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ANCIENT HYBRIDIZATION AND SUBSEQUENT MITOCHONDRIAL CAPTURE IN GROUND SQUIRRELS (GENUS *ICTIDOMYS*)

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ABSTRACT

The sister species *Ictidomys parvidens* and *I. tridecemlineatus* exhibit hybridization in southeastern New Mexico and western Texas. Presumably, hybridization was due to secondary contact as a result of recent anthropogenic changes to the landscape. However, recent phylogenetic studies of *Spermophilus*, which previously included *Ictidomys*, indicated the possibility of widespread introgression due to paraphyly of haploytypes in the mitochondrial cytochrome-*b* (*Cyt*b) gene. Samples of *Ictidomys* were obtained from 38 populations ($N = 211$) in the presumed zone of sympatry, as well as samples from 58 populations ($N = 208$) throughout the distribution of both sister species. DNA sequences from the *Cyt*b gene ($N = 419$) and the Y-linked structural maintenance of chromosomes (*SmcY*) gene ($N = 129$) were used to determine the origin of paraphyly among *Cyt*b haplotypes. In addition, divergence date estimates were calculated to determine the association between hybridization and climate oscillations. Analyses of *Cyt*b sequences indicated a unique mitochondrial haplotype in both parental types and putative hybrids within the zone of sympatry but provided no evidence of contemporary mitochondrial introgression. However, analyses of the *SmcY* gene supported contemporary hybridization at six localities. These data, along with estimates of time since divergence, suggested an ancient hybridization event during the Pleistocene, followed by capture of the *I. tridecemlineatus* mitochondrial haplotype by *I. parvidens*. Glacial oscillations during this period of time would have provided multiple opportunities for sympatry between these two species, increasing the potential for ancient hybridization. Whether contemporary hybridization is the result of a recent warming climate or an ancillary effect of anthropogenic habitat alterations is yet to be determined.

Key words: *Cyt*b, hybridization, *Ictidomys*, mitochondrial capture, Pleistocene, *SmcY*

INTRODUCTION

Hybridization is the process where two lineages interact and produce offspring of mixed ancestry (Barton and Hewitt 1985). Depending on the extent of isolation, the potential for interbreeding may diminish over time and limit introgression between each lineage. However, recurrent, or contemporary, hybridization may be sufficient to maintain gene flow and inhibit reproductive isolation. In the case of ancient (historic) hybridization, both lineages have evolved sufficient differences to severely hinder nuclear introgression, but patterns of introgression often persist in uniparentally-inherited markers (Good et al. 2008). Therefore, evaluating independent genetic markers is essential to understand the evolutionary history of hybridizing taxa (Coyner et al. 2015).

Hybridization has been reported in many rodent species, including ground squirrels (Shurtliff 2013). For example, the genus *Spermophilus* (sensu lato; Helgen et al. 2009) hybridizes under natural conditions in at least nine pairs of species (Nadler et al. 1971; Zimmerman and Cothran 1976; Koeppl et al. 1978; Hafner and Yates 1983; Ermakov et al. 2002; Spiridonova et al. 2005; Tsvirka et al. 2006). A case in point is the sister species *Ictidomys parvidens* and *I. tridecemlineatus* (formerly *Spermophilus mexicanus* sensu lato and *S. tridecemlineatus*, respectively; Helgen et al. 2009). *Ictidomys parvidens* tends to be a larger animal with a buffy brown coat and nine rows of whitish spots on its dorsum (Young et al. 1982). *Ictidomys tridecemlineatus* typically is smaller than its cousin, named for its distinct pattern of thirteen alternating dark and light dorsal stripes (Streubel and Fitzgerald 1978). It is only at areas of contact where these morphologies become difficult to discern. However, allozymic (Cothran et al. 1977), karyotypic (Nadler and Hughes 1966; Zimmerman and Cothran 1976; Cothran and Honeycutt 1984), and detailed morphologic studies (Cothran 1983) have suggested that the two species hybridize and form a zone of sympatry across southeastern New Mexico and western Texas where their distributions abut (Cothran et al. 1977; Cothran 1983; Schmidly 2004).

Recent studies of the phylogenetic relationships of the genus *Spermophilus* (sensu lato; Harrison et al. 2003; Herron et al. 2004) also indicated the possibility of widespread introgression between members of the genus *Ictidomys*. Examination of the mitochondrial cytochrome-*b* (*Cyt*b) gene revealed a paraphyletic arrangement of sequences between these taxa. Typically, paraphyletic arrangements of alleles are attributed to hybridization events (contemporary or ancient) or incomplete lineage sorting and often occur in species with widespread geographic distributions that have close relatives with restricted distributions (Harrison 1998), such as the case with *I. parvidens* and *I. tridecemlineatus* (Harrison et al. 2003).

More recently, Stangl et al. (2012) reevaluated the potential for hybridization between these two taxa and identified several localities where *I. parvidens* and *I. tridecemlineatus* appeared to produce hybrid offspring. Sampling sites were limited to human-modified habitats (e.g., cemeteries, golf courses, parks) within the eastern portion of the presumed zone of sympatry. Comparisons of hindfoot length and pelage characteristics of putative wild-caught hybrids with laboratory-reared F_1 hybrids revealed a similarity in morphology, indicating several populations with putative hybrids. Hybrids only co-occurred with a single parental type, and parental types never were found in sympatry. Instead, parental types appeared to interdigitate parapatrically throughout the presumed zone of sympatry. Stangl et al. (2012) noted that the current distributions of each species, as well as hybridization, might be a product of the development of modern roadways in the early 1900s, which may have functioned as dispersal corridors across unfavorable habitats (Cothran 1983).

Using genetic markers, Thompson et al. (2013) were able to corroborate the results of Stangl et al. (2012). Their data indicated that hybrid populations were limited to small isolated areas where the two species' distributions came into contact. As a result of this isolation, gene flow between these species was restricted to hybrid populations and did not persist in adjacent parental populations. The fragmented nature of the geographic landscape likely contributed to the limited gene flow and the sporadic location of hybrid and parental populations, suggesting a mosaic hybrid zone. However, a common haplotype for the mitochondrial *Cyt*b gene was found within hybrid and adjacent populations (Thompson et al. 2013). Although Thompson et al. (2013) thoroughly examined hybridizing populations, they did not examine the geographic extent of this unique haplotype, nor the paraphyletic arrangement of *Cyt*b sequences noted in recent phylogenetic studies of the ground squirrels (Harrison et al. 2003; Herron et al. 2004).

Therefore, the primary objective of this study was to evaluate the phylogenetic and phylogeographic history of *I. parvidens* and *I. tridecemlineatus* to determine whether the previously reported paraphyletic arrangement of these taxa was the result of hybridization (contemporary or ancient) or incomplete lineage sorting, as well as determine the geographic extent of the unique haplotype of the mitochondrial *Cyt*b gene. In addition, divergence dates were estimated to determine the possible association between hybridization and historic oscillations in the climate. To address these objectives, the maternally-inherited *Cyt*b gene and the paternally-inherited (Y-linked) structural maintenance of chromosomes (*SmcY*) gene were sequenced to examine the contribution of the maternal and paternal lineages of these two species.

METHODS

Sampling.—Samples were obtained from 96 localities across the distributions of each species focusing primarily in the presumed zone of sympatry, which extends from western Texas into areas of southeastern New Mexico (Fig. 1 and Table 1). Tissue samples were obtained from 415 individuals (220 *I. parvidens*, 171 *I. tridecemlineatus*, and 24 morphologically identified hybrids; see Appendix) from natural history collections (Angelo State Natural History Collections; Midwestern State University; Natural Science Research Laboratory, Museum of Texas Tech University; New Mexico Museum of Natural History), as well as through collecting efforts for this study (132 individuals). Individuals collected for this study were classified tentatively by coat color and pattern using characteristics described by Hall (1981) and by hindfoot length (mm) using criteria described by Stangl et al. (2012; *I. parvidens*, mean of 40.6 ± 2.6; *I. tridecemlineatus*, mean of 34.3 ± 2.6). Voucher specimens and corresponding tissues were deposited in the Natural Science Research Laboratory, Museum of Texas Tech University. Animal care and use methods conformed to guidelines of the American Society of Mammalogists (Sikes et al. 2011) and were approved by the Texas Tech University Animal Care and Use Committee (ACUC protocol 11009-03).

PCR and sequencing methods.—DNA sequences for the *Cyt*b and *SmcY* genes were either generated in this study or obtained from GenBank. For data generated herein, total genomic DNA was isolated from approximately 0.1 g of frozen liver, kidney, or muscle tissue using the Puregene DNA isolation kit (Gentra, Minneapolis, Minnesota). For *Cyt*b, the entire gene (1,140 base pairs [bp]) was amplified with the polymerase chain reaction (PCR; Saiki et al. 1988) using primers L14725 and H15915* (Steppan et al. 1999; Harrison et al. 2003). The thermal profile was as follows: initial denaturation at 95°C for 2 min; 35 cycles of denaturation at 95°C for 30 s, annealing of 52°C for 30 s, and extension at 72°C for 2 min; and a final elongation of 72°C for 10 min. For *SmcY*, the first 382 bp of the 5' end of intron 8 were amplified using primers SmcF (5'-GTAAGTCTTGTCCATCATAGATGTTT-3') and Smc380R (5'-AACGTATTTTAGCAAAACAAAAGCA-3') developed for this study. The thermal profile was as follows: initial denaturation at 95°C for 2 min; 35 cycles of denaturation at 95°C for 30 s, annealing of 52°C for 30 s, and extension at 72°C for 2 min; and a final elongation of 72°C for 10 min.

