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RE-EVALUATION OF THE GEOGRAPHIC DISTRIBUTION AND PHYLOGEOGRAPHY OF THE *SIGMODON HISPIDUS* COMPLEX BASED ON MITOCHONDRIAL DNA SEQUENCES

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Abstract

Geographic distribution among members of the *Sigmodon hispidus* complex (*Sigmodon hirsutus*, *S. hispidus*, and *S. toltecus*) were examined using DNA sequences from the mitochondrial cytochrome-*b* gene. Geographic distribution of each taxon was defined based on DNA sequences obtained from 69 samples (19 newly obtained and 50 from previous studies) collected from North, Central, and South America. These data indicated that *S. hispidus* is restricted to the southern one-half of the United States and northeastern Mexico (Nuevo León and Tamaulipas), *S. toltecus* occupies the eastern one-third of Mexico (central Tamaulipas) to northern Honduras, and *S. hirsutus* is distributed from central Chiapas and southeastern Oaxaca to northern South America (Venezuela). The newly collected data extend distributions of *S. hispidus* from the southern United States southward into northeastern Mexico and that of *S. toltecus* from Chiapas, Mexico, southward to Honduras. Genetic divergence and patterns of phylogeography were examined within each taxon.

DNA-sequence data from the mitochondrial cytochrome-*b* gene (Peppers and Bradley, 2000; Peppers et al., 2002; Carroll et al., 2005) and intron 7 of the beta-fibrinogen gene (Carroll and Bradley, 2005) indicated that the taxon, historically referred to as *S. hispidus*, was a composite and paraphyletic assemblage that consisted of three distinct species (*S. hirsutus*, *S. hispidus*, and *S. toltecus*). Peppers and Bradley (2000) applied the term cryptic species to these taxa to illustrate the lack of morphological divergence accompanying the high level of genetic divergence among the three species. To date, genetic data provide the only means of identifying the three species. Carroll et al. (2005) further attempted to describe distributions and hypothesized in general that *S. hispidus* was restricted to southern portions of the United States north of the Rio Grande, *S. toltecus* was restricted to regions south of the Rio Grande along the eastern coastal regions of Mexico to central Chiapas, and *S. hirsutus* occupied regions of southern Mexico (Chiapas and Oaxaca) to Venezuela. Additionally, Carroll et al. (2005) considered possible explanations (habitat preferences and phylogeography) for the proposed distributions of these three species.

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Concerning separation of *S. hispidus* and *S. toltecus* along boundaries of the United States and Mexico, individuals from Brownsville, Texas, and Calabazas, Tamaulipas, represented peripheral samples of each respective taxon. These individuals were separated by ca. 400 km and genetically differed by genetic-distance values that were typical for any comparison between *S. hispidus* or *S. toltecus*. Carroll et al. (2005) offered two hypotheses to explain the distribution of these two species. First, the Rio Grande historically has been considered to be a formidable barrier separating various species (Toledo and Ordonez, 1993; Edwards et al., 2001) and may be responsible for limiting the distribution of these two species in this geographic region. Second, *S. hispidus* from southern Texas occupied a more arid-plains-grassland habitat (Arid/Semi Arid Terrestrial Ecological Zones; Toledo and Ordonez, 1993), whereas *S. toltecus* appeared to occupy more humid grasslands of the coastal lowlands in eastern and southeastern Mexico (Subhumid Tropical and Humid Tropical Terrestrial Ecological Zones; Toledo and Ordonez, 1993). Unfortunately, Carroll et al. (2005) could not determine whether the Rio Grande played a role in the distributional limits of *S. hispidus* and *S. toltecus*, or if habitat was a more influential component.

Concerning the separation of *S. hirsutus* and *S. toltecus* in southern Mexico, Carroll et al. (2005) proposed the distribution of *S. toltecus* to include the coastal grasslands east of the Sierra Madre Oriental, south to grasslands associated with the Yucatan Peninsula and lowlands of Chiapas (Subhumid Tropical and Humid Tropical Ecological Zones; Toledo and Ordonez, 1993); whereas, *S. hirsutus* occupied medium- to high-elevation-grassland habitats associated with the Guatemalan Highlands. In central Chiapas (near Ocozocoautla, Chiapas) the two species were reported as being sympatric (Carroll et al., 2005), with two individuals of *S. toltecus* and three individuals of *S. hirsutus* being collected in the same trapline. In reference to habitat, this locality represents an interface between lowland grasslands and more montane grasslands associated with the Guatemalan Highlands. However, an individual of *S. hirsutus* from the coastal lowlands of southern Mexico (La Blanca, Oaxaca) complicated this view.

In this study, DNA sequences from the mitochondrial cytochrome-*b* gene (*Cyt*b) were obtained from 19 additional specimens of the three species of *Sigmodon* to increase sample sizes in geographic regions where taxa were parapatric or sympatric. These data were combined with data from Carroll et al. (2005) and Peppers and Bradley (2000) to assess phylogenetic patterns within taxa, evaluate habitat associations and preferences, and determine species boundaries.

Materials And Methods

Samples

Nineteen individuals were collected from natural populations through-out the ranges of *S. hirsutus, S. hispidus*, and *S. toltecus* (Fig. 1). Animal care and use procedures followed guidelines approved by the American Society of Mammalogists (Animal Care and Use Committee 1998). In addition, 50 DNA sequences reported in Carroll et al. (2005),Peppers and Bradley (2000),Peppers et al. (2002), and Smith and Patton (1999) were included in this study. Initial identification of specimens as *S. hispidus*-like was confirmed by morphology and karyology following diagnostic characteristics listed in Bailey (1902),Zimmerman (1970),Elder (1980),Hall (1981),Elder and Lee (1985), and Carleton et al. (1999); however, final identification was based on DNA sequences. Specimen numbers and collection localities are listed in Appendix I.

