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MOLECULAR SYSTEMATICS OF POCKET GOPHERS OF THE GENUS *GEOMYS*

PHILIP D. SUDMAN,* JEFFREY K. WICKLIFFE, PEGGY HORNER, MICHAEL J. SMOLEN, JOHN W. BICKHAM, AND ROBERT D. BRADLEY

Department of Biological Sciences, Tarleton State University, Stephenville, TX 76402, USA (PDS)
Department of Biological Sciences, Texas Tech University, Lubbock, TX 79409, USA (JKW)
Texas Parks and Wildlife Department, Wildlife Diversity Branch, 4200 Smith School Road, Austin, TX 78744, USA (PH)
World Wildlife Fund, 1250 24th Street, Washington, DC 20037-1175, USA (MJS)
Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, TX 77843-2258, USA (JWB)
Department of Biological Sciences and Museum of Texas Tech University, Lubbock, TX 79409-3131, USA (RDB)
Present address of PH: Missouri Department of Conservation, 2901 Truman Boulevard, Jefferson City, MO 65109, USA

Complete DNA sequences obtained from the mitochondrial cytochrome-*b* gene were examined in 41 individuals representing 11 currently recognized species of *Geomys*. Similar tree topologies were obtained from parsimony, genetic distance, likelihood, and Bayesian methods of data analyses. Phylogenetic relationships indicated 4 major clades that are referred to as species groups (*bursarius*, *breviceps*, *personatus*, and *pinetis*). The results also suggest the existence of several cryptic species that warrant further investigation.

Key words: cytochrome *b*, *Geomys*, phylogenetics, pocket gophers

Pocket gophers (Rodentia: Geomyidae) are fossorial herbivores, sister to the New World Heteromyidae, and, with one exception (*Orthogeomys*), are restricted in distribution to North America. Because they occur in small, isolated demes and possess an overall lack of vagility as well as conservative morphology, these rodents pose many problems with respect to their taxonomy, systematics, evolutionary history, and intrageneric affinities. Members of the genus *Geomys* are no exception. Species of *Geomys* occur in the southeastern United States and throughout much of the central and southern Great Plains westward to the eastern slope of the Rocky Mountains and south into northern Mexico (Fig. 1). Considerable uncertainty exists concerning the number of species present and the historical relationships among described taxa. Much of the confusion involves the presence of sibling species, with little or no morphological differentiation and, in some instances, high degrees of chromosomal diversification and allozymic variation.

Merriam (1895), in his revision of pocket gophers of the family Geomyidae (exclusive of *Thomomys*), recognized 3 species groups within *Geomys*: the *tuza* (= *pinetis*) group including what is today recognized as *G. pinetis*; the *bursarius* group including only *G. bursarius*; and the *texensis—breviceps* group including *G. arenarius*, *G. breviceps*, *G. lutescens*, *G. personatus*, and *G. texensis*. During the subsequent 80 years, results from several studies (Alvarez 1963; Baker 1950; Baker and Genoways 1975; Baker and Glass 1951; Bangs 1898; Davis 1938, 1940; Hall 1932; Hooper 1940; Jackson 1957; Komarek and Spencer 1931; McLaughlin 1958; Russell 1968; Sherman 1940, 1944; Swenk 1939, 1940; Villa-R. and Hall 1947) led to the addition of 4 new species (*colonus*, *cumberlandius*, *fontanelus*, and *tropicalis*) and 20 subspecies. Hall (1981), based on these efforts, recognized 8 species (*arenarius*, *bursarius*, *colonus*, *cumberlandius*, *fontanelus*, *personatus*, *pinetis*, and *tropicalis*) and 35 subspecies. However Hall's (1981) synthesis did not include the recommendation of Williams and Genoways (1980) that *G. colonus*, *G. cumberlandius*, and *G. fontanelus* should be synonymized into *G. pinetis*.

With the advent of chromosomal and molecular techniques, *attwateri*, *breviceps*, *knoxjonesi*, *lutescens*, and *texensis* were elevated to species status (Baker et al. 1989; Block and Zimmerman 1991; Bradley et al. 1991; Burt and Dowler 1999; Dowler 1989; Heaney and Timm 1983, 1985; Sulentich et al. 1991; Tucker and Schmidly 1981). However, Patton (1993) recognized only 5 of these putative species (*arenarius*, *bursarius*, *personatus*, *pinetis*, and *tropicalis*). In the most recent phylogenetic study of *Geomys*, Jolley et al. (2000) examined 12S ribosomal RNA (rRNA) sequence data and argued for the recognition of 11 species (*arenarius*, *attwateri*,

