

MOLECULAR SYSTEMATICS OF BAIRD'S POCKET GOPHER

(Geomys breviceps)

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Baird's pocket gopher (*Geomys breviceps*) is found in eastern Texas, eastern Oklahoma, central and western Arkansas, and western Louisiana. The cytochrome-*b* gene was sequenced and analyzed for 16 pocket gophers from throughout the range of the species. Similar phylogenetic trees were obtained using maximum-parsimony, maximum-likelihood, neighbor-joining, and Bayesian analyses. Two major clades were formed with northern individuals belonging to clade I and southern individuals belonging to clade II. *G. b. sagittalis* was paraphyletic in relation to *G. b. breviceps* in all analyses. Based on inconsistencies between the taxonomic classification and systematic relationships within Baird's pocket gopher, a taxonomic restructuring appears warranted.

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MOLECULAR SYSTEMATICS AND BIOGEOGRAPHY OF BAIRD'S POCKET GOPHER

(Geomys breviceps)

Introduction

Baird's pocket gopher, *Geomys breviceps*, is a species of fossorial rodent located in eastern Texas, eastern Oklahoma, central and western Arkansas, and western Louisiana (Sudman et al. 2006; Sulentic et al. 1991). Pocket gophers inhabit fine sandy loam soils and tend to avoid areas with high clay content. This restricted habitat preference often leads to small isolated demes. Genetic drift, often observed in isolated populations, is further exacerbated by inbreeding and small effective population sizes (due to unequal sex ratios; Zimmerman and Gayden 1981). Additionally, low vagility limits range expansion and migration between populations (Davis 1938; Honeycutt and Schmidly 1979; Zimmerman and Gayden 1981). The above factors result in high rates of genetic divergence between pocket gopher populations (Penney and Zimmerman 1976). Conversely, convergent adaptation to fossorial life results in cryptic morphology (Honeycutt and Schmidly 1979; Mauk et al. 1999). These natural history traits present a challenge for differentiating taxa and elucidation of the systematics of pocket gophers. A wide range of research methods, including fossil records, morphology, and molecular techniques, have been used to focus on better understanding the biogeography and systematics of pocket gophers.

Geomys first appeared in the fossil record during the Pliocene and were common by the Pleistocene. The Great Plains region served as the center of differentiation for pocket gophers with range expansion when conditions were favorable. During the Illinoian, *Geomys* extended to the Atlantic coast in southeastern U.S. These populations were isolated from western populations during the late Pleistocene resulting in two extant species-groups as defined by Russell (1968).

The southeastern populations (Russell's *pinetis* group) have been synonymized into a single species, *G. pinetis*, which is the most divergent *Geomys* species (Sudman et al. 2006). Based on sequence data of the cytochrome-*b* gene, the remaining species to the west (the *bursarius* species-group according to Russell, 1968) have been further separated into three species groups, the *breviceps* group, the *bursarius* group, and the *personatus* group (Sudman et al. 2006).

Geomys breviceps, the most basal clade of the western groups, was first described in 1855 (Baird; Sudman et al. 2006). By 1938, five subspecies were recognized, *G. b. attwateri*, *G. b. breviceps*, *G. b. llanensis*, *G. b. sagittalis*, and *G. b. texensis*, (Davis 1938; Merriam 1895). Baker and Glass (1951) identified *G. breviceps* as conspecific with *G. bursarius* and demoted *breviceps* to subspecies level based on morphological features.

In the 1970's, several chromosomal studies were conducted on *Geomys bursarius*. The populations inhabiting the currently accepted range of Baird's pocket gopher were identified as the *breviceps* chromosomal group (2N=74, FN=72). This group included the following subspecies: *G. b. brazensis*, *G. b. breviceps*, *G. b. dutcheri*, *G. b. ludemani*, *G. b. pratincolus*, *G. b. sagittalis*, and *G. b. terricolus* (Hart 1978; Honeycutt and Schmidly 1979; Kim 1972). Honeycutt and Schmidly's (1979) examination of chromosomal variation in relation to morphological variation was one of the early indicators that changes in genetic traits were not always reflected in displayed characters for *Geomys*.

Several studies stemming from the chromosomal analyses focused on the contact zone between the *breviceps* group and its bordering chromosomal groups (Bohlin and Zimmerman 1982; Burt and Dowler 1999; Cothran and Zimmerman 1985; Tucker and Schmidly 1981; and

Zimmerman and Gayden 1981). These studies culminated in the re-elevation of several species including *G. breviceps*.

Recent studies involving Baird's pocket gopher have uncovered inconsistencies between the accepted taxonomy of the species and the systematic relationships within the species (Demastes 1994; Demastes and Hafner 1993; Kieschnick 2008; Sudman 2006). *G. b. breviceps* and *G. b. sagittalis* are the two recognized subspecies of Baird's pocket gopher (Sulentich et al. 1991). *G. b. breviceps* is found in a single parish in Louisiana (Morehouse Parish). *G. b. sagittalis* is found throughout the remainder of the range where habitat is suitable. No less than 17 miles (approximately 27 kilometers) separates these two subspecies (Demastes and Hafner 1991). In 1993, Demastes and Hafner, examined allozyme data, and found *G. b. sagittalis* to be paraphyletic in relation to *G. b. breviceps*. *G. b. sagittalis* in southeast Texas were found to be more similar to *G. b. breviceps* than to other *G. b. sagittalis* in northeast Texas or eastern Louisiana. In 1994, Demastes examined a segment of the cytochrome-*b* gene for six *G. breviceps* populations in Texas and Louisiana. The mean percent sequence divergence between *G. b. breviceps* and *G. b. sagittalis* (~8%) was in the range of between species comparisons for other cytochrome-*b* gene studies of pocket gophers. In 2006, Sudman et al. also found the average genetic distance between *G. b. breviceps* and *G. b. sagittalis* cytochrome-*b* sequences (~9%) to be in the range of between species comparisons. However, the small sample size (n=3) limited conclusions. In 2008, Kieschnick, using amplified fragment length polymorphism (AFLP) analysis, defined six populations of Baird's pocket gopher. His findings support *G. b. breviceps* as a unique population. However, based on his data, the Ouachita River did not appear to be a barrier to distribution, and accordingly Kieschnick recommended expanding the range of *G. b. breviceps* to include adjacent localities to the north and west of Morehouse Parish, Louisiana.

As evidenced by the above, further clarification of the genetic diversity within *Geomys breviceps* is needed. This study examined sequence divergence of the cytochrome-*b* gene for Baird's pocket gophers throughout their range. The cytochrome-*b* gene is a protein coding mitochondrial gene (Bradley and Baker 2001). It's stable yet rapidly evolving nature makes the cytochrome-*b* gene highly useful in phylogenetic studies of recently evolved animals (including within species and sister species) (Johns and Avise 1998; Prychitko and Moore 2000). Additionally, its widespread use throughout vertebrate taxa provides an abundance of data for comparison (Bradley and Baker 2001). The objective of this study was to examine sequence diversity in the cytochrome-*b* gene of *G. breviceps* from throughout the species distribution to determine if the genetic diversity within the species warrants taxonomic revision.