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Sequence Data

Genomic DNA was extracted from frozen samples of liver (0.1 g) using the Qiagen DNeasy Tissue Kit (Qiagen, Valencia, California). The complete *Cyt*b gene (1,143 base pairs) was amplified using the following polymerase-chain-reaction (PCR) parameters modified from Saiki et al. (1988): 35 cycles of 95°C denaturation (1 min), 50°C annealing (1 min), 72°C extension (2 min), and 1 final 72°C extension cycle (7 min). Primers used in the PCR reactions were MVZ05 (5′ end; Smith and Patton, 1993) or H15915 (3′ end; Irwin et al., 1991) and P3′ (Tiemann-Boege et al., 2000) or MVZ14 (3′ end; Smith and Patton, 1993). The resulting PCR product was purified using the QIAquick PCR purification kit (Qiagen, Valencia, California). The PCR primers and following five primers were used in cycle sequencing reactions to amplify three fragments (ca. 400 base pairs each) on the forward and reverse strands, respectively: F1 (Whiting et al., 2003), 400R (Tiemann-Boege et al., 2000), 700H and 700L (Peppers and Bradley, 2000), and 870R (Peppers et al., 2002). Cycle sequencing was conducted using the ABI Big Dye terminator v3.1 reaction mix (PE Applied Biosystems, Foster City, California) and samples were analyzed on an ABI 3100-Avant automated sequencer (PE Applied Biosystems, Foster City, California). Sequencher 3.0 software (Gene Codes, Ann Arbor, Michigan) was used to align and proof nucleotide sequences. All *Cyt*b sequences obtained in this study were deposited in GenBank and are listed in Appendix I.

Data Analyses

Based on phylogenetic relationships presented in Peppers et al. (2002), *S. alstoni* was used as the outgroup taxon in all analyses. In addition, representatives of *S. alleni* (*n* = 1), *S. arizonae* (*n* = 2), *S. fulviventer* (*n* = 2), *S. leucotis* (*n* = 1), *S. mascotensis* (*n* = 5), *S. ochrognathus* (*n* = 2), and *S. peruanus* (*n* = 2), were included as references. These taxa were identified by Peppers et al. (2002) as being either sister taxa or as being closely related to certain members of the ingroup taxa. Bayesian and parsimony models (PAUP*; Swofford, 2002; MrBayes 2.01; Huelsenbeck and Ronquist, 2001) were used to generate hypotheses concerning phylogenetic relationships of taxa.

Parsimony analyses were constructed using equally weighted characters and the heuristic search option with tree-bisection-reconnection, random stepwise addition of taxa, and 100 repetitions to obtain the most-parsimonious tree-set. Phylogenetically uninformative characters were excluded from analyses and variable nucleotide positions within the dataset were treated as unordered, discrete characters with four possible states; A, C, G, or T. Due to computational limitation, a Fast-step-wise-addition bootstrap analysis (PAUP*; Swofford, 2002) with 1,000 iterations was used to assess nodal support.

A Bayesian analysis (MrBayes 2.01; Huelsenbeck and Ronquist, 2001) using a GTR+I+G model with rates set equal to the proportion of invariable sites and sites partitioned by codon was used with the following options: 4 Markov-chains (one cold and three heated), 10 million generations, sample frequency = every 1,000th generation. *S. alstoni* was designated as the outgroup and two runs were conducted simultaneously. The GTR+I+G model was chosen so that data analyses were comparable to those conducted by Carroll et al. (2005). After a visual inspection of likelihood scores, convergence statistics, and potential scale-reduction factors the first 100,000 trees were discarded and a consensus tree (50% majority rule) was constructed from remaining trees. Clade probabilities were estimated and values ≥95 were used as indication of nodal support.

Kimura two-parameter genetic distances (Kimura, 1980) were estimated for individuals within each of the three respective taxa. The Kimura two-parameter was chosen so that genetic-distance values were comparable to those reported in Peppers and Bradley (2000)

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and Carroll et al. (2005). These distances were used to evaluate genetic divergences relative to geographic regions based on structure of clade obtained from parsimony and Bayesian analyses.

Results

Complete nucleotide sequences (1,143 base pairs) from the mitochondrial *Cyt*b gene were included from 69 individuals, representing *S. hirsutus* (*n* = 29), *S. hispidus* (*n* = 25), and *S. toltecus* (*n* = 15). Both phylogenetic analyses (parsimony and Bayesian) produced similar topologies. Given the levels of molecular evolution relative to branch lengths, only the topology recovered from the Bayesian analysis was depicted (Fig. 2). Clade probability and bootstrap support values were superimposed on the tree recovered from the Bayesian analysis. DNA sequences were included for *S. alleni*, *S. alstoni*, *S. arizonae*, *S. mascotensis*, *S. ochrognathus*, and *S. peruanus* solely for the purpose of reference; therefore, the phylogenetic relationships of these taxa were not described in detail.

In the parsimony analysis, 394 phylogenetically informative characters generated 172,798 equally parsimonious trees (1,428 steps, consistency index = 0.401, retention index = 0.864). A strict consensus tree generated a topology (not shown) similar to that obtained in the Bayesian analysis described below. Bootstrap values (Fig. 2) were high for the *S. hirsutus* clade (91), the *S. toltecus* clade (100), and the *S. hispidus* clade (100).