PCR products were purified using the Exosap-II PCR purification kit (USB Corporation, Cleveland, Ohio). Amplified gene products were sequenced on an ABI 3100-Avant using ABI Prism Big Dye v3.1 terminator technology (Applied Biosystems, Foster City, California). For *Cyt*b, the two PCR primers were used as sequencing primers, along with primers (L14931, L15135, L15318, L15519, and L15702) reported in Harrison et al. (2003). In addition, the following primers were developed and used for sequencing: Spero300R (5'-CTCGGCCTACATGAAGGAAA-3'), Spero440R (5'-GTAATTACGGTTGCCCCTCA-3'), Spero665R (5'-GGGTGAAAGGGGATTTTGTC-3'), and Spero785R (5'-AAAGGATTTGCGGGTGTGTA-3'). For the *SmcY* gene, the PCR primers (listed above) were used as sequencing primers. Cycle sequencing reactions were purified using isopropanol cleanup protocols. Sequences were contiged and proofed using Sequencher 4.10.1 software (Gene Codes, Ann Arbor, Michigan) and chromatograms were examined to verify nucleotide bases and to inspect sequences for heterozygous sites, which were coded following the International Union of Biochemistry (IUB) polymorphic code. The ClustalW algorithm (Thompson et al. 1994) in MEGA 5.05 software (Tamura et al. 2011) was used to align sequences. Sequences were examined for the presence of stop codons and pseudogenes. GenBank accession numbers and corresponding catalog numbers are provided in the Appendix.

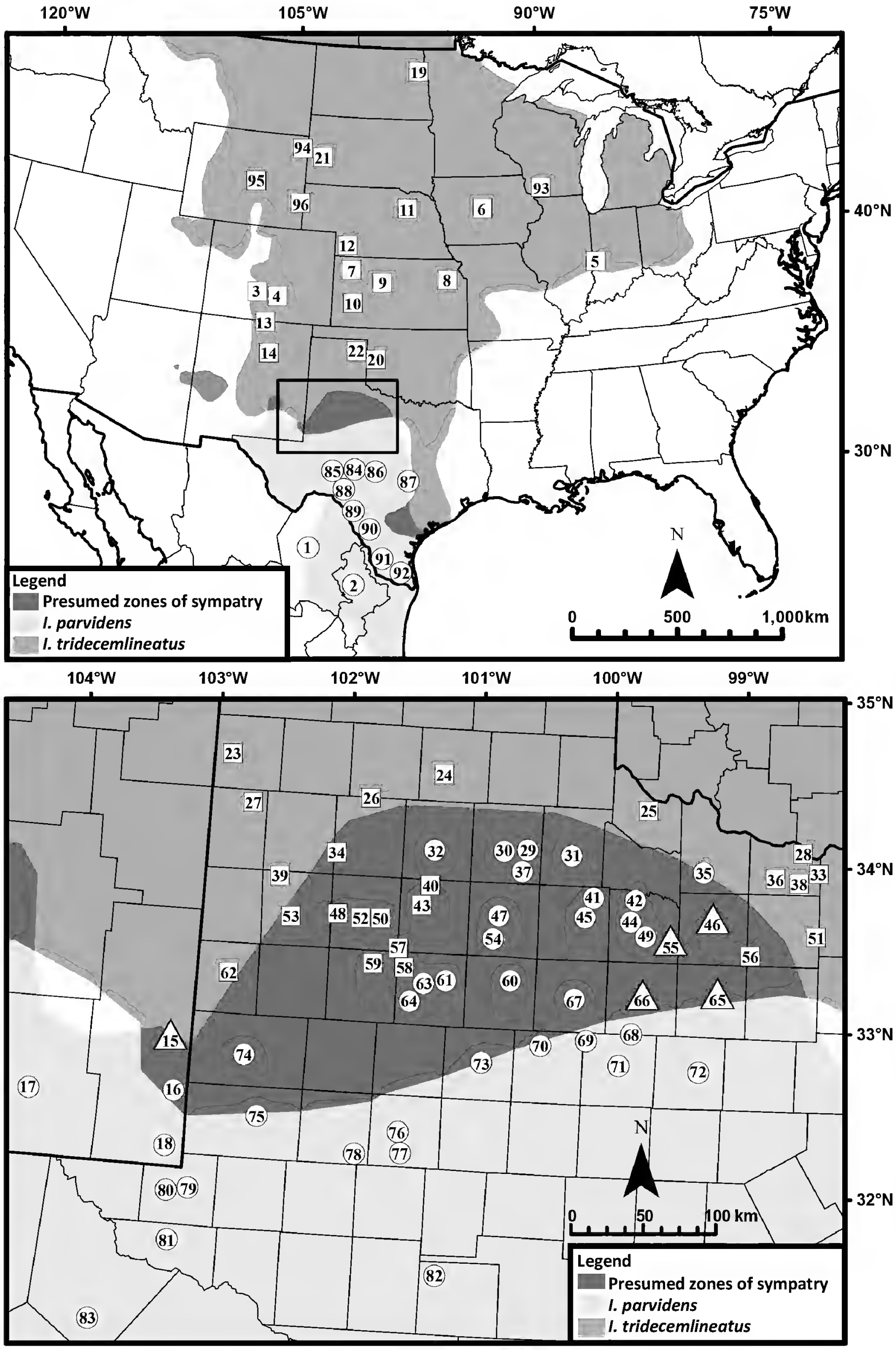


Figure 1. Maps illustrating localities of the populations of ground squirrels (genus *Ictidomys*) examined in this study. Top map shows locations of populations examined across the entire distribution of both species. Bottom map shows the area indicated within the black box in the top map. In both maps, circles indicate locations where all specimens were identified morphologically as *I. parvidens*. Squares indicate locations where all specimens were identified morphologically as *I. tridecemlineatus*. Triangles indicate locations containing both species and where specimens were identified morphologically as hybrids. Numbers within the respective shapes indicate the locality number referenced in Table 1 and in the Appendix.

Table 1. Summary of specimens examined based on field, mitochondrial cytochrome-*b* gene (*Cyt*b), and Y-linked structural maintenance of chromosomes gene (*SmcY*) identifications. Locality # corresponds to the numbers depicted on the map in Fig. 1. Numbers of specimens identified by each method is indicated for each locality. *SmcY* haplotypes indicated in Fig. 3 are listed for corresponding localities (e.g., P0, T23, etc.). Abbreviations used were as follows: COA = Coahuila; COL = Colorado; IA = Iowa; IN = Indiana; KS = Kansas; ND = North Dakota; NE = Nebraska; NL = Nuevo Leon; NM = New Mexico; OK = Oklahoma; SD = South Dakota; TX = Texas; WI = Wisconsin; WY = Wyoming; Field ID = field identification; *Cyt*b = *Cyt*b identification; *SmcY* = *SmcY* identification; hyb = morphological hybrid; par = *Ictidomys parvidens*; tri = *I. tridecemlineatus*; tri-like = *I. tridecemlineatus*-like *Cyt*b haplotype; P(plus number) = *I. parvidens SmcY* haplotype; T(plus number) = *I. tridecemlineatus SmcY* haplotype; and N/A = data not available.

Locality #	State	Locality Name	Field ID	*Cyt*b	*SmcY*
1	COA	1.5 mi. NW Ocampo	3 par	3 par	3 par (P0)
2	NL	3 km NE Apodaca	2 par	2 par	N/A
3	COL	5 mi. S, 5 mi. E Villa Grove	1 tri	1 tri	N/A
4	COL	Baca Grande Golf Course	4 tri	4 tri	1 tri (T22)
5	IN	Rose Lawn Cemetery	6 tri	6 tri	N/A
6	IA	Oaks Golf Course	5 tri	5 tri	1 tri (T25)
7	KS	Colby	1 tri	1 tri	N/A
8	KS	Topeka	5 tri	5 tri	1 tri (T5)
9	KS	Hays	6 tri	6 tri	1 tri (T5)
10	KS	Garden City	2 tri	2 tri	N/A
11	NE	0.7 mi. W Oakdale	1 tri	1 tri	N/A
12	NE	Wauneta Country Club	1 tri	1 tri	1 tri (T5)
13	NM	Urraca State Wildlife Area	2 tri	2 tri	2 tri (T5)
14	NM	Las Vegas	5 tri	5 tri	2 tri (T5)
15	NM	Hobbs	29 par, 9 tri	38 tri-like	3 par (P1, P4); 1 tri (T5)
16	NM	Eunice	2 par	2 tri-like	1 par (P1)
17	NM	Carlsbad	13 par	13 par	4 par (P1, P2, P4); 1 tri (T3)
18	NM	Jal	3 par	3 tri-like	2 par (P1)
19	ND	2 mi. N Arvilla	4 tri	4 tri	2 tri (T25, T29)
20	OK	Cheyenne Cemetery	2 tri	2 tri-like	N/A
21	SD	Rapid City	1 tri	1 tri	N/A
22	TX	Gene Howe Wildlife Management Area	1 tri	1 tri-like	1 tri (T7)
23	TX	Bovina	1 tri	1 tri-like	N/A
24	TX	Silverton Cemetery	4 tri	4 tri-like	1 tri (T17)

Table 1. (cont.)

Locality #	State	Locality Name	Field ID	*Cyt*b	*SmcY*
25	TX	Quanah Cemetery	4 tri	4 tri-like	N/A
26	TX	Alton Painter Ranch	9 tri	9 tri-like	4 tri (T7)
27	TX	Muleshoe	1 tri	1 tri-like	N/A
28	TX	Burkburnett Cemetery	4 tri	4 tri-like	N/A
29	TX	11 mi. E Matador	5 par	5 tri-like	4 par (P1)
30	TX	Matador Cemetery	5 par	5 tri-like	1 par (P1)
31	TX	Paducah Cemetery	6 par	6 tri-like	3 par (P1, P6)
32	TX	Floydada	5 tri	5 tri-like	1 par (P1); 1 tri (T18)
33	TX	River Creek Golf Course	4 tri	4 tri-like	1 tri (T7)
34	TX	2 mi. S, 5 mi. W Cotton Center	4 tri	4 tri-like	2 tri (T7, T5)
35	TX	0.4 mi. NE Lockett	3 tri	3 tri-like	N/A
36	TX	Electra Cemetery	9 tri	9 tri-like	3 tri (T7, T24)
37	TX	8 mi. S, 9 mi. E Matador	9 par	9 tri-like	2 par (P1)
38	TX	Wichita Falls	13 tri	13 tri-like	7 tri (T7, T23)
39	TX	14 mi. N, 6 mi. W Levelland	1 tri	1 tri-like	N/A
40	TX	2 mi. E Cone	1 tri	1 tri-like	1 tri (T7)
41	TX	11.8 mi. N, 18.3 mi. W Benjamin	1 par	1 tri-like	1 par (P1)
42	TX	Truscott Cemetery	2 par	2 tri-like	1 par (P1)
43	TX	Ralls Cemetery	3 tri	3 tri-like	1 tri (T7)
44	TX	Benjamin Cemetery	4 par	4 tri-like	1 par (P1)
45	TX	Guthrie	2 par	2 tri-like	N/A
46	TX	Seymour	15 hyb, 1 par, 3 tri	19 tri-like	2 par (P1, P12); 2 tri (T5, T7)
47	TX	Dickens Cemetery	2 par	2 tri-like	1 par (P1)
48	TX	Reese Air Force Base Golf Course	1 tri	1 tri-like	N/A
49	TX	3.5 mi. E Benjamin	1 par	1 tri-like	N/A
50	TX	2 mi. S, 5 mi. E Tahoka	1 tri	1 tri-like	1 tri (T21)
51	TX	Windthorst	4 tri	4 tri-like	2 tri (T7)
52	TX	Lubbock	7 tri	7 tri-like	2 tri (T7)
53	TX	5 km S Levelland	10 tri	10 tri-like	4 tri (T5, T20)

Table 1. (cont.)

