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Molecular systematics of the genus *Sigmodon*: results from mitochondrial and nuclear gene sequences

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Abstract

Phylogenetic relationships within the genus *Sigmodon* Say and Ord, 1825 were examined using sequence data from multiple gene regions, including exon 1 of the nuclear-encoded interphotoreceptor retinoid binding protein, intron 7 of the nuclear beta-fibrinogen gene, and the mitochondrial cytochrome *b* gene from 27 individuals representing 11 species of *Sigmodon*. Nuclear genes were analyzed independently, combined with each other, and combined with the mitochondrial data. Topologies were constructed using parsimony and Bayesian methods, with nodal support provided by bootstrap and posterior probability values. All analyses recovered four independent clades (I–IV), each representing unique species groups: *hispidus*, *fulviventer*, *peruanus*, and *alstoni*. The analyses from the combined data also provided support for relationships previously proposed within those species groups.

Introduction

Members of the genus *Sigmodon* Say and Ord, 1825 (cotton rats) are distributed throughout the grasslands and savannas of south-central United States, Mexico, Central America, and northern South America. Although cotton rats are abundant in the field and museum collections, a deficiency in morphologically distinct characteristics among species (Allen 1897, 1906; Bailey 1902; Nelson and Goldman 1933; Baker 1969) and the difficulties associated with obtaining taxa from throughout their range have resulted in a poor understanding of cotton rat relationships (Peppers et al. 2002). Several studies have examined relationships of species from North and Central America (Allen 1897, 1906; Bailey 1902; Nelson and Goldman 1933; Baker 1969; Zimmerman 1970; Elder 1980; Elder and Lee 1985); however, few have examined species from South America, and even fewer have sampled species from throughout their collective ranges.

Early systematic studies focused on North American species of *Sigmodon* and on the placement of morphologically similar taxa into species groups. Initially, Bailey (1902) defined the *hispidus* species group (*S. alleni* Bailey, 1902 and 14 subspecies of *S. hispidus* Say and Ord, 1825) and the *fulviventer* species group (*S. fulviventer* J.A. Allen, 1889, *S. ochragnathus* Bailey, 1902, and *S. leucotis* Bailey, 1902). Baker (1969) suggested a classification similar to that proposed by Bailey (1902), postulating that *S. ochragnathus*, *S. fulviventer*, and *S. leucotis* were descendents of a Mexican plateau form of *S. hispidus*,

whereas *S. alleni* was derived from a Pacific lowland *S. hispidus* like ancestor. In a later study using chromosomal data, Zimmerman (1970) elevated *S. arizonae* Mearns, 1890 and *S. mascotensis* J.A. Allen, 1897 to species status. Additional chromosomal studies by Elder (1980) and Elder and Lee (1985) provided new evidence and redefined the *hispidus* and *fulviventer* species groups with the *hispidus* species group containing *S. alleni*, *S. arizonae*, *S. hispidus*, *S. leucotis*, *S. mascotensis*, and *S. ochragnathus*, placing only *S. fulviventer* in the *fulviventer* species group.

Only a few studies (Martin 1979; Dickerman 1992; Voss 1992) have examined the taxonomic and phylogenetic relationships of South American cotton rats. Martin (1979) studied dental characteristics, and separated the genus into two species groups: *S. hispidus* (*S. alleni*, *S. arizonae*, *S. fulviventer*, *S. hispidus*, *S. mascotensis*, and *S. ochragnathus*) and *S. leucotis* (*S. alstoni* (Thomas, 1881), *S. leucotis*, and *S. peruanus* J.A. Allen, 1897). Voss (1992) concluded that there were four South American species (*S. alstoni*, *S. hispidus*, *S. inopinatus* Anthony, 1924, and *S. peruanus*) based on extensive morphological analyses. Dickerman (1992) examined DNA hybridization to evaluate inter-relationships among 39 New World rodents, which included five species of cotton rats (*S. alstoni*, *S. arizonae*, *S. fulviventer*, *S. hispidus*, and *S. ochragnathus*). This analysis suggested that populations of *S. hispidus* formed a group, and *S. arizonae*, *S. ochragnathus*, and *S. fulviventer* joined in a stepwise manner. *Sigmodon alstoni* was positioned as the most basal taxon of the genus based on the hybridization data.