Methods

Samples. Specimens were collected from throughout the recognized range of *Geomys breviceps* using Victor® gopher traps (Victor, Lititz, Pennsylvania). Sixteen individuals were examined, and, when available, specimens that represented the corners of populations defined by Kieschnick (2008) were used (Fig. 1). Locations were as follows: Ashley County, Arkansas; Union County, Arkansas; Ouachita County, Arkansas; Calhoun County, Arkansas; Jefferson County, Arkansas; Cleburne County, Arkansas; Little River County, Arkansas; Atoka County, Oklahoma; Lincoln County, Oklahoma; Lamar County, Texas; Van Zandt County, Texas; Anderson County, Texas; Rusk County, Texas; Vernon Parish, Louisiana; Union Parish, Louisiana; and Morehouse Parish, Louisiana. See Appendix I for additional information on all specimens examined.

Data collection. Muscle and liver tissue were collected and stored in lysis buffer (Longmire et al. 1997). DNA was extracted from liver tissue using a QIAGEN® DNeasy® Kit

(QIAGEN, Valencia, California) following their protocol for animal tissue. DNA concentration was measured for each sample using a NanoDrop™ ND-1000 UV-Vos Spectrophotometer (NanoDrop Technologies, Wilmington, DE) and portion of each extraction was diluted with sterile water to a concentration of 50 ng/μL.

Polymerase chain reaction (PCR; Saiki et al. 1986, 1988) was performed to amplify the entire cytochrome-*b* gene with the following parameters: 1 cycle of 94°C (2 min); 33 cycles of 94°C (45 s) denaturing, 56°C (1 min) annealing, and 72°C (1 min, 10 s) extension; followed by 1 cycle of 72°C (10 min). Amplification reactions contained 0.5 μL of diluted extraction product, 1.25 μL of 5X PCR buffer, 2.5 mM MgCl₂, 0.6 μM primer concentrations, 1.25 U of *Taq* DNA polymerase, and sterile diH₂O to bring the total volume to 25 μL. The primers used to amplify the cytochrome-*b* gene were L14735 (5'-TGAAAAACCATCGTTGTTAATTCAACT-3') and H15906 (5'-CATCTCCGGTTTACAAGACCTAAGTAAT-3'; Elrod et al. 2000). The double-stranded amplicons were purified using Promega® Wizard® PCR Clean-up kit (Promega®, Madison, Wisconsin) or ExoSAP-IT® For PCR Product Clean-Up (USB®, Cleveland, Ohio).

Sequences were obtained for both directions using dye-labeled chain terminators and 1.0 μl of amplified product. In addition to the above primers, LGL 765 (5'-GAAAAACCAAYCGTTGTWATTCAACT-3') and LGL 766 (5'-GTTTAATTAGAAATYTYAGCTTTGGG-3'; Harlin-Cognato et al. 2006) were used in sequencing reactions. Sequencing parameters were 95°C (20 s) denaturing; 50°C (20 s) annealing, and 60°C (4 min) extension for 30 cycles. Reactions were ethanol-precipitated before being analyzed with a Beckman-Coulter® CEQ™ 8000 Automated Genetic Analysis System (Beckman-Coulter®, Inc., Fullerton, California). Single-stranded sequence fragments were aligned and proofed using BioEdit v5.0.6 software (Hall 1999).

Data analyses. The program PAUP 4.0b10 (Swofford 2002) was used to obtain genetic distances as well as to perform neighbor-joining, maximum-parsimony, and maximum-likelihood analyses. A Bayesian analysis was performed using MRBAYES (Huelsenbeck and Ronquist 2001). *G. pinetis floridanus* was used as the outgroup for all analyses based on the close affinity it has to *G. breviceps* (Block and Zimmerman 1991).

Genetic distances were calculated using the Kimura two-parameter model of evolution (Kimura 1980). This model of evolution was chosen so that the distances could be compared to other pocket gopher studies which used this model.

For maximum-parsimony analysis, nucleotides were coded as discrete, unordered characters with equal weight. There were four possible character states: A, C, G, and T. Uninformative characters were excluded. Starting trees were obtained via stepwise addition. The tree-bisection-reconnection (TBR) branch-swapping algorithm and heuristic search option were used for tree construction. Reliability of clades was evaluated using 1,000 bootstrap iterations.

Prior to maximum-likelihood analysis, the program jModelTest (Posada 2008) identified the GTR + I + G model of evolution as the best fit given the data set. In the general time-reversible model (GTR), there are six substitution rates (A to C, A to G, A to T, C to G, C to T, and G to T), and the rate of change from i to j is equal to the rate of change from j to i (Tavare 1986). For this model, the estimated substitution rates were $r_{AC} = 3.46679$, $r_{AG} = 16.2114$, $r_{AT} = 1.69424$, $r_{CG} = 0.17453$, $r_{CT} = 17.66515$, and $r_{GT} = 1$; and the estimated base frequencies were $f_A = 0.3113$, $f_C = 0.2553$, $f_G = 0.1396$, and $f_T = 0.2938$. Additional model parameters included gamma (G) distributed rate variation among sites ($\alpha = 1.043$) with an assumed proportion of invariable (I) sites, $\text{pinvar} = 0.389$. The maximum-likelihood analysis was performed using the above evolutionary model parameters. The likelihood criterion under the

heuristic tree search options and the tree-bisection-reconnection (TBR) branch-swapping algorithm were used for tree construction.

The above GTR + I + G evolutionary model parameters were also used to perform the Bayesian analysis. One cold and three incrementally heated Markov chains were run for two million generations. Random starting trees were used for each chain, and trees were saved every fiftieth generation. A consensus tree was created using 50% majority rule. The first 300 trees were not used in creating the consensus tree; this allowed the likelihood values of the trees to stabilize. Two independent Bayesian phylogenetic analyses were performed to assess the reliability of the results.

Results

The cytochrome-*b* gene was examined for 16 in-group samples of *Geomys breviceps* and one outgroup sample (*Geomys pinetis floridanus*). A total of 1,140 base pairs were sequenced for each sample with mean base frequencies of A = 0.31, C = 0.25, G = 0.14, and T = 0.30.

