genus *Perognathus* have resulted in mixed conclusions regarding the conspecificity of *P. flavus* and *P. merriami*. These two taxa have identical karyotypes with the same diploid number, chromosomal morphology, and banding patterns resulting in the conclusion that they represent a single species (Patton, 1967; Williams, 1978). Allozyme data resulted in identifi-

TABLE 3—Kimura two-parameter, corrected, percentage sequence divergences for comparisons within and between subclades of *Perognathus flavus*. Number of pairwise comparisons is given in parentheses.

Taxon	Between subclades			
	Within subclade	1	2	3
1. Central Mexico subclade	0.234 (3)			
2. Eastern and northern Mexico and New Mexico subclade	1.546 (3)	8.784 (9)		
3. Arizona, northern Mexico, and New Mexico subclade	2.745 (6)	8.432 (12)	7.870 (12)	
4. Arizona, New Mexico, Oklahoma, and Texas subclade	0.765 (171)	7.667 (57)	7.722 (57)	5.574 (76)

cation of fixed differences at five loci (NP, 6-PGD, EST, LDH, and SOD-1) supporting recognition of *P. flavus* and *P. merriami* as distinct species (Lee and Engstrom, 1991). Conclusions based upon morphology have varied from no significant difference and the recommendation that the two taxa be recognized as a single species (Wilson, 1973) to the recent identification of five cranial (bullar length, mastoid breadth, interorbital breadth, interparietal breadth, and interparietal length) and two external characters (lengths of tail and hind foot) that differed significantly and support recognition of these two taxa as valid species (Brant and Lee, 2006). Previous sequence data are limited to studies that were focused at higher taxonomic levels, which recovered a strongly supported sister relationship between *P. flavus* and *P. merriami* (Riddle, 1995; Alexander and Riddle, 2005; Hafner et al., 2007). Limited sampling in each study assumes that *P. flavus* and *P. merriami* are monophyletic, and no conclusion about validity of *P. flavus* and *P. merriami* as distinct species was made in these molecular studies.

Results obtained in this study support the recognition of two species. Clades corresponding to *P. flavus* and *P. merriami* were recovered in all four analyses and their hypothesized sister relationship was well supported in all analyses. Despite superficial morphological similarity of *P. flavus* and *P. merriami*, the relatively high sequence-divergence value between these two clades, together with fixed allozyme differences (Lee and Engstrom, 1991) and significant morphological differences (Brant and Lee, 2006), is strong support for a species-level separation and is indicative of a relatively ancient split between the two taxa.

Although useful at evaluating interspecific relationships, the cytochrome-*b* data were less

useful in resolving intraspecific relationships within the three densely sampled species of *Perognathus* (Fig. 2). Some hierarchical structuring is apparent in the *P. flavus* clade with specimens collected in southern regions being more basal than specimens collected in northern areas. Three strongly supported subclades are present within *P. merriami*. The most basal, a south-central Texas subclade, consists of animals collected in Dimmit, Maverick, and Val Verde counties. Remaining samples of *P. merriami* are divided between a southern clade consisting of individuals from western Texas and a northern clade containing animals collected from central and northern Texas and Oklahoma.

As the first study to include specimens of *Perognathus* from Oklahoma, the secondary objective was to broadly define species limits of *P. flavus*, *P. flavescens*, and *P. merriami* to determine which of the three species occurred within the state. Following the taxonomy of Hall (1981) and Caire et al. (1989), only *P. flavus* and *P. flavescens* are recognized as occurring within Oklahoma. Based upon results of this study, *P. merriami* is present in Oklahoma with populations documented from Cimarron and Texas counties of the panhandle and in the southwestern portion of the state. Although *P. merriami* also may be present in the northwestern part of the state, only *P. flavescens* was collected in this area. In Oklahoma, *P. flavus* is known only from Rita Blanca Wildlife Management Area in extreme southwestern portions of the Oklahoma Panhandle. In Cimarron County, both *P. flavus* and *P. merriami* were collected within ca. 1 km of each other, indicating the two species may be sympatric in western portions of the panhandle. In west-central Oklahoma, *P. flavescens* and *P. merriami* have been collected within 18 km of each other. These two species are not known to hybridize and generally are believed to partition micro-

TABLE 4—Kimura two-parameter, corrected, percentage sequence divergences for comparisons within and between subclades of *Perognathus merriami*. Number of pairwise comparisons are in parentheses.