* *Correspondent: sudman@tarleton.edu*

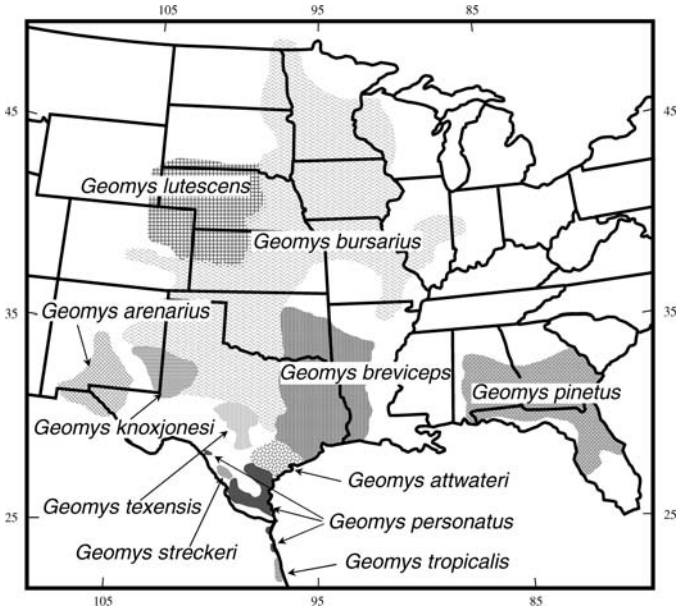

Fig. 1.—Geographic range of species of *Geomys*.

breviceps, *bursarius*, *knoxjonesi*, *lutescens*, *personatus*, *pinetis*, *streckeri*, *texensis*, and *tropicalis*), and it is this proposed taxonomy that is used herein.

Similar to the difficulties in determining the alpha taxonomy of *Geomys*, resolving phylogenetic relationships within *Geomys* has proven to be a laborious challenge. Conservation or convergence of morphological characters, presumably resulting from adaptations to a fossorial lifestyle, has led to difficulties in differentiating species based on morphometric variation (Mauk et al. 1999). Conversely, extensive variation in heterochromatic regions of chromosomes allows for distinction among most species but has hampered identification of synapomorphies that reflect relationships among species (Qumsiyeh et al. 1988; Smolen and Bickham 1994, 1995). Although examination of data from the 12S rRNA gene (Jolley et al. 2000) supported many of the earlier phylogenetic relationships, the slow rate of evolution in this gene hindered resolution of relationships among certain taxa. Specifically, several well-established relationships were poorly supported.

In this study, we examined DNA sequences from the mitochondrial cytochrome-*b* gene to ascertain the phylogenetic relationships within *Geomys*. This mitochondrial gene region evolves at a more rapid rate than the 12S rRNA gene and has been used successfully in reconstructing phylogenetic relationships among several genera of rodents (Bell et al. 2001; Carroll et al., in press; Edwards and Bradley 2003; Peppers et al. 2002; Smith and Patton 1993; Tiemann-Boege et al. 2000), including pocket gophers (Demastes et al. 2002; DeWalt et al. 1993; Smith 1998; Wickliffe et al., in press). The purpose of this research was to 1) examine the genetic divergence among various taxa of *Geomys* to better understand species and subspecies boundaries and 2) construct a comprehensive phylogeny of the genus to assess species and subspecies relationships.

Materials and Methods

Samples.—Forty-one specimens were examined (Appendix I) representing all 11 recognized species and 21 of the 24 recognized subspecies of pocket gophers, including *G. arenarius* ($n = 1$), *G. attwateri* ($n = 3$), *G. breviceps* ($n = 3$), *G. bursarius* ($n = 10$), *G. knoxjonesi* ($n = 1$), *G. lutescens* ($n = 2$), *G. personatus* ($n = 10$), *G. pinetis* ($n = 3$), *G. streckeri* ($n = 3$), *G. texensis* ($n = 3$), and *G. tropicalis* ($n = 2$). Nucleotide sequences are deposited in GenBank under accession numbers AY393935–AY393971. *Pappogeomys bulleri* and *Cratogeomys castanops* were used as outgroup taxa in all analyses (sequences for outgroups were taken from GenBank, accession numbers L11900 and L11902, respectively) based on the close affinity of these taxa to *Geomys* within Geomyinae (Hafner et al. 1994; Honeycutt and Williams 1982; Russell 1968).

Data collection.—The DNA was isolated from liver tissue following either phenol–chloroform extraction techniques as described by Hillis et al. (1990) or the lysis buffer extraction protocol of Longmire et al. (1997). Alternatively, mitochondrial DNA was extracted and purified using Wizard Miniprep kits (Promega, Madison, Wisconsin). The entire cytochrome-*b* gene was amplified via polymerase chain reaction (Saiki et al. 1988) with the following parameters: 39 cycles of 92°C (15 s) denaturing, 50°C annealing (1 min), and 72°C (1 min, 10 s) extension; followed by 1 cycle of 72°C (4 min). Amplification reactions were performed in 50-μl volumes, 10 mM Tris-HCl pH 8.3, 50 mM KCl, 2 mM $MgCl_2$, 1 μM primer concentration, and 1.25 U of *Taq* (Fisher Scientific, Fairlawn, New Jersey). Polymerase chain reaction primers used to amplify the cytochrome-*b* gene were either H15915 and MVZ05 (Irwin et al. [1991] and Smith and Patton [1993], respectively) or L14735 and H15906 (Elrod et al. 2000). After initial amplification, the resulting amplicons were used as templates for a subsequent asymmetric reaction and the entire region was sequenced as described by Sudman and Hafner (1992), using the primers listed above as well as internal primers L14841, L15049, H15149, H15408, H15513 (Irwin et al. 1991), and H15275 (reverse and complement of L15299—Edwards et al. 1991). Alternatively, the amplified products were purified with silica gel using QIAquick PCR Purification Kit (Qiagen, Valencia, California) and prepared for automated sequencing.