For the maximum-parsimony (MP) analysis there were 155 informative characters. The analysis resulted in 12 equally most parsimonious trees with tree lengths of 481, consistency indices of 0.7505, and retention indices of 0.6809. A bootstrap consensus tree (Fig. 2) revealed two major clades, I and II. Clade I was an unresolved polytomy with six subclades. Subclade A contained the sample from Union Parish, Louisiana; subclade B contained the sample from Morehouse Parish, Louisiana (the representative *G. breviceps breviceps* sample); and subclade C contained the Jefferson County, Arkansas sample. Subclade D contained five samples forming an unresolved trichotomy. The Cleburne County, Arkansas sample formed one branch of the trichotomy, while the Atoka County, Oklahoma sample with the Lincoln County, Oklahoma sample and the Little River County, Arkansas sample with the Lamar County, Texas sample

formed the other two branches. Subclade E was composed of samples from Ashley County, Arkansas and Union County, Arkansas, and subclade F was composed of samples from Calhoun County, Arkansas and Ouachita County, Arkansas. Clade II included two subclades, G and H. Subclade G contained samples from Vernon Parish, Louisiana and Rusk County, Texas and subclade H contained samples from Anderson County, Texas and Van Zandt County, Texas. All 12 most parsimonious trees contained clades I and II, and the organization of clade II was consistent through all 12 trees as well as with the consensus tree. Also in all most parsimonious trees, the *G. b. breviceps* sample from Morehouse Parish, Louisiana (subclade B) was the most basal for clade I, followed by the sample from Union Parish, Louisiana (subclade A), except for tree seven in which the Union Parish, Louisiana sample was not basal. Subclades C-F were consistently present in all trees, but these subclades varied greatly in their relationships among each other with no consistencies between the trees. However, subclade D showed greater resolution in the 12 most parsimonious trees than in the consensus tree. In the 12 most parsimonious trees, the Atoka County, Oklahoma sample and Lincoln County, Oklahoma sample formed one half of a dichotomy and the Cleburne County, Arkansas sample was basal to Little River County, Arkansas and Lamar County, Texas samples in the other half of the dichotomy.

The neighbor-joining tree (Fig. 3) was consistent with the MP consensus tree. There were two clades, and clade II was identical to that seen in the MP trees. Also identical to the 12 most parsimonious trees, the Morehouse Parish, Louisiana sample was the most basal of clade I. The remainder of the samples in clade I of the neighbor-joining tree formed a dichotomy between two subclades. The first subclade was identical to subclade D from the most parsimonious trees. The Union Parish, Louisiana sample was the most basal in the second subclade followed by the Jefferson County, Arkansas sample. As with the MP consensus tree, the Ashley County,

Arkansas sample paired with the Union County, Arkansas sample and the Calhoun County, Arkansas sample paired with the Ouachita County, Arkansas sample. These two pairs formed a dichotomy completing the second subclade.

Pair-wise Kimura 2-parameter genetic distances (Table 1) ranged from 0.7062% (between Atoka County, Oklahoma and Lincoln County, Oklahoma) to 12.6557% (between Ouachita County, Arkansas and Van Zandt County, Texas). The genetic distance between clade I and clade II was 8.96% (Table 2).

Using the GTR + I + G model of evolution, the maximum-likelihood (ML) analysis produced a tree with $-\ln L = 3924.20994$ (Fig. 4). The major difference between the ML tree and previously described trees was that subclade H (the Anderson County, Texas and Van Zandt County, Texas samples) was basal to clade I organisms, while subclade G (Vernon Parish, Louisiana and Rusk County, Texas samples) remained in a separate clade. The remainder of clade I samples had similar organization to that seen in the most parsimonious trees. The Morehouse Parish, Louisiana individual was most basal followed by the Union Parish, Louisiana sample. The Jefferson County, Arkansas sample was basal to an unresolved trichotomy between subclades D, E, and F described above.

The Bayesian analysis consensus tree (Fig. 5) showed little resolution. Subclade G formed a trichotomy with the outgroup and the remaining samples. Like in the maximum-likelihood tree, subclade H was most basal of the remaining samples, and subclades A-F described above, all formed an unresolved polytomy.

Discussion

Due to their unique natural history traits, such as low vagility, specific habitat preference, and small effective population sizes, pocket gopher populations have high rates of genetic

divergence. However, their convergent morphological adaptations to fossorial life leads to phenotypic similarities causing distinct taxa to be cryptic. Following the advent of molecular techniques pocket gophers were determined to be more diverse than previously recognized, and accordingly, several taxa of pocket gophers were elevated to higher taxonomic levels. In the Great Plains region of the United States, what was previously thought to be a single species, *G. bursarius*, has since been divided into nine distinct species, *G. arenarius*, *G. attwateri*, *G. breviceps*, *G. bursarius*, *G. knoxjonesi*, *G. lutescens*, *G. personatus*, *G. streckeri*, and *G. texensis* (Baker et al. 1989; Block and Zimmerman 1991; Bohlin and Zimmerman 1982; Heaney and Timm 1983, 1985; Jolley et al. 2000; Sudman et al. 2006; Tucker and Schmidly 1981). Similarly, based on the results of this study and other studies (Demastes 1994; Demastes and Hafner 1993; Kieschnick 2008; Sudman 2006) pocket gophers occupying the *G. breviceps* range are more diverse than previously thought and a taxonomic revision appears warranted.

Early taxonomists classified pocket gophers using morphological variation (Baird 1855; Baker and Glass 1951; Davis 1940; Merriam 1895). After the development and use of relevant statistical analyses, the morphological traits that were used in classifying pocket gophers were not statistically significant between distinct taxa, and accordingly many taxa were merged (Honeycutt and Schmidly 1979). However, results of this study correspond with early morphologically-based taxonomic distinctions and demonstrate that, while morphological techniques may not be sensitive enough to withstand statistical analyses for use in pocket gopher systematics, they may be valuable in identifying differences worthy of testing with more sensitive techniques.

In 1940, Davis used morphological variation to identify ten subspecies of *Geomys breviceps*. Two of Davis's (1940) subspecies, *G. b. attwateri* and *G. b. ammophilus* were later

combined and elevated to species status (*G. attwateri*; Tucker and Schmidly 1981). *G. b. texensis* was also elevated to species status (*G. texensis*; Block and Zimmerman 1991). Davis's (1940) remaining seven subspecies of *Geomys breviceps* (*pratincolus*, *brazensis*, *breviceps*, *dutcheri*, *ludemani*, *terricolus*, and *sagittalis*) were located throughout the present day range of the species.

Based on morphological variation, Honeycutt and Schmidly (1979) found that individuals belonging to Davis's previously mentioned seven subspecies (Honeycutt and Schmidly's *breviceps* chromosomal group) were paraphyletic in relation to other chromosomal groups. Based on their larger size, the Morehouse Parish, Louisiana pocket gophers grouped separately from other members of the *breviceps* chromosomal group. Honeycutt and Schmidly (1979) maintained the subspecific status of the Morehouse Parish, Louisiana pocket gophers (*G. bursarius breviceps*) due to its morphological distinctness and peripheral distribution. However, based on the lack of significant morphological and karyological differences, they combined *brazensis*, *sagittalis*, *terricolus*, *ludemani*, *pratincolus*, and *dutcheri* into a single subspecies, *G. bursarius sagittalis*. This classification was carried over when the *breviceps* chromosomal group was elevated to species status (*G. breviceps*; Bohlin and Zimmerman 1982). Honeycutt and Schmidly (1979) concluded that while genetic variation is not always reflected by similar morphological variation, the two may partially correlate. Their conclusion is supported by the results of this study. Statistical analyses of the cytochrome-*b* gene supports a taxonomic restructuring similar to the distinctions made using morphological techniques (Davis 1940).