In the Bayesian analyses, the two simultaneous runs generated identical topologies (Fig. 2) and clade probability values differed only slightly; therefore, if support values differed at a node, both were reported. In this analysis, a monophyletic clade (I, clade probability value = 98) was formed containing 29 individuals that were referable to *S. hirsutus*. Within this clade, four supported subclades were identified (A through D): subclade A contained 20 individuals from Central America (Costa Rica, El Salvador, Honduras, and Nicaragua), B contained six individuals from Mexico (Chiapas and Oaxaca), C was comprised of individuals from clades A and B plus two individuals from Chiapas, Mexico, and two individuals from Panama, and D contained one individual from South America (Venezuela). The *S. toltecus* clade (II) included 15 individuals that formed a monophyletic clade (clade probability = 100) and two subclades (E and F). Subclade E contained seven individuals from southeastern Mexico (Campeche, Chiapas, Quintana Roo, Tabasco, and Yucatán) and four individuals from Guatemala and Honduras, whereas subclade F contained four individuals from northeastern Mexico (Tamaulipas and Veracruz). The 25 individuals formed a monophyletic clade (III, clade probability = 100) and were referred to as *S. hispidus*. Two supported subclades (G and H) were present with subclade G contained 15 individuals from the western United States and 2 individuals from northern Mexico (Tamaulipas and Nuevo León); whereas F represented individuals primarily restricted to the southeastern United States.

Kimura two-parameter (Kimura, 1980) genetic distances were estimated within each species and selected clades and are presented in Table 1. Genetic distances between the three species ranged from 12.55% (*S. hirsutus* and *S. hispidus*) to 12.90% (*S. hirsutus* and *S. toltecus*). Genetic distances within the three species ranged from 2.34% (*S. hirsutus*) to 2.81% (*S. toltecus*). Genetic distance values within and between clades ranged from 0.56% (within clade A) to 4.89% (between clades C and D).

Discussion

Phylogenetic analyses (parsimony and Bayesian) support the overall conclusions of Peppers et al. (2002) and Carroll et al. (2005) that *S. hirsutus*, *S. hispidus*, and *S. toltecus* represent

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valid species. Individuals of *S. hirsutus* formed a monophyletic group sister to *S. alleni*. The 15 individuals representing *S. toltecus* formed a monophyletic clade sister to the *S. alleni* and *S. hirsutus* clade. Individuals of *S. hispidus* formed a single clade; however, the sister taxon to *S. hispidus* was not resolved with this dataset, nor has it been resolved in other molecular studies (Carroll and Bradley, 2005; Carroll et al., 2005). Phylogenetic relationships of other members of the genus *Sigmodon* are depicted in Fig. 2, but are not discussed herein as they are beyond the scope of this study. Below, the geographic distribution and biogeographic parameters for each taxon are discussed in light of the newly examined samples.

Sigmodon hirsutus

The distribution of *S. hirsutus* remains relatively unchanged from that described by Carroll et al. (2005). Additional samples examined herein document the presence of *S. hirsutus* in Honduras and Nicaragua. These samples provide evidence for a distribution from southern Mexico (Chiapas and Oaxaca) into northern South America (Venezuela); however, four observations prevent this from being a simple and straightforward geographic range. First, as discussed by Carroll et al. (2005), *S. hirsutus* primarily occurs in medium-to-highelevation grasslands of the Central America Highlands or Guatemalan Highlands (Ryan, 1963; Carleton et al., 2002), whereas *S. toltecus* appears to be restricted to the coastal regions (Subhumid Tropical and Humid Tropical Terrestrial Ecological Zones; Toledo and Ordonez, 1993) of eastern Mexico (discussed below). However, both species were recorded in sympatry in north-central Chiapas, Mexico (near Ocozocoautla), where lowland grasslands of the Depression Central de Chiapas transition into those occurring at more moderate elevations in the Sierra Madre de Chiapas (Ferrusquía-Villafranca, 1993). Therefore, it is possible that one or both species may be encountered in the foothills in central Chiapas. Second, samples of *S. hirsutus* from southeastern Oaxaca (La Blanca) and southwestern Chiapas (Mapastepec) are from coastal grassland habitats, more typically associated with *S. toltecus*. Third, samples of *S. hirsutus* from Capiro y Calenturo, Honduras, were collected within 1 km of the coast; however, these were collected from a foothill region (a few hundred meters in elevation). Fourth, although our study included only two samples from Panama and did not include samples from Costa Rica, other authors (Goodwin, 1946; Handley, 1966; McPherson, 1985) have reported cotton rats from low-elevation scrubland and savanna habitats. These cotton rats formerly were assigned to *S. hispidus*, but given more recent interpretations of this species complex they presumably are referable to *S. hirsutus*.

In general, it appears that the key to interpreting the distribution of *S. hirsutus* lies between central Chiapas and northern Honduras. In Chiapas, two mountain ranges (Montañas del Norte de Chiapas and Sierra Madre de Chiapas) provide suitable habitat (moderate-elevation grasslands) for *S. hirsutus* and presumably restrict the distribution of *S. toltecus* to the northeast along the coastal regions of the Yucatan Peninsula. However, in north-central Chiapas, a lowelevation trough (Meseta Central de Chiapas and Depression Central de Chiapas) is formed between the two mountain regions and allows for an interdigitation between the coastal grasslands and the grasslands associated with medium-to-high elevations as described for the area of sympatry between *S. hirsutus* and *S. toltecus* near Ocozocoautla. The southern boundary of this trough (Sierra Madre de Chiapas) presumably prevents *S. toltecus* from occupying the lowelevation grasslands of southwestern Chiapas and southern Oaxaca, but provided an opportunity for *S. hirsutus* to expand its distribution from more southern regions; although it is not clear whether this would have been a recent or older invasion.