Automated sequencing used dye-labeled terminators and approximately 60–80 ng of DNA and cycle sequencing conditions of 95°C (30 s) denaturing, 50°C (20 s) annealing, and 60°C (3 min) extension. Eight primers were used in the sequencing protocol: 2 (H15915 and MVZ05) that were used in the polymerase chain reaction amplification 3 (400R, 700L, and WDRAT 1100) that were reported in Peppers and Bradley (2000), and 3 (400F, WDRAT 650, and CWE1) that were designed specifically for members of *Neotoma* (Edwards et al. 2001). After 25–29 cycles, reactions were ethanol-precipitated. Sequences for the heavy and the light strand were analyzed using an ABI-Prism 310 Genetic Analyzer (PE Applied Biosystems, Foster City, California). Sequences were aligned and proofed using Sequencher 5.0 software (Bromberg et al. 1995).

Data analysis.—A maximum-parsimony analysis was conducted using PAUP* 4.0b10 software (Swofford 2002). Variable nucleotide positions were treated as unordered, discrete characters with 4 possible character states: A, C, G, and T. Uninformative characters were excluded and characters were weighted equally. The tree-bisection-reconnection branch swapping algorithm and heuristic search option were used for tree construction. Robustness and nodal support was evaluated using 1,000 bootstrap iterations (Felsenstein 1985) and Bremer support indices (Bremer 1994) were calculated with the Autodecay Analysis program (Eriksson 1997).

Genetic distances were calculated using the Kimura-2 parameter model of evolution (Kimura 1980). This model was selected so that values could be compared to those of other studies involving rodents. In addition, genetic distances were obtained from the Tamura–Nei+I+Γ model of evolution (Tamura and Nei 1993) to construct a neighbor-joining tree (Saitou and Nei 1987). This model of evolution was identified by the MODELTEST program (Posada and Crandall 1998) and the hierarchical likelihood rate test criterion (to avoid nesting of models) as the most appropriate model of DNA evolution that best fit the data. Estimated parameters for this model included unequal base frequencies (A = 0.3348, C = 0.2706, G = 0.1000, T = 0.2946), 1 transversion rate but different transition rates (rAG = 15.337, rCT = 12.75, all others = 1.00), a proportion of invariant sites (pinv = 0.518), a gamma shape parameter (a = 1.44), and no molecular clock.

Maximum-likelihood parameters also were examined using the Tamura–Nei+I+Γ model of evolution and parameters (see above) obtained from the MODELTEST program (Posada and Crandall 1998). Heuristic tree searches under the maximum-likelihood criterion were preformed with these parameters fixed, 10 random input orders, and tree-bisection-reconnection branch swapping.

A Bayesian analysis was performed using the MrBayes 2.01 program (Huelsenbeck and Ronquist 2001). Four Markov chains were run simultaneously, each for 2,000,000 generations, and every 50th tree was sampled. The first 300 trees were discarded to allow for stabilization of likelihood scores. A consensus tree (50% majority rule) was generated from the remaining trees and clade probabilities were calculated. The analysis was rerun (same conditions as above) to insure convergence to the initial analysis.

Results

The DNA sequences from the mitochondrial cytochrome-*b* gene were obtained for 41 ingroup taxa (species and subspecies of *Geomys*) and 2 outgroup taxa (*P. bulleri* and *C. castanops*). This region encompassed 1,140 base pairs with the following nucleotide frequencies (estimated from the data): A = 33.5%, C = 27.1%, G = 10.0%, and T = 29.5%. The average transition : transversion ratio (estimated from the data) for the ingroup taxa was 5.2 for all characters.

The parsimony analysis of equally weighted characters (371 informative characters) generated 4 trees of equal length (1,492), consistency index (0.3861), and retention index (0.7076). A bootstrap consensus tree of these 4 trees depicted 5 primary clades (I–V; Fig. 2); relationships among 3 of which (I–III) were unresolved. The 1st, clade I, was subdivided into 5 subclades (A–E) that were unresolved. Subclade A contained *G. arenarius* and *G. knoxjonesi*, subclade B contained 9 taxa representing various subspecies of *G. bursarius*, subclade C contained 1 individual representing *G. l. lutescens*, subclade D contained *G. b. jugossicularis* and *G. l. halli*, and subclade E contained the 3 subspecies of *G. texensis*. Clade II contained 2 subclades (F, samples of *G. attwateri*; and G, *G. personatus* and *G. tropicalis*). Clade III contained samples of *G. streckeri*. Clade IV contained samples of *G. breviceps* and clade V contained samples of *G. pinetis*. Bootstrap and Bremer support values are shown in Fig. 2 and, with the exception of clade II, showed high levels of support for the primary clades.

The neighbor-joining method, using genetic distances obtained from the Tamura–Nei+I+Γ model of evolution, generated a tree (not shown) similar to the topology obtained from the equally weighted parsimony analysis. The only difference between the neighbor-joining and parsimony tree involved relationships within clade I (subclades A–E were resolved) and among clades I–III; all were resolved in a stepwise fashion. Relationships within and between clades II–V were identical to that depicted in the equally weighted parsimony tree.