More recent studies (Carroll et al. 2005; Peppers and Bradley 2000; Peppers et al. 2002) examined the phylo-genetic relationships of both North and South American taxa using mitochondrial DNA markers such as the cytochrome *b* (*Cytb*) gene. Results of these DNA sequence studies led to the recognition of two species groups: the *hispidus* species group with seven species (*S. alleni*, *S. arizonae*, *S. hirsutus* Burmeister, 1854, *S. hispidus*, *S. mascotensis*, *S. ochragnathus*, and *S. toltecus* Saussure, 1860) and the *fulviventer* species group with four species (*S. fulviventer*, *S. leucotis*, *S. inopinatus*, and *S. peruanus*). *Sigmodon alstoni* was basal to the other two species groups; however, it was unclear whether *S. alstoni* represented a separate species group or should be included within the *hispidus* or *fulviventer* species group. Additionally, Peppers et al. (2002) recognized three cryptic species within the *S. hispidus* group (*S. hirsutus*, *S. hispidus*, and *S. toltecus*). Carroll and Bradley (2005) compared *Cytb* sequences with results from the intron 7 of the nuclear beta-fibrinogen gene (*Fgb-I7*), and recovered a similar topology, even though *Fgb-I7* contained little nucleotide variation. The combined sequence data did provide better resolution than the single gene tree for the species that were included in the study (*S. peruanus* was not included). Consequently, Carroll and Bradley (2005) suggested that three species groups (*alstoni*, *fulviventer*, and *hispidus*) should be recognized.

The primary objective of this study is to analyze sequence data from additional nuclear markers to test and provide information concerning the phylogenetic relationships within the genus *Sigmodon*. Two nuclear genes were evaluated for phylogenetic reconstruction in this study. First, exon 1 of the interphotoreceptor retinoid binding protein (RBP3 = IRBP) has been used effectively for rodent phylogenetics at intra-generic levels (Serizawa et al. 2000; Weksler 2003). Second, this study added to the existing *Fgb-I7* data set (Carroll et al. 2005), as 14 new samples were included for this study. With sequence data from additional nuclear genes, this study re-examined hypotheses concerning the phylogeny of the genus *Sigmodon* previously proposed by Carroll and Bradley (2005) and Peppers et al. (2002).

Materials and methods

Taxonomic sampling

To provide consistency of samples between this and previous studies, efforts were made to sample the same individual specimens that were used by Carroll and Bradley (2005) and Peppers et al. (2002). Sequences from these previous studies (*Cytb* and *Fgb-17*) were included and analyzed (Gen-Bank accession numbers provided in Appendix A). Whenever possible, at least 2 individuals of each species were represented, and >2 individuals were examined for species that were more widely distributed to address intraspecific variation. *Cytb* data were obtained for 3 additional samples not included in previous studies (*S. alstoni*, *n* = 1; *S. arizonae*, *n* = 1; *S. leucotis*, *n* = 1) and 14 *Fgb-17* samples (*S. alstoni*, *n* = 1; *S. arizonae*, *n* = 1; *S. fulviventer*, *n* = 1; *S. hirsutus*, *n* = 2; *S. hispidus*, *n* = 3; *S. leucotis*, *n* = 1; *S. mascotensis*, *n* = 1; *S. ochragnathus*, *n* = 1; *S. peruanus*, *n* = 2; *S. toltecus*, *n* = 1).

Molecular methods

Genomic DNA was extracted from frozen tissue using the DNeasy Tissue Kit (Qiagen Inc.). Specific gene regions were amplified using a standard polymerase chain reaction (PCR) protocol. Thermal profiles for each gene and primers are listed below.

Rbp3

A partial sequence of exon 1 of the nuclear gene encoding *Rbp3* (1266 bp) was amplified using primers A1 (Stanhope et al. 1992), B2 (Weksler 2003), and Promega GoTaq. Thermal profiles were based on the following standard profile: 1 cycle of 95 °C for 10 min, 3 stages of 5 cycles each with denaturation at 95 °C for 20 s, annealing at 58, 56, 54 °C for 15 s extension, at 72 °C for 60 s, 1 stage of 23 cycles of denaturation at 95 °C for 20 s, annealing at 52 °C for 15 s, and extension at 72 °C for 60 s, 1 cycle of 72 °C for 7 min (Weksler 2003). PCR products were then cycle-sequenced using primers 395R (J.D. Hanson and R.D. Bradley, unpublished data), 125F and 609F (DeBry and Sagel 2001), and D2, E2, and B2 (Weksler 2003).

Cytb

The complete mitochondrial *Cytb* (1143 bp) was amplified using primers MVZ05 (Smith and Patton 1993) and CB40 (J.D. Hanson and R.D. Bradley),² and Promega Taq. Thermal profiles were modified from the following standard profile: 35 cycles of 95 °C for 45 s, 50–52 °C for 1 min 30 s, 72 °C for 1 min 45 s, and 1 cycle of 72 °C (Smith and Patton 1993). PCR products were then cycle-sequenced using primers MVZ05, 400R (Tiemann-Boege et al. 2000), F1 (Whiting et al. 2003), 700H (Peppers and Bradley 2000), 870R (Peppers et al. 2002), and CB40 (J.D. Hanson and R.D. Bradley).² For some samples, 400F (Tiemann-Boege et al. 2000) replaced F1, and 700H (Peppers and Bradley 2000) replaced 870R.