Sigmodon hirsutus appears to be the primary inhabitant of Central America except for the occurrence of *S. toltecus* along the northern coast of Honduras. The presence of *S. toltecus*

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in this region depends upon availability of suitable habitat (coastal or lowland grasslands) along the Yucatan Peninsula and Belize to about Tela and La Ceiba in northern Honduras. The Cordillera Nombre de Dios range may restrict the distribution of *S. toltecus* to the east and provides suitable habitat for *S. hirsutus* to the south. This hypothesis is supported by the presence of *S. toltecus* at Tela (Jardin Botanico Lancetilla) and samples of *S. hirsutus* 150 km E Tela (Trujillo, Parque Nacional Capiro y Calentura) and 100 km S Tela (La Guama).

Specimens comprising clades A, B, and D conform to three general geographic regions discussed above. Clade A contained 18 individuals from the Central American Highlands (Costa Rica, El Salvador, Honduras, and Nicaragua), clade B contained 6 individuals from the foothills and low-elevation grasslands of southern Mexico (southeastern Oaxaca and central and western Chiapas), whereas clade D contained the single individual from South America (Venezuela). However, two caveats hinder this simplistic interpretation. First, the two individuals from Panama were not included in clade A and second, two individuals from Chiapas were not contained in clade B, although, these four individuals are part of the larger clade C, which contains all specimens from Mexico and Central America.

The delineation and divergence between clades C and D may be explained by the influences of an emerging and disappearing Panamanian Land Bridge along the Isthmus of Panama, which may have acted to isolate the more northern forms from those to the south. Average genetic distances between those two clades (4.89%, Table 1) indicated a molecular divergence of the two clades ca. 1.5–2 million years ago (based on an approximation of 2.5–3.5%/million year divergence rate in the mammalian mitochondrial *Cyt*b gene; Arbogast and Slowinski, 1998). If these rates and estimates are valid, then clades C and D may represent independent evolutionary trajectories. Voss (1992) examined the available specimens of *S. hirsutus* from South America and concluded that they are not morphologically different from more northern samples. Unfortunately, lack of sufficient genetic data from South America precludes a satisfactory test of this hypothesis.

Phylogenetic differentiation between clades A and B is more complex. Genetic distances (Table 1) between these two clades (3.99%) suggest a substantial divergence, although geographically some members of each group are in proximity. It is further complicated if genetic variation within clades is considered. For example, within clade A average genetic distance among individuals was low (0.56%) compared to that for members of clade B (3.92%). It appears that individuals from the Central American Highlands are genetically homogenous, whereas individuals from foothills and lowlands of Oaxaca and Chiapas may have been isolated by factors associated with establishment of the Isthmus of Tehuantepec.

Sigmodon toltecus

Evidence obtained from this study alters the conclusions of Carroll et al. (2005) concerning the distribution of *S. toltecus* and refutes the possibility that the Rio Grande forms a barrier between *S. toltecus* and *S. hispidus*. Based on newly acquired samples, *S. toltecus* appears to be distributed from northeastern Mexico (Tamaulipas and Nuevo León) to the northern coast of Honduras. In Mexico, *S. toltecus* typically is found in the coastal grasslands east of the Sierra Madre Oriental range. As discussed previously, the distribution of *S. toltecus* in Chiapas is complex and an area of sympatry with *S. hirsutus* has been described (Carroll et al., 2005). In southeastern Mexico, *S. toltecus* occurs throughout the Yucatan Peninsula to Belize and the northern coast of Honduras (see above). The distribution of *S. toltecus* in northern Mexico is difficult to discern, as few samples were available for study and the fact that individuals of *S. hispidus* were collected in proximity to individuals of *S. toltecus* (La Carbonera, Tamaulipas, and Doctor Arroyo, Nuevo León). To date, the northern-most sample of *S. toltecus* is from Calabazas, Tamaulipas (near Cuidad Victoria), located to the east of the Sierra Madre Oriental range. Doctor Arroyo, Nuevo León, is located ca. 100 km

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W of Calabazas and is located on the western side of the Sierra Madre Oriental range. It is plausible that this mountain range serves to isolate *S. hispidus* and *S. toltecus*. The individual of *S. hispidus* from La Carbonera is located ca. 16 km NE Calabazas and is on the coast of central Tamaulipas. It may be that some of the smaller mountain ranges (Sierra de San José de las Rusias, Sierra de Tamaulipas, and Sierra de San Carlos) affect the distribution of *S. hispidus* and *S. toltecus* in east-central Tamaulipas.

Individuals of *S. toltecus* formed two clades that correspond to a southern geographical group (E) and a northern group (F). Clade E consists of a genetically homogenous series of individuals (average genetic distance between samples = 1.46%, Table 1) from coastal lowlands of southern Mexico and northeastern Central America. Clade F was comprised of individuals from Tamaulipas and Veracruz, Mexico, that also were genetically similar (average genetic distance between samples = 1.42%). However, the two clades differed by a value of 4.69% indicating some degree of historical isolation that may correspond to the Isthmus of Tehuantepec.

