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Genetic divergence of *Microtus pennsylvanicus chihuahuensis* and conservation implications of marginal population extinctions

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Microtus pennsylvanicus is represented in Mexico only by the Chihuahuan meadow vole (*M. p. chihuahuensis*), known from only 1 disjunct population in a small and isolated marsh in the arid lands of northern Chihuahua. Livetrapping conducted between 2000 and 2004 provided no specimens of *M. p. chihuahuensis*, nor was any sign of this vole observed. By the end of this study the marsh providing water had been drained, thereby destroying the vole's habitat. Surveys of other marshes in northern Chihuahua also failed to produce evidence of the species. We therefore conclude that *M. p. chihuahuensis* has been extirpated from its only known locality. Using ''ancient'' DNA from museum specimens we evaluated genetic divergence between museum specimens of *M. p. chihuahuensis* and 46 extant *Microtus* species and subspecies. Our results support the subspecific status of *M. p. chihuahuensis*. The loss of this subspecies is an example of population extinction, a very severe form of biodiversity loss. Until recently such losses have been mostly neglected. DOI: 10.1644/09-MAMM-A-168.1.

Key words: ancient DNA, biodiversity loss, Chihuahua, marginal populations, meadow vole, *Microtus*, mitochondrial DNA, population extinctions

The importance of marginal populations for the conservation of species has been widely debated. Marginal populations are separated spatially from central or core populations and tend to be found in suboptimal habitats. These populations have a greater risk of extinction than core populations (Lienert et al. 2002; Tomback et al. 2005). However, investment of resources for conservation of continuous marginal populations is criticized in favor of conservation efforts in core distribution areas (Bunnell et al. 2004). This contradicts available evidence from some species in which contraction and fragmentation of their geographic ranges has affected both marginal and core populations at global scales. Furthermore, focusing on core populations does not take into account the loss of populations, which at local scales is as important as species loss (Ceballos and Ehrlich 2002). Yet, populations with peripheral distribution are more tolerant of disturbance and tend to be substantially different from core populations in traits such as genetics, behavior, life history, or size (Gaines et al. 1997; Garavello et al. 1998; Snyder and Peterson 1999). Sometimes these differences accrue rapidly, over centuries or even decades rather than geologic time (Ashley et al. 2003; Hendry and Kinnison 1999; Pergams and Kareiva 2009). This means that even relatively recently diverged populations may deserve conservation attention for their intrinsic value (Ehrlich 1988; Joyal et al. 2000). More important, marginal populations often differ genetically from other populations of the same species, even after a relatively short time of separation, playing a role in allopatric speciation (Fritz et al. 2006; Huang et al. 2005; Waters et al. 2000). This is particularly relevant for conservation because loss of marginal populations implies the loss of genetic diversity, the loss of that portion of the gene pool that is not present in other parts of the range of a species (Eckstein et al. 2006; Johannesson and André 2006). Some studies have reported range contraction (Channell and Lomolino 2000). Overall, however, we ignore the magnitude of genetic diversity we lose when marginal populations disappear as a consequence of geographic range contraction due to anthropogenic causes. We use the Chihuahuan meadow vole (*M. pennsylvanicus chihuahuensis*) as a case study of a marginal population to assess genetic divergence and determine current conservation status at the southern portion of the species' range.

The meadow vole (*Microtus pennsylvanicus*) inhabits moist habitats (Reich 1981). It is represented in Mexico by 1 subspecies, the Chihuahuan meadow vole (*M. p. chihuahuensis*), known from only 1 disjunct population. This population is found in a small and isolated marsh in the arid lands of northern Chihuahua, approximately 700 km south of the core range of the species and 400 km south-southeast from the closest relict population (both in the United States—Anderson and Hubbard 1971). Like most mammals it was designated a subspecies on the basis of morphology (Bradley and Cockrum 1968). It represents the most geographically restricted species of mammal in Mexico and is considered endangered (Secretaría del Medio Ambiente y Recursos Naturales 2002), but its current status has been considered unknown (Ceballos 2007; Ceballos et al. 1998). Hence, the objectives of our research were to determine its current status, if *M. p. chihuahuensis* maintained its morphologically assigned subspecific status using metrics of genetic divergence and distinction from its most closely related taxa, and a conservation strategy if the subspecies still existed.