Taxon	Between subclades		
	Within subclade	1	2
1. South-central Texas subclade	0.613 (276)		
2. Western Texas and New Mexico subclade	0.698 (21)	9.684 (168)	
3. Central and northern Texas and Oklahoma subclade	0.636 (406)	9.530 (696)	8.755 (203)

habitats with *P. flavescens* restricted to sandy soils and *P. merriami* being a habitat generalist (Best and Skupski, 1994; Monk and Jones, 1996).

An area of overlap between geographic ranges of *P. flavus* and *P. merriami* is known from the Big Bend Region of Texas, southeastern New Mexico, and portions of northern Mexico (Brant and Lee, 2005). No other area of overlap was documented previously and their ranges were believed to diverge from each other in the Texas Panhandle with *P. flavus* occupying the northwestern portion of the panhandle and *P. merriami* occurring in the eastern portions (Hall, 1981; Schmidly, 2004). Identification of multiple samples captured in the Oklahoma Panhandle as *P. merriami* extends the range of this species westward into areas occupied by *P. flavus*. A denser sampling of the Texas and Oklahoma panhandles is likely to result in identifying additional areas where both species occur. Hybrids are known from only one location (Carlsbad, New Mexico) of the six sympatric locations known for *P. flavus* and *P. merriami* (Lee and Engstrom, 1991). Hybrids may occur if the two species are sympatric in the northern portions of their range.

As shown in previous studies (Riddle, 1995; Alexander and Riddle, 2005), cytochrome *b* was sufficient in elucidating intrageneric relationships in *Perognathus* needed to answer questions proposed in this study. However, additional insight could be gained by better understanding relationships within each species as well as by including all nine species of *Perognathus* in a sample-rich evaluation of the genus. To obtain better phylogeographic resolution within each species, a more rapidly evolving mitochondrial gene or the use of microsatellites would be useful. Additionally, a denser geographic sampling is warranted to better define species boundaries and potential areas of sympatry. Future research regarding *Perognathus* should be directed toward these areas together with

evaluating questions regarding hybrid individuals and the areas in which they occur.

We are grateful to R. J. Baker of the Museum of Texas Tech University and B. R. Riddle of the University of Nevada—Las Vegas for loans of tissues. We thank C. E. Palladino and T. Maxwell for laboratory assistance and the Oklahoma State University Recombinant DNA/Protein Resource Facility and the DNA Sequencing Center at Brigham Young University for use of equipment. Permits for field work were issued by the Arizona Game and Fish Department, Secretaria de Medio Ambiente, Recursos Naturales y Pesca, New Mexico Department of Game and Fish, Oklahoma Department of Wildlife Conservation, Texas Parks and Wildlife Department, and Utah Division of Wildlife Resources. Financial support was provided by the National Science Foundation (DEB 06-10844 to RAVDB) and Brigham Young University (Professional Development Grants, Departments of Zoology and Integrative Biology, the M. L. Bean Life Science Museum to DSR, and the Office of Research and Creative Activities to T. Maxwell). The National Science Foundation Graduate Research Fellowship Program provided stipend and educational support to BSC. Finally, we thank the numerous individuals who aided in collection of specimens, especially E. Arellano, J. G. Brant, F. X. González-Cózatl, Z. P. Roehrs, and R. E. Sherwin, without which this project would not have been possible.

LITERATURE CITED

- ALEXANDER, L. F., AND B. R. RIDDLE. 2005. Phylogenetics of the New World rodent family Heteromyidae. *Journal of Mammalogy* 86:366–379.
- ALLEN, J. A. 1892. Description of a new species of *Perognathus* from southeastern Texas. *Bulletin of the American Museum of Natural History* 4:45–46.
- ANIMAL CARE AND USE COMMITTEE. 1998. Guidelines for the capture, handling, and care of mammals as approved by the American Society of Mammalogists. *Journal of Mammalogy* 79:1416–1431.
- BAIRD, S. F. 1855. Characteristics of some new species of Mammalia, collected by the U.S. and Mexican Survey, Major W. H. Emory, USA Commissioner. *Proceedings of the National Academy of Science, Philadelphia* 7:331–333.

