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**GEOGRAPHIC DISTRIBUTION, GENETIC DIVERSITY, AND
CONSERVATION STATUS OF THE SOUTHERN FLYING SQUIRREL
(*GLAUCOMYS VOLANS*) IN MÉXICO**

Front cover: Three southern flying squirrels (*Glaucomys volans*) emerging from a nest cavity in a mature dead pine tree (*Pinus* sp.). Old growth trees, especially oaks and snags, are important nesting sites for flying squirrels in south-central Mexico. Photograph by Gerardo Ceballos.

GEOGRAPHIC DISTRIBUTION, GENETIC DIVERSITY, AND CONSERVATION STATUS OF THE SOUTHERN FLYING SQUIRREL (*GLAUCOMYS VOLANS*) IN MÉXICO

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ABSTRACT

Ecological surveys were conducted in central México to assess parameters associated with the distribution, habitat preference, and nesting sites of the southern flying squirrel (*Glaucomys volans*). These surveys indicated that deforestation and harvesting of mature oak trees has depopulated flying squirrels from a large portion of their historic range. DNA sequences obtained from the mitochondrial control region were used to evaluate genetic divergence among four populations in central and southern México. Levels of intrapopulation genetic variation were greatest in the Chapa de Mota, México population (average genetic distance = 2.8%), whereas interpopulational comparisons showed the Chapa de Mota, México population to be the most different from the Floresta, Chiapas population (average genetic distance = 4.1%). Results indicate that substantial genetic variation exists between populations in southern México and suggests either a historical bottleneck associated with the Isthmus of Tehuantepec in Chiapas or more widespread gene flow across central México influenced by the Sierra Madre Oriental and Trans-Mexican Volcanic Zone. Conservation efforts are needed, especially in southern México, to preserve populations of southern flying squirrels threatened by habitat loss.

Key words: conservation, flying squirrels, genetic diversity, *Glaucomys volans*, México

RESUMEN

Se condujeron inventarios ecológicos en la parte central de México, con el fin de evaluar los parámetros asociados a la distribución, preferencia de hábitat, y sitios de crianza de la ardilla voladora sureña (*Glaucomys volans*). Estos inventarios indicaron que la deforestación y la recolección de encinos maduros han despoblado a las ardillas voladoras de una gran porción de su distribución histórica. Secuencias de ADN obtenidas de la región control mitocondrial fueron utilizadas para evaluar la divergencia genética entre cuatro poblaciones del centro y sur de México. Los niveles de variación genética fueron mayores en Chapa de Mota, México (distancia genética media = 2.8%), mientras que las comparaciones interpopulacionales muestran que Chapa de Mota, México es muy diferente que la población de Floresta, Chiapas (distancia genética media = 4.1%). Los resultados indican que existe una variación genética substancial entre poblaciones en el sur de México y sugiere que esto podría ocurrir por cuellos de botella históricos asociados con el istmo de Tehuantepec en Chiapas o por flujo génico a lo largo del centro de México influenciado por la Sierra Madre Oriental y la Zona Volcánica Transversal de México. Esfuerzos de conservación son necesarios, especialmente en el sur de México, para preservar las poblaciones de ardilla voladora del sur amenazadas por pérdida de hábitat.

Palabras clave: ardillas voladoras, conservación, diversidad genética, *Glaucomys volans*, México

INTRODUCTION

The southern flying squirrel (*Glaucomys volans*) is distributed from southeastern Canada through the eastern United States to central Honduras (Hall 1981). Typically, *G. volans* is associated with deciduous-hardwood forests, relying on mature oak trees (*Quercus*) for food and nest cavities (Dolan and Carter 1977). This reliance on mesophytic forests coupled with Pleistocene climatic changes has resulted in a disjunct distribution for *G. volans* (Braun 1988; Arbogast 1999; Ceballos et al. 2010). Three allopatric distributions have been documented, with the largest occurring in the eastern United States and southern Canada, the second occupying southern México and northern Central America, and the third including a small area in southwestern portion of the Mexican State of Chihuahua (Goldman 1936). Information on the present distribution and habitat use of flying squirrels in México is sparse (Ceballos and Oliva 2005). The species is considered threatened in México due to a restricted distribution and habitat loss (SEMARNAT 2002).

Flying squirrels in México and Central America probably originated from a population in the southeastern United States, following corridors of suitable habitat during the Pleistocene, but the timing and dynamics of the interchange are unknown (Ceballos et al. 2010; Kerhoulas and Arbogast 2010). Arbogast (1999) suggested that the patterns of genetic variation in the southern flying squirrel were the result of Pleistocene shifts in the distribution of oak (*Quercus*) and hickory (*Carya*) forests. Historically, six subspecies of flying squirrels were documented in México and Central America (*chontali*, *goldmani*, *herreranus*, *madrensis*, *oaxacensis*, and *underwoodi*; Hall 1981); however, only three subspecies currently are recognized (*goldmani*, *guerreroensis*, and *oaxacensis*;

Diersing 1980). Recently, Kerhoulas and Arbogast (2010) examined five populations from México and Guatemala and reported that genetic divergence values in the mitochondrial cytochrome-*b* gene ranged from 0.71% (Oaxaca Highlands and Sierra Madre de Chiapas) to 4.38% (Chiapas Highlands and Sierra Madre del Sur). These five populations differed genetically from populations located in eastern North America by divergence values ranging from 2.3-4.9%. Kerhoulas and Arbogast (2010) concluded that two primary genetic groups existed - one from the Sierra Madre del Sur and another that included all other populations from México and Guatemala, although the later group potentially could be subdivided into additional groups. These two groups were hypothesized to have diverged from one another approximately 0.75-0.50 million years ago. No definitive taxonomic conclusions could be reached because of incomplete sampling of some subspecies and geographic regions.

In this paper, we evaluate the following issues regarding flying squirrels in México: 1) geographic distribution and habitat use; 2) genetic diversity; and 3) conservation status. We specifically assessed the genetic diversity of four populations of *G. volans* from the states of Chiapas, México, and Querétaro. These populations represent different geographic regions, including the Sierra Madre Oriental and the Trans-Mexican Belt, which were not available for study by Kerhoulas and Arbogast (2010). Nucleotide sequences from the mitochondrial DNA control region (D-loop) were used to assess genetic divergence within and among populations. The D-loop region evolves at a sufficient rate (Petersen and Stewart 2006) to detect genetic divergence among populations in flying squirrels.