The topology of trees in this study corresponds to Davis's (1940) morphologically-based classification. Davis's classification is delineated in Fig. 6. Clade I, excluding the Morehouse Parish, Louisiana individual, corresponds to *G. breviceps dutcheri*, and also with Kieschnick's

(2008) northern population (population one). Davis (1940) found *G. b. dutcheri* ranging from eastern Oklahoma to central Arkansas and south to northeast Texas and northwest Louisiana. The Morehouse Parish, Louisiana individual corresponds to *G. b. breviceps* (Davis 1940). These two groups are 4.09% divergent from each other, which is within the range of between subspecies comparisons (Elrod et al. 2000, Sudman et al. 2006). Kieschnick (2008) found pocket gophers previously classified as *G. b. sagittalis* (from Ashley County, Arkansas; Union County, Arkansas; and Union Parish, Louisiana) to belong to the same population as *G. b. breviceps*, and accordingly he suggested expanding the range of *G. b. breviceps* to include those localities. In this study, those same individuals did not form a monophyletic relationship with *G. b. breviceps* in relation to other members of clade I, and the average genetic distance between those samples and the Morehouse Parish, Louisiana sample is in the range of between subspecies comparisons (Table 1; Elrod et al. 2000). While results from this study support maintaining the Morehouse Parish, Louisiana sample as a unique subspecies (*G. b. breviceps*), combined with Kieschnick's results (2008) it appears that the two subspecies are only partially isolated with occasional genetic exchange occurring.

Clade II also corresponds to the classification defined by Davis (1940). Subclade G (Vernon Parish, Louisiana and Rusk County, Texas) corresponds to *G. b. pratincolus*. Davis (1940) defined the distribution of *G. b. pratincolus* as east of the Trinity River in the piney woods area of southeastern Texas, north of the coastal prairie, and east as far as the Red River in Louisiana. Subclade H (Anderson County, Texas and Van Zandt County, Texas) corresponds to *G. b. brazensis*. Davis (1940) found *G. b. brazensis* from the Sabine River in Kaufman County, Texas and Panola County, Texas south and west to the Colorado River in Bastrop County, Texas and Colorado County, Texas. This would have included the organisms from Rusk County,

Texas; however, based on this study, individuals from Rusk County, Texas group more closely to those from the adjacent *pratincolus* range.

G. breviceps sagittalis was paraphyletic in relation to *G. breviceps breviceps* in all analyses of this study. The results of this study combined with the results from several other studies (Demastes 1994; Demastes and Hafner 1993; Kieschnick 2008; Sudman 2006) show a discrepancy between the taxonomic distinctions and systematic relationships of Baird's pocket gopher. Based on this discrepancy along with observed genetic distances, a taxonomic revision appears warranted. The genetic distance seen between clades I and II (approximately 9%) is extremely high when compared to other within species distances, and is more similar to between species comparisons. Elrod et al. (2000) found genetic distances of approximately 1-6% between subspecies (this excludes comparisons between taxa that have since been separated). Sudman et al. (2006) found between species comparisons as low as approximately 8%. The above evidence suggests that individuals in clade I form a distinct taxonomic entity from individuals in clade II. This division is further supported by Kieschnick (2008). All individuals sampled from Kieschnick's populations one and six (in Oklahoma, Arkansas, northern Texas, and northern Louisiana) belong to clade I, and all individuals sampled from Kieschnick's populations two, three, and five (in western Louisiana and eastern Texas) belong to clade II. Clade I appears to occupy the range of *G. b. dutcheri* and *G. b. breviceps* as described by Davis (1940) with *G. b. breviceps* isolated to a patch of sandy soil in Morehouse Parish, Louisiana, and the remainder of the northern range of *G. breviceps* occupied by *G. b. dutcheri*. Davis described *G. b. dutcheri* as extending south to the Red River in Louisiana, and to the Sabine and Trinity rivers in Texas. He further explains that ranges of *G. b. dutcheri* and *G. b. brazensis* are not clearly separated in the area south of the Sabine River and north of the Trinity River in Texas. In this study however, all

individuals south of the Sabine River in Texas belong to clade II, the southern clade, while all individuals north of the Sabine River in Texas belong to clade I, the northern clade. This suggests that the Sabine River may delineate the boundary in Texas between individuals in clades I and II.

While results of this study also show high sequence divergence between subclades G and H (Table 2), their relationship to each other is unclear. Kieschnick (2008) grouped Anderson County, Texas from subclade H and Rusk County, Texas from subclade G in the same population (population three) separate from Van Zandt, Texas and Vernon Parish, Louisiana which were assigned to populations two and five, respectively. Additionally, there was variation in the placement of subclades G and H between the various trees of this study (Fig.s 2-5). Although clades I and II appear to represent distinct taxa, additional research is necessary to clarify the relationship between subclades G and H, and to delineate the boundaries between taxa before taxonomic revisions can be made.

Higher genetic divergence was observed within clade II (southern samples) versus the divergence observed within clade I (northern samples; Table 2), and the southern samples were more basal suggesting a recent dispersal northward. Kieschnick (2008) also suggested a recent dispersal northward based on higher genetic divergence in the southern localities. Pocket gopher populations are thought to have retreated south during glacial advancements followed by expansion north during more favorable conditions (Russell 1968). This could explain the recent dispersal northward of pocket gophers observed by this study and Kieschnick's study (2008). Furthermore, *G. breviceps* has been identified as the basal species to the western group of *Geomys* (Sudman et al. 2006). This suggests that *G. breviceps* populations are derived from

refugial populations of pocket gophers separately from the rest of the present day western *Geomys*.

Based on the evidence that southern populations tend to have greater genetic diversity, future research should include additional samples from the southernmost parts of the *G. breviceps* range. In particular, additional research should focus on the Galveston Bay area of Texas, an area excluded from this study. This area included Davis's (1940) three remaining subspecies (*terricolus*, *sagittalis*, and *ludemani*). Considering that pocket gophers are restricted to habitats of sandy soils, and these habitats are especially patchy in the coastal plains, the Galveston Bay area is expected to have greater diversity, as was suggested by early morphologically based taxonomic descriptions (Davis 1940).

Results of this study indicate a distinct paraphyletic relationship within the *Geomys breviceps* complex as well as genetic distances that are in the range of between species comparisons of *Geomys*. A taxonomic revision of pocket gophers found in the range of *Geomys breviceps* appears warranted. A more extensive assessment of populations that occur between the Red River and the Sabine River is necessary to further support differentiation between northern populations (in the former *G. b. dutcheri* and *G. b. breviceps* ranges) and southern populations (in the former *G. b. brazensis* and *G. b. pratincolus* ranges). Additional research should also include populations south of the Neches and Trinity rivers with special attention focused on the area surrounding Galveston Bay.