Locality #	State	Locality Name	Field ID	*Cyt*b	*SmcY*
54	TX	Spur	10 par	10 tri-like	6 par (P1, P8)
55	TX	Goree Cemetery	2 hyb, 2 par	4 tri-like	2 par (P1)
56	TX	Megargel Cemetery	1 tri	1 tri-like	N/A
57	TX	Southland	2 tri	2 tri-like	N/A
58	TX	3 km S, 0.6 km E Southland	7 tri	7 tri-like	2 tri (T7, T19)
59	TX	Green Memorial Park	4 tri	4 tri-like	1 tri (T7)
60	TX	Clairemont Fairgrounds	3 par	3 tri-like	2 par (P1)
61	TX	Roadside Park	4 par	4 tri-like	1 par (P1)
62	TX	1 mi. E Plains	1 tri	1 tri-like	N/A
63	TX	Post	8 par	8 tri-like	2 par (P1, P10)
64	TX	Wayne Stewart Ranch	2 par	2 tri-like	1 tri (T)
65	TX	Throckmorton Cemetery	3 hyb, 8 par	11 tri-like	3 par (P12, P15, P16); 1 tri (T7)
66	TX	Haskell	1 hyb, 2 par	3 tri-like	N/A
67	TX	Aspermont	6 par	6 tri-like	2 par (P1, P10)
68	TX	Stamford Cemetery	2 par	2 tri-like	1 par (P10)
69	TX	Hamilton Cemetery	4 par	4 tri-like	2 par (P1, P13)
70	TX	Rotan Cemetery	2 par	2 tri-like	N/A
71	TX	1 mi. E Anson	11 par	11 tri-like	7 par (P1, P12)
72	TX	Albany	7 par	7 tri-like	5 par (P1, P14)
73	TX	Snyder Cemetery	3 par	3 tri-like	N/A
74	TX	Gaines County Cemetery	5 par	5 tri-like	1 par (P1)
75	TX	Andrews	13 par	13 tri-like	4 par (P1)
76	TX	Big Spring	4 par	4 tri-like	2 par (P1)
77	TX	Trinity Memorial Park	1 par	1 tri-like	N/A
78	TX	Stanton	4 par	4 tri-like	2 par (P1)
79	TX	Kermit	6 par	1 par, 5 tri-like	N/A
80	TX	Winkler County Airport	2 par	1 par, 1 tri-like	2 par (P1)
81	TX	Pyote	1 par	1 par	1 par (P1)
82	TX	11.5 mi. N, 20 mi. W Mertzon	1 par	1 tri-like	N/A

Table 1. (cont.)

Locality #	State	Locality Name	Field ID	*Cyt*b	*SmcY*
83	TX	Saragosa Cemetery	1 par	1 par	N/A
84	TX	Sonora	2 par	2 tri-like	N/A
85	TX	Eugene Miller Ranch	1 par	1 tri-like	N/A
86	TX	Junction	10 par	10 tri-like	N/A
87	TX	Austin	2 par	2 tri-like	N/A
88	TX	Turkey Track Canyon	1 par	1 par	N/A
89	TX	1 mi. S Highway 90 on Kingsway in Del Rio	1 par	1 par	N/A
90	TX	B & M Ranch	1 par	1 par	1 par (P9)
91	TX	FM Road 649	1 par	1 par	N/A
92	TX	Bentsen Rio Grande Valley State Park	1 par	1 par	1 par (P11)
93	WI	Ludden Lake Golf Course	5 tri	5 tri	N/A
94	WY	Sundance	1 tri	1 tri	N/A
95	WY	Engel King Ranch	2 tri	2 tri	2 tri (T5)
96	WY	Torrington Country Club	2 tri	2 tri	N/A
N/A	N/A	Laboratory F_1 Hybrids	3 hyb	3 tri-like	1 tri (T7)

Data analyses.—Estimates of nucleotide composition, polymorphism, and nucleotide diversity (π) were generated using MEGA 5.05. Tajima's D (Tajima 1989), which tests for departure from equilibrium, was estimated for each gene using MEGA 5.05. A positive Tajima's D indicates a decrease in population size and/or balancing selection, whereas a negative value is indicative of a population size expansion and/or purifying selection.

Unique haplotypes were identified using the program TCS 1.21 (Clement et al. 2000). Sequences from all individuals were combined within the analysis of each gene. TCS also was used to create statistical parsimony networks, using a 95% connection limit with gaps treated as the 5th state. Each dataset was reduced to include only unique haplotypes for maximum-likelihood and Bayesian analyses. Maximum-likelihood analyses were conducted using the program MEGA 5.05 using the nearest-neighbor-interchange (NNI) heuristic search method. Models of substitution selected for the maximum likelihood analyses inferred by Modeltest version 3.7 (Posada and Crandall 1998) were HKY + I + Γ and F81 + I for the *Cyt*b and *SmcY* datasets, respectively. MrBayes 3.2 (Ronquist et al. 2012) was used to obtain Bayesian phylogenies for each dataset. The Akaike information criterion was used to determine the appropriate model of substitution for each individual gene using the program MrModeltest version 2.2 (Nylander 2004). Models of substitution chosen for the *Cyt*b and *SmcY* datasets were HKY + I + Γ and F81 + I, respectively. Each Bayesian analysis was run for 1.0 X 10^7 generations with 1,000 generations sampled and 10% burn-in. Tracer v1.5 (Drummond and Rambaut 2003) was used to check for sufficient mixing (efficiency of sampling of the Markov chain Monte

Carlo algorithm of a parameter or set of parameters) and convergence stability for all parameters within each Bayesian analysis. *Cynomys ludovicianus* (GenBank AF157890), *I. mexicanus* (GenBank AF157848), *Poliocitellus franklinii* (GenBank AF157893), and *Xerospermophilus spilosoma* (GenBank AF157911) were used as outgroup taxa for the *Cyt*b dataset in both analyses. *Spermophilus fulvus* (GenBank AY898765) was used as the outgroup taxon for the *SmcY* dataset. Genetic distances within and between major clades for both genes (using the reduced datasets) were estimated with the program MEGA 5.05 following the Kimura 2-parameter distance model (Kimura 1980) and p-distance for the *Cyt*b and *SmcY* datasets, respectively.

Interspecific divergence dating of major clades of *Cyt*b sequences (produced during Bayesian analysis) and estimation of intraspecific historic demographic patterns was determined using the program BEAST v1.7 (Drummond et al. 2012). The molecular clock test in the program MEGA 5.05 was used to determine whether to accept or reject a strict molecular clock for each analysis. To determine divergence times for nodes among major clades of *Cyt*b sequences, a Yule tree prior using the sequences of five individuals chosen randomly from each major clade to maximize geographic representation was used. Outgroup taxa were the same as above.

Fossil calibrations were placed on the *Ictidomys* node (~0.3 mya; Dalquest and Shultz 1992) and the *C. ludovicianus*/*X. spilosoma* split (~2.5 mya; Goodwin 1995). Both calibrations used prior lognormal distributions to reflect the uncertainty in the fossil record. Means and standard deviations for each distribution were adjusted to have an upper bound of ~7.3 mya (Harrison et al. 2003), reflecting the most recent dated fossil predating the taxa in question. The model of substitution chosen for the interspecific analyses was HKY + Γ to minimize the effects of overparameterization on effective sample size (ESS). Test runs of 1.0 x 10^7 generations with a 10% burn-in were used to check for bias in sequences chosen for the analysis and to optimize for the final analysis. Results of test runs were compared using Bayes factors (Kass and Rafferty 1995; Suchard et al. 2001) calculated in Tracer v1.5 to determine parameters for final analyses. Two final runs of 2.0 x 10^7 generations were analyzed with log and tree files combined for final divergence estimates.

To estimate historic demographic patterns within each species, the piecewise-constant Bayesian skyline plot (BSP; Drummond et al. 2005) was used. Bayesian skyline plots estimate effective population size over time and allow for an estimation of the time to most recent common ancestor (TMRCA) for haplotypes included in the analysis (Lack et al. 2010). To account for possible mitochondrial introgression due to hybridization, only haplotypes from clades exclusively represented by a single taxon were used in the demographic analyses. For each species, a prior lognormal distribution was placed on root height to account for the estimated divergence between *I. parvidens* and *I. tridecemlineatus* as predicted by the interspecific BEAST analyses, following a similar calibration scheme as used for the *Ictidomys* node in the interspecific analyses. The model of substitution identified by the program MrModeltest version 2.2 was HKY + I for the intraspecific analyses. Test runs of 1.0 X 10^7 generations with a 10% burn-in were used to optimize the final analysis. One final simulation of 5.0 x 10^7 generations was run to produce a BSP for each species. All BEAST analyses were examined for sufficient mixing, convergence stability, and ESSs > 200 for all parameters using the program Tracer v1.5.

RESULTS

Patterns of sequence variation.—DNA sequences for the entire or a partial portion of *Cyt*b gene were obtained from each individual sampled (415 individuals; Table 1). Two hundred-five haplotypes were identified. Composition of nucleotides was 28.0% adenine, 26.9% cytosine, 12.9% guanine, and 32.3% thymine. Nucleotide substitutions were present at 204 variable sites (78 singletons and 126 parsimony informative sites). Nucleotide diversity for the *Cyt*b dataset was 0.02810. Tajima's D statistic (-2.36356) indicated population size expansion and/or purifying selection.