METHODS

Geographic distribution and habitat use.—The historical distribution of *G. volans* in México was determined on the basis of published and unpublished records. Localities where species have been recorded were characterized for geographic coordinates, vegetation type, and elevation. Localities were grouped

together in eight more or less discrete mountain regions. The area of each region and the distance between closest regions were calculated.

To determine habitat requirements of flying squirrels, we compared four adjacent areas in Pinal de

Amoles, Querétaro. This locality possesses a rough topography, covered by pine, oak and pine-oak forests surrounded by an arid zone. Vegetation associated with the zone has been highly fragmented due to agricultural and forestry practices. We randomly selected 12 plots (100 x 50 m) known to harbor flying squirrels and four plots without squirrels. In each plot, tree diversity, density, height, and DBH (diameter at breast height) of all the trees > 10 cm of DBH were measured. Each time a nest was located we recorded the following variables: tree species, DBH, tree height, number of dens per tree, diameter and height of den, height and width of the entrance of the den, content of the den (e.g., feathers, moss, hair, lichen), and number of animals found inside the den. Tree diversity was calculated using the Shannon-Wiener diversity index (Zar 1984).

Stepwise discriminant functions analysis (DFA) was used to separate plots that were occupied by squirrels from those without squirrels and to identify variables contributing to the distribution of squirrels in the zone. Prior to the multivariate analysis and univariate screen procedure (ANOVA), variables were selected having an F value with at least $p < 0.10$. An F-to-enter of 3.84 and an F-to-remove of 2.71 were utilized. Distribution of squirrels was investigated using a DFA analysis, habitat variable, plots possessing squirrels (e.g., nests, squirrels present), and plots with no evidence of squirrels.

Sampling.—Flying squirrels were collected from four localities in central and southern México (Fig. 1) by either wiring a Sherman live-trap (H. B. Sherman

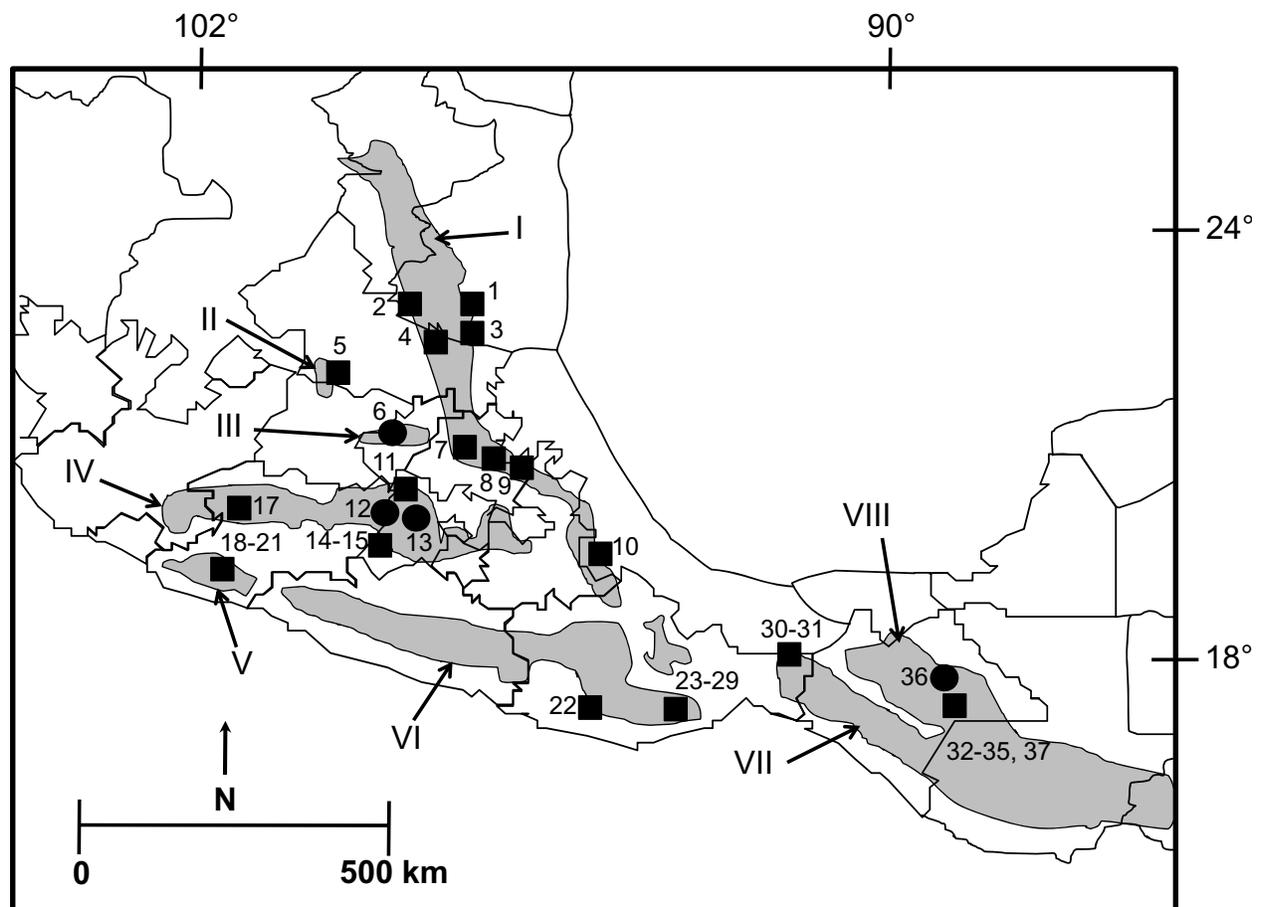


Figure 1. Geographic distribution of *Glaucomys volans* in México (see Table 1 and Appendix I for details). Localities (1-37) utilized in the habitat and nest cavity analyses are represented by boxes; in addition, localities 6, 12, 13, and 36 were used in the genetic analyses and are indicated by circles. Roman numerals (I-VIII) reflect the major mountain ranges discussed in this study.