Arkansas

1. Ashley Co.
2. Union Co.
3. Ouachita Co.
4. Calhoun Co.
5. Jefferson Co.
6. Cleburne Co.
7. Little River Co.

Oklahoma

8. Atoka Co.
9. Lincoln Co.

Texas

10. Lamar Co.
11. Van Zandt Co.
12. Anderson Co.
13. Rusk Co.

Louisiana

14. Vernon Pa.
15. Union Pa.
16. Morehouse Pa.

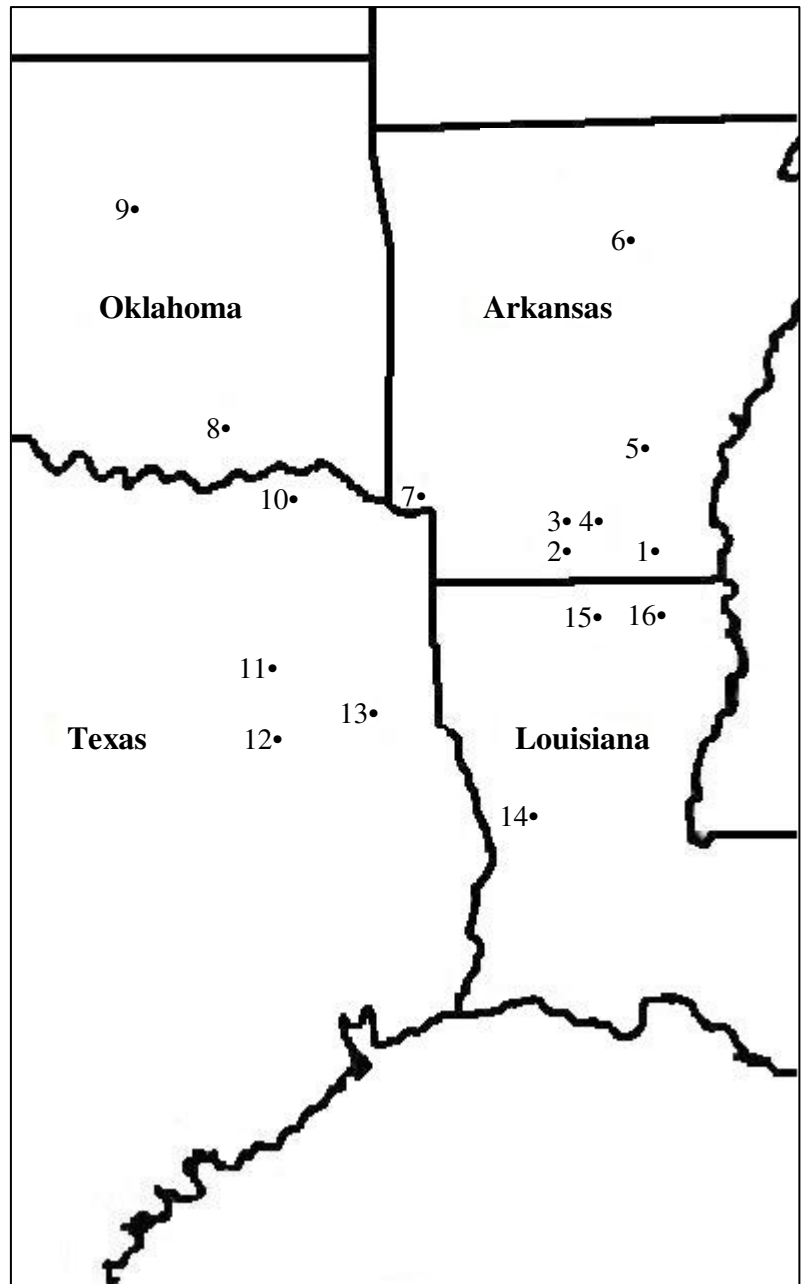


FIG. 1.—Locations of pocket gopher specimens examined.

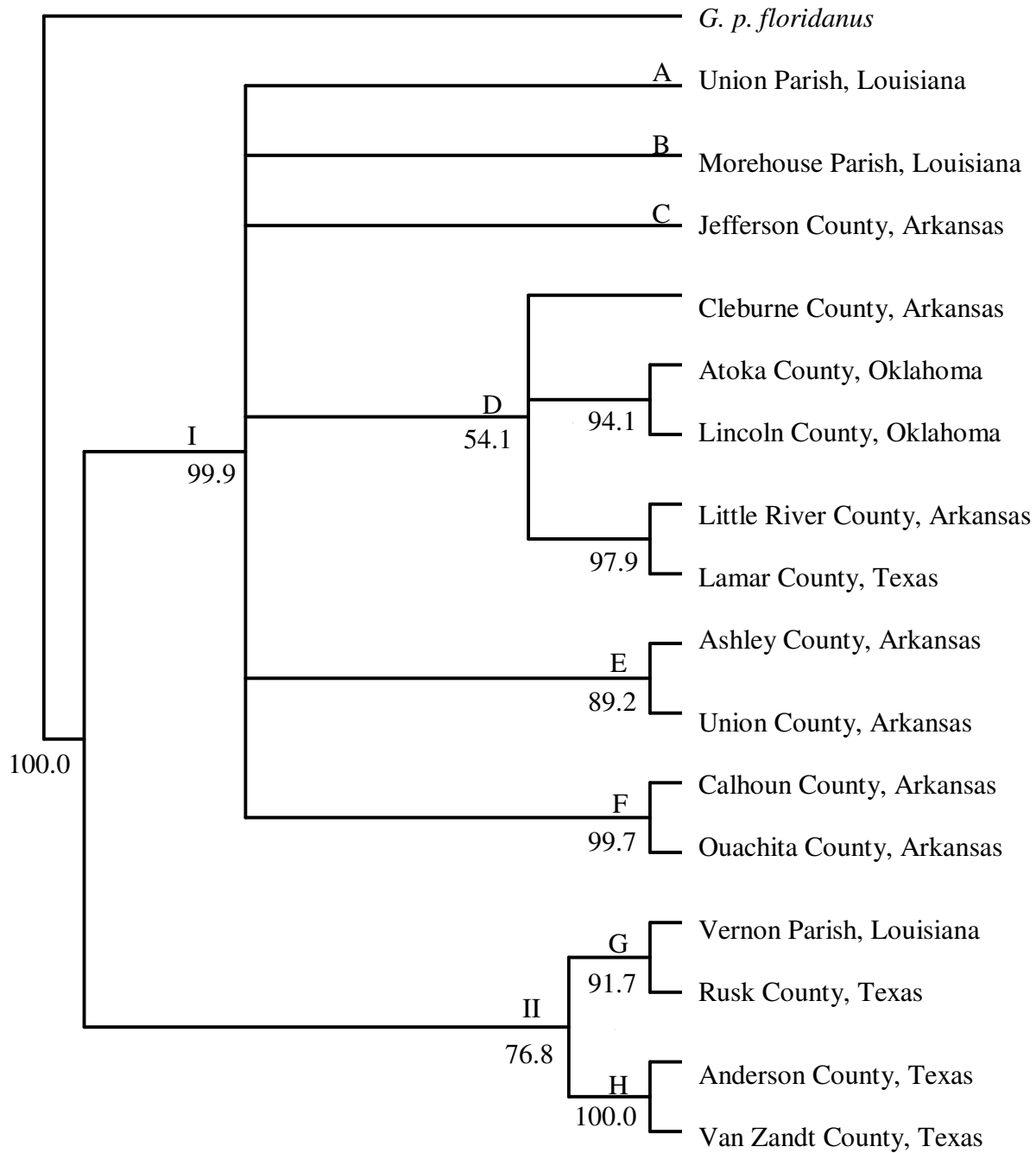


FIG. 2. Maximum parsimony consensus tree of 12 most parsimonious trees for cytochrome-*b* sequences in *Geomys breviceps*. Major clades are labeled with Roman numerals and minor clades are labeled with capital letters. Bootstrap support values are displayed at nodes.