- BEST, T. L., AND M. P. SKUPSKI. 1994. *Perognathus merriami*. Mammalian Species 473:1–7.
- BRANT, J. G., AND T. E. LEE, JR. 2006. Morphological analysis of *Perognathus flavus* and *P. merriami* (Rodentia: Heteromyidae). Southwestern Naturalist 51:79–86.
- CAIRE, W., J. D. TYLER, B. P. GLASS, AND M. A. MARES. 1989. Mammals of Oklahoma. University of Oklahoma Press, Norman.
- FETZNER, J. W. 1999. Extracting high quality DNA from shed reptile skins: a simplified method. Biotechniques 26:1052–1054.
- HAFNER, J. C., J. E. LIGHT, D. J. HAFNER, M. S. HAFNER, E. REDDINGTON, D. S. ROGERS, AND B. R. RIDDLE. 2007. Basal clades and molecular systematics of heteromyid rodents. Journal of Mammalogy 88:1129–1145.
- HALL, E. R. 1981. The mammals of North America. John Wiley and Sons, New York.
- HUELSENBECK, J. P., AND F. RONQUIST. 2001. MRBAYES: Bayesian inference of phylogeny. Bioinformatics 17: 754–755.
- IRWIN, D. M., T. D. KOCHER, AND A. C. WILSON. 1991. Evolution of the cytochrome *b* gene in mammals. Journal of Molecular Evolution 2:37–55.
- LEE, T. E. JR., AND M. D. ENGSTROM. 1991. Genetic variation in the silky pocket mouse (*Perognathus flavus*) in Texas and New Mexico. Journal of Mammalogy 72:273–285.
- LONGMIRE, J., M. MALTBIE, AND R. J. BAKER. 1997. Use of “lysis buffer” in DNA isolation and its implication for museum collections. Occasional Papers, The Museum, Texas Tech University 163:1–3.
- MADDISON, W. P., AND D. R. MADDISON. 2000. MacClade, version 4: analysis of phylogeny and character evolution. Sinauer Associates, Inc., Publishers, Sunderland, Massachusetts.
- MARTIN, R. E. 1977. Species preferences of allopatric and sympatric populations of silky pocket mouse, genus *Perognathus* (Rodentia: Heteromyidae). American Midland Naturalist 98:124–136.
- MONK, R. R., AND J. K. JONES, JR. 1996. *Perognathus flavescens*. Mammalian Species 525:1–4.
- OSGOOD, W. H. 1900. Revision of the pocket mice of the genus *Perognathus*. North American Fauna 18:1–63.
- OXFORD MOLECULAR GROUP PLC. 1998. AssemblyLIGN 1.0.9. Oxford Molecular Group, PLC, Oxford, United Kingdom.
- PALUMBI, S. R. 1996. Nucleic acids I: the polymerase chain reaction. Pages 205–247 in Molecular systematics (D. M. Hillis, C. Moritz, and B. K. Mable, editors). Second edition. Sinauer Associates, Inc., Publishers, Sunderland, Massachusetts.
- PATTON, J. L. 1967. Chromosomes and evolutionary trends in the pocket mouse subgenus *Perognathus* (Rodentia: Heteromyidae). Southwestern Naturalist 12:429–438.
- PATTON, J. L. 2005. Family Heteromyidae. Pages 844–858 in Mammal species of the world: a taxonomic and geographic reference (D. E. Wilson and D. M. Reeder, editors). Third edition. John Hopkins University Press, Baltimore, Maryland.
- PEPPERS, L. L., AND R. D. BRADLEY. 2000. Molecular systematics of the genus *Sigmodon*. Journal of Mammalogy 81:332–343.
- POSADA, D., AND K. A. CRANDALL. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14: 817–818.
- RIDDLE, B. R. 1995. Molecular biogeography in the pocket mice (*Perognathus* and *Chaetodipus*) and grasshopper mice (*Onychomys*): the late Cenozoic development of a North American aridlands rodent guild. Journal of Mammalogy 76:283–301.
- SCHMIDL, D. J. 2004. Mammals of Texas. Revised Edition. University of Texas Press, Austin.
- SCHMIDL, D. J., K. T. WILKINS, AND J. N. DERR. 1993. Biogeography. Pages 319–365 in Biology of the Heteromyidae (H. H. Genoways and J. H. Brown, editors). Special Publication, American Society of Mammalogists 10:1–719.
- SMITH, M. F., AND J. L. PATTON. 1993. The diversification of South American murid rodents: evidence of mitochondrial DNA sequence data for the akodontine tribe. Biological Journal of the Linnean Society 50:149–177.
- SMITH, M. F., AND J. L. PATTON. 1999. Phylogenetic relationships and the radiation of sigmodontine rodents in South America: evidence from cytochrome *b*. Journal of Mammalian Evolution 6: 89–128.
- SWOFFORD, D. L. 2000. PAUP*: phylogenetic analysis using parsimony (*and other methods). Version 4.02b. Sinauer Associates, Inc., Publishers, Sunderland, Massachusetts.
- THOMPSON, J. D., T. J. GIBSON, F. PLEWNIAC, F. JEANMOUGIN, AND D. G. HIGGINS. 1997. The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 24:4876–4882.
- WHITING, A. S., A. M. BAUER, AND J. W. SITES, JR. 2003. Phylogenetic relationships and limb loss in sub-Saharan African scincine lizards (Squamata: Scincidae). Molecular and Phylogenetic Evolution 29: 583–598.
- WILLIAMS, D. F. 1978. Karyological affinities of the species group of silky pocket mice (Rodentia: Heteromyidae). Journal of Mammalogy 59:599–612.
- WILSON, D. E. 1973. The systematic status of *Perognathus merriami* Allen. Proceedings of the Biological Society of Washington 86:175–192.