Trap Company, Tallahassee, Florida) to a tree limb (baited with bacon and apple bits, oats, or chicken meat) or by placing a net over a tree cavity and striking the tree and capturing individual squirrels in a hand net. Flying squirrels were anesthetized with ketamine (following methods approved in the guidelines of the American Society of Mammalogists Animal Care and Use Committee 1998) and hair, blood, or toe samples were collected and the animal released. In some cases, animals were euthanized and tissue samples (muscle, liver, heart, and kidney) were obtained.

Genetic diversity.—Mitochondrial DNA was extracted either from hair, blood, or tissue samples and purified using the Wizard Miniprep kit (Promega, Madison, Wisconsin), or genomic DNA was isolated following the methods of Smith and Patton (1999). Depending on the quality of DNA, the entire D-loop region (approximately 1,000 bp) and a portion of the 5'-proline tRNA (approximately 85 bp) were amplified using PCR primers specific to the D-loop (Castro-Campillo et al. 1999) or smaller regions of the D-loop (200--300 bp) were amplified using internal D-loop primers (Méndez-Harclerode et al. 2005).

The following thermal profile was used for PCR amplification: an initial cycle of 93.5° C for 1 min, followed by 33 cycles of 93.5° C for 40 sec, 49° C for 40 sec, and 72° C for 1 min 30 sec, and a final cycle of 72° C for 2 min. PCR products were purified with a QIAquick kit (Qiagen Inc., Chatsworth, California). Sequencing reactions used primers reported in Méndez-Harclerode et al. (2005). Cycle sequencing was conducted using the ABI Prism dRhodamine terminator ready reaction mix (Applied Biosystems, Foster City, California) or ABI Big Dye version 3.0 ready reaction mix and samples were analyzed on an ABI Prism 310 or ABI 3100-*Avant* automated sequencer (Applied Biosystems, Foster City, California). Both forward and reverse sequences were obtained. Sequences initially were aligned using Sequencher 3.1 software (Gene Codes, Ann Arbor, Michigan) and then adjusted manually. All sequences were deposited into GenBank (accession numbers are provided in Appendix I).

Two samples of *G. volans* obtained from GenBank (Petersen and Stewart 2006) were used as outgroups in all analyses. Variable nucleotide positions were treated as unordered, discrete characters with five possible states: A, C, G, T, or gaps. Several alignments, reflecting differences in the placement of gaps, were examined using the heuristic search option in PAUP* (Swofford 2002). The alignment that produced the shortest tree was assumed to have incorporated the best placement of gaps and was used in all analyses.

The Tamura-Nei model of evolution (Tamura and Nei 1993), developed specifically for analysis of DNA regions with different rates of evolution, was used to calculate genetic distances. These values were then used to assess levels of genetic divergence within and among populations. A network was constructed using the software program TCS (version 1.18; Clement et al. 2000) under the method of statistical parsimony (Templeton et al. 1992). Twenty-two individuals (outgroup excluded) were included in this analysis and potentially unsampled haplotypes were estimated.

Conservation Status.—To evaluate the present conservation status of flying squirrels in central México, surveys were carried out in five localities in three states (Hidalgo, Querétaro, and México). Localities where relatively healthy populations of flying squirrels were found in the last decade were selected for this study (Ceballos and Galindo 1983; Ceballos and Miranda 1985). We searched for flying squirrels in pine-oak and oak forests using a simple searching technique that has been very useful to locate and capture flying squirrels (Ceballos and Galindo 1983; Ceballos and Miranda 1985). The technique consisted of establishing transects in dense mature forests and searching all trees for crevices and nests. Once a hollow or nest was found, squirrels were captured with either a hand net or trapped as described previously. At each locality we recorded the number of nests and flying squirrels found, general conditions of the habitat, and the presence of human activities impacting the forests.

RESULTS

Geographic distribution and habitat use.—*Glaucomys volans* has a scattered distribution in eastern, central, and southern México (Fig. 1). Knowledge of the species' distribution in México has become more detailed in the last two decades, where most localities along the Trans-Mexican Volcanic Belt in central México have been recorded, including new records from Villa Victoria, San Cayetano, Chapa de Mota, and Dexcaní (México), Zitácuaro (Michoacán), and Santa Elena (Hidalgo). In México, flying squirrels are known from 37 localities in 11 states (Fig. 1 and Table 1), with most records from the southern states of Oaxaca and Chiapas. The northernmost record is from Rancho del Cielo (Tamaulipas; 23°04' N, 99°11' W), southernmost from San Sebastián Rio Hondo (Oaxaca; 16°10' N, 96°27' W), easternmost from Ocosingo (Chiapas; 16°54' N, 92°06' W), and westernmost from Pátzcuaro (Michoacán; 19°31' N, 101°36' W).

Flying squirrels have been found at elevations from 840 m in Acahuizotla (Guerrero) to 2,800 m in Tepozán (Hidalgo) and 3,048 m in Cofre de Perote (Veracruz). They have a disjunct distribution, and populations are confined to the following eight, more or less well-defined, isolated mountain ranges: I) Sierra Madre Oriental; II) San Luis Potosí; III) Sierra Gorda; IV) Eje Neovolcánico; V) Sierra Madre del Sur de Guerrero; VI) Sierra Madre del Sur de Oaxaca; VII) Sierra Madre de Chiapas; and VIII) Macizo Central de Chiapas (Fig. 1). Such mountain ranges vary in size, ranging from 801 to 67,142 km², and are isolated from each other by belts of xeric (arid) or mesic (tropical) vegetation. The number of populations in each mountain range was weakly but positively correlated with their size ($r = 0.87$), and most localities were in the larger mountain ranges (VI - Sierra Madre de Oaxaca, and IV - Eje Neovolcánico).