Kimura (1980) 2-parameter genetic distance values for selected clades and samples are shown in Table 1. Values for comparisons of currently recognized subspecies ranged from 0.88% (*G. bursarius bursarius* and *G. bursarius majusculus*) to 9.28% (*G. breviceps breviceps* and *G. breviceps sagittalis*); values for comparisons of currently recognized species ranged from 8.14% (*G. bursarius* and *G. lutescens*) to 21.03% (*G. personatus* and *G. pinetis*).

The maximum-likelihood (−lnL = 8,796.39) and Baysian analyses generated tree topologies that were identical to each other and similar to parsimony and neighbor-joining trees. Differences involved the placement of *G. bursarius major* and *G. bursarius ozarkensis* within subclade B and the placement of *G. streckeri* within clade II. The topology and clade probabilities obtained from the Bayesian analysis are shown in Fig. 3.

Discussion

All analyses (parsimony, genetic distance, likelihood, and Baysian) depicted similar topologies, with minor differences resulting from the placement of 2 subspecies of *G. bursarius* (*major* and *ozarkensis*) and *G. streckeri*. Given the similarity of topologies among analyses, we have used the Bayesian analysis (Fig. 3) as a reference for discussing relationships among taxa. Additionally, we refer to each of the primary clades identified in our study as ''groups'' following the terminology of Merriam (1895) and Davis (1940). These groups (*bursarius*, *personatus*, *breviceps*, and *pinetis*) are identical in composition to those proposed by Davis (1986) based on analyses of ribosomal DNA (rDNA) restriction site data, and the branching order of all major taxa is in agreement with rDNA and mitochondrial DNA (mtDNA) restriction site analyses. Although we use our cytochrome-*b* phylogeny as the basis for discussion, it should be noted that most of the relationships depicted, including those suggesting the elevation of specific taxa, are supported by chromosomal, allozymic, or parasitic data, or a combination of these. Although our results indicate the potential addition of new species, we do not formally elevate or describe them at this time, but instead point out the need for additional work within this genus.

Geomys bursarius group (clade I).—Depending on one's systematics philosophy and interpretation, this group contains between 1 and 6 species. If differences among branching patterns (subclades) and genetic distances are ignored, then the argument could be made for combining all taxa into a single species referred to as *G. bursarius*. However, if branching patterns are used as the criterion, then 3 species probably should be recognized: *G. arenarius* (including *G. arenarius*