Submitted 18 August 2008. Accepted 22 May 2009.
Associate Editor was Richard T. Stevens.

APPENDIX 1—*Specimens Examined*—Voucher and tissue samples for individuals included in this study are deposited in the Abilene Christian University Natural

History Collection (ACUNHC), M. L. Bean Life Science Museum at Brigham Young University (BYU), University of Nevada—Las Vegas (LVT), Oklahoma State University Collection of Vertebrates (OSU), and Museum of Texas Tech University (TTU).

Chaetodipus baileyi ($n = 1$): MEXICO: Sonora, 2 km N Puerto de la Libertad (LVT1210/AY926393).

Chaetodipus hispidus ($n = 1$): MEXICO: Durango, 7 miles NNW La Zarca (LVT1099/AY926291).

Chaetodipus intermedius ($n = 1$): MEXICO: Chihuahua, 5 km NNW Chihuahua (LVT1063/AY926389).

Perognathus amplus ($n = 3$): ARIZONA: Maricopa County, 14.5 miles S, 80 miles E Yuma (ACUNHC 22–23); Pima County, 0.5 mile N Organ Pipe Cactus National Monument (LVT 403/AY926403).

Perognathus fasciatus ($n = 1$): WYOMING: Carbon County, 10 miles S Seminoe (LVT 2525/AY925410).

Perognathus flavescens ($n = 16$): NEBRASKA: Sheridan County, 27 miles N Lakeside (LVT 2527/AY926411); NEW MEXICO: Socorro County, Rio Solado (LVT 3703/AY926412); OKLAHOMA: Beaver County, 2.25 miles N, 4.1 miles W Beaver in Beaver River Wildlife Management Area, UTM 14S 0348941 4078048, elevation 767 m (OSU 13171); Blaine County, 4.5 miles N, 1 mile E Canton in Canton Wildlife Management Area 14S 0538582 3997702, elevation 504 m (OSU 13181); Blaine County, 5.6 miles N, 4.8 miles W Canton in Canton Wildlife Management Area, UTM 14S 0529504 3999278, elevation 499 m (OSU 13182); Caddo County, 6.3 miles S, 7.6 miles W Binger in Fort Cobb Wildlife Management Area just north of EW124 road, UTM 14S 0548178 3897999, elevation 421 m (OSU 13170); Dewey County, 6.6 miles N, 7.9 miles W Canton in Canton Wildlife Management Area, UTM 14S 0524393 4001020, elevation 496 m (OSU 13183–13185); Ellis County, 17.3 miles S, 6.8 miles E Arnett in Packsaddle Wildlife Management Area, UTM 14S 0441177 3971262, elevation 676 m (OSU 13179); Ellis County, 17.2 miles S, 4.3 miles E Arnett in Packsaddle Wildlife Management Area, UTM 14S 0437369 3971402, elevation 648 m (OSU 13177–13178); Woodward County, 3.3 miles S, 2 miles E Fort Supply in Fort Supply Wildlife Management Area, UTM 14S 0450771 4043138, elevation 626 m (OSU 13188); Woodward County, 8.8 miles S, 0.3 mile E Fort Supply in Fort Supply Wildlife Management Area, UTM 14S 0451357 4043434, elevation 621 m (OSU 13189); Woodward County, 1.1 mile S, 1.2 mile E Fort Supply in Fort Supply Wildlife Management Area, UTM 14S 0450648 4045865, elevation 607 m (OSU 13186); Woodward County, 3.0 miles S, 4.8 miles E Fort Supply in Fort Supply Wildlife Management Area, UTM 14S 0456302 4042908, elevation 636 m (OSU 13187).