Fgb-17

The intron 7 of the beta-fibrinogen gene was amplified using primers *Fgb-17U-Rattus* and *Fgb-17L-Rattus* (Wickliffe et al. 2003) and Promega GoTaq. Thermal profiles were based on the following standard profile: 1 cycle 95 °C for 60 s and 33 cycles of 95 °C for 40 s, 53 °C for 40 s, and 72 °C for 60 s, and 1 cycle of 72 °C for 2 min (Carroll and Bradley 2005). PCR products were cycle-sequenced using primers *Fgb-17U-Rattus*, *Fgb-17L-Rattus*, Bfib300F, and Bfib300R (Carroll and Bradley 2005).

²J.D. Hanson and R.D. Bradley. Molecular divergence in the *Oryzomys palustris* complex: evidence for multiple cryptic species. Submitted for publication.

Amplified gene products were purified using a QIAquick PCR purification kit (QIAGEN), and then cycle-sequenced using BigDye version 3.1 terminator technology and primers described above. Cycle-sequencing reactions were purified using isopropanol clean-up protocols. The purified product was sequenced on an ABI 3100-*Avant* automated sequencer. Sequences were aligned and proofed using Sequencher version 4.0 software (Gene Codes, Ann Arbor, Michigan). MEGA3.0 (Kumar et al. 2004) software was used to detect the presence of stop codons and pseudogenes.

Data analysis

Based on phylogenetic relationships established in Smith and Patton (1999), *Holochilus chacarius* Thomas, 1906, *Oryzomys couesi* (Alston, 1877), *Zygodontomys brevicauda* (J.A. Allen and Chapman, 1893), and *Reithrodon auritus* (G. Fischer, 1814) were used as outgroup taxa for data analyses. Bayesian and parsimony criteria (MRBAYES, Huelsenbeck and Ronquist 2001; PAUP*, Swofford 2002) were used to generate hypotheses concerning phylogenetic relationships of taxa. Each data set was analyzed separately and then the data sets were analyzed in a concatenated fashion.

Parsimony analyses used 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 all analyses and variable nucleotide positions within the data set were treated as unordered, discrete characters with four possible states: A, G, C, or T. To assess nodal support, bootstrap analysis (PAUP*; Swofford 2002) with 1000 replicates was utilized. Nucleotide sites exhibiting heterozygous bases were coded following International Union of Biochemistry (IUB) codes. Nodal support was evaluated using bootstrap support values (Felsenstein 1985), with values ≥ 70 considered indicative of nodal support.

A Bayesian analysis (MRBAYES; Huelsenbeck and Ronquist 2001) using a GTR + I + G model (determined from the MODELTEST program; Posada and Crandall 1998) with rates set equal to the proportion of invariable sites, and sites partitioned by codon (coding regions) or individually (noncoding), was used with the following options: 4 Markov chains (1 cold and 3 heated), 10 million generations, and sample frequency every 1000th generation. 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. Posterior probability values were estimated and values ≥ 95 were used as indication of nodal support.

Kimura two-parameter genetic distances (Kimura 1980) were estimated for each gene region between each of the 11 respective taxa. These genetic distances were used to compare the rates of evolution between the three gene regions.

Results

Rbp3

Sequence data of the nuclear exon 1 of *Rbp3* (1266 bp) from 27 ingroup individuals yielded 134 phylogenetically informative characters, and possessed a nucleotide composition of 22.1% A, 28.1% C, 28.3% G, and 21.5% T. The transition to transversion ratio was 3.4:1 and 13 heterozygous sites were identified.

In the Bayesian analysis (Fig. 1), four well-supported clades were recovered. Clade I (posterior probability = 1.00) contained individuals of *S. alleni*, *S. arizonae*, *S. hirsutus*, *S. hispidus*, *S. mascotensis*, *S. ochragnathus*, and *S. toltecus*. Within clade I, a smaller clade containing *S. alleni*, *S. arizonae*, *S. hispidus*, *S. toltecus*, and *S. mascotensis* was supported.

Clade II consisted of samples of *S. fulviventer* and *S. leucotis* (posterior probability = 1.00), and individual clades of each species formed separate, supported subclades. Clade III contained individuals of *S. peruanus*, and clade IV comprised individuals of *S. alstoni*. Both clades III and IV were supported by posterior probabilities of 1.00. Despite strong support for species and species group clades, the Bayesian analysis did not provide support for relationships among major clades.

The maximum parsimony analysis (not shown) generated 856 most parsimonious trees (length = 182, consistency index (CI) = 0.8077, and retention index (RI) = 0.9077) and revealed four well-supported clades (I–IV) similar to those recovered in the Bayesian analysis. Clade I received strong nodal support (bootstrap = 97) and contained seven species (*S. alleni*, *S. arizonae*, *S. hirsutus*, *S. hispidus*, *S. mascotensis*, *S. ochragnathus*, and *S. toltecus*), although most relationships between members within this clade were not supported. Clade II (bootstrap = 99) contained individuals of two species, *S. fulviventer* and *S. leucotis*. Clade III (bootstrap = 100) contained two individuals of *S. peruanus*. Clade IV also was strongly supported (bootstrap = 100) and contained two individuals of *S. alstoni*. Although clades (I–IV) were all strongly supported, there was no support for phylogenetic relationships among clades.