Materials and Methods

Study area.—The study area is located in the northwest portion of the Chihuahuan Desert, at 30°03′32″N, 107°35′29″W and an altitude of 1,461 m (Galeana marsh; Fig. 1). The climate is arid with hot summers and cold winters. Mean annual temperature is 16.9°C (García 1973), with extremes ranging between −12°C in winter and 48°C in summer. Annual precipitation averages 294 mm, most of it occurring in July and August and, to a lesser extent, during the winter (Rzedowski 1981). The area is a small (34-ha), isolated marsh surrounded by grasslands and desert scrub dominated by *Prosopis* sp. The vegetation of the marsh consists of sedges and rushes, partly immersed in water in the lowest parts of the marsh (Bradley and Cockrum 1968). In 2000 the marsh was fed from 2 springs that surfaced near the base of a hill and were separated from each other by 300 m. The longest spring formed a natural pond 45 m long and 32 wide and ran into a stream for 2,340 m, the marsh being 30–260 m wide along the stream. The shortest stream was channeled at the spring into 3 consecutive artificial pools built by local people. After leaving the pools the water continued along a stream for 320 m, blending with the marsh, which ranged from 87 to 200 m wide. The depth of the ponds surpassed 80 cm in some places. In 2004 the only water available was in 2 small ponds approximately 50 and 120 cm in diameter and separated by 15 m, with an average water depth of 3.5 cm and greatest depth of 15 cm. In 2005 the marsh, streams, and springs had completely disappeared. Three additional marshes exist in the region. One was transformed into a recreational area (Casas Grandes marsh; Fig. 1), but trapping for voles was conducted in the other 2 (Ojitos and Ojo Caliente marshes; Fig. 1) despite the absence of vole records.

Small mammal sampling.—We conducted our survey to capture meadow voles during 5 sampling periods in May 2000, November 2002, February 2003, November 2003, and September 2004. We used 120 Sherman traps (7.5 × 9 × 23 cm; H. B. Sherman Traps, Inc., Tallahassee, Florida) set in linear transects within the flooding area. Trapping effort consisted of a system of linear transects arranged in parallel lines separated by 20 m. Trap interval was 10 m, and traps were baited with a mixture of rolled oats and peanut butter. All of the marsh was sampled. Traps were set from 3 to 5 consecutive days. All individuals collected or observed in the area were identified in situ and then released. No mark–recapture techniques were used, because the objective of the study was solely to locate the Chihuahuan vole. The total trapping effort consisted of 1,920 trap days. We complemented our trap data with direct observations, conducting random walks across the marsh and searching for runways. Animal trapping and handling was conducted according to guidelines approved by the American Society of Mammalogists (Animal Care and Use Committee 1998).

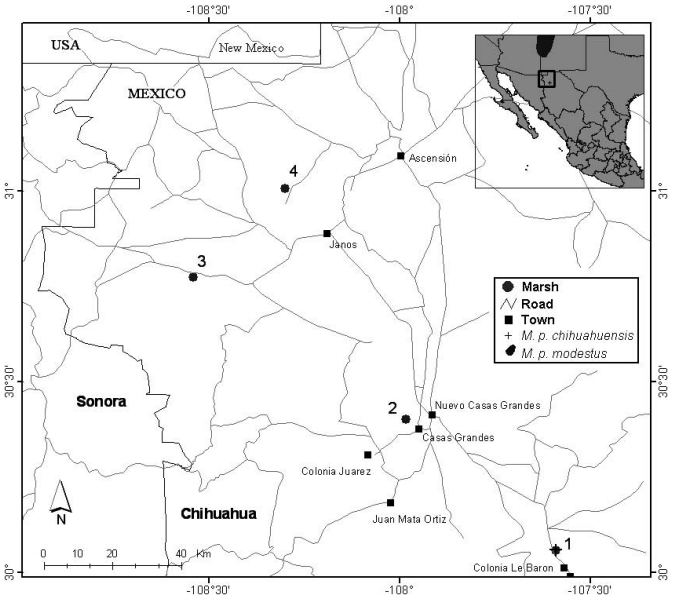

Fig. 1.—Map of northwestern Chihuahua, Mexico, showing the region's marshes: 1) Galeana, 2) Casas Grandes, 3) Ojitos, and 4) Ojo Caliente. The reported distribution of *Microtus pennsylvanicus chihuahuensis* was limited to the Galeana marsh.