The distribution of flying squirrels is restricted to temperate plant communities, especially oak (*Quercus*) and mixed (oak-pine) forests (Table 2). All records were from oak (31%), pine-oak (56%), cloud (10%), and mixed (pine-fir-oak; 3%) forests. These forests have an annual precipitation between 600 and 1,200 mm and average annual temperatures ranging from 12

to 20°C. In Pinal de Amoles (Querétaro), flying squirrels were found exclusively in oak forests and were recorded in 11 species of trees (five oaks and six pines; Table 2). The most abundant tree was the “escobillo” oak (*Quercus laurina*), which accounted for 55% of all individuals sampled. A comparison between areas with and without flying squirrels showed that they differed in plant structure and plant species composition (Table 3). The diversity of the species of trees was significantly higher in the areas without squirrels ($t = -2.563$, $n = 11$, $p = 0.02$). Habitats possessing flying squirrels were dominated by *Q. laurina* (84% of all trees); in contrast, in areas without squirrels, the dominant species were “roble” oaks (*Quercus crassifolia*; 26.4%), “lacio” pines (*Pinus patula*, 24%), and “prieto” pines (*Pinus montezumae*, 20%). Additionally, tree height, DPA, density and diversity of herbaceous plants, trees with cavities, and total number of cavities had lower values in areas without squirrels (Table 3). There was a positive significant classification of both sites with and without squirrels ($X^2 = 27.89$, d.f. = 4, $P < 0.0000$), based on DPA tree values, and greater density and diversity of herbaceous vegetation.

Few flying squirrels nests have been recorded in México, and all have been recorded in cavities of either oak or pine trees (see also Ceballos and Galindo 1983; Ceballos and Miranda 1985). At Chapa de Mota, México, all nests ($n = 20$) were found in dead pine trees in a pine-oak forest. In Pinal de Amoles, there were more trees with cavities and more cavities in plots with flying squirrels (Fig. 2, Table 3). The highest percentage of hollows was found in escobillo oaks (*Q. laurina*, 74%), followed by roble oaks (*Q. crassifolia*, 16%), and black oaks (*Q. rugosa*, 7.2%). Most (92%) nests were located in escobillo oaks and the remaining in roble oaks. All nests were lined exclusively with moss and possessed an average of two individuals. At Pinal de Amoles, the presence of oak trees seems to be a limiting factor for the distribution of *G. volans*, as we found nests only in the hollows of a single species of oak. The forest of escobillo oak presented the largest availability of hollows, as hollows naturally are formed in this species of oak. The number of hollows, in relation with the total number of trees, is very small in the

Table 1. Localities where flying squirrels (*Glaucomys volans*) have been recorded in México. Localities are organized by mountain range (Regions I-VIII) and indicate the primary plant communities and elevation. For forest type, the dominant tree group is listed first. Abbreviations are as follows: Latitude (Lat), longitude (Long), and elevation (Elev).

State	Locality	Lat/Long	Forest	Elev (m)	Reference
Region I (Sierra Madre Oriental)					
Tamaulipas	1) Rancho El Cielo	23°50' 99°12'	Cloud	1,320	Koopman and Martin 1959
	2) Aserradero Paraiso	23°50' 99°17'	Cloud	420	Koopman and Martin 1959
	3) Aserradero Infiernillo	23°03' 99°13'	Cloud	1,050	Koopman and Martin 1959
San Luis Potosí	4) Santa Barbarita	22°28' 99°23'	Oak	1,020	Dalquest 1953
Region II (San Luis Potosí)					
San Luis Potosí	5) Xilitla	21°20' 98°58'	Oak	2,420	Ceballos, G. unpubl. data; Dalquest 1953
Region III (Sierra Gorda)					
Querétaro	6) Pinal de Amoles	21°06' 99°38'	Oak	2,520	Nelson 1904
Region IV (Eje Neovolcánico)					
Hidalgo	7) Rancho Santa Elena	N/A	Oak-pine	N/A	Ceballos, G. unpubl. data
	8) Tepozán	19°47' 98°14'	Pine-oak-fir	2,800	Ceballos and Galindo 1983
Puebla	9) Santa Rita Tlahuapan	19°22' 98°32'	Oak	2,600	Castillo-Meza et al. 1997
Veracruz	10) Cofre de Perote	19°34' 97°09'	Oak-cloud	3,000	Hooper 1952
México	11) Dexcani El Alto	19°56' 99°26'	Oak	2,500	Sanchez, O. unpubl. data
	12) Chapa de Mota	19°49' 99°40'	Oak	N/A	Chavez and Ceballos 1998
	13) San Bartolo Morelos	19°49' 99°37'	Oak	2,100	Ceballos and Miranda 1985
	14) Villa Victoria	19°26' 99°59'	Pine-oak	2,500	Ceballos, G. unpubl. data
	15) San Cayetano	N/A	Pine-oak	N/A	Cervantes et al. 1995
Michoacán	16) Zitácuaro	19°26' 100°21'	Oak	2,500	Ceballos, G. unpubl. data
	17) Pátzcuaro	19°31' 101°35'	Oak-pine	2,500	Hooper 1952

Table 1. (cont.)

State	Locality	Lat/Long	Forest	Elev (m)	Reference
Region V (Sierra Madre del Sur de Guerrero)					
Guerrero	18) Acahuizotla	17°23' 99°27'	Pine-oak	900	Goodwin 1961
	19) Agua de Obispo	17°17' 99°22'	Pine-oak, oak	960	Goodwin 1961
	20) Coapongo	17°23' 99°34'	Pine-oak, oak	2,200	Goodwin 1961
	21) Omiltemi	17°31' 99°40'	Pine-oak, oak	2,190	Goodwin 1961
Region VI (Sierra Madre del Sur de Oaxaca)					
Oaxaca	22) Cerro San Felipe	17°30' 96°43'	Oak	900	Goodwin 1961
	23) Cerro Yucunaca	16°49' 97°35'	Pine-oak	2,100	Goodwin 1961
	24) Santo Domingo Chonteco- matlán	16°15' 96°01'	Pine-oak	2,100	Goodwin 1961
	25) San Pedro Jilotepec	16°32' 95°36'	Pine-oak	1,500	Goodwin 1961
	26) Santo Domingo Nejapa	16°37' 95°38'	Pine-oak	1,000	Goodwin 1961
	27) San. Sebastian Jilotepec	16°34' 95°38'	Pine-oak	1,200	Goodwin 1961
	28) San Sebastian Rio Hondo	16°10' 96°27'	Oak	1,000	Goodwin 1961
	29) Tenango	16°16' 95°36'	Oak	1,500	Goodwin 1961
	Region VII (Sierra Madre del Sur de Chiapas)				
Oaxaca	30) Zanatepec	16°37' 94°16'	Oak	1,500	Goodwin 1961
Chiapas	31) Ocozocuahtla	16°52' 93°22'	Pine-oak	1,800	Goodwin 1961
Region VIII (Macizo Central de Chiapas)					
Chiapas	32) Comitán	16°15' 92°07'	Pine-oak	2,400	Goodwin 1961
	33) Ocosingo	16°54' 92°06'	Pine-oak	1,500	Barrera 1958
	34) San Cristobal de las Casas	16°45' 92°30'	Oak	2,460	Goodwin 1961
	35) Huitepec	16° 45' 92° 28'	Oak	2,400	Espinoza-Medimilla et al. 2004
	36) Floresta	16°28' 92°18'	Oak	2,140	This study
	37) Teopisca	16°20' 92°14'	Pine-oak	1,800	Nelson 1904