A partial sequence of the *SmcY* gene was obtained for 129 individuals, representing 57 localities across both species' ranges (Table 1). Twenty-seven haplotypes were identified. Composition of nucleotides was 34.2% adenine, 14.5% cytosine, 17.0% guanine, and 34.3% thymine. Nucleotide substitutions were present at 32 variable sites (24 singletons and 8 parsimony informative sites). Nucleotide diversity for the *SmcY* dataset was 0.07609. Tajima's D statistic was -1.44210, indicating a population size expansion and/or purifying selection.

Phylogenetic analyses.—Three major clades with posterior probabilities ≥ 95% were obtained from the maximum-likelihood and Bayesian analyses (Fig. 2A). One of these clades (Clade I; $N = 27$) consisted of individuals identified as *I. parvidens*. This clade included individuals from northern Mexico and southern Texas (1.07% average within-group genetic distance). The second clade (Clade II; $N = 338$) consisted of a group of individuals that were identified as *I. parvidens*, *I. tridecemlineatus*, or hybrids (0.73% average within-group genetic distance). Individuals in this group were mostly from the presumed zone of sympatry and adjacent areas in New Mexico, Oklahoma, and Texas (Table 1). A third clade (Clade III; $N = 50$) consisted of *I. tridecemlineatus* individuals found in localities primarily north of New Mexico and Texas (0.80% average within-group genetic distance). Mean genetic distances between clades were 4.10% between Clades I and II, 4.28% between Clades I and III, and 1.92% between Clades II and III. Parsimony network analysis identified two unconnected networks (not shown), producing the same relationships found with the maximum-likelihood and Bayesian analyses (i.e., 1st network = Clade I, 2nd network = Clades II and III).

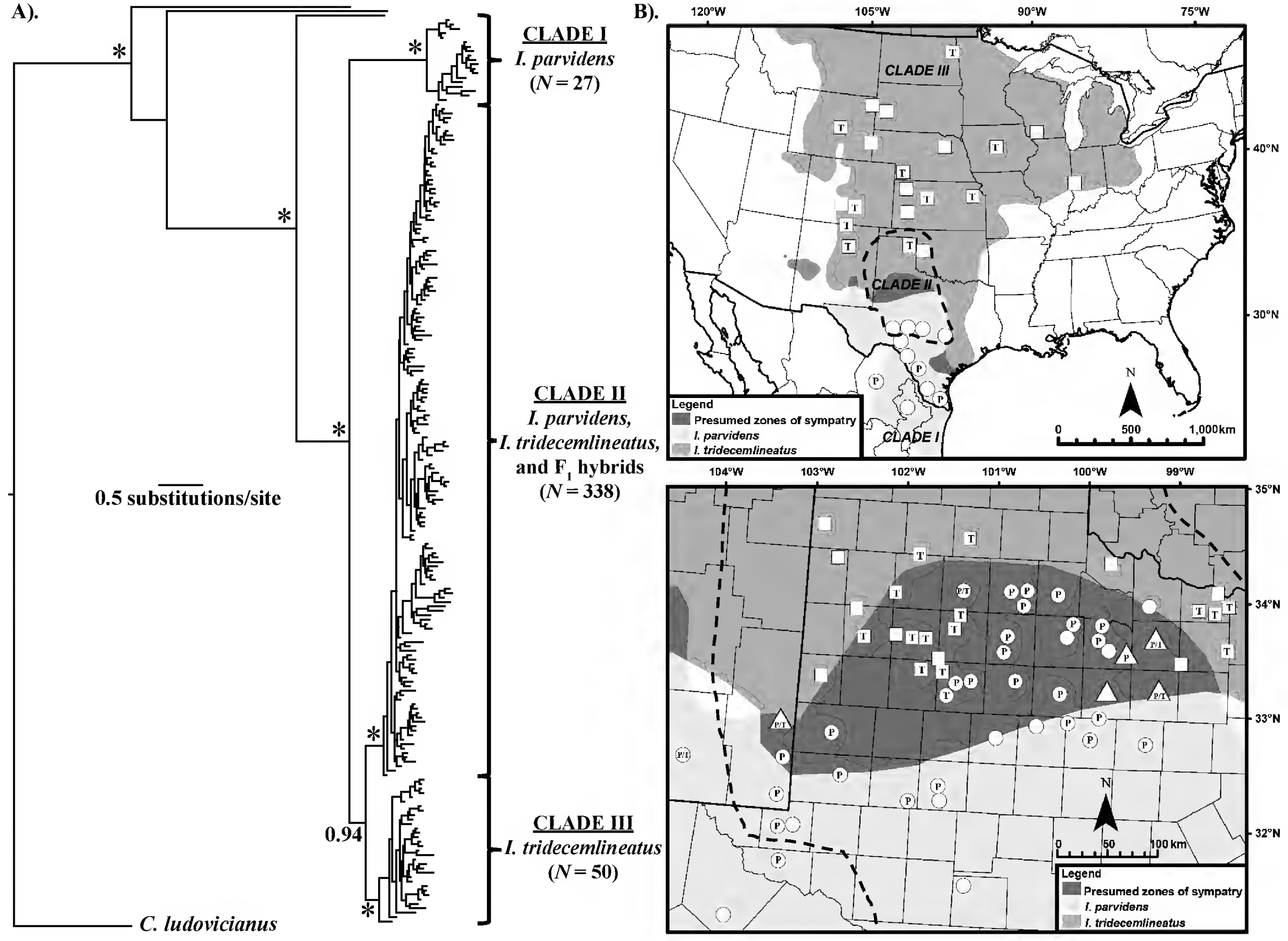
The maximum-likelihood and Bayesian analysis of the *SmcY* dataset resulted in an unresolved polytomy with no significant support values; therefore, these results were not considered further. Parsimony network analysis resulted in three unconnected networks (Fig. 3). Network 1 contained individuals that were identified primarily as *I. parvidens* based on morphology; whereas Network 2 and Network 3 represented mostly individuals morphologically identified as *I. tridecemlineatus*. Seven individuals identified by morphology from the alternate species were included within Network 1 (TTU115588, TTU115592, and TTU115600) and Network 2 (MWSU22285, MWSU22380, TTU54340, and TTU115524). Three of these individuals (TTU115524, TTU115588, and TTU115592) were from localities previously known to contain hybrid individuals (localities 15 and 46), but four individuals (MWSU22285, MWSU22380, TTU54340, and TTU115600) were from localities in close proximity to the boundary of each species' distribution (localities 17, 32, 64, and 65), suggesting the possibility of hybridizing populations (see Fig. 2B and Table 1). Five of these localities (15, 17, 32, 46, and 65) had haplotypes of both species. Network 3 did not have any individuals identified as the alternate species.

Further support for these groupings was obtained from the examination of diagnostic indels (insertions or deletions). Specifically, both *I. parvidens* and *I. tridecemlineatus* (excluding individuals from Iowa and North Dakota, i.e., Network 3) have two deletions compared to the outgroup (*S. fulvus*). Each species has a single diagnostic deletion (i.e., *I. parvidens*, 16-base deletion; *I. tridecemlineatus*, 9-base deletion) and share a single 2-base deletion. Individuals from Network 3 have a single base insertion.

Divergence dating and historic demographic patterns.—A strict molecular clock model was rejected for the interspecific BEAST analyses for divergence dating. Interspecific dating for major *Cyt*b clades yielded a mean rate of evolution of 0.02 substitutions per site per million years (95% highest posterior density [HPD]: 0.01–0.03). The Yule birth rate was estimated to be 0.77 (95% HPD: 0.36–1.24). The divergence dates of these clades indicated that the initial radiation of species within *Ictidomys* began approximately 2.20 mya (95% HPD: 1.22–3.74) with the split of *I. mexicanus* from the *I. parvidens*/*I. tridecemlineatus* complex during the Lower Pleistocene (see Fig. 4). The initial divergence between *I. parvidens* and *I. tridecemlineatus* began approximately 1.43 mya (95% HPD: 0.75–2.50). This split led to the divergence of individuals of *Cyt*b Clade I from individuals of *Cyt*b Clades II and III. Divergence of individuals of Clade II from individuals of Clade III occurred approximately 0.80 mya (95% HPD: 0.34–1.64).

For the intraspecific BEAST analyses, the *Cyt*b dataset was truncated to only include haplotypes identified as *I. parvidens* ($N = 19$) and *I. tridecemlineatus* ($N = 32$). A strict molecular clock was rejected for I. *parvidens* but not for *I. tridecemlineatus*. The BSP for

Figure 2. A) Tree obtained from the Bayesian analysis of the mitochondrial *Cyt*b dataset for *Ictidomys*. Asterisks (*) above branches represent Bayesian posterior probabilities ≥ 95%, except where indicated. Branch lengths reflect genetic divergence among taxa. B) Maps illustrating the geographic distribution of *Cyt*b clades shown in Fig. 2A and the geographic distribution of *SmcY* haplotypes. Bottom map shows the relative area indicated by the dashed line in the top map. This area represents the general distribution of *Cyt*b haplotypes found in Clade II. In both maps, circles indicate locations where all specimens were identified morphologically as *I. parvidens*. Squares indicate locations where all specimens were identified morphologically as *I. tridecemlineatus*. Triangles indicate locations containing both species and where specimens were identified morphologically as hybrids. *SmcY* haplotypes are indicated as P (*I. parvidens*), T (*I. tridecemlineatus*), or P/T (*I. parvidens* and *I. tridecemlineatus*) at each location where data is available.