Genetic analyses.—Four museum skins of *M. p. chihuahuensis*—numbers 7231, 7134, 7138, and 7139—borrowed from the University of Nevada at Las Vegas were sequenced for this study. Museum tags show that all were collected by R. Mauer and G. Austin 3 miles south of Galeana, Chihuahua, Mexico, in 1964. Mitochondrial DNA (mtDNA) cytochrome-*b* gene (*Cytb*) sequences from all *Microtus* species present on GenBank on 17 August 2004 were downloaded. A total of 46 *Microtus* species with complete (1,143 base pairs [bp]) *Cytb* sequences was deposited in GenBank, plus *Cytb* sequences of 5 *Myodes* species used as outgroups. Fortuitously, the GenBank *M. pennsylvanicus* sequence (accession number AF119279) came from the subspecies geographically closest to *M. p. chihuahuensis*, *M. p. modestus*, caught in San Juan County, New Mexico (Conroy and Cook 2000) about 400 km north of our study site (Hoffmann and Koeppl 1985).

For further comparison an individual of subspecies *M. p. pennsylvanicus* was sequenced. This specimen was collected in 2003 near State College, Pennsylvania, the approximate center of the ranges of both the subspecies and the entire species and about 3,000 km northeast of the location of *M. p. chihuahuensis*.

Museum skins were sampled as follows. An approximately 1.5 × 12-mm strip was removed from ventral seam of the skin. Because we found that proteins produced from hair during the Chelex extraction interfered with the polymerase chain reaction (PCR) process (Pergams et al. 2003; Pergams and Lacy 2007), we shaved the strips of skin with single-edged razors. Each strip was then minced into approximately 1.5-mm squares. Museum DNA extraction was performed using a Chelex 100 protocol (Walsh et al. 1991) modified by the addition of proteinase K as in Steinberg (1999). However, we performed trials to determine what proportion of proteinase K was most effective and found that this proportion was 10 μl of 20 mg/ml proteinase K solution added to 490 μl of 5% Chelex solution (Pergams et al. 2003; Pergams and Lacy 2007). We also found that freezing the resulting DNA-laden supernatant to 20°C in a Boekel Polar Block model 260012 benchtop cooler/heater (Boekel Scientific, Feasterville, Pennsylvania), thawing the supernatant fully, and repeating the process 1 or 2 more times helped to separate out residual proteins and other substances that seemed to inhibit PCR (Pergams et al. 2003).

We subjected the supernatant that remained after the freeze–thaw process to phenol–chloroform extraction (Maniatis et al. 1982). The DNA pellet was resuspended in 20 μl of 1 mM TE buffer. Amplification was performed by PCR using 2 sets of 2 nested primers. All primers were designed using the program Primer3 (Rozen and Skaletsky 2000) from GenBank *M. pennsylvanicus* sequence AF119279. Primers MPC1 (5′-TCT TCG CCT TCC ACT TCA TT-3′ and MPC2 (5′-CCT GCG ATT GGC ATA AAG AT-3′) were designed to amplify 577 bp. PCR was performed for 20 cycles with each 2-part cycle 93°C for 1 min and 60°C for 20 min. We then performed a nested PCR, moving in 25–100 bp from the ends of the previous segment and using the previous PCR product as a template. Primers MPC5 (5′-TCC CAC CGG TCT AAA CTC AG-3′) and MPC8 (5′-GGT TGA CCA CCG ATT CAT GT-3′) amplified 405 bp. Nested PCR is useful with very low numbers of target templates (van Pelt-Verkuil et al. 2008). PCR was performed for 22 cycles with each cycle 93°C for 1 min and 65°C for 20 min. Important were the use of KlenTaq LA DNA polymerase and PCR buffer (DNA Polymerase Technology, Inc., St. Louis, Missouri), because 5′-exonuclease–deficient Taq polymerase provides improved fidelity and thermostability (Barnes 1992, 1995; Cheng et al. 1994; Korolev et al. 1995), the long extension cycles (Barnes 1994), and the use of betaine (Barnes 1994). Bovine serum albumin was added to prevent proteins from further inhibiting PCR (Pääbo et al. 1988; Thomas et al. 1990). PCR products in agarose check gels were stained with ethidium bromide and viewed under an ultraviolet transilluminator. Single bands resulted for most runs for most samples, but not when bands of the appropriate width (based on size standards) were cut from the gels with a single-edge razor blade. These gel slices were processed using Montage Gel Extraction Kits (Millipore, Inc., Billerica, Massachusetts). PCR product was cleaned with QIAquick PCR Purification Kits (Qiagen, Inc., Valencia, California) and quantified using a GeneQuant RNA/DNA Calculator spectrophotometer (Amersham Pharmacia Biotech, GE Healthcare UK Ltd., Buckinghamshire, United Kingdom).