Table 2. Habitat characteristics at 16 sites (100 m X 50 m each) near Pinal de Amoles, Querétaro where *Glaucomys volans* were recorded as either present or absent. Flying squirrels were found in sites differing in tree species composition and abundance when compared with adjacent sites without flying squirrels. Percentages are provided in parentheses.

Tree Species	<i>Glaucomys</i> Present	<i>Glaucomys</i> Absent	Tree Abundance
<i>Alnus jorullensis</i>	6 (1)	1 (<1)	9 (1)
<i>Arbutus glandulosa</i>	1 (< 1)	44 (5)	51 (2)
<i>Cercocarpus macrophyllus</i>	0	9 (1)	9 (1)
<i>Pinus montezumae</i>	14 (2)	13 (2)	37 (1)
<i>Pinus nelsonii</i>	7 (1)	180 (20)	204 (6)
<i>Pinus patula</i>	46 (7)	217 (24)	523 (16)
<i>Quercus crassifolia</i>	18 (3)	237 (27)	455 (14)
<i>Quercus laurina</i>	572 (84)	95 (11)	1,812 (55)
<i>Quercus mexicana</i>	1 (< 1)	54 (6)	56 (2)
<i>Quercus praineana</i>	2 (< 1)	1 (< 1)	7 (1)
<i>Quercus rugosa</i>	144 (3)	11 (2)	42 (5)
Total	678	893	3,207

Table 3. Habitat characteristics of 16 sites (100 m X 50 m each) near Pinal de Amoles, Querétaro where *Glaucomys volans* were recorded as either present or absent. Abbreviations are as follows: H' (Shannon Weiner Diversity Index), H_{max} (maximum diversity), and J' (evenness of species abundance).

Characteristics	<i>Glaucomys</i> Present	<i>Glaucomys</i> Absent
Number of tree species	10	11
Tree density	169.5	223.2
Number of individual trees	678	893
H' (H_{max})	0.3 (1)	0.8 (1.04)
J'	0.303	0.771
Average tree height (m)	15.9	12.1
Diameter at breast height (cm)	35.9	24.2
Number of hollows	50	3
Shrub density	193	116
Number of individuals	770	464
Herb density	117	182
Number of individuals	468	726
Slope	32.2	39.8

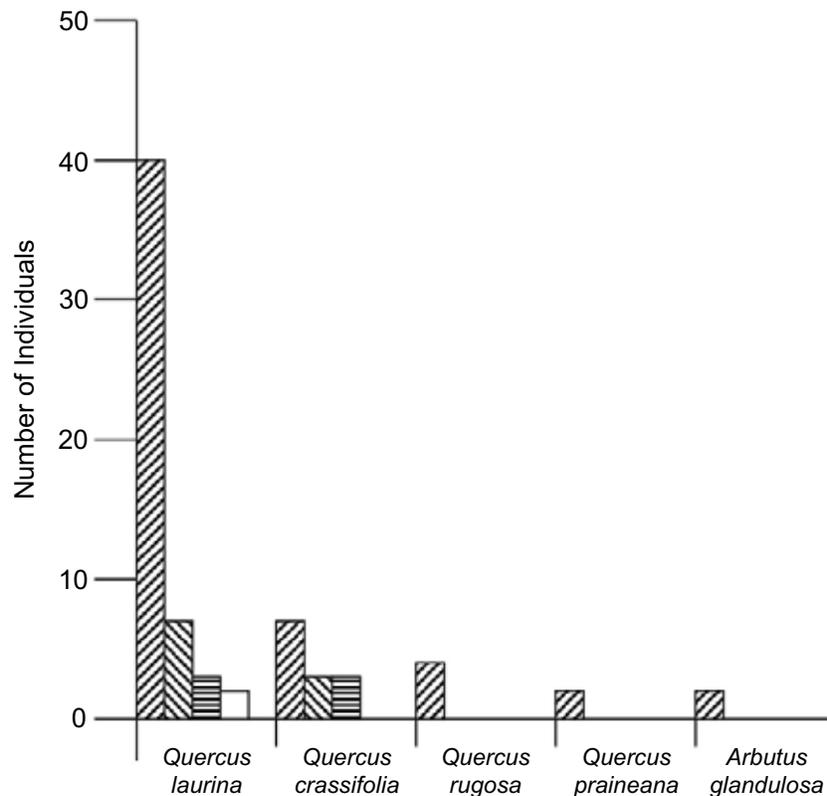


Figure 2. Number of cavities suitable for flying squirrel nests in various species of oak trees at Pinal de Amoles, Querétaro. The different bars indicate one (diagonal hatched to the right), two (diagonal hatched to the left), three (hatched horizontally) or four (open) cavities per tree.

area of Pinal de Amoles and is probably due to different natural and anthropological factors. For example, sawmills prefer trees of 30 cm dbh and 25 m of height, thereby limiting the number of trees that reach maturity and large size. Consequently, the number of den sites are limited, which in turn directly limits the utilization of habitat by flying squirrels (Muul 1968, 1974).

Genetic diversity.—DNA sequences were obtained from 23 samples of *G. volans* representing the four populations examined in central and southern México (Appendix I). Due to poor quality and quantity of some DNA samples, amplified sequences ranged in size from 243 to 1,165 bp. As a consequence of insertion/deletions, that were not a product of incomplete sequences, gaps were inserted to align sequences and resulted in a total alignment of 1,181 bp (including a portion of the proline tRNA region).