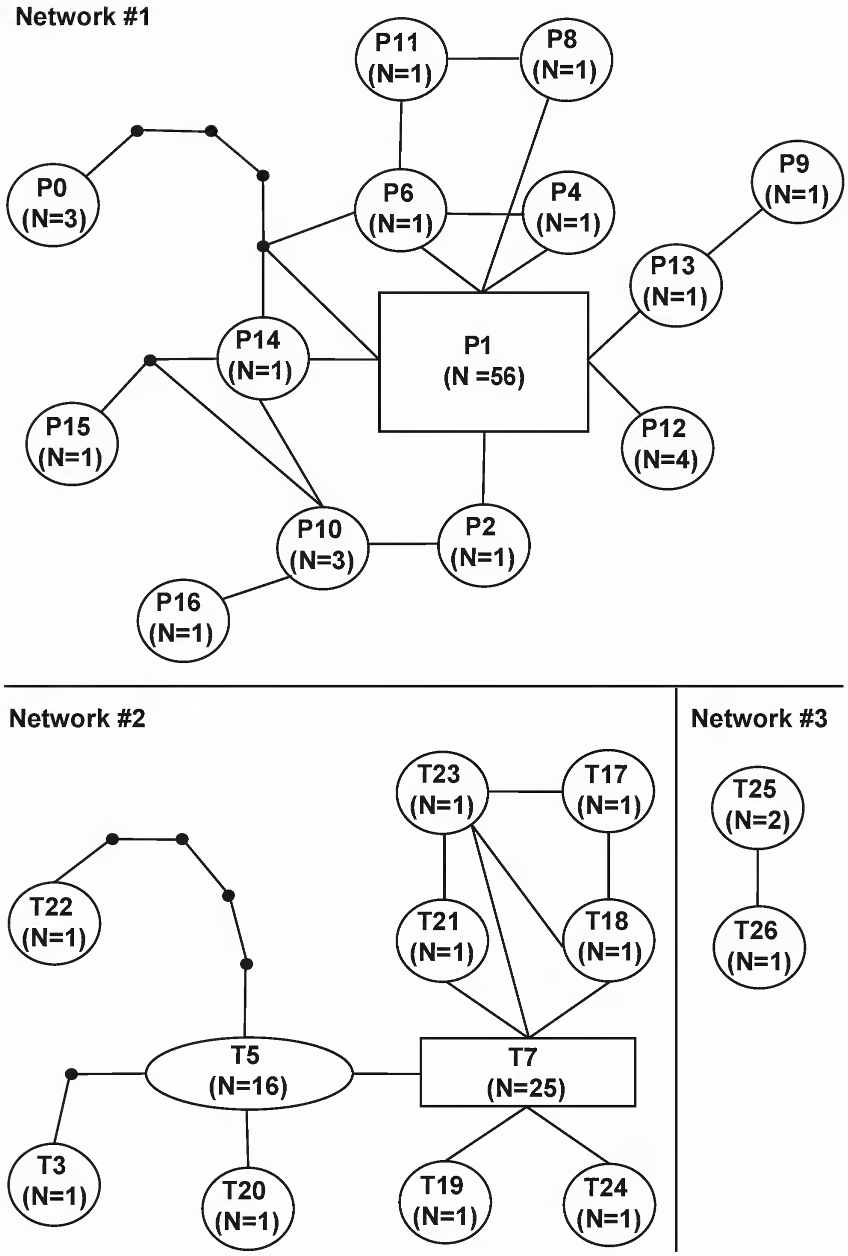


Figure 3. Parsimony networks of the *SmcY* dataset for *Ictidomys*. Network 1 represents haplotypes of specimens predominately identified as *I. parvidens*, and Networks 2 and 3 represent haplotypes of specimens predominately identified as *I. tridecemlineatus*. Sampled haplotypes are represented by ovals or squares (likely outgroup for each network) and are distinguished by P (*I. parvidens*) and a number or T (*I. tridecemlineatus*) and a number, corresponding to Table 1. Total number of individuals (N) of a particular haplotype is indicated within each shape. Closed circles represent unsampled haplotypes.

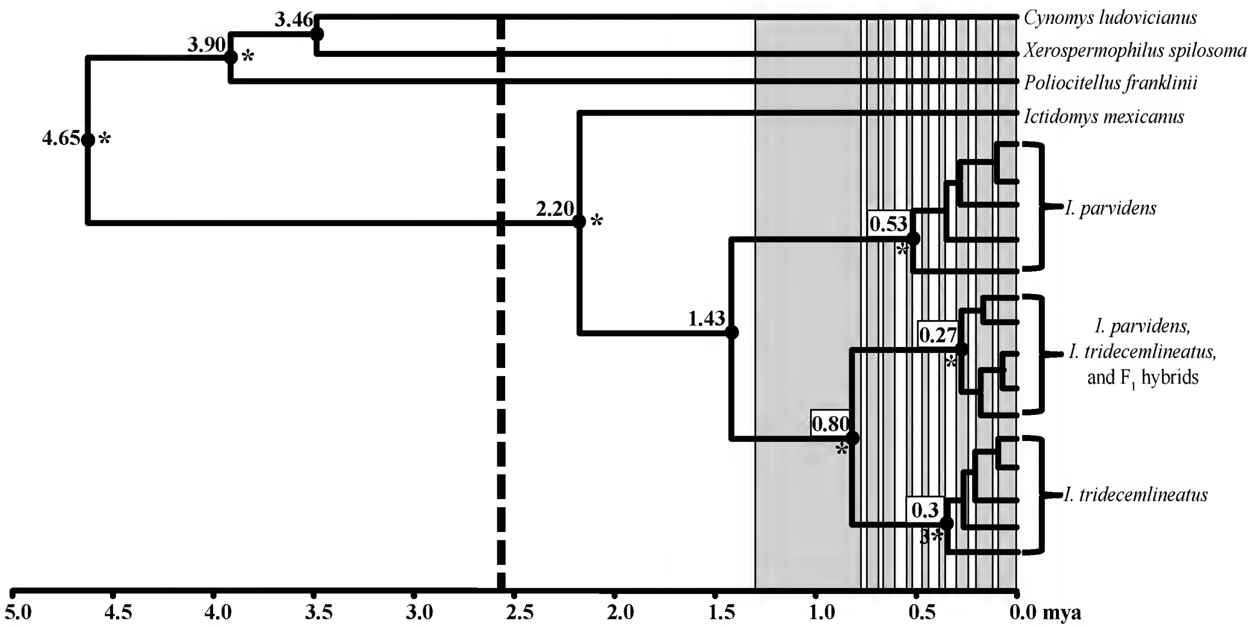


Figure 4. Maximum-credibility tree depicting divergence estimates within the genus *Ictidomys*. Outgroup taxa are *Cynomys ludovicianus*, *Poliocitellus franklinii*, and *Xerospermophilus spilosoma*. Ingroup taxa are representatives of the *Cyt*b clades (I, II, and III) determined by maximum-likelihood and Bayesian analyses. Divergence dates are indicated next to corresponding nodes (enclosed circles). Asterisks (*) next to these nodes represent Bayesian posterior probabilities ≥ 95%. Gray bars indicate approximate dates of known glacial periods within the Quaternary Period (dashed line).

both species (not shown) indicated a constant effective population size (Ne) since the divergence date of the *I. parvidens*/*I. tridecemlineatus* complex (1.43 mya). The BSPs also indicated that both species underwent a population expansion beginning approximately 0.10 to 0.50 mya, which coincides with the interspecific BEAST analysis.

Discussion

Ground squirrels of the genus *Spermophilus* (sensu lato) exhibit female philopatry (Mandier and Gouat 1996; Ermakov et al. 2002, 2006) and are known to be male-biased dispersers (Holekamp and Sherman 1989; Devillard et al. 2004). As a result, mitochondrial introgression likely occurs in actively hybridizing sympatric populations (Funk and Omland 2003; Petit and Excoffier 2009). However, in this study, *Cyt*b haplotypes within the mixed ancestry clade (Clade II) were more similar to haplotypes of the more northern *I. tridecemlineatus* clade (Clade III) than to haplotypes of the more southern *I. parvidens* clade (Clade I). This suggests a common ancestry between Clades II and III. These clades also are geographically distinct from one another, indicating some structuring in the geographic distribution of mitochondrial haplotypes (Fig. 2). This eliminates the likelihood of incomplete lineage sorting (Kulikova et al. 2005). Therefore, the similarities of haplotypes within Clades II and III most likely are due to the capture of the *I. tridecemlineatus* mitochondrial haplotype by northern populations of *I. parvidens* as a result of ancient hybridization and not the result of contemporary hybridization. The patchiness and parapatric nature of breeding populations observed by Stangl et al. (2012) and Thompson et al. (2013) may have facilitated the fixation and subsequent divergence of a mitochondrial haplotype by limiting gene flow and introgression between these populations (Patton and Smith 1994; Jones et al. 1995). Thus, any level of mitochondrial introgression as a result of contemporary hybridization would not be detected within the presumed zone of sympatry (Takahata and Slatkin 1984; Funk and Omland 2003).

The *SmcY* dataset provided some evidence for contemporary introgression. Overall, this dataset supported the division of the two species following their morphological identifications (*I. parvidens*, network 1; *I. tridecemlineatus*, networks 2 and 3). The distinct groupings in the *SmcY* dataset most likely are a result of male-dominated dispersal in ground squirrels. Because males are the heterogametic sex in mammals, introgression of genes found on the Y-chromosome is less likely to occur due to the constant dispersal of males into populations (Petit and Excoffier 2009) and subsequent dilution of their nuclear genomes. This would limit the likelihood of the haplotypes of Y-linked genes persisting beyond the area of contact. If females were contributing equally to gene flow, the introgression of mitochondrial haplotypes would occur rapidly.

The incongruence of morphological identifications and haplotypes from the *SmcY* dataset indicated the presence of seven putative hybrids among localities 15, 17, 32, 46, 64, and 65 (Fig. 2B and Table 1). In addition, five of these localities (15, 17, 32, 46, and 65) contained haplotypes representing each species. Only three of these localities (15, 46, and 65) contained potential morphological hybrids or both parental types; therefore, the utility of morphology to identify hybrids may be limited. As suggested by previous authors (Cothran 1983; Stangl et al. 2012; Thompson et al. 2013), most of these localities were within or in close proximity to human-populated areas and showed no obvious associations to natural boundaries found in the surrounding landscape (e.g., ecoclines, ecotones, etc.). However, localities 32 and 64 were found in close proximity to the edge of the Llano Estacado in Texas (Choate 1997), where the more northern short-grass prairie borders the mesquite savannahs to the south. The interdigitating nature of the boundary between these two habitats may be associated with the apparent isolation of populations observed by this study and others (Stangl et al. 2012; Thompson et al. 2013). The overall spatial orientation of "hybrid" localities compared to other localities distinctly *I. parvidens* or *I. tridecemlineatus* (as determined by their morphology and *SmcY* haplotypes) follow this pattern, indicating a parapatric relationship between these two species. Nonetheless, the limited extent of putative hybrids indicated by the *SmcY* dataset (both in numbers and spatial orientation) and the uniqueness of the *Cyt*b clades support the ancient hybridization hypothesis.