Sequencing was performed on an ABI PRISM 3100 Genetic Analyzer (Life Technologies Corp., Carlsbad, California) at the University of Illinois at Chicago DNA Sequencing Facility. Sequences were aligned with the program ClustalW (Higgins et al. 1994). The program DnaSP (Rozas et al. 2003) was used to format sequence files and to examine synonymous and nonsynonymous substitutions. *Microtus* species genetically closest to *M. pennsylvanicus* (based on the entire *Cytb* sequence) were determined by constructing phylogenetic trees using all *Microtus* species in GenBank. Unweighted pair-group method with arithmetic mean, neighbor-joining, minimum-evolution, and maximum-parsimony methods (using 2-parameter distances—Kimura 1980) were constructed using Mega2 (Kumar et al. 2001). These genetically closest species were then compared to the 3 *M. pennsylvanicus* subspecies, again using 4 tree-building methods. However, simple *p*-distances instead of Kimura 2-parameter distances were used because of the reduced amount of divergence.

Results

Disappearance of Microtus pennsylvanicus chihuahuensis.—In June of 1988 we visited the type locality and saw 2 *M. pennsylvanicus*. At that time the marsh had been partly transformed into a recreation area with 2 swimming pools. In 2000 the swimming pools were still filled by water from the spring that fed the marsh, but the surrounding vegetation, where the specimens of *M. p. chihuahuensis* had been collected in 1968 and observed in 1988, was heavily overgrazed. That year we observed what seemed to be runways of this species, but no specimens were captured in >120 trap days (day being a 24-h period). In addition, despite intensive trapping efforts, no specimens were captured in 2002, 2003, or 2004. In 2003 the condition of the marsh had improved and the flooded vegetation had recovered, because cattle had been excluded from the swimming pool and the surrounding areas. In 2004, however, several center-pivot irrigation systems had been installed in croplands located >2 km away from the marsh. As a result the stream had completely disappeared, and the marsh was reduced to a few small and scattered ponds. The recreational area was closed and decaying. In 2005 the former marsh was entirely gone; exposed soil and dry grasses had replaced it. No trapping was conducted, because appropriate habitat for the meadow vole was absent. We saw no sign of the species (Fig. 2). Our effort in the other marshes was more limited: 400 trap days in Ojo Caliente in September 2000 and 800 trap days in Ojitos in 2000 and 2003.

Taxonomic status.—Bradley and Cockrum (1968) described *M. p. chihuahuensis* on the basis of morphology. We tested

Fig. 2.—Habitat transformation of the Chihuahuan meadow vole marsh in Galeana, Chihuahua, Mexico, from A) 2000 to B) 2003 to C) 2006.

whether genetic differences corroborated a subspecies designation. A neighbor-joining tree was constructed from 46 *Microtus* species in GenBank, plus 5 *Myodes* species used as outgroups. *M. p. modestus* and *M. montanus* form a clade, and with *M. canicaudus* and *M. townsendii* form a larger clade (Fig. 3). Similar to what Conroy and Cook (2000) reported, identical topologies for these 4 species were obtained using unweighted pair-group method with arithmetic mean, minimum-evolution, and maximum-parsimony methods. These 4 genetically most similar species then were compared to *M. p. chihuahuensis* and *M. p. pennsylvanicus*.