Genetic distance values for selected populations are listed in Table 4. Values for within population comparisons ranged from 0.18% (Floresta, Chiapas) to 2.8% (Chapa de Mota, México), and interpopulation comparisons ranged from 3.0% (Pinal de Amoles, Querétaro and Chapa de Mota, México) to 5.5% (Pinal de Amoles, Querétaro and Floresta, Chiapas).

The statistical parsimony network (Fig. 3) indicated that the haplotype shared by seven individuals from Floresta, Chiapas appeared to be the most basal haplotype based on the “star-like” topology with other haplotypes radiating from it. Based on the number of substitutions from the Floresta, Chiapas haplotype, samples from Chapa de Mota, México appear to be unique and more divergent than samples from Pinal de Amoles, Querétaro or San Bartolo Morelos, México.

Table 4. Average genetic distances estimated using the Tamura-Nei model of evolution (Tamura and Nei 1993) for selected comparisons of populations of *Glaucomys*. Ranges are indicated in parentheses, where appropriate.

Comparison	Average Genetic Distance
Within populations	
Floresta, Chiapas	0.18% (0.00-1.11%)
Pinal de Amoles, Querétaro	0.42% (0.00-0.83%)
Chapa de Mota, México	2.81% (1.40-4.20%)
Between populations	
Floresta, Chiapas – Pinal de Amoles, Querétaro	2.56% (0.75-8.19%)
Floresta, Chiapas – Chapa de Mota, México	4.10% (1.02-8.41%)
Floresta, Chiapas – San Bartolo Morelos, México	1.70% (1.36-2.34%)
Pinal de Amoles, Querétaro – Chapa de Mota, México	2.92% (0.94-4.30%)
Pinal de Amoles, Querétaro – San Bartolo Morelos, México	1.12% (0.74-1.52%)
San Bartolo Morelos, México – Chapa de Mota, México	1.86% (1.53-2.68%)
Chapa de Mota, México – Oklahoma	5.80% (5.48-6.10%)
Oklahoma – All samples from México	5.40% (4.10-7.30%)
Oklahoma – Nova Scotia	5.20% (NA)
All samples from México – Nova Scotia	10.10% (NA)

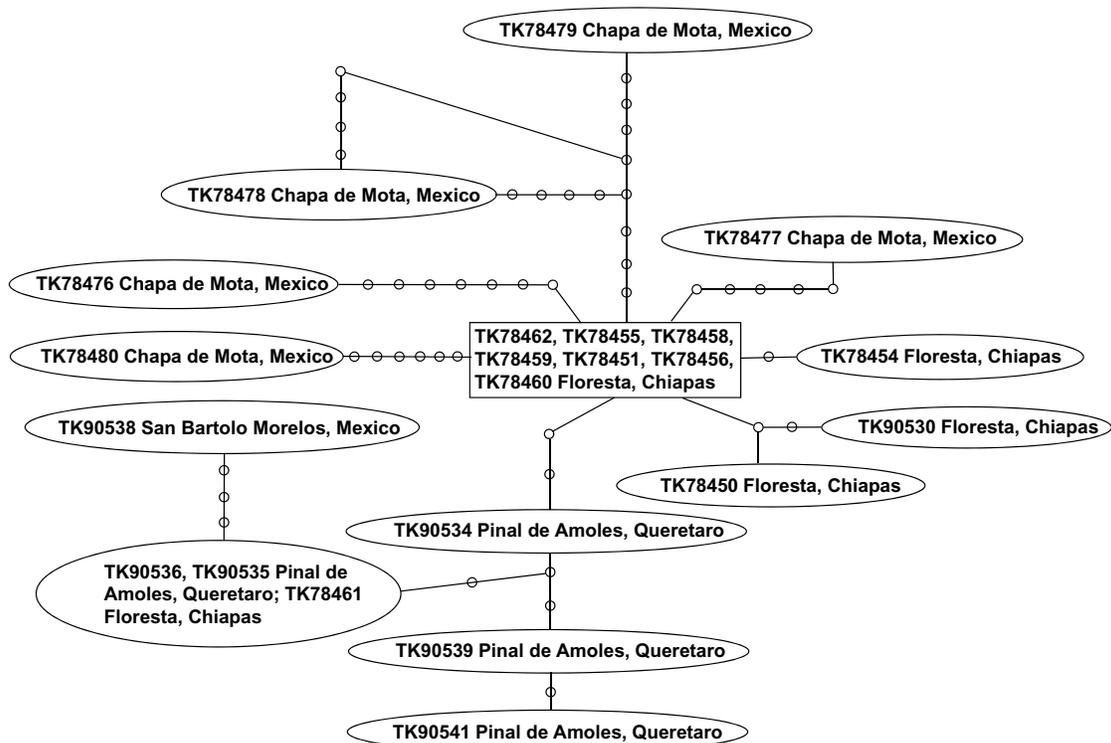


Figure 3. Network constructed using statistical parsimony methods. Individuals contained within the rectangle represent the most basal grouping. Large ellipses depict groupings of identical haplotypes. Small circles depict the number of base changes between adjacent haplotypes.

DISCUSSION

Geographic distribution and habitat use.—The dispersion route obtained for *G. volans* suggests that they invaded México and Central America from the eastern United States, following the Sierra Madre Oriental through the center and south of México to Central America (Ceballos et al. 2010). In México, the subspecies of *G. volans* are endangered because of their restricted distribution, as they are found only in oak and oak-pine forest along the mountain range (Ceballos and Navarro 1991). We support this conclusion, as our results show that the species has disappeared in several regions as a result of anthropogenic activities, especially deforestation. The natural distribution of *G. volans* is limited primarily to availability of cavities. There is a relationship between the availability of cavities and the density of squirrels (Sawyer and Rose 1985). This situation could explain the scarcity of flying squirrels in most localities in México, where the deforestation and changes in land use have reduced the surface cover of forests, eliminating trees that can be used as a refuge for this species.

Genetic Diversity.—Based on the statistical parsimony network (Fig. 3), it appears that the ancestral haplotype may have originated in southern México (Chiapas). Samples from Chapa de Mota, México, were the most divergent based on the number of mutational steps separating them from other haplotypes. This divergence suggests that this population is more fragmented and has experienced less gene flow than populations from Querétaro, Morelos, and Chiapas. The samples from Floresta (with the exception of TK78461) grouped together as do the samples from Pinal de Amoles, Querétaro.