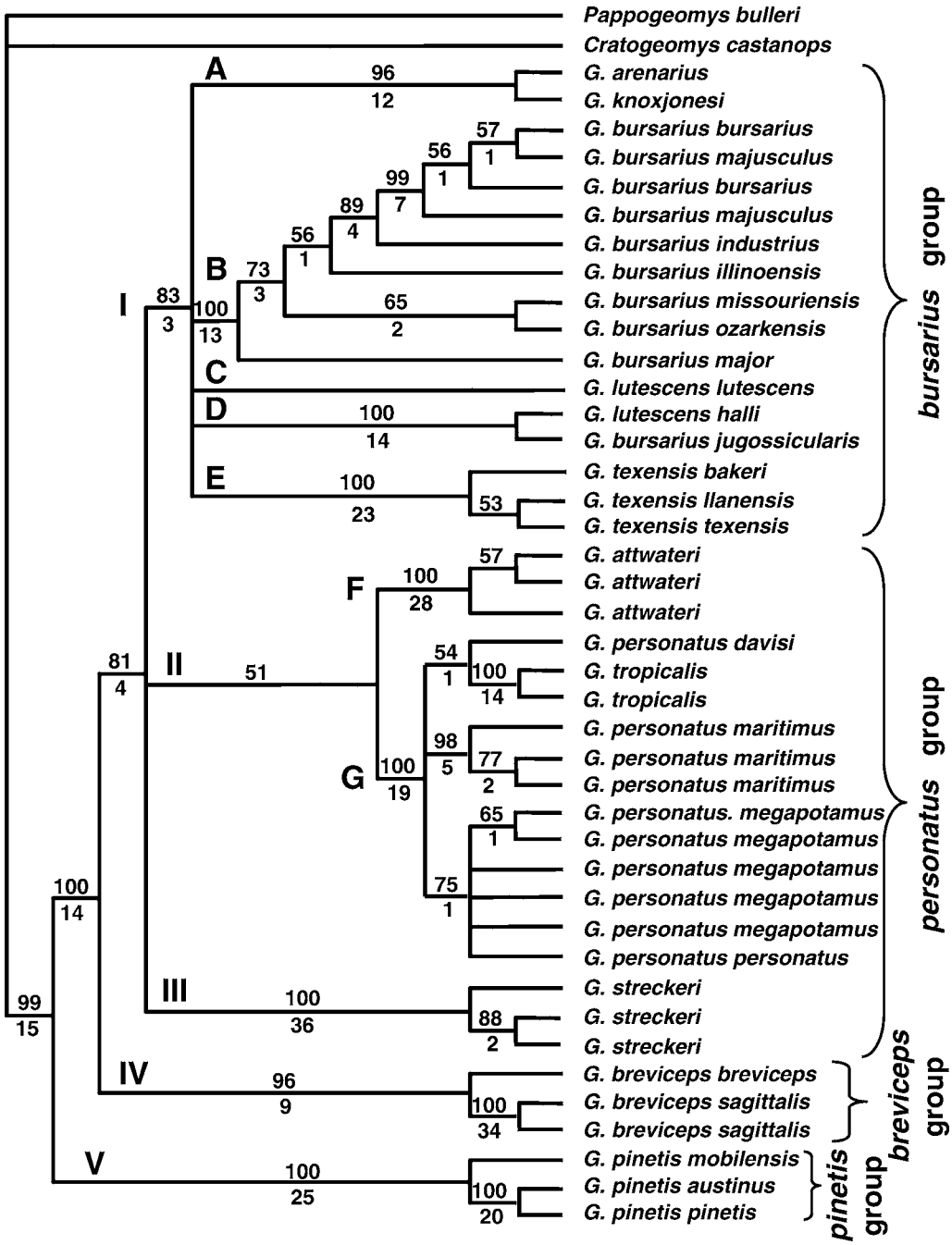

Fig. 2.—Parsimony tree depicting the phylogenetic relationships among 41 samples of *Geomys*. This tree represents a strict consensus of 4 most-parsimonious trees obtained from the analysis of equally weighted characters and the heuristic search option of PAUP* (Swofford 2002). Major clades are labeled with Roman numerals and minor clades are represented by capital letters (see text for a discussion). Bootstrap support values are above branches and Bremer support indices are below.

and *G. knoxjonesi*), *G. bursarius* (including all subspecies of both *G. bursarius* and *G. lutescens*), and *G. texensis* (including 3 subspecies). If genetic distances are used in conjunction with branching patterns (Bradley and Baker 2001), then the argument could be made for the additional recognition of *G. lutescens* (including only the sample of *G. l. lutescens*), *G. jugossicularis* (samples currently referred to as *G. b. jugossicularis* and *G. l. halli*), and *G. knoxjonesi* as valid species.

This latter interpretation is supported by allozymic and chromosomal data. Using the taxon names suggested above, the members of subclade B (*G. bursarius*) have been shown to have a diploid number (2n) = 70–72 and fundamental number (FN) = 68–74 (Hart 1978) with little or no gene flow among members of this subclade and those located further west (Burns et al. 1985). *G. lutescens* (subclade C) possesses a distinctive karyotype (2n = 72, FN = 86–98—Hart 1978) with no apparent gene flow between *G. lutescens* to the north and

members of subclade D, *G. jugossicularis* (*lutescens*) *halli* (2n = 70, FN = 72), to the south (Sudman et al. 1987). *G. texensis*, subclade E, is geographically isolated from other *Geomys*, and has been shown to be genetically more similar to members of subclade A (*G. knoxjonesi*) than to other, geographically closer, *Geomys* (Block and Zimmerman 1991) that are clearly affiliated with *G. bursarius* and its allies. Finally, *G. knoxjonesi* and *G. arenarius*, both in subclade A, possess distinctive chromosomal differences (2n = 70, FN = 68–70 and 2n = 70, FN = 88–102, respectively—Baker et al. 1989; Hafner and Geluso 1983) and are highly differentiated from each other based on genetic distances (see Table 1).

Genetic distances estimated from the cytochrome-*b* sequence data lend support to the possible recognition of 6 species within the *bursarius* group (Table 1), with *G. b. jugossicularis*/*G. l. halli* representing the 6th species (*G. jugossicularis*). Pairwise distances within these 6 taxa are all below 6% and range from 1.3% (between *G. b. bursarius* and *G. b. majusculus*) to 5.8% (between *G. b. major* and *G. b. missouriensis*), whereas distances among species are all 8% or higher. We interpret this dichotomy to represent subspecific as opposed to specific levels of genetic differentiation, as it holds true for most of the other groups within this genus (see below).

Geomys personatus group (clades II and III).—Similar to the *bursarius* group, the number of species in the *personatus* group varies depending on the method of interpretation. Using the genetic distance criteria described above, 3 species should be recognized (*G. attwateri*, *G. personatus*, and *G. streckeri*). *G. attwateri* is well differentiated from all other taxa within the *personatus* group (with a sequence divergence of 11.89% from the next closest taxon), and has been shown to be both allozymically (Block and Zimmerman 1991) and chromosomally (Hart 1978; Smolen and Bickham 1995) distinct. Restriction site analyses of rDNA also indicate that *G. attwateri* forms a separate taxon from other members of the *personatus* group (Davis 1986).

Geomys streckeri (clade III) is sister to a clade containing samples of *G. personatus* and *G. tropicalis* (clade II), but the genetic distance separating these clades is 11.66%. This value represents the 2nd greatest difference between any pairwise comparisons of sister species in the genus. This coupled with chromosomal differences (Smolen and Bickham 1995), rDNA (Davis 1986), and mtDNA (Jolley et al. 2000) argues for recognition of *G. attwateri*, *G. personatus*, and *G. streckeri* as separate species.