The interspecific and intraspecific BEAST analyses provide additional evidence for the ancient hybridization hypothesis. Estimates show that the initial divergence of the genus *Ictidomys* began during the Lower Pleistocene (~2.20 mya). This split corresponds to the separation of *I. mexicanus* from the monophyletic *I. parvidens*/*I. tridecemlineatus* complex recognized by Helgen et al. (2009). The divergence of *I. parvidens* and *I. tridecemlineatus* occurred approximately 1.43 mya, but the divergence between Clade II and Clade III was more recent, approximately 0.80 mya. Therefore, it seems reasonable to hypothesize that an ancestral hybridization event would have occurred after the initial divergence of *I. parvidens* and *I. tridecemlineatus* but prior to any major subsequent divergences between Clade II and Clade III or within each clade. The earlier date (~2.20 mya) corresponds with the Pre-Illinoian glacial period (Gibbard and van Kolfschoten 2004) during the Irvingtonian Land Mammal Age (LMA), which is the most probable timeframe for populations to occur sympatrically in glacial refugia. The latter date (~0.80 mya) is near a glacial/interglacial boundary of the Kansan glacial period (Gibbard and van Kolfschoten 2004) during the Late Irvingtonian LMA, which may have produced the divergence of Clade II and Clade III. Because overall divergence within the genus may be a result of climatic shifts during the Quaternary period (Black 1977; Smith and Coss 1984), the frequency and intensity of the glacial cycle would increase the opportunity for sympatry in glacial refugia and enhance the prospect for ancient hybridization.

In addition, the BSPs indicated that *I. parvidens* and *I. tridecemlineatus* clades began undergoing population expansions approximately 0.10 to 0.50 mya, which corresponds closely with the intraspecific divergence in the Yule dating analysis. The negative values of Tajima's D (Tajima 1989) for each gene support a recent population expansion within each species. This timeframe corresponds to an interglacial period prior to the Illinoian glacial period (Gibbard and van Kolfschoten 2004) during the Late Irvingtonian and Early Rancholabrean LMA. Therefore, the link between genetic divergence (interspecific and intraspecific) within each *Cyt* clade and the periodic fluctuations in climate further support the ancient hybridization hypothesis. Along with the sporadic and isolated nature of populations of *I. parvidens* and *I. tridecemlineatus*, the frequency and intensity of the Quaternary glacial cycle during the Pleistocene epoch would have enhanced the potential for mitochondrial capture due to many possible episodes of ancient hybridization (Patton and Smith 1994; Funk and Omland 2003). Given that *I. tridecemlineatus* is the more northern species, the effect of the Quaternary glaciations on its distribution would have led to a drastic reduction in its range and increase the opportunities of sympatry and ultimately hybridization with *I. parvidens*. This observation could explain the similarity of haplotypes of the *Cyt*b gene in Clades II and III.

Harrison et al. (2003) found a similar pattern of divergence of *I. tridecemlineatus* from *I. parvidens*, attributing paraphyly to contemporary hybridization. However, Harrison et al. (2003) dated the split between these two taxa at approximately 2.9 mya. Harrison et al. (2003) primarily calibrated the nodes of higher-level clades within the marmotines and did not include fossil evidence for *Ictidomys* in their analyses, potentially leading to the overestimation of the divergence dates of this clade. Though we cannot dispute their findings due to the much narrower scope of our study, an overestimation seems likely given comparable nodes for the marmotines are nearly six million years younger in the divergence date estimates by Mercer and Roth (2003). Recent divergence date estimates of extant species of *Xerospermophilus* place the radiation of this group during the Pleistocene (Fernández 2012), indicating that *Spermophilus* (sensu lato) may be younger than indicated previously.

Mitochondrial capture has been reported for a wide array of animal groups (Harrison 1989), suggesting the widespread occurrence of ancient hybridization (Avise 2004). Although the two species of *Ictidomys* studied herein appear to be quite divergent genetically and morphologically, it is of particular interest that limited hybridization is occurring in more recent times, which may be a result of secondary contact due to increased human modification of the study area (Stangl et al. 2012; Thompson et al. 2013). Many semi-fossorial animals, such as ground squirrels, are restricted by their shortened annual activity periods due to the onset of hibernation, which limits dispersal capabilities due to their energetic demands. Therefore, any shift in local climate (acute or chronic) may alter hibernation periods (Inouye et al. 2000). This would allow additional time for natal dispersal and the expansion of male home ranges in a warming climate, thereby increasing the possibility of contact with their congenerics, and consequently, hybridization. However, whether contemporary hybridization is the result of a recent warming climate (Shurtliff 2013) or an ancillary effect of anthropogenic habitat alterations (Stangl et al. 2012; Thompson et al. 2013) is yet to be determined. Further examination will be required to discriminate between these two hypotheses.

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Appendix

List of specimens examined in this study. Individual specimen numbers are ordered as follows: museum catalog number/*Cyt*b GenBank number/*SmcY* GenBank number. Commas separate specimen numbers of localities with multiple individuals. Museum collection acronyms used are as follows: ASK or ASNHC = Angelo State University, Natural History Collection; MWSU = Midwestern State University; NMMNH = New Mexico Museum of Natural History; S = Cornell University; and TTU = Natural Science Research Laboratory, Museum of Texas Tech University.

Ictidomys parvidens

MEXICO.—(Locality 1) Coahuila: Ocampo Municipality; 1.5 mi. NW Ocampo (NMMNH3679/JX902675/JX903039, NMMNH3680/JX902676/JX903040, NMMNH3715/JX902677/JX903041). (Locality 2) Nuevo Leon: Apodaca Municipality; 3 km NE Apodaca, ca. 23 km NE Monterrey (S111/AF157852/—, S112/AF157853/—).

UNITED STATES.—(Locality 15) New Mexico: Lea Co.; Hobbs (TTU35812/JX902678/—, TTU54338/JX902688/—); Hobbs, Bensing Park (TTU115515/JX902744/JX903052, TTU115516/JX902745/—); Hobbs, Ocotilla Park Golf Course (TTU115517/JX902929/JX903105, TTU115525/JX902753/—); Hobbs, Prairie Haven Cemetery (TTU115512/JX902723/—, TTU115512A/JX902724/—, TTU115512B/JX902725/—, TTU115512C/JX902726/—, TTU115512D/JX902727/—, TTU115512E/JX902728/—, TTU115513/JX902729/—, TTU115513A/JX902730/—, TTU115513B/JX902731/—, TTU115513C/JX902732/—, TTU115513D/JX902733/—, TTU115513E/JX902734/—, TTU115513F/JX902735/—, TTU115513G/JX902736/—, TTU115514/JX902737/—, TTU115514A/JX902738/—, TTU115514B/JX902739/—, TTU115514C/JX902740/—, TTU115514D/JX902741/—, TTU115514E/JX902742/—, TTU115514F/JX902743/—, TTU115523/JX902751/—). (Locality 16) New Mexico: Lea Co.; Eunice, Municipal Park (TTU115521/JX902749/JX903055, TTU115522/JX902750/—). (Locality 17) New Mexico: Eddy Co.; Carlsbad (MWSU22377/JX902631/JX903019, MWSU22378/JX902632/—, MWSU22379/JX902633/JX903020); Carlsbad, Pecos River Park (TTU115509/JX902714/JX903050, TTU115510/JX902715/—, TTU115510B/JX902716/—, TTU115510C/JX902717/—, TTU115510D/JX902718/—, TTU115510E/JX902719/—, TTU115510F/JX902720/—, TTU115510G/JX902721/—, TTU115511/JX902722/JX903051). (Locality 18) New Mexico: Lea Co.; Jal, City Park (TTU115518/JX902746/JX903053, TTU115519/JX902747/—, TTU115520/JX902748/JX903054). (Locality 29) Texas: Motley Co.; 11 mi. E Matador, Hwy 62 (TTU54352/JX902690/JX903044, TTU54353/JX902691/JX903045, TTU54354/JX902692/JX903046, TTU54355/JX902693/JX903047, TTU54356/JX902694/—). (Locality 30) Texas: Motley Co.; Matador Cemetery (MWSU22381/JX902634/—, MWSU22382/JX902635/—, MWSU22460/JX902665/—, MWSU22466/JX902669/JX903035, MWSU22467/JX902670/—). (Locality 31) Texas: Cottle Co.; Paducah Cemetery (MWSU22336/JX902597/—, MWSU22337/JX902598/—, MWSU22338/JX902599/JX903003, MWSU22339/JX902600/JX903004, MWSU22464/JX902667/JX903034, MWSU22465/JX902668/—); (Locality 37) Texas: Motley Co.; 8 mi. S, 9 mi. E Matador (TTU42031/JX902684/—, TTU42035/JX902685/—, TTU54357/JX902695/JX903048, TTU54358/JX902696/JX903049, TTU54360/JX902697/—, TTU54361/JX902698/—, TTU54362/JX902699/—, TTU54363/JX902700/—, TTU54364/JX902701/—). (Locality 41) Texas: King Co.; 11.8 mi. N, 18.3 mi. W Benjamin (MWSU22512/JX902671/JX903036). (Locality 42) Texas: Knox Co.; Truscott Cemetery (MWSU22560/JX902672/JX903037, MWSU22561/JX902673/—). (Locality 44) Texas: Knox Co.; Benjamin Cemetery (MWSU22390/JX902640/—, MWSU22434/JX902659/—, MWSU22435/JX902660/—, MWSU22436/JX902661/KC011083). (Locality 45) Texas: King Co.; Guthrie, high school campus (MWSU22456/JX902662/—, MWSU22457/JX902663/—). (Locality 46) Texas: Baylor Co.; Seymour, Masonic Cemetery (MWSU22327/JX902596/—). (Locality 47) Texas: Dickens Co.; Dickens Cemetery (MWSU22458/JX902664/—, MWSU22463/JX902666/JX903033). (Locality 49) Texas: Knox Co.; 3.5 mi. E Benjamin (TTU40789/JX902683/—). (Locality 54) Texas: Dickens Co.; Spur, South Plains Electric Cooperative (TTU115539/JX902767/JX903060, TTU115540/