Fgb-17

Sequence data from intron 7 of the nuclear beta-fibrinogen (*Fgb-17*) gene were obtained from 35 individual cotton rats. The 606 bp *Fgb-17* yielded 122 phylogenetically informative characters with a nucleotide composition of 32.9% A, 21.8% C, 16.8% G, and 28.5% T. There were five heterozygous sites, and the transition to transversion ratio was 3.83:1.

The Bayesian analysis (Fig. 2) generated a topology with four well-supported clades (posterior probabilities >0.95) similar to those depicted in the *Rbp3* analysis. Clade I contained the same seven taxa (*S. alleni*, *S. arizonae*, *S. hirsutus*, *S. hispidus*, *S. mascotensis*, *S. ochragnathus*, and *S. toltecus*), although relationships among many taxa were not supported. Clade II contained *S. fulviventer* and *S. leucotis* (posterior probability = 0.99), clade III contained two individuals of *S. peruanus*, and clade IV contained two individuals of *S. alstoni*. Even though each of the four clades was well supported, there was no support for relationships between clades.

The maximum parsimony analysis (not shown) generated 24 most parsimonious trees (length = 174, CI = 0.7874, and RI = 0.8966) and revealed the same four strongly supported clades (I–IV) depicted in the parsimony analysis of the *Rbp3* data. Specifically, clade I contained the same taxa as in the *Rbp3* analysis, clade II contained samples of *S. fulviventer* and *S. leucotis*, clade III contained individuals of *S. peruanus*, and clade IV contained only *S. alstoni*. The parsimony analysis of *Fgb-17* was unable to provide support for relationships between any of the four clades.

Rbp3 and *Fgb-17* combined

The nuclear gene sequence data from 27 individuals were combined and analyzed using 255 phylogenetically informative characters, and a transition to transversion ratio of 3.54:1. The nucleotide composition was 25.6% A, 26.0% C, 24.6% G, and 23.7% T.

The Bayesian analysis (not shown) recovered the same four clades as in the separate analyses of the two genes. Clade I contained seven taxa with *S. hispidus* and *S. toltecus* forming a sister relationship with *S. arizonae*, and *S. mascotensis* and *S. alleni*, and *S. hirsutus* attaching in a stepwise fashion followed by addition of *S. ochragnathus* as the most basal member of clade I. Clade II consisted of *S. fulviventer* and *S. leucotis*. Clade III contained individuals of *S. peruanus* and clade IV contained individuals of *S. alstoni*.

Although the combined data showed better support for relationships within each clade, support was not provided for relationships between clades.

The maximum parsimony analysis of the combined nuclear data recovered 225 parsimonious trees (length = 355, CI = 0.7972, and RI = 0.7799) and recovered the same well-supported clades (I–IV) as in parsimony analyses of individual genes. Moderate support (bootstrap = 73) was provided for *S. ochragnathus* as the most basal member of clade I; however, little support (bootstrap = 59) was provided for phylogenetic relationships of the remaining members of this clade. Individuals of *S. alleni* and *S. mascotensis* formed a clade with moderate support (bootstrap = 74), whereas individuals of *S. hirsutus* formed a moderately supported clade (bootstrap = 83) and individuals of *S. arizonae* formed a clade (bootstrap = 93). The sister relationship of *S. hispidus* and *S. toltecus* was well supported (bootstrap = 97) and individuals of *S. toltecus* formed a well-supported clade (bootstrap = 100). Clade II had a bootstrap support value of 100 and contained *S. fulviventer* and *S. leucotis*. Clade III consisted of individuals of *S. peruanus* (bootstrap = 100) and clade IV (bootstrap = 100) contained individuals of *S. alstoni*.

Cytb and nuclear combined

The combined 3015 nucleotide analysis of *Rbp3*, *Cytb*, and *Fgb-I7* yielded 679 phylogenetically informative characters. The nucleotide composition was 24.2% A, 36.0% C, 12.8% G, and 26.9% T. The transitions to transversions ratio was 3.35:1.

The Bayesian analysis (Fig. 3) recovered four major clades (I–IV). Within clade I, subclade A (containing samples of *S. alleni*, *S. hirsutus*, and *S. toltecus*) was well supported, as well as subclade B (containing samples of *S. arizonae* and *S. mascotensis*). Samples of *S. hispidus* formed a well-supported clade C. This analysis also provided support for clade II, which contained members of *S. fulviventer* and *S. leucotis*. Clade III (containing *S. peruanus*) and clade IV (containing *S. alstoni*) were well supported in this analysis. The Bayesian analysis did not provide support for phylogenetic relationships between clades I–IV.