Examination of intrapopulation variation (Table 4) revealed that the population from Floresta, Chiapas, possessed the least amount of genetic divergence (average genetic divergence, AGD = 0.18%), whereas the population from Chapa de Mota, México, was the most diverse (AGD = 2.8%). Interpopulational comparisons showed the Chapa de Mota, México, population to be the most different from the Floresta, Chiapas, population (average genetic distance = 4.1%). Although this study is limited by small sample sizes and geographic representation, it appears that substantial genetic variation exists among populations in southern México.

Levels of genetic divergence approach that between samples in Canada and the central United States (AGD = 5.2%) and between the United States and México (AGD = 5.4%). Intrapopulation levels ranged from 0.18% (Floresta, Chiapas) to 2.8% (Chapa de Mota, México), indicating either a historical bottleneck in Chiapas or more widespread gene flow across central México. Both hypotheses have their merits, and it is difficult to favor one over the other. First, the Floresta, Chiapas, population is located south of the Isthmus of Tehuantepec, long considered to have been isolated from southern México (Ceballos et al. 2010). Second, the high levels of genetic divergence in the Pinal de Amoles, Querétaro, population may have been influenced by gene flow across the Sierra Madre Oriental and Transverse Volcanic Zone. No evidence of isolation by distance was detected.

These findings support those of Kerhoulas and Arbogast (2010) who depict a separate evolutionary trajectory for North American and Mesoamerican populations of *Glaucomys*. Kerhoulas and Arbogast (2010) proposed that populations of *Glaucomys* from Chiapas, México, are genetically divergent and perhaps isolated from other populations in México, especially those from the Sierra Madre del Sur mountain ranges. Our data suggest that in addition to being divergent from populations in southwestern México, populations from Chiapas are genetically different from populations located in the Trans-Mexican Volcanic Belt (Table 4).

Conservation Status.—Currently, there are four protected zones with flying squirrel populations: the ecological reserve of Huitepec, in San Cristóbal de las Casas; El Sepulcro, southeast to Ocozocouautla, both in the state of Chiapas; the Benito Juárez National Park in Oaxaca; and the ecological state park of Omiltemi, in Chilpancingo, Guerrero. However, these reserves represent only a small portion of the geographic distribution of *G. volans*. The current presence of flying squirrels has not been confirmed in other reserves such as El Cielo biosphere reserve in Tamaulipas, where large tracks of cloud forests occur and flying squirrels are documented from subfossil remains (Martin 1955).

A feasible strategy to maintain viable populations of flying squirrels in México will be to designate

protected areas throughout its geographic range. This especially is urgent in Chiapas, where suitable habitat is being destroyed at a rapid rate. Increasing the size of the Huitepec, Chiapas, reserve or other oak forests in the state is fundamental to maintain current populations. Important areas for the conservation of the species, because of the extent of the remaining habitat, are located

in central México, in the states of México, Michoacán, and Hidalgo. The selection of new reserves for flying squirrels can be combined with initiatives already established to complement the national protected areas system in México (Ceballos et al. 1998; Chavez and Ceballos 1998; Ceballos 2007).

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LITERATURE CITED

- Animal Care and Use Committee. 1998. Guideline for the capture, handling, and care of mammals as approved by the American Society of Mammalogists. *Journal of Mammalogy* 79:1416-1431.
- Arbogast, B. S. 1999. Mitochondrial DNA phylogeography of the New World flying squirrels (*Glaucomys*): implications for Pleistocene biogeography. *Journal of Mammalogy* 80:142-155.
- Barrera, A. 1958. Insectos parasitos de mamíferos salvajes de Omiltemi, Gro., y descripción de un nuevo sifonaptero: *Leochaetisso beronni* sv. sp. *Anales de la Escuela Nacional de Ciencias Biológicas* 9:89-96.
- Braun, J. 1988. Systematics and biogeography of the southern flying squirrel, *Glaucomys volans*. *Journal of Mammalogy* 69:422-42.
- Castillo-Meza L., S. Gaona, and J. García-Chávez. 1997. La ardilla voladora *Glaucomys volans goldmani* (Nelson, 1904) en Puebla, México. *Revista Mexicana de Mastozoología* 2:119-121.
- Castro-Campillo, A. A., H. R. Roberts, D. J. Schmidly, and R. D. Bradley. 1999. The systematic status of *Peromyscus boylii ambiguus* based on morphological and molecular data. *Journal of Mammalogy* 80:1214-1231.
- Ceballos, G. 2007. Conservation priorities for mammals in megadiverse Mexico: the efficiency of reserve Networks. *Ecological Applications* 17:569-578.
- Ceballos, G., and C. Galindo. 1983. *Glaucomys volans goldmani* (Rodentia: Sciuridae) in central Mexico. *The Southwestern Naturalist* 28:375-376.
- Ceballos, G., and A. Miranda. 1985. Notes on the biology of Mexican flying squirrels (*Glaucomys volans*) (Rodentia: Sciuridae). *The Southwestern Naturalist* 30:449-450.
- Ceballos, G., and D. Navarro. 1991. Diversity and conservation of Mexican mammals. Pps. 167-198 in *Latin American Mammalogy: History, Biodiversity, and Conservation* (M. A. Mares and D. J. Schmidly, eds). University of Oklahoma Press, Norman.
- Ceballos, G., and G. Oliva. 2005. Los mamíferos silvestres de México. CONABIO - Fondo de Cultura Económica, México D.F.
- Ceballos, G., J. Arroyo-Cabrales, and E. Ponce. 2010. Effects of Pleistocene environmental changes on the distribution and community structure of the mammalian fauna of Mexico. *Quaternary Research* 73:464-473.
- Ceballos, G., P. Rodríguez, and R. Medellín. 1998. Assessing conservation priorities in megadiverse Mexico: mammalian diversity, endemism, and endangerment. *Ecological Applications* 8:8-17.
- Cervantes, F. A., G. Matamoros-Trejo, and I. Martínez-Mateos. 1995. Mamíferos silvestres de la unidad de evaluación y monitoreo de la biodiversidad "Ing. Luis Macías Arellano", San Cayetano, Es-