Perognathus flavus ($n = 29$): ARIZONA: Cochise County, 8 miles E Portal (ACUNHC 779); Navajo County, 3 miles S Keyenta (LVT 702/AY926405); MEXICO: Coahuila: 66 miles SW Cuatro Ciénegas

(TTU 35363/DQ168551); Chihuahua: 4 km S Parrita (LVT 1050); Durango: 7 miles NNW La Zarca (LVT 1109); Durango, Municipio Vicente Guerrero, 2.1 km E, 5.8 km N Vicente Guerrero, elevation 1,937 m (BYU 15783–15785); San Luis Potosí: 3 miles S Matehu (LVT 1198); Sonora: Punte La Poza, 18 km S (by road) Hermosillo, 28°49'692"N, 110°57'500"W, elevation 250 m (BYU 17883); NEW MEXICO: Grant County, 15 miles SE Silver City (ACUNHC 452); Lincoln County, Coyote (TTU 38444); OKLAHOMA: Cimarron County, 2.5 miles S, 6.8 miles W Felt in Rita Blanca Wildlife Management Area Unit 103, UTM 13S 0686584 4044008, elevation 1,419 m (OSU 13194–13195); Cimarron County, 2.5 miles S, 6.85 miles W Felt in Rita Blanca Wildlife Management Area Unit 103, UTM 13S 0686584 4044008, elevation 1,419 m (OSU 13192–13193, OSU 13196–13197); Cimarron County, 3.5 miles S, 7.25 miles E Felt in Rita Blanca Wildlife Management Area Unit 137, UTM 13S 0709475 4043605, elevation 1,300 m (OSU 13200–13203); Cimarron County, 3.25 miles S, 13.2 miles E Felt in Rita Blanca Wildlife Management Area Unit 142, 13S 0718926 4044545, elevation 1,276 m (OSU 13199); Cimarron County, 0.6 mile N, 2.4 miles W Felt in Rita Blanca Wildlife Management Area Unit 112, UTM 13S 0693293 4049892, elevation 1,387 m (OSU 13190); Cimarron County, 2.9 miles S, 2.6 miles W Felt in Rita Blanca Wildlife Management Area Unit 115, elevation 1,383 m (OSU 13198); Cimarron County, 0.4 mile N, 4.6 miles E Felt in Rita Blanca Wildlife Management Area Unit 130, UTM 13S 0704464 4050018, elevation 1,342 m (OSU 13191); TEXAS: Culberson County, Sierra Diablo Wildlife Management Area (TTU 75817, TTU 75790); Ward County, 1.5 mile SE Barstow (ACUNHC 195).

Perognathus inornatus ($n = 1$): CALIFORNIA: Madera County (LVT 601/AY026404).

Perognathus longimembris ($n = 3$): ARIZONA: Yuma County, 4.5 miles S Yuma (ACUNHC 35); CALIFORNIA: Imperial County, 2 miles S, 7.5 miles W Winterhaven (ACUNHC 36); MEXICO: Baja California, 27 km S Punta Prieta (LVT 2191/AY026408).