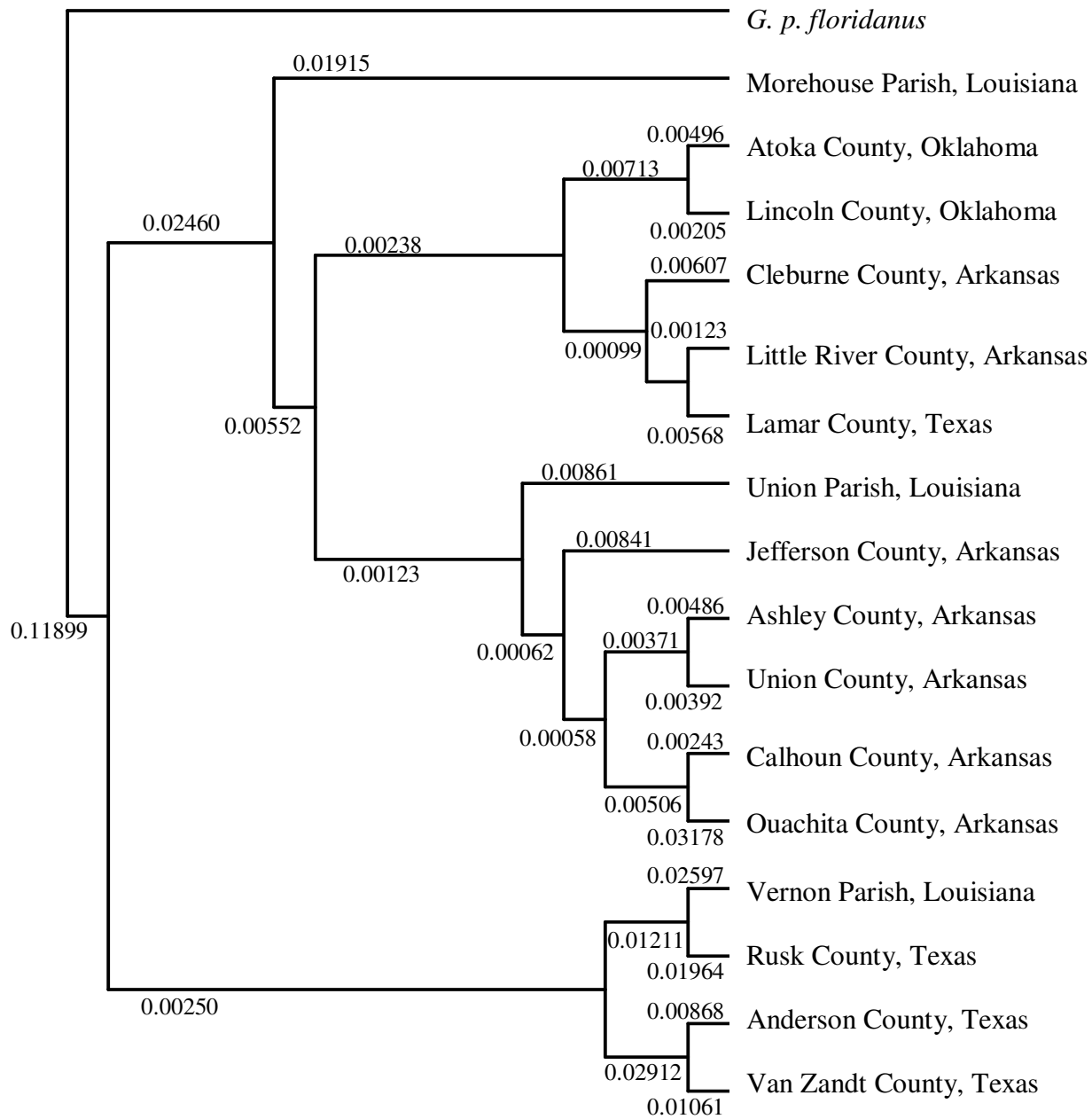


FIG. 3.—Neighbor-joining tree for cytochrome-*b* sequences in *Geomys breviceps* with branch lengths labeled.