The maximum parsimony analysis (not shown) retained one most parsimonious tree (length = 2006, CI = 0.4751, and RI = 0.7038). The combined analysis provided four well-supported clades within members of *Sigmodon*. These four clades (I–IV) included the same members as the individual and combined nuclear analyses.

Genetic distance

Kimura two-parameter (Kimura 1980) genetic distances were estimated between species (Table 1). The *Cytb* sequences possessed the highest genetic distances between species (mean = 15.36%), with the lowest reported distance between *S. arizonae* and *S. mascotensis* (8.54%) and the greatest distance between *S. alstoni* and *S. fulviventer* (20.78%). The range of genetic distances of *Fgb-I7* was 0.00% (*S. alleni* to *S. arizonae*) to 6.38% (*S. fulviventer* to *S. ochragnathus*), and the mean value was 4.25% between species. *Rbp3* possessed the lowest levels of variation with a range from 0.67% (*S. alleni* to *S. arizonae* / *S. hispidus*) to 2.90% (*S. leucotis* to *S. peruanus*), with a mean value of 1.62%. Genetic distances (Table 1) also were calculated between individual clades (I–IV). In the *Cytb* sequence data, genetic distances between clades ranged from 20.3% (clade II to clade IV) to 17.1% (clade I to clade III). Both of the nuclear genes exhibited much lower genetic distances. The *Fgb-I7* genetic distances ranged from 3.91% (clade I to clade II / clade IV) to 5.56% (clade II to clade IV), and *Rbp3* reported distances of 2.00% (clade I to clade IV) to 2.78% (clade II to clade III).

Discussion

Topologies obtained from independent analyses of nuclear genes (*Rbp3* and *Fgb-17*) recovered the same four clades (I–IV) with high nodal support values. Relationships of species composing each clade were not resolved or supported at terminal nodes, given the slow rate of genetic divergence associated with each gene (Table 1). Combination of both nuclear genes provided better nodal support, as a result of an increase in the number of informative characters. The most strongly supported topology was obtained from the combination of the mitochondrial *Cytb* and the two nuclear sequences, and was well supported at the terminal and middle nodes. Therefore, this topology (Fig. 3) will be referred to throughout the discussion.

This study recovered a topology similar to that reported in previous studies by Peppers et al. (2002) and Carroll and Bradley (2005). These previous studies recovered three independent lineages within the genus, which were defined as species groups (*hispidus*, *fulviventer*, and *alstoni*). Results of the present study, however, depict four independent clades as *S. peruanus* (clade III) was removed from the *fulviventer* species group. The *hispidus* and *alstoni* species groups proposed by Peppers et al. (2002) were recovered in all analyses (clades I and IV, respectively).

Sigmodon hispidus species group

Clade I contained individuals of *S. alleni*, *S. arizonae*, *S. hirsutus*, *S. hispidus*, *S. mascotensis*, *S. ochragnathus*, and *S. toltecus*. Relationships among members of this group also corresponded to relationships recovered by Peppers et al. (2002), Carroll and Bradley (2005), and Carroll et al. (2005); our additional sequence data provided increased support at several nodes. Within clade I, two major well-supported subclades were recovered. In the first subclade, *S. alleni* and *S. hirsutus* formed a sister relationship that was joined in a stepwise manner by *S. toltecus*. The second subclade depicted a sister relationship between *S. arizonae* and *S. mascotensis*, which then joined with a clade containing individuals of *S. hispidus*. Of note, within the *S. hispidus* clade, support was recovered for two lineages with one consisting of specimens from Tennessee and Florida, and the other containing specimens from Kansas, Texas, and northern Mexico. These two lineages correspond to the eastern and western forms of *S. hispidus* discussed by Carroll et al. (2005) and Phillips et al. (2007). Similarly, support was identified for separate lineages among individuals of *S. toltecus*. Bradley et al. (2008) recovered two clades within *S. toltecus* that corresponded to northern and southern groups. *Sigmodon ochragnathus* was depicted as the basal-most member of the *hispidus* species group.

Sigmodon fulviventer and S. leucotis

In all analyses, the sister relationship between *S. fulviventer* and *S. leucotis* (clade II) was recovered with high nodal support values. This result corroborates previous studies that suggested *S. fulviventer* and *S. leucotis* may belong to a clade separate from that of the other North American species. Although Bailey (1902), Baker (1969), Elder and Lee (1985), Fuller et al. (1984), and Martin (1979) differed in their arrangements of *S. fulviventer* and *S. leucotis*, all arrangements excluded an association with the *S. hispidus* group. The molecular data of Peppers et al. (2002) provided evidence for the sister relationship of *S. fulviventer* and *S. leucotis*, with *S. peruanus* being the basal-most member, although there was little support for this relationship, and hypothesized that *S. inopinatus* would be included in this group based on morphological interpretations by Voss (1992). Although all analyses in this study recovered support for the clade containing *S. fulviventer* and *S. leucotis*, none supported the inclusion of *S. peruanus*.