The status of the remaining *personatus* group taxa (*G. p. davisi*, *G. p. maritimus*, *G. p. megapotamus*, *G. p. personatus*, and *G. tropicalis*) is more complex. Based on these samples, *G. p. maritimus* is basal to the clade formed by *G. tropicalis*, *G. p. megapotamus*, *G. p. personatus*, and *G. p. davisi*. These 5 taxa could be recognized as subspecies of *G. personatus* based on the oldest available name (True 1889). The low levels of genetic differentiation within this clade (5.3%) support this view, although chromosomal and mtDNA restriction site data for *G. tropicalis* would refute this position (Davis 1986; Davis et al. 1971; Qumsiyeh et al. 1988). Alternatively, *G. tropicalis* and *G. p. maritimus* could be recognized as species, leaving *G. p. megapotamus* to represent *G. personatus*. If *G. tropicalis* is recognized as a species, as most evidence suggests it should be, then what is the status of *G. p. davisi*? Two choices are appropriate: either synonymize *G. p. davisi* into *G. tropicalis* or elevate it to species level. However, *G. p. davisi* is not distinct karyotypically from *G. p. personatus* and *G. p. megapotamus*, although it possesses a unique chromosomal polymorphism (Smolen and Bickham 1995). We believe that it is premature to suggest taxonomic changes based upon such a small data set. Perhaps inclusion of the remaining subspecies of *G. personatus* (*fallax* and *fuscus*) will help, but clearly better population sampling is necessary to finally resolve these taxonomic issues.

Table 1.—Average genetic distances (Kimura 1980) for selected clades and taxa of *Geomys*.

Taxa	Average genetic distance (%)
Within *G. bursarius* (not including *G. bursarius jugossicularis*)	3.78
G. bursarius (not including *G. bursarius jugossicularis*) versus *G. lutescens lutescens*	8.79
G. bursarius jugossicularis versus *G. lutescens halli*	2.88
G. bursarius jugossicularis/*G. lutescens halli* versus *G. lutescens lutescens*	8.14
Within *G. texensis*	2.85
G. texensis versus *G. bursarius*/*G. lutescens lutscens*/*G. lutescens halli*	10.02
G. arenarius/*G. knoxjonesi* versus *G. bursarius*/*G. lutescens*/*G. texensis*	11.89
G. arenarius versus *G. knoxjonesi*	10.48
G. attwateri versus *G. streckeri*	11.91
G. streckeri versus *G. personatus davisi*/*G. personatus maritimus*/*G. personatus megapotamus*/*G. personatus personatus*/*G. tropicalis*	11.66
G. personatus davisi versus *G. tropicalis*	4.89
G. tropicalis versus *G. personatus megapotamus*/*G. personatus personatus*	4.97
G. breviceps breviceps versus *G. breviceps sagittalis*	9.28
Within *G. pinetis*	3.44
G. pinetis pinetis versus *G. pinetis* "*mobilensis*"	8.10

Geomys breviceps group (clade IV).—Only 3 samples of *G. breviceps* (1 *breviceps* and 2 *sagittalis*) were included in this study. The genetic distance between *G. b. breviceps* and *G. b. sagittalis* was 9.28%, whereas the 2 samples of *G. b. sagittalis* differed by only 2.33%. This comparison suggests an extremely high rate of differentiation compared to differences observed between other recognized subspecies (*G. bursarius* = 3.78% and *G. texensis* = 2.85%). Although it is premature to formally recognize these taxa as distinct species, it is important to recognize that the level of genetic divergence exceeds levels observed among other species. Additional studies currently are underway to examine the genetic structuring within and between the 2 currently recognized subspecies of *G. breviceps*. Examination of preliminary data suggests that at least 2 species are present within the currently accepted range of *breviceps*, with additional sampling needed to delineate the boundaries of these taxa.

Geomys pinetis group (clade V).—Three samples of *G. pinetis* were examined in this study, representing the sub-