Sigmodon hispidus

As discussed above, data collected from newly acquired samples of *S. hispidus* refute the hypothesis of Carroll et al. (2005) concerning the possibility of the Rio Grande forming a barrier to these two taxa. As discussed above, it now appears that *S. hispidus* is distributed from central-southern United States into northern Mexico (Tamaulipas), although there are few available samples from northern Mexico to assist in a thorough characterization of the southern portion of its range. Samples from Coahuila, Nuevo León, San Luis Potosí, and Tamaulipas will be crucial in determining the ranges of *S. hispidus* and *S. toltecus*. This especially will be true where the interdigitation of mountain ranges and intervening valleys may complicate the issue, or even provide opportunities for sympatry.

Carroll et al. (2005) and Phillips et al. (2007) proposed that samples of *S. hispidus* genetically could be separated into eastern and western clades. Based on genetic-distance values (4.80%, Table 1), these two clades are divergent, whereas there is little divergence within each clade (1.17% western, 0.76% eastern). Carroll et al. (2005) postulated that boundaries of the two genetic forms could be tentatively explained by habitat preferences with western clade members being associated with plains grasslands and eastern clade members being associated with southeast hardwood grasslands. Alternatively, the eastern and western forms may have been isolated as a result of a some vicariant event and rejoined at a latter date. Phillips et al. (2007) provided evidence that the two genetic lineages hybridized in eastern Texas and that the contact zone coincided with the interface of the post oak savannah and piney woods. The possibility of two ecological species (eastern and western) remains to be resolved.

Based on data from this study, individuals of *S. hispidus* from northern Mexico (Nuevo León and Tamaulipas) phylogenetically are more similar to individuals from the western United States. It is not clear whether samples in northern Mexico represent an extension of populations across the Rio Grande from southern Texas or are they connected, via the Mesa Central, to samples from farther west. More sampling is needed in northern Mexico where *S. hispidus* and *S. toltecus* are in proximity. Also, a broader sampling scheme is needed in southern Mexico and northern Central America to determine the distribution and habitat affiliation of *S. toltecus* and *S. hirsutus*, as well as the possible relationship of *S. zanjonensis* (Carleton et al., 1999; Musser and Carleton, 2005). Comparison of genetic-distance values between major clades (A and B, C and D, E and F, and G and H) produced high levels of intraspecific genetic divergence that should be further investigated in light of the possibility of genetic isolation without substantial morphological differentiation (genetic species concept, sensu Baker and Bradley, 2006). Genetic-distance values (Table 1) between these

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clades were ca. 4–5% indicating a substantial divergence time. In addition, clades corresponded to either different habitats or regions potentially separated by geographic barriers.

Appendix I

Specimens Examined

The specimens examined are listed below by museum acronym and catalogue number (Hafner et al., 1997) and GenBank accession number. In some cases, no museum catalogue number was available so a collector number was reported. All localities are in the United States unless otherwise specified. Abbreviations for museum catalogue or collector numbers are as follows: Abilene Christian University Natural History Collection (ACUNHC); Angelo State Natural History Collections (ASU); Brigham Young University (BYU); Centro Interdisciplinario de Investigacion para el Desarrollo Integral Regional Unidad Durango (CRD), Collection of Tissues, Oklahoma State University (OSU); John C. Patton (JCP); Midwestern State University (MWSU), Museum of Texas Tech University (TTU and TK); Museum of Natural Science, Louisiana State University (LSUMZ); Museum of Vertebrate Zoology (MVZ); Royal Ontario Museum (ROM), Museum of Southwestern Biology, (MSB); and University of Texas Medical Branch at Galveston (T and FSH). In some cases, localities are provided with Universal Transverse Mercator (UTM) coordinates. Sequences generated in this study are identified by GenBank accession numbers EU073164-EU073182 and EU078398; all others were reported by Peppers and Bradley (2000), Peppers et al. (2002), Carroll et al. (2005), or were obtained from GenBank.

Sigmodon alleni vulcani

MEXICO: Michoacán; 3.5 km N Tancotaro, 2,353 m (TK45276, AF155425).

Sigmodon alstoni

VENEZUELA: Portuguesa; Municipality Guanare, Gato Negro (just S Guanare) (T2140, AF293396).

Sigmodon arizonae major

MEXICO: Durango; San Juan de Camarones, UTM 13-385011E-2775718N (TTU81699, AF155423); Sonora; 20 km W Alamos (by road) (MSB55566, AF293398).

Sigmodon fulviventer dalquesti

Texas; Jeff Davis County, 2.4 km W Point of Rocks Park (MWSU17910, AF293399).

Sigmodon fulviventer fulviventer

MEXICO: Durango; 2.2 km S, 2.5 km E Vicente Guerrero (TK48915, AF293400).

Sigmodon hirsutus borucae

COSTA RICA: Puntarenas; Finca Mamos, Chomes, 60 m (BYU15259, AF108702).

Sigmodon hirsutus chiriquensis

PANAMA: Chiriquí; Hotel La Siesta, by airport (TTU39163, AF155416); Canal Zone; Gamboa (ROM104228, AF425191).