Sigmodon peruanus

As discussed above, *S. peruanus* was placed with *S. fulviventer* and *S. leucotis* in the *fulviventer* species group by Peppers et al. (2002). All analyses performed herein did not support this relationship. Instead, individuals of *S. peruanus* formed a well-supported monotypic clade (clade III) separate from the other taxa. Based on the relationship between *S. inopinatus* and *S. peruanus* discussed by Voss (1992) and Peppers et al. (2002), it would be reasonable to hypothesize that *S. inopinatus* would be a member of the *S. peruanus* clade.

Sigmodon alstoni

In each of the analyses, individuals of *S. alstoni* formed a well-supported monotypic clade (clade IV) separate from other species, although the relationship of *S. alstoni* to other species of *Sigmodon* has been difficult to establish. Owing to the presence of grooved incisors, *S. alstoni* has been suggested to be the most divergent (Voss 1992) or basal member of the genus (Peppers et al. 2002). None of the analyses herein provided support for *S. alstoni* as the basal-most member of the genus, as all clades were unresolved.

Taxonomic implications

The most recent synopsis of the genus *Sigmodon* (Musser and Carleton 2005) recognized 14 species, 11 of which were included in this study. The inclusion of additional samples and additional nuclear sequence data has provided different perspectives concerning aspects of the genus *Sigmodon*.

Historically, *S. hispidus* was thought to be a single species that occurred from the United States to South America. Using *Cytb* sequence data, Peppers and Bradley (2000) suggested that *S. hispidus* was paraphyletic and actually represented three distinct species, based on a genetic divergence of 12%–13% between the three species, even though the North American and South American species do not exhibit morphological variation (Voss 1992). The genetic species concept (Baker and Bradley 2006) argues that under the Bateson–Dobzhansky–Muller model of speciation, morphological divergence is often not congruent with genetic isolation, and genetic divergence estimates can indicate speciation events. With this in mind, several other studies (Bradley et al. 2008; Carroll and Bradley 2005; Carroll et al. 2005; Peppers et al. 2002) have supported the recognition of the three species (*S. hispidus*, *S. hirsutus*, and *S. toltecus*) based on mitochondrial data. The nuclear sequence data provided by Carroll and Bradley (2005) and this study also suggests the recognition of three species, owing to nonsister relationships shown between the three species in the individual and combined nuclear data.

All analyses performed in this study recovered four independent clades (I–IV); however, relationships between clades were not supported. Examination of genetic variation between the four independent clades identified in this study depicted similar divergence values between each clade (Table 1), based on Kimura two-parameter genetic distances (Kimura 1980). For *Cytb*, the largest genetic distance values (20.3%) were obtained between clade II (*fulviventer* species group) and clade IV (*alstoni* species group), whereas the smallest genetic distance (17.1%) was between clades I (*hispidus* species group) and III (*S. peruanus*). Owing to similar genetic distance values between these clades, it appears that all four clades warrant the same level of recognition. Musser and Carleton (2005) suggested that *Sigmodon* be partitioned into two subgenera, *Sigmomys* and *Sigmodon*, with *S. alstoni* placed in *Sigmomys* because of the distinct morphological character of grooved incisors. The remaining members of *Sigmodon* were placed into the subgenus *Sigmodon* and divided into the *hispidus* and *fulviventer* species groups. The data herein would modify the arrangement by Musser and Carleton (2005) by the additional recognition of the *peruanus* species group.

Although this is the most comprehensive view of this genus to date, further studies are necessary that include missing taxa that may provide support for relationships between the proposed species groups within the genus *Sigmodon*. Molecular data are needed for three currently recognized species (*S. inopinatus*, *S. planifrons* Nelson and Goldman, 1933, and *S. zanzonensis* Goodwin, 1932) that were not available for this study. Inclusion of *S. inopinatus* may provide insight as to the relationships of the South American species of *Sigmodon* to the North American species, and provide evidence for relationships between species groups. *Sigmodon planifrons* and *S. zanzonensis* were suggested to belong to the *S. hispidus* species group (Carleton et al. 1999), and inclusion of these taxa may lead to a better understanding of this group. The addition of nuclear genes by Carroll and Bradley (2005) and this study have provided greater resolution of relationships within *Sigmodon*. Additional nuclear genes will likely continue this trend and provide a more completely resolved phylogeny for the genus *Sigmodon*, especially in resolving the phylogenetic relationships of North American and South American taxa.