JX902768/—, TTU115541/JX902769/—, TTU115542/JX902769/—); Spur, Swenson Park (TTU115543/JX902771/—, TTU115544/JX902772/JX903061); Spur Cemetery (MWSU22383/JX902636/JX903021, MWSU22384/JX902637/—, MWSU22385/JX902638/JX903022, MWSU22386/JX902639/JX903023). (Locality 55) Texas: Knox Co.; Goree Cemetery (MWSU22369/JX902625/—, MWSU22370/JX902626/—). (Locality 60) Texas: Kent Co.; Clairemont Fairgrounds (MWSU22421/JX902652/JX903029, MWSU22427/JX902657/—, MWSU22428/JX902658/JX903032). (Locality 61) Texas: Garza Co.; 2.5 km N, 5 km W Post, Roadside Park (TTU115552/JX902780/JX903063, TTU115553/JX902781/—, TTU115554/JX902782/—, TTU115555/JX902783/—). (Locality 63) Texas: Garza Co.; Post, Nichols Park (TTU115550/JX902778/—, TTU115551/JX902779/—); Post Cemetery (MWSU22419/JX902650/—, MWSU22420/JX902651/—, MWSU22422/JX902653/—, MWSU22423/JX902654/JX903030, MWSU22424/JX902655/JX903031, MWSU22426/JX902656/—). (Locality 64) Texas: Garza Co.; 4 mi. W Post, Wayne Stewart Ranch (TTU54339/JX902689/—). (Locality 65) Texas: Throckmorton Co.; Throckmorton Cemetery (MWSU22291/JX902595/—, MWSU22357/JX902615/JX903012, MWSU22358/JX902616/JX903013, MWSU22359/JX902617/—, MWSU22361/JX902618/—, MWSU22362/JX902619/—, MWSU22793/JX902674/JX903038). (Locality 66) Texas: Haskell Co.; Haskell (MWSU22282/JX902593/—, MWSU22283/JX902594/—). (Locality 67) Texas: Stonewall Co.; Aspermont Cemetery (MWSU22406/JX902643/JX903026, MWSU22407/JX902644/—, MWSU22408/JX902645/—, MWSU22409/JX902646/—); City of Aspermont (MWSU22410/JX902647/—, MWSU22411/JX902648/JX903027). (Locality 68) Texas: Jones Co.; Stamford Cemetery (MWSU22347/JX902607/JX903007, MWSU22348/JX902608/—). (Locality 69) Texas: Jones Co.; Hamilton Cemetery (MWSU22342/JX902603/—, MWSU22343/JX902604/JX903005, MWSU22344/JX902605/—, MWSU22345/JX902606/JX903006). (Locality 70) Texas: Fisher Co.; Rotan Cemetery (MWSU22340/JX902601/—, MWSU22341/JX902602/—). (Locality 71) Texas: Jones Co.; 1 mi. E Anson (MWSU22350/JX902610/JX903009, MWSU22351/JX902611/JX903010, MWSU22352/JX902612/—, MWSU22353/JX902613/—, MWSU22354/JX902614/JX903011, MWSU22363/JX902620/JX903014, MWSU22364/JX902621/—, MWSU22365/JX902622/—, MWSU22366/JX902623/JX903015, MWSU22367/JX902624/JX903016); Anson (MWSU22349/JX902609/JX903008). (Locality 72) Texas: Shackleford Co.; Albany (MWSU22374/JX902628/JX903017, MWSU22375/JX902629/—, MWSU22376/JX902630/JX903018); Albany Cemetery (MWSU22373/JX902627/—, MWSU22403/JX902641/JX903024, MWSU22404/JX902642/JX903025, MWSU22415/JX902649/JX903028). (Locality 73) Texas: Scurry Co.; 1.75 km N, 1.75 km W Snyder, Snyder Cemetery (TTU115577/JX902794/—, TTU115578/JX902795/—, TTU115579/JX902796/—). (Locality 74) Texas: Gaines Co.; 2.5 km S, 1.0 km W Seminole, Gaines County Cemetery (TTU115545/JX902773/—, TTU115546/JX902774/JX903062, TTU115547/JX902775/—, TTU115548/JX902776/—, TTU115549/JX902777/—). (Locality 75) Texas: Andrews Co.; Andrews, Andrews County Golf Course (TTU115526/JX902754/—, TTU115527/JX902755/—, TTU115528/JX902756/—, TTU115529/JX902757/—, TTU115530/JX902758/—, TTU115531/JX902759/JX903056, TTU115532/JX902760/JX903057, TTU115533/JX902761/—, TTU115534/JX902762/—, TTU115535/JX902763/JX903058, TTU115536/JX902764/JX903059, TTU115537/JX902765/—, TTU115538/JX902766/—). (Locality 76) Texas: Howard Co.; Big Spring, Comanche Trail Park Golf Course (TTU115558/JX902785/JX903064, TTU115559/JX902786/—, TTU115561/JX902787/—, TTU115562/JX902788/JX903065). (Locality 77) Texas: Howard Co.; 6.3 mi. S, 1 mi. E Big Spring, Trinity Memorial Park (TTU115557/JX902784/—). (Locality 78) Texas: Martin Co.; Stanton, Shelbourne Park (TTU115564/JX902789/JX903066, TTU115565/JX902790/JX903067, TTU115566/JX902791/—, TTU115567/JX902792/—). (Locality 79) Texas: Winkler Co.; Kermit, Kermit ISD Baseball Field (TTU115581/JX902798/—, TTU115585/JX902802/—); Kermit, Winkler County Park (TTU115582/JX902799/—, TTU115583/JX902800/—, TTU115584/JX902801/—, TTU115586/JX902803/—). (Locality 80) Texas: Winkler Co.; 0.5 mi. NW Winkler County Airport (TTU40683/JX902682/JX903043); Winkler County Airport (TTU35815/JX902681/JX903042). (Locality 81) Texas: Ward Co.; Pyote, City Park (TTU115580/JX902797/JX903068). (Locality 82) Texas: Irion Co.; 11.5 mi. N, 20 mi. W Mertzon (ASNHC7728/JX902590/—). (Locality 83) Texas: Reeves Co.; 0.6 km N, 0.25 km E Saragosa, Saragosa Cemetery (TTU115576/JX902793/—). (Locality 84) Texas: Sutton Co.; Sonora (TTU44454/JX902686/—, TTU44455/JX902687/—). (Locality 85) Texas: Crockett Co.; Eugene

Miller Ranch, Gonzalo Pasture, 7.5 mi. W of Hwy 167 and 5.1 mi. N FM 1973 (TTU97999/JX902711/—). (Locality 86) Texas: Kimble Co.; 1.0 mi. S Texas Tech Junction Center (TTU76653/JX902703/—); Junction, Texas Tech University Center (TTU77844/JX902704/—, TTU77845/JX902705/—, TTU82585/JX902709/—, TTU107866/JX902713/—); Junction Golf Course (TTU76651/JX902702/—, TTU82581/JX902706/—, TTU82583/JX902707/—, TTU82584/JX902708/—); Walter Buck Wildlife Management Area (TTU98434/JX902712/—). (Locality 87) Texas: Travis Co.; Austin (TTU35813/JX902679/—, TTU35814/JX902680/—). (Locality 88) Texas: Val Verde Co.; 0.5 mi. W Devil's River State Natural Area, Turkey Track Canyon (ASNHC10697/JX902589/—). (Locality 89) Texas: Val Verde Co.; 1 mi. S Hwy 90 on Kingsway in Del Rio (TTU83634/JX902710/—). (Locality 90) Texas: Dimmit Co.; 13 mi. W Artesia Wells, B & M Ranch (ASNHC11371/JX902591/JX903001). (Locality 91) Texas: Starr Co.; FM Road 649 (ASK8188/JX902588/—). (Locality 92) Texas: Hidalgo Co.; Bentsen Rio Grande Valley State Park (ASNHC13686/JX902592/JX903002).

Ictidomys tridecemlineatus

UNITED STATES.—(Locality 3) Colorado: Saguache Co.; 5 mi. S, 5 mi. E Villa Grove (TTU54368/JX902880/—). (Locality 4) Colorado: Saguache Co.; 0.6 mi. N, 3.5 mi. E Crestone, Baca Grande Golf Course (TTU54369/JX902881/JX903095, TTU54370/JX902882/—, TTU54371/JX902883/—, TTU54372/JX902884/—). (Locality 5) Indiana: Vigo Co.; 6 mi. N Terre Haute, Rose Lawn Cemetery (TTU49336/JX902877/—, TTU54373/JX902885/—, TTU54374/JX902886/—, TTU54375/JX902887/—, TTU54376/JX902888/—, TTU54377/JX902889/—). (Locality 6) Iowa: Story Co.; 0.6 mi. N Ames, Oaks Golf Course (TTU49341/JX902878/—, TTU49342/JX902879/—, TTU54396/JX902890/—, TTU54397/JX902891/JX903096, TTU54401/JX902892/—). (Locality 7) Kansas: Thomas Co.; Colby (TTU54425/JX902904/—). (Locality 8) Kansas: Shawnee Co.; Topeka, Dillard Airport (TTU54417/JX902899/JX903098, TTU54418/JX902900/—, TTU54422/JX902901/—, TTU54423/JX902902/—, TTU54424/JX902903/—). (Locality 9) Kansas: Ellis Co.; Hays (TTU54402/JX902893/JX903097, TTU54403/JX902894/—, TTU54404/JX902895/—, TTU54405/JX902896/—, TTU54406/JX902897/—, TTU54407/JX902898/—). (Locality 10) Kansas: Finney Co.; Garden City (S13/AF157870/—, S14/AF157877/—). (Locality 11) Nebraska: Antelope Co.; 0.7 mi. W Oakdale, Gopher Site (TTU75920/JX902928/—). (Locality 12) Nebraska: Chase Co.; 2 mi. W Wauneta, Wauneta Country Club (TTU54426/JX902905/JX903099). (Locality 13) New Mexico: Taos Co.; Urraca State Wildlife Area, 14 mi. N Questa (NMMNH2312/JX902858/JX903087, NMMNH2314/JX902859/JX903088). (Locality 14) New Mexico: San Miguel Co.; Las Vegas (TTU54434/JX902906/JX903100, TTU54435/JX902907/KC011085, TTU54436/JX902908/—, TTU54437/JX902909/—, TTU54438/JX902910/—). (Locality 15) New Mexico: Lea Co.; Hobbs, Ocotilla Park Golf Course (TTU115587/JX902930/—, TTU115587A/JX902931/—, TTU115587B/JX902932/—, TTU115587C/JX902933/—, TTU115587D/JX902934/—, TTU115587E/JX902935/—, TTU115587F/JX902936/—, TTU115587G/JX902937/—). (Locality 19) North Dakota: Grand Forks Co.; 2 mi. N Arvilla (ASNHC9109/JX902804/JX903069, ASNHC9110/JX902805/JX903070, ASNHC9111/JX902806/—, ASNHC9148/JX902807/—). (Locality 20) Oklahoma: Roger Mills Co.; 0.5 mi. S, 1.75 mi. W Cheyenne, Cheyenne Cemetery (TTU115589/JX902938/—, TTU115590/JX902939/—). (Locality 21) South Dakota: Pennington Co.; Rapid City, golf course (TTU54439/JX902911/—). (Locality 22) Texas: Hemphill Co.; Gene Howe Wildlife Management Area (TTU115611/JX902958/KC011087). (Locality 23) Texas: Parmer Co.; Bovina, Ridgelea Elementary (TTU115635/JX902973/—). (Locality 24) Texas: Briscoe Co.; 1 km S, 1.5 km E Silverton, Silverton Cemetery (TTU115595/JX902944/—, TTU115596/JX902945/—, TTU115598/JX902947/—, TTU115599/JX902948/JX903106). (Locality 25) Texas: Hardeman Co.; Quanah Cemetery (MWSU22308/JX902817/—, MWSU22309/JX902818/—, MWSU22310/JX902819/—, MWSU22311/JX902820/—). (Locality 26) Texas: Hale Co.; 2 mi N, 2.5 mi. E Edmonson, Alton Painter Ranch (TTU39699/JX902864/JX903091, TTU39700/JX902865/—, TTU39701/JX902866/JX903092, TTU39702/JX902867/—, TTU39703/JX902868/JX903093, TTU39704/JX902869/—, TTU54443/JX902913/—, TTU54444/JX902914/—, TTU54445/JX902915/JX903102). (Locality 27) Texas: Bailey Co.; Muleshoe, Youth Baseball Complex (TTU115591/JX902940/—). (Locality 28) Texas: Wichita Co.; Burkburnett Cemetery (MWSU22317/JX902826/—, MWSU22318/JX902827/—, MWSU22319/JX902828/—, MWSU22320/