- tado de México. Anales del Instituto de Biología, Universidad Nacional Autónoma de México. Serie Zoología 66:233-239.
- Chavez, C., and G. Ceballos. 1998. Diversidad y conservación de los mamíferos del Estado de México. *Revista Mexicana de Mastozoología* 3:113-134.
- Clement, M., D. Posada, and K. A. Crandall. 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology* 9:1657-1660.
- Dalquest, W. W. 1953. Mammals of the Mexican state of San Luis Potosí. Louisiana State University, Studies in Biology Series 1:1-229.
- Diersing, V. E. 1980. Systematics of flying squirrels, *Glaucomys volans* (Linnaeus), from Mexico, Guatemala, and Honduras. *The Southwestern Naturalist* 25:157-172.
- Dolan, P. G., and D. C. Carter. 1977. *Glaucomys volans*. *Mammalian Species* 78:1-6.
- Espinoza-Medinilla E., E. Cruz, I. Lira, and I. Sánchez. 2004. Mamíferos de la Reserva de la Biosfera “La Sepultura”, Chiapas, México. *Revista Biología Tropical* 52:249-259.
- Goldman, E. A. 1936. Two new flying squirrels from Mexico. *Journal of the Washington Academy of Sciences* 26:462-464.
- Goodwin, G. G. 1961. Flying squirrels (*Glaucomys volans*) of middle America. *American Museum Novitates* 2059:1-22.
- Hall, E. R. 1981. *The Mammals of North America*. 2nd ed. John Wiley & Sons, New York.
- Hooper, E. T. 1952. Records of the flying squirrels (*Glaucomys volans*) in Mexico. *Journal of Mammalogy* 33:109-110.
- Kerhoulas, N. J., and B. S. Arbogast. 2010. Molecular systematics and Pleistocene biogeography of Mesoamerican flying squirrels. *Journal of Mammalogy* 91:654-667.
- Koopman, K. F., and P. S. Martin. 1959. Subfossil mammals from the Gomez Farías region and the tropical gradient of eastern México. *Journal of Mammalogy* 40:1-12.
- Martin, P. S. 1955. Zonal distribution of vertebrates in a Mexican cloud forest. *American Naturalist* 89:347-361.
- Méndez-Harclerode, F. M., J. D. Hanson, C. F. Fulhorst, M. L. Milazzo, D. C. Ruthven III, and R. D. Bradley. 2005. Genetic diversity within the southern plains woodrat (*Neotoma micropus*) in southern Texas. *Journal of Mammalogy* 86:189-190.
- Muul, I. 1968. Behavioral and physiological influences on the distribution of the flying squirrel, *Glaucomys volans*. *Miscellaneous Publications, Museum of Zoology, University of Michigan* 134:1-66.
- Muul, I. 1974. Geographic variation in the nesting habits of *Glaucomys volans*. *Journal of Mammalogy* 55:840-844.
- Nelson, E. W. 1904. Descriptions of new squirrels from Mexico. *Proceedings of the Biological Society of Washington* 17:147-150.
- Petersen, S. D., and D. T. Stewart. 2006. Phylogeography and conservation genetics of southern flying squirrels (*Glaucomys volans*) from Nova Scotia. *Journal of Mammalogy* 98:153-160.
- Sawyer, S. L., and R. K. Rose. 1985. Homing in and ecology of the southern flying squirrel *Glaucomys volans* in southwestern Virginia. *The American Midland Naturalist* 113:238-244.
- SEMARNAT (Secretaría de Medio Ambiente y Recursos Naturales). 2002. Norma Oficial Mexicana NOM-059-ECOL-2001. Protección ambiental-Especies nativas de México de flora y fauna silvestres-Categoría de riesgo y especificaciones para su inclusión, exclusión o cambio-Lista de especies en riesgo. *Diario Oficial de la Federación*, Marzo 6 2002, Ciudad de México, México.
- Smith, M. F., and J. L. Patton. 1999. Phylogenetic relationships and the radiation of sigmodontine rodents in South America: evidence from cytochrome *b*. *Journal of Mammalian Evolution* 6:89-128.
- Swofford, D. L. 2002. PAUP: Phylogenetic analysis using parsimony (* and other methods), Version 4.0b10. Sinauer Associates, Inc., Sunderland, Massachusetts.
- Tamura, K., and M. Nei. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* 10:512-526.
- Templeton, A. R., K. A. Crandall, and C. F. Sing. 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data III. Cladogram estimation. *Genetics* 132:619-633.
- Zar, J. H. 1984. *Biostatistics*. Prentice Hall, Englewoods Cliffs.

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APPENDIX I

Specimens examined.—Some samples used in this study were obtained from specimens that subsequently were released, and therefore voucher and museum catalogue numbers are not available for all specimens. All tissue samples were deposited in the Natural Science Research Laboratory, Museum of Texas Tech University, and are referenced with a museum identification number (TK). All specimens are from México unless otherwise indicated. GenBank accession numbers are provided in parentheses following the TK number.

Locality 6 in Table 1 and Figure 1 (N = 5).—Querétaro; Pinal de Amoles (TK90534, FJ376438; TK90535, FJ376440; TK90536, FJ376439; TK90539, FJ376437; TK90541, FJ376441).

Locality 12 in Table 1 and Figure 1 (N = 5).—México; Chapa de Mota (TK78476, FJ376456; TK78477, FJ376454; TK78478, FJ376455; TK78479, FJ376457; TK78480, FJ376458).

Locality 13 in Table 1 and Figure 1 (N = 1).—México; San Bartolo Morelos (TK90538, FJ376453).

Locality 36 in Table 1 and Figure 1 (N = 11).—Chiapas; Municipio Comitán, 2 km S Floresta 16°28'32" N, 92°18'0" W, 2140 m (TK78450, FJ376444; TK78451, FJ376450; TK78454, FJ376445; TK78455, FJ376447; TK78456, FJ376451; TK78458, FJ376448; TK78459, FJ376449; TK78460, FJ376452; TK78461, FJ376446; TK78462, FJ376442; TK90530, FJ376443).

Reference localities and outgroup samples.—UNITED STATES: Oklahoma; Seminole (TK26069, FJ376459); CANADA: Nova Scotia (AY928943; AY928973).

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