Perognathus merriami ($n = 60$): NEW MEXICO: Hidalgo County, 10 miles NE Portal Arizona, Granite Gap (ACUNHC 504); OKLAHOMA: Beckham County, 9.25 miles S, 1.7 mile E Erick Post Office in Sandy Sanders Wildlife Management Area, UTM 14S 0423855 3881003, elevation 620 m (OSU 13206); Beckham County, 9.6 miles S, 1.5 mile E Erick Post Office in Sandy Sanders Wildlife Management Area, UTM 14S 0423497 3880487, elevation 588 m (OSU 13207–13209); Beckham County, 8.75 miles S, 2 miles W Erick Post Office in Sandy Sanders Wildlife Management Area, UTM 0417421 3882169, elevation 636 m (OSU 13204–13205); Beckham County, 10.3 miles S, 1.3 mile E Erick Post Office in Sandy Sanders Wildlife Management Area, UTM 14S 0423377 3879476, elevation 616 m (OSU 13211); Beckham County, 12.7 miles

S, 3.9 miles E Erick Post Office in Sandy Sanders Wildlife Management Area, UTM 14S 0427002 3876666, elevation 590 m (OSU 13214–13215); Cimarron County, 3.4 miles S, 2.4 miles W Felt in Rita Blanca Wildlife Management Area Unit 116, UTM 13S 0693405 4043393, elevation 1,377 m (OSU 13212); Cimarron County, 4.3 miles S, 2.4 miles E Kenton (TTU 39792); Greer County, 10.2 miles S, 6.6 miles E Erick Post Office in Sandy Sanders Wildlife Management Area, UTM 14S 0431455 3879780, elevation 568 m (OSU 13210); Greer County, 11.4 miles S, 5.4 miles E Erick Post Office in Sandy Sanders Wildlife Management Area, UTM 14S 0429561 3877754, elevation 559 m (OSU 13213); Greer County, 15.2 miles S, 2.5 miles E Erick Post Office in Sandy Sanders Wildlife Management Area, UTM 14S 0424759 3873453, elevation 523 m (OSU 13216); Greer County, 15.4 miles S, 2.4 miles E Erick Post Office in Sandy Sanders Wildlife Management Area, UTM 14S 0424603 3872369, elevation 546 m (OSU 13217); Roger Mills County, 8.4 miles N, 2.7 miles W Cheyenne in Black Kettle Wildlife Management Area, UTM 14S 0434586 3955225, elevation 659 m (OSU 13218–13220); Roger Mills County, 2.1 miles S, 0.5 mile E Cheyenne in Black Kettle Wildlife Management Area, UTM 14S 0440460 3938120, elevation 651 m (OSU 13221); Texas County, 12.1 miles S, 0.7 mile E Hooker in Optima Wildlife Management Area, UTM 14S 0303608 4061935, elevation 849 m (OSU 13180); TEXAS: Brewster County, 50 miles S Marathon, Black Gap Wildlife Management Area (ACUNHC 175); Brewster County, 26 miles S Alpine, Elephant Mountain Wildlife Management Area (ACUNHC 809–810); El Paso County, 24 miles E El Paso (ACUNHC 505); Dimmit County, 34 miles E Eagle Pass (ACUNHC 598); Garza County, 4.6 miles E Southland (TTU 54553, TTU 54557, TTU 54571); Jeff Davis County, 10 miles W Fort Davis (ACUNHC 451); Kimble County, Texas Tech University Center at Junction (TTU 71105–71106); Loving County, 1 mile E Mentone (ACUNHC 501–502); Maverick County, 4 miles E Eagle Pass (ACUNHC 616); Maverick County, 6 miles E Eagle Pass (ACUNHC 594); Maverick County, 12 miles E Eagle Pass (ACUNHC 597); Maverick County, 15 miles N Eagle Pass (ACUNHC 618–619, ACUNHC 622, ACUNHC 624–625, ACUNHC 628–630, ACUNHC 642, ACUNHC 657, ACUNHC 662, ACUNHC 697, ACUNHC 702–703); Taylor County, 2 miles N Lake Abilene (ACUNHC 780); Val Verde County, Langtry (Eagle Nest Canyon) (ACUNHC 257); Val Verde County (LVT 603/AY926409); Val Verde County, 12 miles N Del Rio (ACUNHC 585, ACUNHC 588–591, ACUNHC 635).