JX902829/—). (Locality 32) Texas: Floyd Co.; Floydada, Floydada Park (TTU115601/JX902949/—); Floydada Cemetery (MWSU22429/JX902843/—, MWSU22430/JX902844/JX903084, MWSU22431/JX902845/—). (Locality 33) Texas: Wichita Co.: 10 mi. SE Burkburnett, River Creek Golf Course (MWSU22321/JX902830/—, MWSU22322/JX902831/—, MWSU22323/JX902832/—, MWSU22324/JX902833/JX903078). (Locality 34) Texas: Hale Co.; 2 mi. S, 5 mi. W Cotton Center (TTU39691/JX902860/JX903089, TTU39693/JX902861/JX903090, TTU39696/JX902862/—, TTU39697/JX902863/—). (Locality 35) Texas: Wilbarger Co.; 0.4 mi. NE Lockett (MWSU22790/JX902855/—, MWSU22794/JX902856/—, MWSU22795/JX902857/—). (Locality 36) Texas: Wichita Co.; Electra Cemetery (MWSU22301/JX902810/JX903073, MWSU22302/JX902811/JX903074, MWSU22303/JX902812/JX903075, MWSU22304/JX902813/—, MWSU22305/JX902814/—, MWSU22306/JX902815/—, MWSU22307/JX902816/—, MWSU22400/JX902838/—, MWSU22562/JX902854/—). (Locality 38) Texas: Wichita Co.; Wichita Falls (MWSU22396/JX902836/JX903079, MWSU22397/JX902837/JX903080); Wichita Falls, Kiwanis Park (MWSU22312/JX902821/—, MWSU22313/JX902822/—, MWSU22314/JX902823/JX903076, MWSU22315/JX902824/—, MWSU22316/JX902825/JX903077, MWSU22416/JX902839/JX903081, MWSU22417/JX902840/JX903082, MWSU22418/JX902841/JX903083, MWSU22516/JX902853/—); Wichita Falls, Memorial Stadium (MWSU22325/JX902834/—, MWSU22326/JX902835/—). (Locality 39) Texas: Hockley Co.; 14 mi. N, 6 mi. W Levelland (TTU39705/JX902870/—). (Locality 40) Texas: Crosby Co.; 2 mi. E Cone (TTU54442/JX902912/JX903101). (Locality 43) Texas: Crosby Co.; Ralls Cemetery (MWSU22425/JX902842/—, MWSU22432/JX902846/—, MWSU22433/JX902847/JX903085). (Locality 46) Texas: Baylor Co.; 2.5 km S, 1.25 km E Seymour, Masonic Cemetery (TTU115593/JX902942/—, TTU115594/JX902943/KC011086). (Locality 48) Texas: Lubbock Co.; Lubbock, Reese Air Force Base Golf Course (TTU54450/JX902916/—). (Locality 50) Texas: Lynn Co.; 2 mi. S, 5 mi. W Tahoka (TTU75430/JX902927/JX903104). (Locality 51) Texas: Archer Co.; Windthorst (MWSU22437/JX902848/—, MWSU22438/JX902849/—, MWSU22439/JX902850/KC011084, MWSU22443/JX902852/JX903086). (Locality 52) Texas: Lubbock Co.; Lubbock (TTU42039/JX902871/—, TTU43244/JX902874/—, TTU43245/JX902875/JX903094, TTU43247/JX902876/—, TTU57663/JX902926/JX903103); Lubbock, Texas Tech University (TTU43241/JX902872/—, TTU43243/JX902873/—). (Locality 53) Texas: Hockley Co.; 5 km S Levelland, Levelland Airport (TTU115620/JX902959/JX903109, TTU115621/JX902960/JX903110, TTU115622/JX902961/JX903111, TTU115623/JX902962/—, TTU115624/JX902963/—, TTU115625/JX902964/—, TTU115626/JX902965/—, TTU115627/JX902966/—, TTU115628/JX902967/JX903112); 5 km S Levelland, RV Park (TTU115629/JX902968/—). (Locality 56) Texas: Archer Co.; Megargel Cemetery (MWSU22441/JX902851/—). (Locality 57) Texas: Garza Co.; Southland, Southland Schools (TTU115602/JX902950/—, TTU115603/JX902951/—). (Locality 58) Texas: Garza Co.; 3 km S, 0.6 km E Southland (TTU115604/JX902952/JX903107, TTU115605/JX902953/—, TTU115606/KC011081/JX903108, TTU115607/JX902954/—, TTU115608/JX902955/—, TTU115609/JX902956/—, TTU115610/JX902957/—). (Locality 59) Texas: Lynn Co.; 1.6 km S, 1.5 km W Wilson, Green Memorial Park (TTU115631/JX902969/JX903113, TTU115632/JX902970/—, TTU115633/JX902971/—, TTU115634/JX902972/—). (Locality 62) Texas: Yoakum Co.; 1 mi. E Plains (TTU54516/JX902917/—). (Locality 93) Wisconsin: Iowa Co.; 2 mi. E Mineral Point, Ludden Lake Golf Course (TTU54523/JX902918/—, TTU54524/JX902919/—, TTU54525/JX902920/—, TTU54526/JX902921/—, TTU54527/JX902922/—). (Locality 94) Wyoming: Crook Co.; Sundance (TTU54528/JX902923/—). (Locality 95) Wyoming: Natrona Co.; 6.5 mi. W Casper, Engel King Ranch (ASNHC15065/JX902808/JX903071, ASNHC15066/JX902809/JX903072). (Locality 96) Wyoming: Goshen Co.; 1 mi. S Torrington, Torrington Country Club (TTU54529/JX902924/—, TTU54531/JX902925/—).

Ictidomys parvidens* x *Ictidomys tridecemlineatus (putative morphological hybrids)

UNITED STATES.—(Locality 46) Texas: Baylor Co.; 2.5 km S, 1.25 km E Seymour, Masonic Cemetery (TTU115595/JX902944/—); Seymour, Catholic Cemetery (MWSU22440/JX902992/—); Seymour, Masonic Cemetery (MWSU22328/JX902978/—, MWSU22329/JX902979/—, MWSU22330/JX902980/—, MWSU22331/JX902981/—, MWSU22332/JX902982/—, MWSU22333/JX902983/—, MWSU22334/JX902984/—, MWSU22335/JX902985/—, MWSU22413/JX902990/—, MWSU22414/JX902991/—); Seymour, Old Seymour

Cemetery (MWSU22412/JX902989/—); Seymour, Salt Creek Golf Course (MWSU22788/JX902994/JX903118, MWSU22789/JX902995/JX903119). (Locality 55) Texas: Knox Co.; Goree Cemetery (MWSU22371/JX902987/JX903115, MWSU22372/KC011082 /KC011088). (Locality 65) Texas: Throckmorton Co.; Throckmorton Cemetery (MWSU22286/JX902976/—, MWSU22287/JX902977/—, MWSU22360/JX902986/—). (Locality 66) Texas: Haskell Co.; Haskell (MWSU22284/JX902974/—). (Laboratory Bred) Texas (MWSU22564/JX902998/—, MWSU22565/JX902999/—, MWSU22566/JX903000/JX903122).

Ictidomys parvidens* x *Ictidomys tridecemlineatus (putative genetic hybrids)

UNITED STATES.—(Locality 15) New Mexico: Lea Co.; Hobbs, Ocotilla Park Golf Course (TTU115524/JX902752/KC011089, TTU115588/JX902996/JX903120). (Locality 17) New Mexico: Eddy Co.; Carlsbad (MWSU22380/JX902988/JX903116). (Locality 32) Texas: Floyd Co.; Floydada, Floydada Park (TTU115600/JX902997/JX903121). (Locality 46) Texas: Baylor Co.; 2.5 km S, 1.25 km E Seymour, Woodmen Cemetery (TTU115592/JX902941/KC011090). (Locality 64) Texas: Garza Co.; 4 mi. W Post, Wayne Stewart Ranch (TTU54340/JX902993/JX903117). (Locality 65) Texas: Throckmorton Co.; Throckmorton Cemetery (MWSU22285/JX902975/JX903114).

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