For all museum specimens 379 bp were sequenced. All 4 museum skins yielded the same haplotype (GenBank accession number GU177626). A comparison with the sequence for *M. p. modestus* shows only 3 substitutions along this length ($\pi = 0.00782$), all of which are 3rd-position and synonymous. The genetic distances between *M. p. modestus* or *M. p. chihuahuensis* to *M. p. pennsylvanicus* are about 3.5 times as great (range of pairwise $\pi = 0.026$–0.029), but the substitutions are still all synonymous. The distances from *M. p. chihuahuensis* to *M. montanus*, *M. canicaudus*, and *M. townsendii* are about 10 times as great (range of pairwise $\pi = 0.071$–0.090). A neighbor-joining tree using only these taxa further illustrates these relationships (Fig. 4). Again, identical topologies for these 6 taxa were obtained using unweighted pair-group method with arithmetic mean, minimum-evolution, and maximum-parsimony methods. Also, we note that there were 1 or 2 nonsynonymous substitutions between *M. p. chihuahuensis* and *M. montanus* and *M. townsendii*, although none were found between *M. p. chihuahuensis* and *M. canicaudus*.

Discussion

The genetic divergences we found are consistent with divergences noted in coding mitochondrial genes among other, known murid rodent subspecies. Using mtDNA restriction fragment length polymorphisms, Plante et al. (1989) found divergences of 0.007–0.045 in *M. p. pennsylvanicus*, *M. p. drummondi*, and *M. p. aphorodemus* from Canada. However, restriction fragment length polymorphism divergence, even when corrected, is not fully comparable to nucleotide sequence divergence. Fink et al. (2004) found a divergence range of 0.007–0.248 in 1,044 bp of *Cytb* among populations of *Mictotus arvalis* spanning almost all of Europe, which must have included a number of subspecies (26 total subspecies are found in Europe—Niethammer and Krapp 1982). A slightly reduced subset of these specimens resulted in a slightly narrower divergence range of 0.010–0.019 (Haynes et al. 2003). Within the species *Peromyscus maniculatus*, 6 known California Channel Island and adjoining mainland subspecies exhibited a mean π of 0.00714 using 603 bp of cytochrome c oxidase subunit II (Pergams and Ashley 2000; Pergams et al. 2000), a value essentially identical to the 0.00782 divergence we calculated between *M. p. chihuahuensis* and *M. p. modestus*.

Were *M. p. chihuhuensis* to be considered a separate species, divergence probably should have been greater. For comparison we have 2 recent and comprehensive works on interspecific *Cytb* divergence between a large number of *Microtus* species, Conroy and Cook (2000), cited earlier, and Jaarola et al. (2004). Among the 46 *Microtus* species used in Conroy and Cook (2000), divergence in 1,143 bp of *Cytb* ranged from 0.015 (*M. abbreviatus* and *M. miurus*) to 0.18 (*M.*