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Sigmodon hirsutus griseus

EL SALVADOR: La Pax; 4.8 km NW San Luis Talpa (TK34810, AF155417). HONDURAS: Colon; Trujillo, Parque Nacional Capiro y Calentura, UTM 16-612297E-1758742N (TTU104184, EU073174; TTU104185, EU073176; TTU103971, EU073175); Cortez; La Guama, UTM 16-395886E- 1649092N (TTU104420, EU073170; TTU104422, EU073171); Francisco Morazan; El Picacho Zoological Parque, UTM 16-497451E-1561275N (TTU83741, AY517528); Olancho; 4 km E Catacamas, Escuela de Sembrador, UTM 16-624523E-1637511N (TTU84767, AY517529; TTU84634, EU073172); Valle; 3 km N, 9 km SW San Lorenzo, UTM 16-442952E-1486788N (TTU83788, AY517530); 3 km N, 12.5 km SW San Lorenzo, UTM 16-441040E-1484097N (TTU83794, EU073173). NICARAGUA: Nueva Segovia; El Balsamo (TTU101442, EU073164; TTU100560, EU073168); Boaco; El Paraiso (TTU100570, EU073166; TTU100579, EU073165); Matagalpa; El Tigre (TTU105136, EU073169); Jinotega; El Cua (TTU108154, EU073167).

Sigmodon hirsutus hirsutus

VENEZUELA: Lara; Finca Santa María Sarano (T4632, AF155419).

Sigmodon hirsutus ssp

MEXICO: Chiapas; 14.4 km N Ocozocoaut la, UTM 15 -451772E-1864243N (TTU82801, AF425193; TTU82798, AF425196; TTU82799, AF425192; TTU82798, AF425196); Ixtapa, 12 km SE of Ixtapa (ROM97578, AF425197); Soyalo (ROM97584, AF425195); Mapastepec, Tutuan, Rancho El Trebol, UTM 15-491419E-1703719N (TK138046, EU078398); 2.1 km SW La Sombra (TTU108157, AF425198); Oaxaca; 2 km S La Blanca UTM 15-318523E-1833293N (TTU82796, AF425194).

Sigmodon hispidus berlandieri

MEXICO: Tamaulipas; 12.8 km S la Carbonera, UTM 14-627591E-2711834N (TTU108155, EU073177); Nuevo León; 8.7 km W Doctor Arroyo, UTM 14-369819E-2622301N (TTU108156, EU073178). New Mexico; Otero County, Holloman Air Force Base (TTU79008, AF188198). Texas; Cameron County, Brownsville (TK32481, AF425199); Dimmit County, Chaparral Wildlife Management Area UTM 14-458437E-3134279N (TTU80759, AF425200); Lubbock County, Lubbock Lake Landmark State Historical Park (TTU76231, AF435110); McMullen County, James Daughtrey Wildlife Management Area UTM 14-555436E-3153833 (TTU82847, AF425201); Refugio County, Guadalupe Delta Wildlife Management Area UTM 14-709976E-3146702N (TTU75154, AF155415).

Sigmodon hispidus eremicus

Arizona; Yuma County, 3.2 km S, 1 km W Gadsden UTM 11-707004E-3600561N (TTU78493, AF155421).

Sigmodon hispidus hispidus

Florida; Walton County, 16 km E Destin, Topsail Hill State Recreation Area (TTU77517, AF425206). Louisiana; Bossier Parish, Wright Island (LSUMZ23836, AF425205); East Baton Rouge Parish, (LSUMZ28519, AF425204). Missouri; Howard County, Booneville (TK28256, AF425203). Tennessee; Shelby County, Meeman Biological Station (TTU79181, AF425202).

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Sigmodon hispidus littoralis

Florida; Brevard County, Sebastion Inlet State Park (TTU97859, AF425207; TTU97861, AF425208).

Sigmodon hispidus spadicipygus

Florida; Dade County, Homestead Airforce Reserve Base golf course (FSH33, AF155420).

Sigmodon hispidus texianus

Kansas; Ellis County, Hays (OSU13231, AF425213; OSU13227, AF425209). Okla Oklahoma; Caddo County, 3.2 km S, 1.6 km W Anadarko (TTU54962, AF155414). Texas; Cottle County, Matador Wildlife Management Area UTM 14-374841E- 3778551N (TTU78822, AF425212); Freestone County, Richland Creek Wildlife Management Area UTM 14-775688E-3542219N (TTU75347, AF425210); Kimble County, Junction, Texas Tech University Center UTM 14-425829E-3373218N (TTU40477, AF425211); Lamar County, Pat Mayse Wildlife Management Area (TTU80626, AF425227); Nacogdoches County, Alazon Bayou Wildlife Management Area (ASU4996, AF425214).

Sigmodon leucotis leucotis

MEXICO: Durango; 12 km E Ojitos, UTM 13-385011E-2775718 (TTU81692, AF293401).

Sigmodon mascotensis inexoratus

MEXICO: Jalisco; 2 km NW Mesconcitos UTM 13-808753E-2375677N (TTU82793, AF425215).

Sigmodon mascotensis mascotensis

MEXICO: Colima; 6 km W Colima (JCP1020, AF155424). MEXICO: Michoacán; Apatzingán (TK45737, AF425216); Patzcuaro (Lake) (JCP1061, AF296188); Oaxaca; Las Minas (TTU82794, AF425217).

Sigmodon ochrognathus

MEXICO: Durango; 24 km N Las Herreras (CRD1033, AF155422). Arizona; Cochise County, 3.2 km W Portal (ACUNHC500, AF155592).

Sigmodon peruanus

EQUADOR: El Oro; Progreso (MSB140112, AF293395); Guayas, Manglares Churute, Cerro Cimalon UTM 17-650092E-9732559N (TTU103494, EU073179).