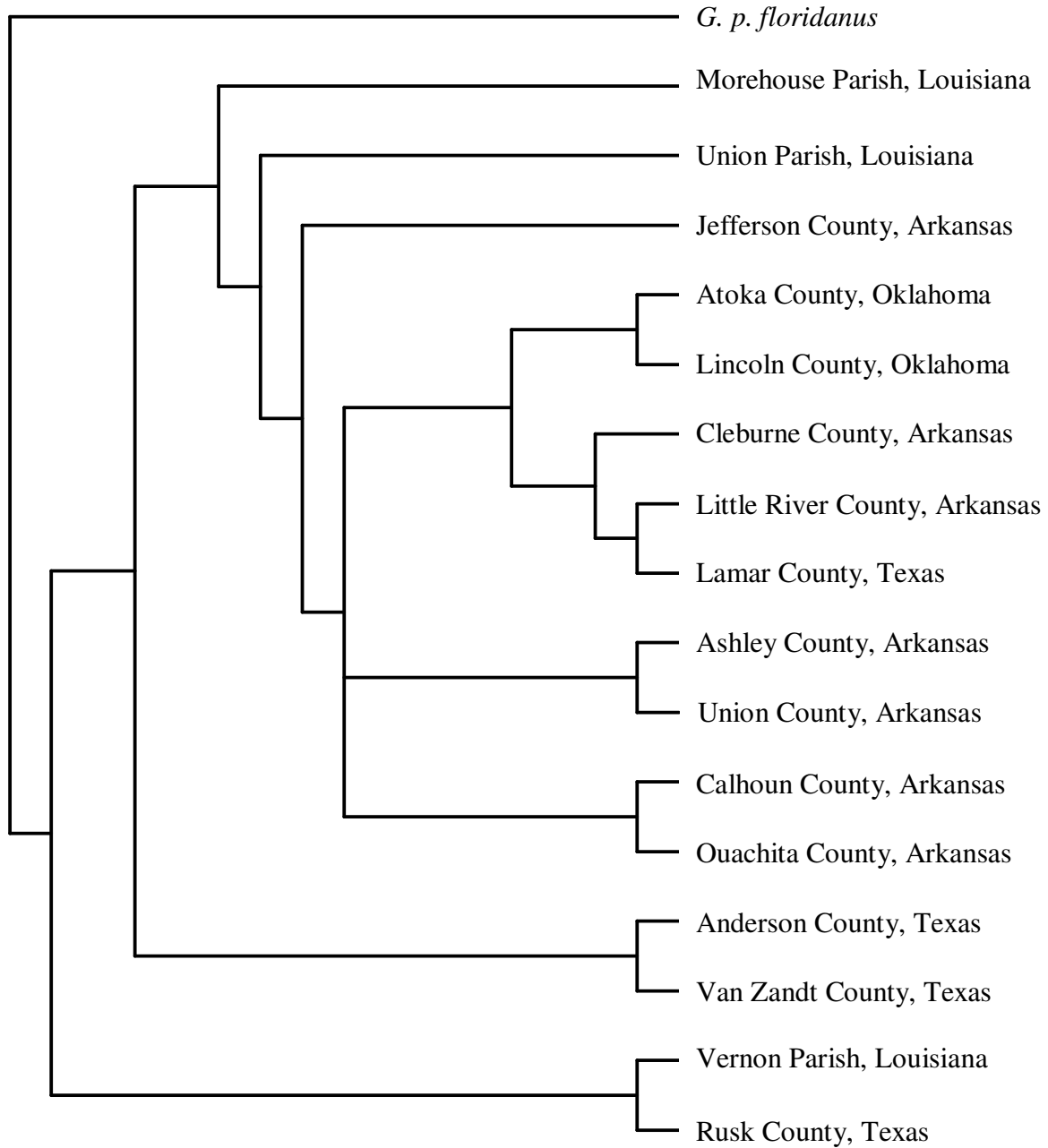


FIG. 4. Maximum-likelihood tree for cytochrome-*b* sequences in *Geomys breviceps*.

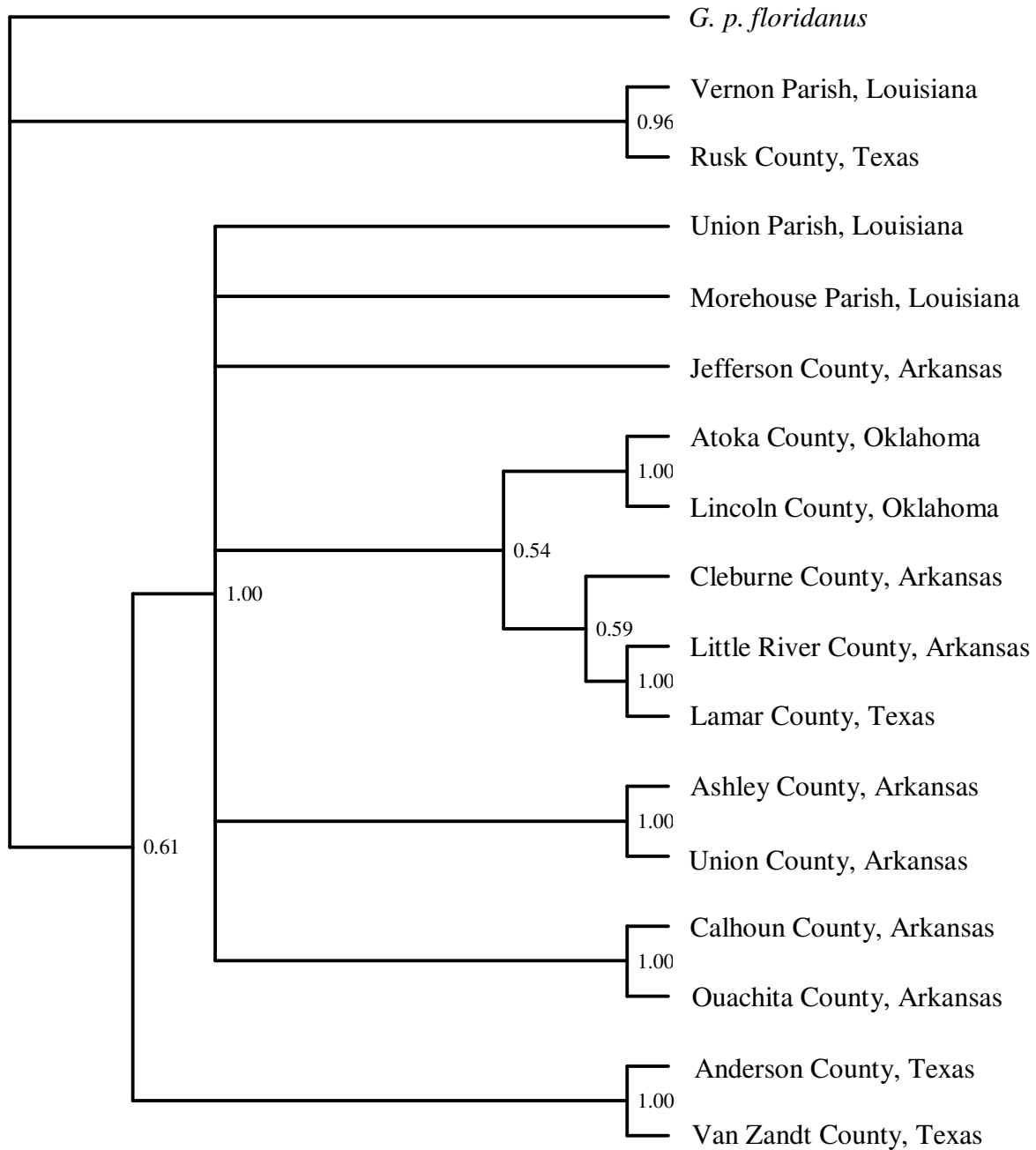


FIG. 5.—Bayesian tree for cytochrome-*b* sequences in *Geomys breviceps* with clade posterior probability values at nodes.