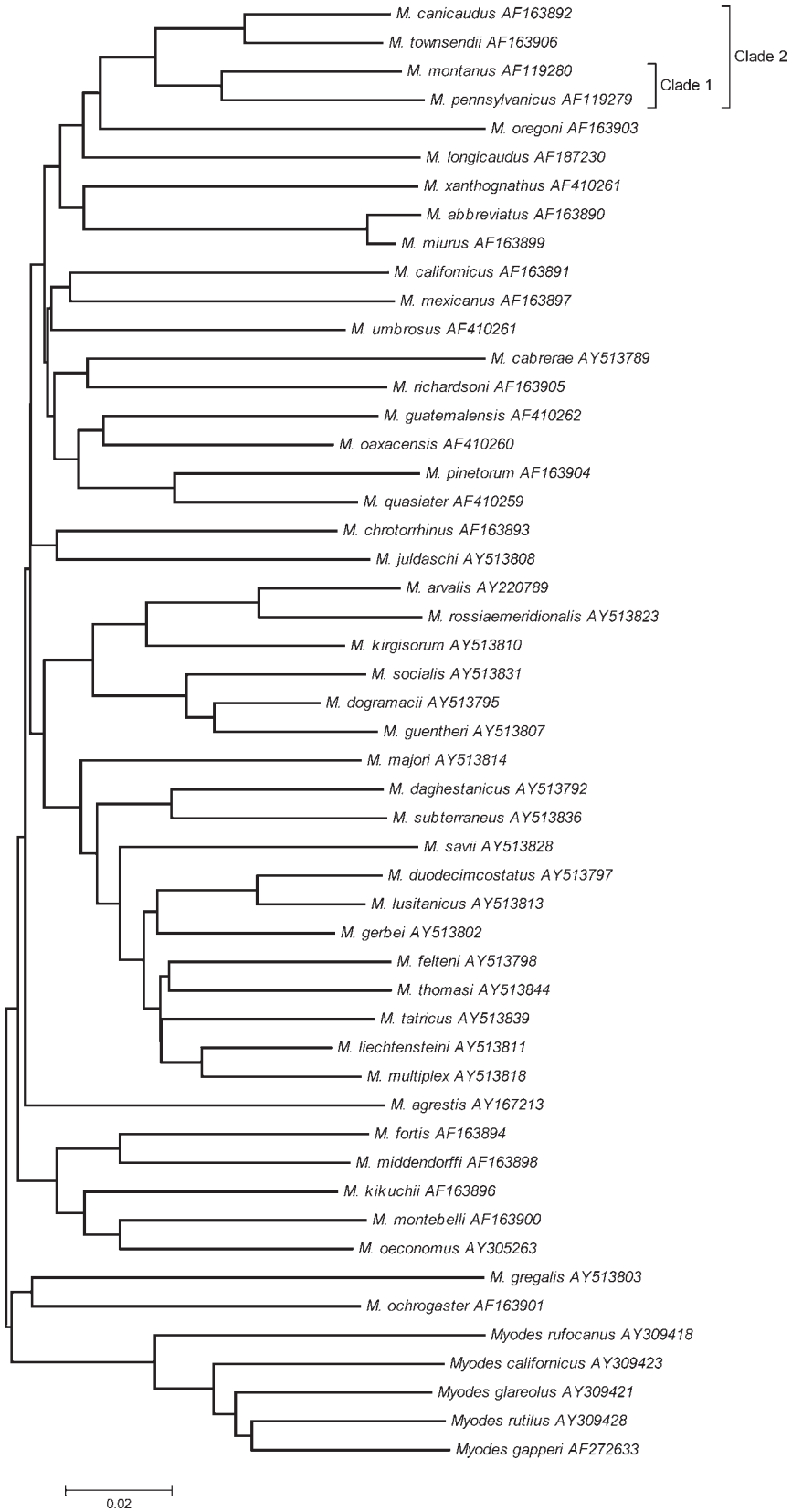

FIG. 3.—Neighbor-joining tree using Kimura 2-parameter distances (scale at bottom) and 1,143 base pairs of the cytochrome-*b* gene from 46 *Microtus* species (with genus abbreviated as *M.*) plus 5 *Myodes* species as outgroups. *Microtus pennsylvanicus* (*modestus*) and *M. montanus* form clade 1, and with *M. canicaudus* and *M. townsendii* form a larger clade 2. These 4 genetically most-similar species were compared to *M. p. chihuahuensis* and *M. p. pennsylvanicus* in this study, and identical topologies for these 4 species were obtained using unweighted pair-group method with arithmetic mean, minimum-evolution, and maximum-parsimony methods. Numbers after species are GenBank accession numbers.

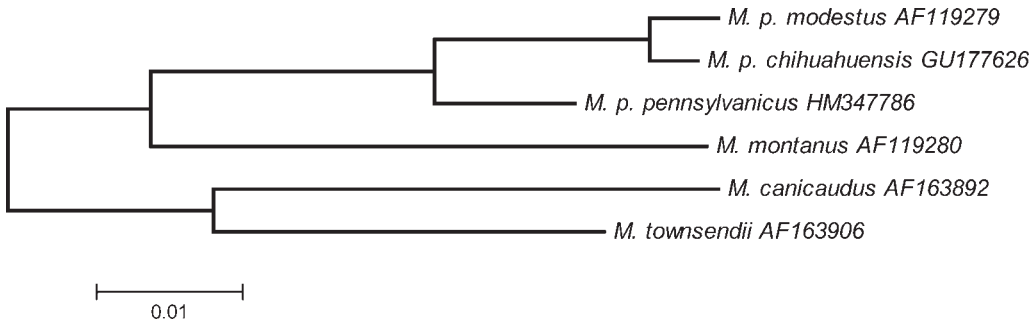

Fig. 4.—Neighbor-joining tree using *p*-distances (scale at bottom) and 379 base pairs of the cytochrome-*b* gene from *Microtus pennsylvanicus chihuahuensis*, *M. p. modestus*, *M. p. pennsylvanicus*, *M. montanus*, *M. canicaudus*, and *M. townsendii*. Numbers after taxa are GenBank accession numbers.

oregoni and *M. gregalis*). Jaarola et al. (2004) found a similar divergence range of 0.042–0.180 in 1,140 bp of *Cytb* among 49 *Microtus* species. Considering the outcomes of these studies, we conclude that *M. p. chihuahuensis* is genetically distinct enough to be considered a subspecies, thereby corroborating its morphological assignment. Although genetically closely related to the geographically closest subspecies, *M. p. modestus*, *M. p. chihuahuensis* nevertheless is genetically irreplaceable.