Sigmodon toltecus furvus

HONDURAS: Atlantida; Jardin Botanico Lancetilla, UTM 16-451012E-1740282N (TTU84378, EU073180; TTU103840, EU073181). MEXICO: Quintana Roo; 6 km S, 1.5 km W Tres Garantias (ASNHC7480, AF293402).

Sigmodon toltecus microdon

MEXICO: Campeche; Escárcega (ASNHC7474, AF425218); LaValeta (ROM95219, AF425221); Tabasco; Jonuta (ROM96256, AF425220); Yucatán; Labna (ROM96474, AF425219).

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Sigmodon toltecus saturatus

GUATEMALA: El Petén; El Remate (ROM99644, AF425223); 10 km N Tikal (ROM99640, AF425222). MEXICO: Chiapas; 14.4 km N Ocozocoautla, UTM 15-451772E-1864243N (TTU108152, AF425228; TTU108153, AF425224).

Sigmodon toltecus toltecus

MEXICO: Tamaulipas; 3.2 km W Calabazas, Rancho Calabazas (TTU44946, AF425225); Veracruz; Estación Biológica Morro de la Mancha, 19°35′23.80″ N, 96°22′49.2″ W, 8 m (MSB75587, AF155418); 30 km N, 3 km E Cardel, Estación Biologica La Mancha (FN29909, AF425226); Paso del Patel , UTM 14-665394E-2282430N (TTU105062, EU073182).

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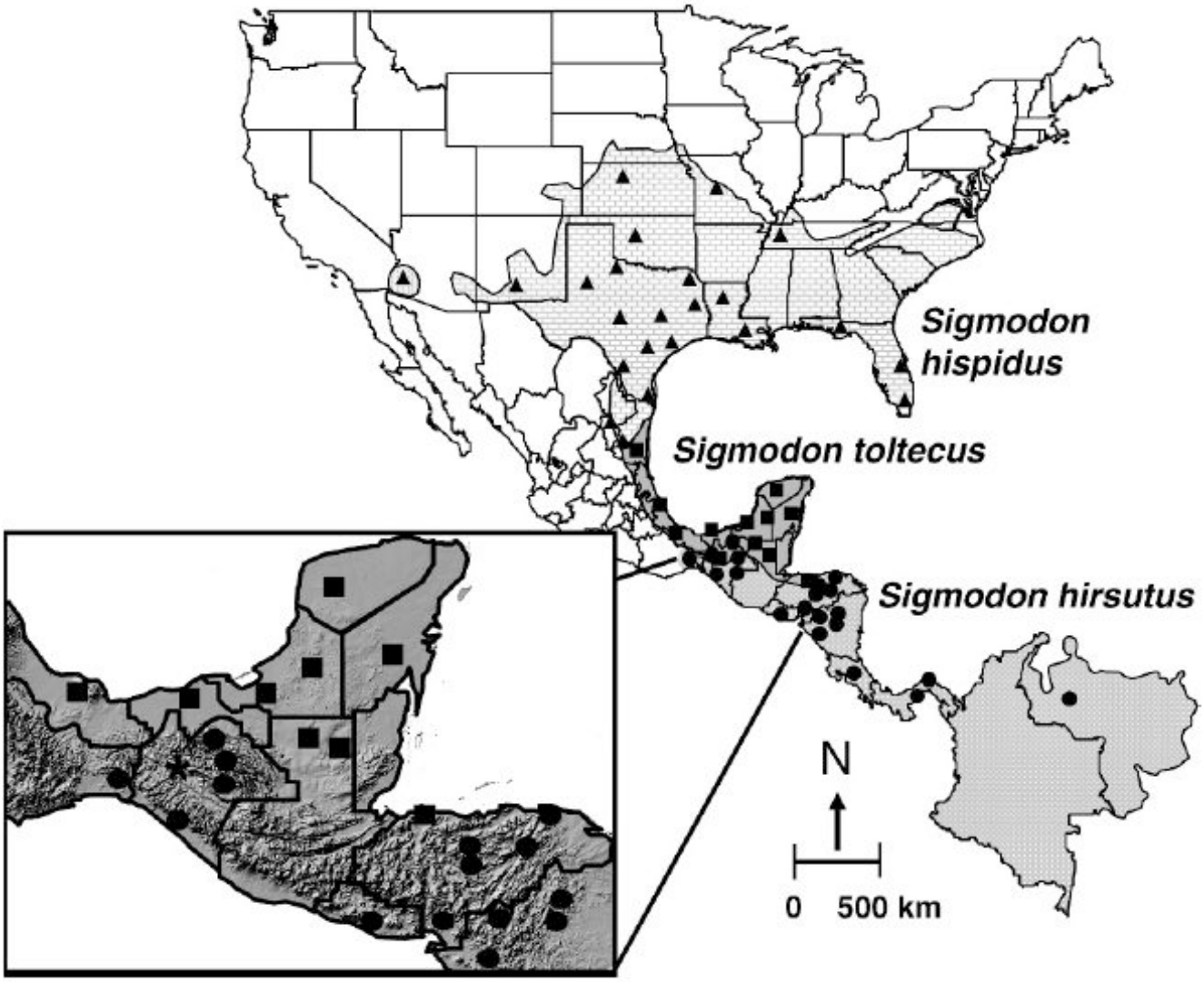

Fig. 1.
Distribution of members of the *hispidus* species group (*Sigmodon hirsutus*, *S. hispidus*, and *S. toltecus*). Localities from which samples were obtained are indicated as follows: *S. hirsutus* (circles), *S. hispidus* (triangles), and *S. toltecus* (squares). An asterisk indicates locality where samples of *S. hirsutus* and *S. toltecus* were sympatric.