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Appendix A

The specimens examined are listed below by museum acronym (Hafner et al. 1997), specific identification numbers, collector numbers, or catalogue numbers, as well as GenBank accession numbers for each separate gene (*Cytb* / *Fgb-I7* / *Rbp3*). Sequence data not included for some individuals are represented by NI. Abbreviations for identification numbers are as follows: Abilene Christian University Natural History Collection (ACUNHC); 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 or TK); Museum of Vertebrate Zoology (MSV); Royal Ontario Museum (ROM); The Museum of Southwestern Biology (MSB); University of Texas Medical Branch at Galveston (T and FSH). In some cases, localities are provided with universal transverse mercator (UTM) coordinates. Sequences generated by this study for *Rbp3* are identified by accession nos. EU635696–EU635722, *Fgb-I7* are identified by accession nos. EU652888–EU652906, and *Cytb* are identified by accession nos. EU652907–EU652909; all other sequences were obtained from Gen-Bank.

Sigmodon alleni

LOCALITY: MÉXICO: Michoacán — 3.5 km N Tancotaro, 2353 m (TK45276, AF155425 / EU652888 / EU635696).

Sigmodon alstoni

LOCALITY: VENEZUELA: Portuguesa — Municipality Guanare, Gato Negro (Just S Guanare) (T2140, AF293396 / EU652889 / EU635697); Municipio de Guanarito, La Arenosa (TK53588, EU652907 / AY459384 / EU635698).

Sigmodon arizonae

LOCALITY: MÉXICO: Durango — San Juan de Camarones, UTM 13, 385011E–2775718N (TTU81699, AF155423 / AY459383 / EU635699). UNITED STATES: Arizona — Maricopa Co., Phoenix Zoological Park (TK115325, EU652908 / EU652890 / EU635700).

Sigmodon fulvivent

LOCALITY: MÉXICO: Durango — 2.2 km S, 2.5 km E Vicente Guerrero (TK48915, AF293400 / EU652891 / EU635701). UNITED STATES: Texas — Jeff Davis County, 2.4 km W Point of Rocks Park (MWSU17910, AF293399 / AY459381 / EU635702).

Sigmodon hirsutus

LOCALITY: HONDURAS: Francisco Morazan — El Picacho Zoological Parque, UTM 16, 497451E–1561275N (TTU83741, NI / AY459368 / NI); Olancho — 4 km E Catcamas, Escuela de Sembrador, UTM 16, 624523E–1637511N (TTU84767, NI / AY459366 / NI); Valle — 3 km N, 9 km SW San Lorenzo, UTM 16, 442952E–1486788N (TTU83794, NI / AY459367 / NI). MÉXICO: Chiapas — 14.4 km N Ocozocoautla, UTM 15, 451772E–1864243N (TK93324, NI / AY459379 / NI); Ixtapa — 12 km SE of Ixtapa (ROM97578, AF425197 / EU652892 / EU635704); Oaxaca — 2 km S La Blanca, UTM 15, 318523E–1833293N (TTU82796, AF425194 / AY459376 / EU635703). PANAMÁ: Chiriquí — Hotel La Siesta, by airport (TTU39163, AF155416 / EU652893 / EU635705).

Sigmodon hispidus

LOCALITY: MÉXICO: Tamaulipas — 12.8 km S la Carbonera, UTM 14, 627591E–2711834N (TTU108155, EU073177 / EU652895 / EU635708). UNITED STATES: Florida — Brevard County, Sebastian Inlet State Park (TTU97861, AF425208 / AY459373 / EU635706); Kansas — Ellis County, Hays (OK5830, NI / AY459372 / NI; OSU13227, AF425209 / EU652894 / EU635707); Louisiana — East Baton Rouge Parish (LSUMZ28519, NI / AY459370 / NI); Tennessee — Shelby County, Meeman Biological Station (TTU79181, AF425202 / EU652896 / EU635709); Texas — Dimmit County, Chaparral Wildlife Management Area, UTM 14, 458437E–3134279N (TTU80759, AF425200 / AY459371 / EU635710).

Sigmodon leucotis

LOCALITY: MÉXICO: Durango — 12 km E Ojitos, UTM 13, 385011E–2775718 (TTU81692, AF293401 / AY459386 / EU635711; TTU81693, EU652909 / EU652897 / EU635712).

Sigmodon mascotensis

LOCALITY: MÉXICO: Jalisco — 2 km NW Mesconitos, UTM 13, 808753E–2375677N (TTU82793, AF425215 / AY459385 / EU635715); Michoacán — Patzcuaro (Lake) (JCP1061, AF296188 / AY459374 / EU635713); Oaxaca — Las Minas (TTU82794, AF425217 / EU652898 / EU635714).

Sigmodon ochrognathus

LOCALITY: MÉXICO: Durango — 24 km N Las Herreras (CRD1033, AF155422 / AY537531 / EU635716). UNITED STATES: Arizona — Cochise County, 3.2 km W Portal (ACUNHC500, AF155592 / EU652899 / EU635717).

Sigmodon peruanus

LOCALITY: ECUADOR El Oro — Progreso (MSB140112, AF293395 / EU652900 / EU635718); Guayas, Manglares Churute, Cerro Cimalon, UTM 17, 650092E–9732559N (TTU103494, EU073179 / EU652901 / EU635719).