Between 2002 and 2004 we found 7 species of small mammals in the Galeana marsh, compared to 5 species found in 1968. Only the deer mouse (*Peromyscus maniculatus*) was trapped within the marsh in both studies (Table 1). We did not catch any Chihuahuan voles, nor did we see any sign of them. Including the marsh and adjacent dry grassland, we trapped 7 species that Bradley and Cockrum (1968) did not trap in either of these habitats (Table 1). One was an introduced species, the black rat (*Rattus rattus*). In the dry grassland we trapped 1 species associated with surrounding scrub, Merriam's kangaroo rat (*Dipodomys merriami*). In addition to the Chihuahuan vole, we did not trap 3 species (*Reithrodontomys montanus*, *Reithrodontomys megalotis*, and *Sigmodon hispidus*) previously reported in the marsh and dry grassland. We also collected 3 species of fish from the springs and streams of Galeana: Tex-Mex gambusia (*Gambusia speciosa*), black bullhead (*Ameiurus melas*), and whitefin pupfish (*Cyprinodon albivelis*), an undescribed species at that time (Minckley et al. 2002). The red shiner (*Cyprinella lutrensis*), which was collected in the springs by Edwards et al. (2003), was not found during our study. During fieldwork we also observed mud turtles (*Kinosternon flavescens*) and many bullfrogs (*Rana catesbeiana*), the latter an introduced species. During the 2005 visit, after the area had dried out, the only signs of the former aquatic life were buried skulls of *A. melas*.

The lack of success in trapping Chihuahuan meadow voles in the Galeana marsh and the other marshes of the region indicates the possibility that the subspecies was not present. The disappearance of the Galeana marsh practically confirms the extirpation of this population. Although an unknown population could exist elsewhere in northwestern Mexico, this possibility is remote, and for practical purposes this subspecies is most likely extinct. The size of the marsh in the early part of our study was similar to that reported by Bradley and Cockrum (1968) 3 decades earlier. The replacement of rodent

Table 1.—Changes in the small mammal community composition in the Ojo de Galeana marsh, Chihuahua, Mexico, from 1968 to 2004.

	Bradley and Cockrum (1968)		This study (2000–2004)	
Rodentia species	Marsh	Adjacent dry grass	Marsh	Adjacent dry grass
Heteromyidae				
Dipodomys merriami				X
Chaetodipus hispidus				X
Chaetodipus intermedius			X	
Chaetodipus penicillatus			X	
Muridae				
Microtus pennsylvanicus	X			
Neotoma albigula			X	
Onychomys torridus				X
Peromyscus leucopus		X	X	X
Peromyscus maniculatus	X	X	X	X
Reithrodontomys megalotis	X	X		
Reithrodontomys montanus	X	X		
Ratus rattus			X	
Sigmodon fulviventer		X	X	
Sigmodon hispidus	X	X		
No. species	5	6	7	5

species (compared to our earliest surveys) indicates that rodent composition had changed to one more characteristic of scrubland habitats. Thus, the decline of the Chihuahuan meadow vole likely began before the drilling of wells for center-pivot irrigation. The loss of a system of desert springs always represents an important loss, but in this case the wetland also was the only known lowland locality of the whitefin pupfish (*C. albivelis*), and the only known locality outside the Río Papigochic drainage (Minckley et al. 2002). The establishment of wells and irrigation channels for the growing town and agricultural area probably caused the loss of the wetland. The diurnal nature of the Chihuahuan meadow vole made it more sensitive to the development of a recreational area in the springs and the increased human activity and presence of pets. A subspecies that was isolated in a very small but stable site for thousands of years was, not surprisingly, sensitive to change. This supports the idea that populations at the margins of species ranges are more susceptible to extinction than core populations (Lienert et al. 2002; Tomback et al. 2005).