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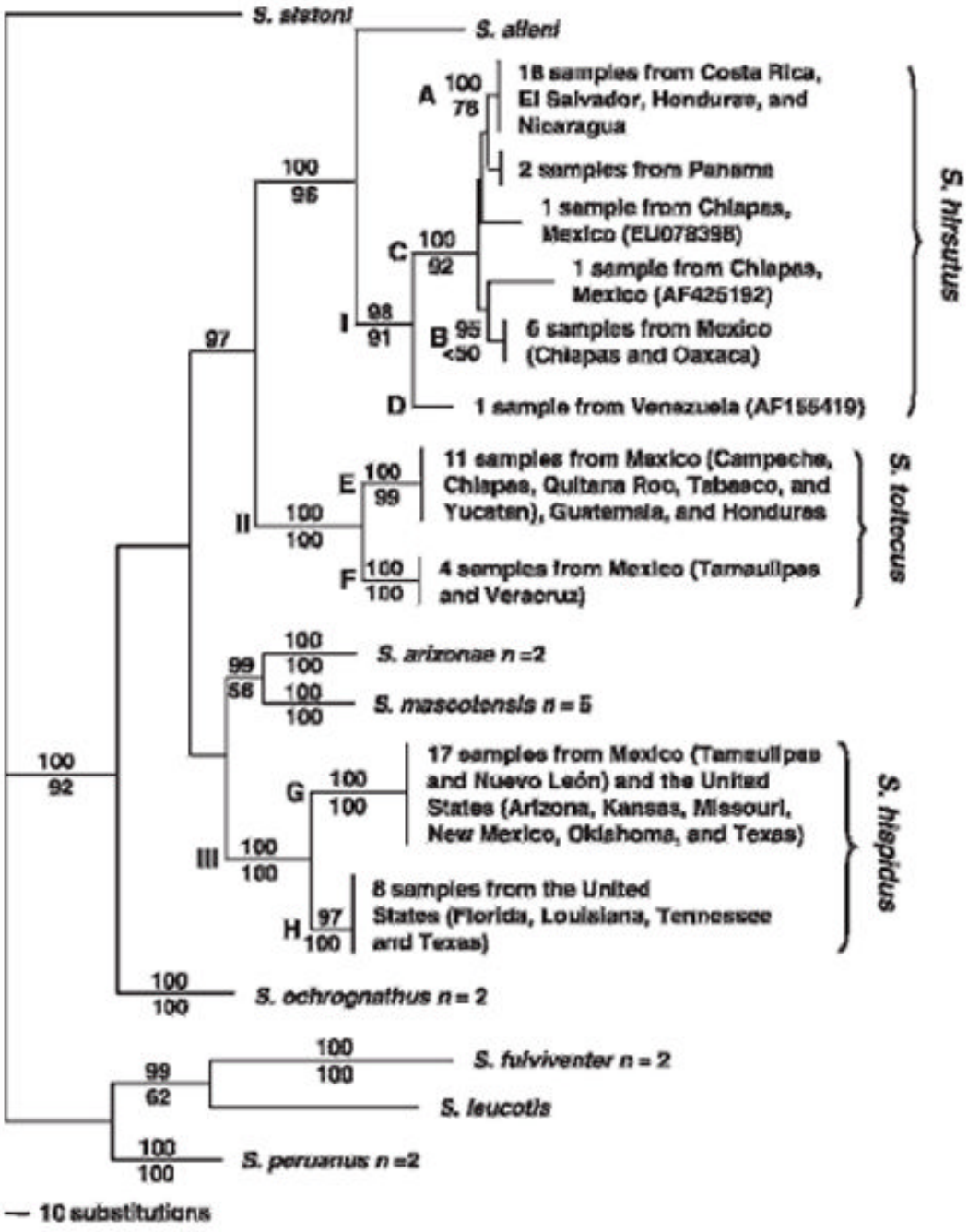

Fig. 2.

Phylogenetic tree obtained from the Bayesian analysis of 69 taxa. Major clades are depicted by Roman numerals (I–III) and subclades by letters (A–H). Clade probability values are indicated above branches and bootstrap values are indicated below branches. Branch lengths for multiple individuals of a species were collapsed for clarity. Vertical bars at terminal nodes indicate that genetic changes are so small that branch lengths are virtually non-existent.

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Table 1

Average genetic distances (Kimura, 1980) for selected taxa and clades of *Sigmodon*.

Taxa	Average genetic distance (%)
S. hirsutus versus *S. alleni*	9.77
S. hirsutus versus *S. toltecus*	12.90
S. hirsutus versus *S. hispidus*	12.55
S. toltecus versus *S. hispidus*	12.81
S. hispidus versus *S. arizonae*	11.32
S. hispidus versus *S. mascotensis*	11.04
S. arizonae versus *S. mascotensis*	8.75
Within *S. hirsutus*	2.34
Within Clade A	0.56
Within Clade B	3.92
Within Clade C	2.15
Within Clade D	—
Clade A versus Clade B	3.99
Clade C versus Clade D	4.89
Within *S. toltecus*	2.81
Within Clade E	1.46
Within Clade F	1.42
Clade E versus Clade F	4.69
Within *S. hispidus*	2.78
Within Clade G	1.17
Within Clade H	0.76
Clade G versus Clade H	4.80