Arkansas

1. Ashley Co.
2. Union Co.
3. Ouachita Co.
4. Calhoun Co.
5. Jefferson Co.
6. Cleburne Co.
7. Little River Co.

Oklahoma

8. Atoka Co.
9. Lincoln Co.

Texas

10. Lamar Co.
11. Van Zandt Co.
12. Anderson Co.
13. Rusk Co.

Louisiana

14. Vernon Pa.
15. Union Pa.
16. Morehouse Pa.

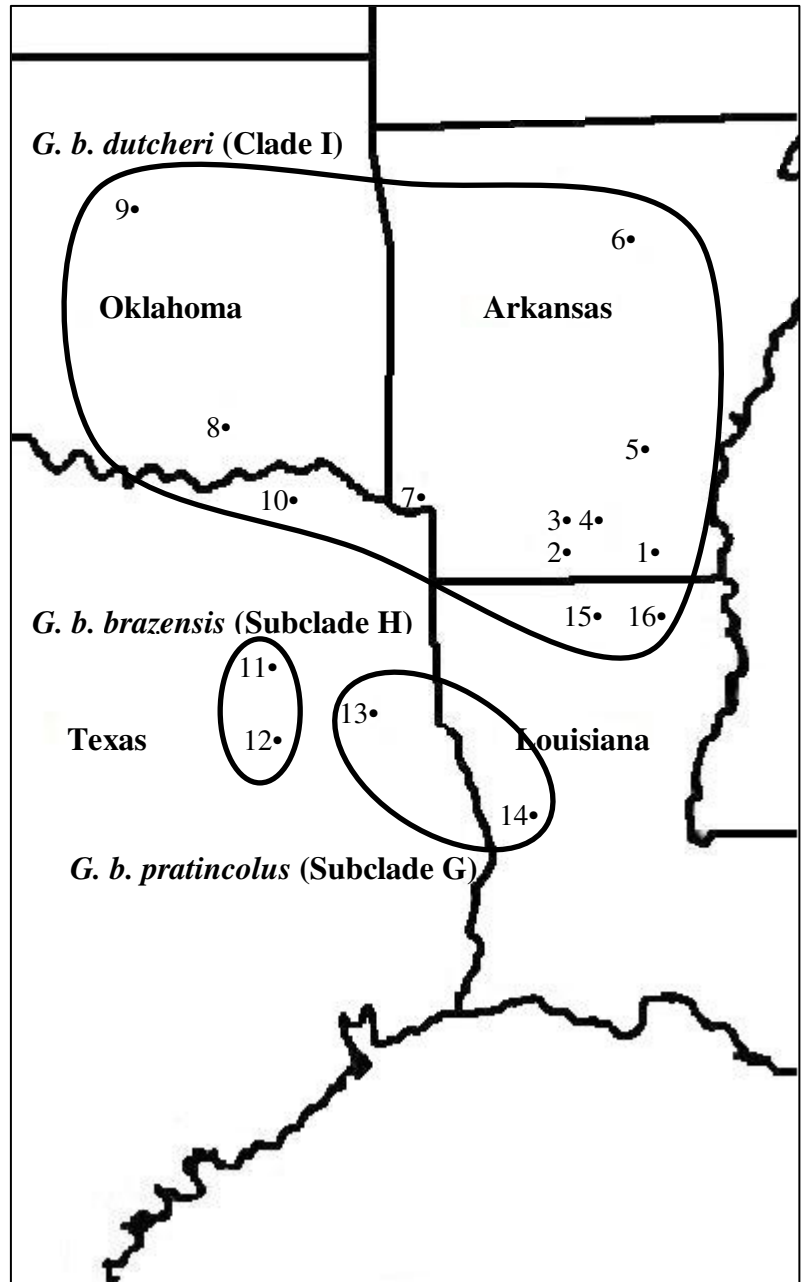


FIG. 6.—Delineation of pocket gophers based on Davis's 1940 classification.

TABLE 1.—Kimura 2-parameter pair-wise genetic distances (%) for cytochrome-*b* sequences in *Geomys breviceps*. Location abbreviations are as follows: AtOK, Atoka County, Oklahoma; UnLA, Union Parish, Louisiana; AsAR, Ashley County, Arkansas; LrAR, Little River County, Arkansas; MhLA, Morehouse Parish, Louisiana; VnLA, Vernon Parish, Louisiana; LmTX, Lamar County, Texas; LnOK, Lincoln County, Texas; CbAR, Cleburne County, Arkansas; UnAR, Union County, Arkansas; AnTX, Anderson County, Texas; RkTX, Rusk County, Texas; VzTX, Van Zandt County, Texas; ChAR, Calhoun County, Arkansas; OaAR, Ouachita County, Arkansas; JfAR, Jefferson County, Arkansas; Gfl, *Geomys pinetis floridanus*.

	AtOK	UnLA	AsAR	LrAR	MhLA	VnLA	LmTX	LnOK	CbAR	UnAR	AnTX	RkTX	VzTX	ChAR	OaAR	JfAR	Gfl
AtOK																	
UnLA	2.6043																
AsAR	2.4222	1.7817															
LrAR	2.6064	2.6043	2.6987														
MhLA	4.3732	3.1494	3.5241	4.2782													
VnLA	8.5683	8.5624	9.0832	9.0964	9.1803												
LmTX	2.6043	2.7869	2.6966	1.2424	4.2752	9.0897											
LnOK	0.7062	2.2354	2.2370	2.4203	3.9913	8.3581	2.2354										
CbAR	1.9664	2.0559	2.0576	1.9664	3.8083	8.4718	1.7830	1.6018									
UnAR	2.4222	1.7817	0.8836	2.6987	3.2488	8.5683	2.5123	2.0544	1.8753								
AnTX	8.2675	8.8766	9.1935	8.5867	10.2351	8.3941	8.1832	8.2618	8.5804	8.6769							
RkTX	7.7405	7.9370	8.4490	8.4601	8.4545	4.7485	8.4545	7.5354	8.0480	7.9419	6.9641						
VzTX	8.6769	9.0832	9.4016	8.7927	10.2351	8.8060	8.3877	8.6708	8.7862	8.8829	1.9620	7.3735					
ChAR	2.6086	1.7830	1.6030	2.7936	3.5266	8.7798	2.6064	2.2387	1.8769	1.6030	9.3043	8.3526	9.5131				
OaAR	5.7621	4.9009	4.6192	5.9551	6.9158	11.7843	5.6636	5.3762	4.9989	4.6192	12.0056	11.3408	12.6557	3.5021			
JfAR	2.4203	1.7805	1.7817	2.7890	3.7091	8.7675	2.6024	2.2354	1.8738	1.7817	8.6708	8.1391	8.7736	1.6018	4.7121		
Gfl	18.8655	18.7312	18.8912	18.6473	18.1510	19.0136	18.7563	18.3923	18.5133	18.5258	18.6098	17.0795	18.1273	18.3923	21.4609	18.3680	

TABLE 2.—Average Kimura 2-parameter genetic distances of cytochrome-*b* sequences in *Geomys breviceps* for selected clades.

Samples	Average genetic distance (%)
Clade II (southwestern samples) versus clade I (remainder of samples)	8.96
Vernon County/Rusk County versus Clade I	8.40
Anderson County/Van Zandt County versus Clade I	9.20
Van Zandt County/Anderson County versus Rusk County/Vernon Parish	7.88
Within clade II	6.37
Within clade I	2.87
Within clade I excluding Morehouse Parish	2.76
Morehouse Parish versus remainder of clade I	4.09
Morehouse Parish versus Ashley County/Union County/Union Parish	3.31

APPENDIX
SPECIMENS EXAMINED

Locality #	Sample/Museum #	State	County/Parish	Specific Locality
1	DLM03	Arkansas	Ashley	5 mi N of Crossett
2	DLM05	Arkansas	Union	3 mi W of Junction 172, Hwy 82
3	SRK138	Arkansas	Ouachita	
4	ASUZ28504	Arkansas	Calhoun	
5	TK151703	Arkansas	Jefferson	
6	TK151557	Arkansas	Cleburne	Heber Springs, Toothfairy Ln, Hwy 5
7	DLM04	Arkansas	Little River	
8	DLM01	Oklahoma	Atoka	4 mi E of Atoka on Hwy 3
9	TK116975	Oklahoma	Lincoln	3 mi E Chandler
10	TK151558	Texas	Lamar	3.6 mi W of Blossom, Hwy 82
11	TK151578	Texas	Van Zandt	E of Canton, Hwy 243
12	TK151510	Texas	Anderson	0.5 mi S FM 499
13	TK151517	Texas	Rusk	2 mi N Henderson
14	LSUMZ30723	Louisiana	Vernon Pa.	2 mi S, 3 mi W Rosepine
15	DLM02	Louisiana	Union	Farmerville
16	LSUMZ31603	Louisiana	Morehouse	3.1 mi E Bastrop

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