We have trapped the few other existing isolated marshes between Galeana and the United States, but we have been unable to trap or find evidence of other isolated populations of *M. p. chihuahensis* in northern Chihuahua. Therefore, we conclude that the Chihuahuan meadow vole has become extinct in Mexico. In this case 1 or 2 deep wells caused the disappearance of the desert spring that maintained the mesic habitat of *M. p. chihuahuensis* in an otherwise arid region. Similar accounts in the literature include that of *Peromyscus guardia* from Estanque Island off Baja California, which became extinct in only a few years as the result of the introduction of a single cat (Vázquez et al. 2004). The extirpation of *M. pennsylvanicus* from the only known locality in Mexico increases to >50 the number of vertebrate species that have become either extirpated or extinct in the country in the last century (Ceballos and Oliva 2005).

The disappearance of *M. p. chihuahuensis* represents the extinction of a subspecies and contraction of the geographic range of the species with the loss of the extreme southern component of its distribution. Because isolation is one of the main sources of allopatric speciation (Bush 1975), the loss of marginal or relict populations such as the Chihuahuan meadow vole reduces not only the species richness of a country or region but also the evolutionary potential of the earth's biota. Although the allocation of resources to the conservation of marginal populations is controversial (Bunnell et al. 2004), in the case of *M. p. chihuahuensis* the absence of conservation efforts resulted in the extinction of an endemic subspecies.

The loss of *M. p. chihuahuensis* is an example of population extinction, a very severe form of biodiversity loss mostly neglected until recently (Ceballos and Ehrlich 2002; Hughes et al. 1997). According to Ceballos and Ehrlich (2002), millions of populations have become extinct in recent decades due to human activities. The loss of those populations reduces morphological, genetic, and ecological diversity. Although relatively little information exists about the magnitude of the negative impacts of such population losses, it is well established that population losses at a local or regional level must be treated as total extinctions. Their disappearance modifies the structure and function of communities and ecosystems and the delivery of ecosystems services (Corlet 2007; Luk et al. 2003; Mayfield et al. 2005; Şekercioğlu et al. 2004), regardless of the persistence of the same species elsewhere.

The disappearance of a population often reduces the geographic range of a species and makes it more vulnerable to extinction both by human and natural causes. Many species are distributed across political boundaries, either state or national (e.g., Bunnell et al. 2004; Manzano-Fischer et al. 2006). This pattern of persistence of marginal populations is repeated in other parts of the world (Burbidge and McKenzie 1989; Channell 1998; Channell and Lomolino 2000). Maintaining marginal populations reduces the risk of global extinction due to policy differences among countries, political instability, economic trends, and other factors that are less likely to manifest themselves similarly in 2 adjacent countries (Ceballos and Ehrlich 2002). The Chihuahuan meadow vole is a good example of the high vulnerability to extinction of subspecies with both restricted geographic ranges and marginal populations. It represents the species with the most restricted geographic range of all mammals in Mexico. Its extremely rapid extinction indicates the vulnerability to extinction of range-restricted subspecies caused by anthropogenic causes.

Resumen

Microtus pennsylvanicus está representado en México sólo por el metorito de Galeana (*M. p. chihuahuensis*), conocido únicamente de 1 población disyunta en un pantano pequeño y aislado en las zonas áridas del norte de Chihuahua. Los muestreos se realizaron entre 2000 y 2004, sin lograr la captura de ningún espécimen de *M. p. chihuahuensis*, ni se observó evidencia alguna de su presencia. Al final de este estudio el pantano se había secado, desapareciendo completamente el hábitat del metorito. Muestreos en otros pantanos en el noroeste de Chihuahua tampoco aportaron prueba de la presencia de esta especie. Por lo que concluimos que *M. p. chihuahuensis* ha sido extirpada de la única localidad conocida en México. Se utilizó ADN de ejemplares de museo para evaluar la divergencia genética entre *M. p. chihuahuensis* y otras 46 especies y subespecies existentes de *Microtus*. Nuestros resultados apoyan el estatus subespecífico de *M. p. chihuahuensis*. La pérdida de esta subespecie es un ejemplo de la extinción de una población y una forma muy severa de la pérdida de la biodiversidad. Hasta hace poco estas pérdidas habían sido menospreciadas.

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