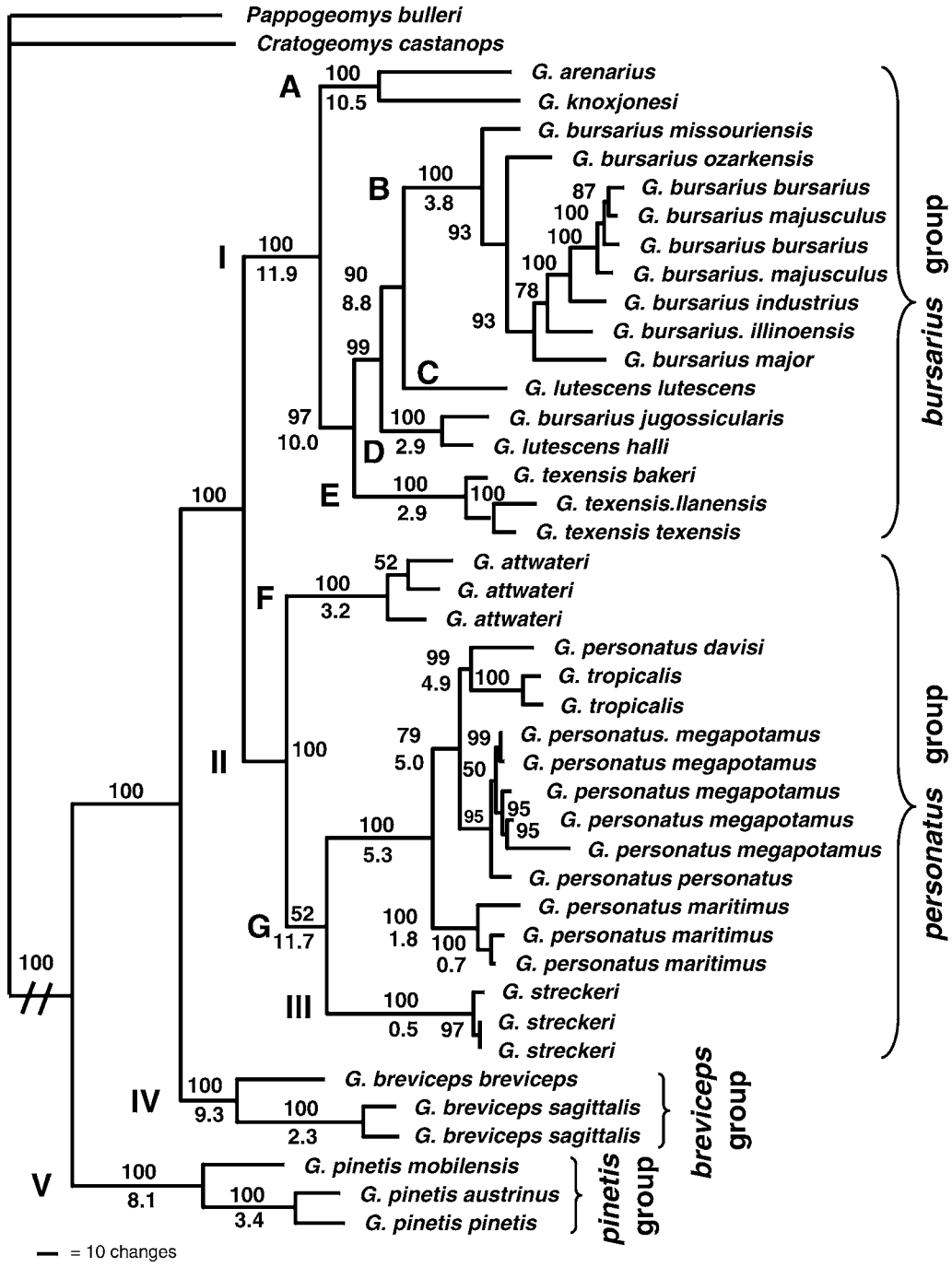

FIG. 3.—Tree obtained using MrBayes (Huelsenbeck and Ronquist 2001). Major clades are labeled with Roman numerals and minor clades are represented by capital letters (see text for a discussion). Clade posterior probability values, expressed as percentages, are above branches and average genetic distances calculated from the Kimura 2-parameter model of evolution (Kimura 1980) are below. Branch lengths are reflective of levels of sequence evolution as estimated in the analysis.

species *pinetis*, *austrinus*, and *mobilensis* as designated by Hall (1981). The *pinetis* group was basal to all other groups. The level of genetic divergence between 2 of the samples (*austrinus* and *pinetis*) was 3.44%; however, the 3rd sample (*mobilensis*) differed substantially from the other 2 (8.10%). Although *G. p. mobilensis* was relegated to *G. p. pinetis* by Williams and Genoways (1980), and these authors failed to identify morphological characters distinguishing *G. p. pinetis* and *G. p. mobilensis*, they pointed out that *G. p. mobilensis* hosted a different species of mallophagan louse than did other forms of *G. pinetis* (*Geomydoecus mobilensis* as opposed to *G. scleritis*). Given the propensity of pocket gophers and lice to cospeciate (Hafner et al. 1994) and the high levels of genetic divergence identified in this study, it may be that *G. p. mobilensis* represents a species distinct from *G. pinetis*. Additional evidence for a split between *G. p. mobilensis* and other *pinetis*

to the east of the Apalachicola River is the report of 2 fixed allelic differences among these gophers in a protein electrophoretic study conducted by Kennedy (1988). Avise et al. (1979) showed that these populations differed in both mtDNA restriction patterns and at 2 allozyme loci. Furthermore, Avise (1992) provided evidence that the Apalachicola River serves as a boundary for other faunal elements within this region of the southeastern United States. It seems plausible that *G. p. mobilensis* might represent a cryptic species.

At this time, it is appropriate to recognize 4 species groups as depicted in Fig. 2 (*bursarius* [including those taxa represented in clade I], *breviceps* [clade IV], *personatus* [clades II and II], and *pinetis* [clade V]) and a minimum of 11 species. Further studies are needed to evaluate 5 additional taxa (*G. b. jugossicularis*, *G. p. davisi*, *G. p. maritimus*, *G. b. sagittalis*, and *G. p. mobilensis*) to determine if they warrant species-level recognition and to define the limits of their ranges. Additionally, evidence is needed from nuclear markers to test these hypotheses to avoid potential gene tree biases. It should be noted that recognition of *G. tropicalis* as a species distinct from the other *G. personatus* subspecies would result in *G. personatus* being a paraphyletic taxon.