Sigmodon toltecus

LOCALITY: GUATEMALA: El Petén — El Remate (ROM99644, NI / AY459378 / NI); 10 km N Tikal (ROM99640, AF425222 / AY459375 / EU635721). MÉXICO: Chiapas — 14.4 km N Ocozocoautla, UTM 15, 451772E–1864243N (TTU108152, AF425228 / AY459380 / EU635722); Veracruz — Paso del Patel, UTM 14, 665394E–2282430N (TTU105062, EU073182 / EU652902 / EU635720).

Outgroup taxa are as follows

Holochilus chacarius

LOCALITY: PARAGUAY: Presidente Hayes — Estancia Loma Pora (TTU104423, DQ227455 / EU652904 / EU649048).

Oryzomys couesi

LOCALITY: HONDURAS: Olancho — 4 km E Catacamas, Escuela de Sembrador (TK 102040, DQ185383 / EU652903 / EU273426).

Reithrodon auritus

LOCALITY: ARGENTINA: Rio Negro — Las Victorias (MVZ182704, EU579474 / EU652906 / AY163634).

Zygodontomys brevicauda

LOCALITY: VENEZUELA: Portuguesa — La Arenosa. (TTU76306, EU579519 / EU652905 / EU649075).

Fig. 1. Phylogenetic tree obtained from Bayesian analysis of *Rbp3* nuclear gene of 27 samples of *Sigmodon*. Major clades are indicated by Roman numerals (I–IV). Posterior probabilities ≥ 95 are represented by asterisks above branches and parsimony bootstrap support values are reported below.

Fig. 2. Phylogenetic tree obtained from Bayesian analysis of *Fgb 17* nuclear gene of 35 samples of *Sigmodon*. Major clades are indicated by Roman numerals (I–IV). Posterior probabilities ≥ 95 are represented by asterisks above branches and parsimony bootstrap support values are reported below.

Fig. 3.

Cytb and nuclear gene phylogenetic tree obtained from Bayesian analysis of 27 samples of *Sigmodon*. Major clades are indicated by Roman numerals (I–IV) and subclades as letters (A–C). Posterior probabilities ≥ 95 are represented by asterisks above branches and parsimony bootstrap support values are reported below.

Table 1

Mean Kimura two-parameter (Kimura 1980) genetic distances for cytochrome *b* (*Cytb*), interphotoreceptor retinoid binding protein (*Rbp3*), and beta-fibrinogen (*Fgb-I7*) genes between species and clades of *Sigmodon*.

Taxa	Mean genetic distance		
	<i>Cytb</i>	<i>Rbp3</i>	<i>Fgb-I7</i>
<i>S. alleni</i> vs. <i>S. hirsutus</i>	9.87	0.73	0.43
<i>S. alleni</i> vs. <i>S. hispidus</i>	13.90	0.67	0.40
<i>S. arizonae</i> vs. <i>S. mascotensis</i>	8.54	0.73	0.36
<i>S. hirsutus</i> vs. <i>S. hispidus</i>	12.30	1.15	0.64
<i>S. hirsutus</i> vs. <i>S. toltecus</i>	11.96	1.24	1.60
<i>S. hirsutus</i> vs. <i>S. ochragnathus</i>	13.76	0.92	2.33
<i>S. hispidus</i> vs. <i>S. toltecus</i>	12.69	0.28	1.37
<i>S. hispidus</i> vs. <i>S. mascotensis</i>	10.80	0.73	0.56
<i>S. hispidus</i> vs. <i>S. fulviventer</i>	19.18	2.02	4.96
<i>S. hispidus</i> vs. <i>S. ochragnathus</i>	12.32	1.05	2.10
<i>S. ochragnathus</i> vs. <i>S. fulviventer</i>	18.19	1.73	6.38
<i>S. fulviventer</i> vs. <i>S. leucotis</i>	19.46	1.34	3.84
<i>S. fulviventer</i> vs. <i>S. alstoni</i>	20.78	1.73	5.81
<i>S. fulviventer</i> vs. <i>S. peruanus</i>	19.24	2.60	5.62
<i>S. peruanus</i> vs. <i>S. hirsutus</i>	17.94	2.59	3.95
<i>S. peruanus</i> vs. <i>S. leucotis</i>	17.72	2.90	4.87
<i>S. alstoni</i> vs. <i>S. hispidus</i>	18.20	2.07	3.67
<i>S. alstoni</i> vs. <i>S. leucotis</i>	19.87	1.92	5.34
Clade I vs. clade II	19.20	2.07	3.91
Clade I vs. clade III	17.10	2.55	5.02
Clade I vs. clade IV	17.70	2.00	3.91
Clade II vs. clade III	18.50	2.78	5.29
Clade II vs. clade IV	20.30	1.99	5.56
Clade III vs. clade IV	18.38	2.19	4.80

Note: Values of selected taxa and clades are shown.