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APPENDIX I

Specimens examined.—The 41 specimens examined in this study are listed below by museum acronym (Hafner et al. 1997) or individual collector number. All localities are in the United States unless otherwise specified. Sample, museum number or collector identification, and GenBank accession numbers are provided in parentheses. Acronyms for identification numbers are as follows: Museum of Natural Science, Louisiana State University (LSUMZ); Texas Cooperative Wildlife Collection, Texas A&M University (AK); University of Arkansas, Little Rock (UALR); Natural Science Research Laboratory, Museum of Texas Tech University (TK); Museum of the High Plains, Fort Hays State University (MHP); Scott B. Block (SBB); and Scott K. Davis (SKD).

Geomys arenarius.—New Mexico: Dona Ana County; east bank Rio Grande; W Las Cruces (LSUMZ 31456, AY393935).

Geomys attwateri.—Texas: Gonzales County; 0.8 miles S Ottine (LSUMZ 29596, AY393936); Wilson County; 10 miles W Floresville (AK 7920, AY393938); Bastrop County; no other data (AK 5455, AY393937).

Geomys breviceps breviceps.—Louisiana: Morehouse Parish; 3.1 miles E Bastrop (LSUMZ 31603, AY393939).

Geomys breviceps sagittalis.—Louisiana: Vernon Parish; 2 miles S, 3 miles W Rosepine (LSUMZ 30723, AY393940). Arkansas: Little River County; 3 miles NW Alleene (UALR 4532, AF158689).

Geomys bursarius bursarius.—Minnesota: Anoka County; Cedar Creek Biological Station (SKD 407, AY393941). Iowa: Jasper County; 2.7 miles N Oakland Acres (MHP 29082, AF158693).

Geomys bursarius illinoensis.—Illinois: Madison County; 1 mile N, 2 miles W Collinsville (LSUMZ 35274, AY393942).

Geomys bursarius industrius.—Kansas: Reno County; 2 miles N, 4 miles W Arlington (MHP 24799, AY393943).

Geomys bursarius major.—Texas: Hood County; 7.5 miles N Granbury (LSUMZ 29606, AY393944).

Geomys bursarius majusculus.—Missouri: Holt County; 6 miles S, 2 miles E Mound City (LSUMZ 31448, AY393945). Nebraska: Saunders County; 1 mile N, 4 miles E Cedar Bluffs (MHP 24869, AF158694).

Geomys bursarius missouriensis.—Missouri: St. Louis County; 1.0 mile S Creve Coeur Lake (LSUMZ 31450, AY393946).

Geomys bursarius ozarkensis.—Arkansas: Izard County; 3 miles W Melbourne (ULAR 4352, AF158697)

Geomys knoxjonesi.—Texas: Winkler County; 3.2 miles S Kermit (SBB 8, AY393947).

Geomys jugosscularis halli.—Nebraska: Harlan County; 2 miles W Alma (LSUMZ 31464, AY393948).

Geomys jugossicularis jugossicularis.—Colorado: Fremont County; 3 miles S, 4 miles E Canon City (LSUMZ 29284, AY393949).

Geomys lutescens lutescens.—Nebraska: Custer County; 8.5 miles N, 0.8 miles W Miller (Buffalo County) (LSUMZ 31447, AY393950).

Geomys personatus davisi.—Texas: Zapata County; 2.5 miles N, 4 miles E San Ignacio (AK 5362, AY393951).

Geomys personatus maritimus.—Texas: Nueces County; Flour Bluff, Graham Road (SKD 176, AY393952; AK 7924, AY393953; AK 5431, AY393954).

Geomys personatus megapotamus.—Texas: Kleberg County; 1.5 miles S Riviera (LSUMZ 31458, AY393959); Willacy County; 6 miles N Raymondville (AK 5242, AY393955; AK 5241, AY393956); Brooks County; 5 miles S Falfurrias (AK 5432, AY393957); Jim Hogg County; 8 miles S Hebbronville (AK 5439, AY393958).

Geomys personatus personatus.—Texas: Nueces County; Mustang Island State Park (AK 7964, AY393960).

Geomys pinetis mobilensis.—Florida: Santa Rosa County; 0.8 miles N Route 90 on Route 87 (LSUMZ 29340, AY393961)

Geomys pinetis pinetis.—Florida: Baker County; 4.5 miles N Maclenny, Route 15 (LSUMZ 29331, AY393963). Georgia: Camden County; 1.7 miles S Kingland, Route 17 (LSUMZ 29327, AY393962).

Geomys streckeri.—Texas: Dimmit County; Carrizo Springs (SKD 47, AY393967; AK 5417, AY393968; AK 4803, AY393969).

Geomys texensis bakeri.—Texas: Uvalde County; 13 miles S Sabinol FM 187 (TK 48998, AY393964).

Geomys texensis llanensis.—Texas: Gillespie County; 9 miles E Fredericksburg (LSUMZ 29604, AY393965).

Geomys texensis texensis.—Texas: Mason County; 2 miles W Mason (LSUMZ 29605, AY393966).

Geomys tropicalis.—MEXICO: Tamaulipas: 3.5 miles SE Altamira (TK 27098, AY393971; SKD 143, AY393970).

Cratogeomys castanops.—GenBank accession L11902.

Pappogeomys bulleri.—GenBank accession L11900.