POPULATION GENETIC STRUCTURE

OF A CAVE-DWELLING BAT, *MYOTIS VELIFER*

THESIS

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ABSTRACT

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Myotis velifer is known to exhibit a wide-range of geographically correlated morphological and behavioral variation, reflecting genetically unexplored ecological subdivisions. Unlike migratory chiropterans, genetic structure is generally found among chiropterans exhibiting non-migratory behavior. Interestingly, both migratory and non-migratory behavior is noted among *M. velifer*. Previous morphometric analyses proposed existing barriers to gene flow among taxonomic subdivisions. Also, abandonment of historical roosts has been used to designate *M. velifer* as a species of concern throughout Texas. Genetic methods were used to evaluate whether population genetic structure is congruent with behavioral or taxonomic subdivisions and, assuming a decline is associated with roost abandonment, whether a population decline in Texas is the result of

a history of demographic contraction. To explore these proposals and concerns, tissue samples were collected from roosts in the Texas Panhandle (3), central and west Texas (13) and the California-Arizona border (4), representing both behavioral designations and three taxonomic subdivisions. Mitochondrial sequence data (982 base pairs of *Cytochrome b*) from 104 individuals recovered 54 haplotypes and three haplogroups. Thirty-four microsatellite loci evaluated for cross-species amplification yielded four suitably polymorphic autosomal loci and one X-linked locus. Mitochondrial structure is not congruent with behavior, taxonomy, or geography and microsatellite data ($n = 192$) recovered no genetic structure. Mitochondrial data indicate weak regional fidelity, nuclear data indicate substantial gene flow, and demographic analyses indicate historic demographic expansion among *M. velifer*. The current subspecies of *M. velifer* are not genetically supported; however, incorporation of (or "accounting for") morphological and behavioral data leads me to conclude that *M. v. magnamolaris* should be maintained for non-migratory populations. California specimens should be assigned to *M. v. incautus* designating *M. v. velifer* to only occur south of the United States border with Mexico. Analysis of Mexican and Central American specimens is needed to determine support for this nominator subspecies. Management of the two proposed United States subspecies should be across areas containing multiple roosts until conclusive evidence suggests causes of roost abandonment.

CHAPTER I

INTRODUCTION

A species genetic structure is determined by the distribution of genetic variation among individuals both within and among populations (Russell 2006). Various molecular markers exist to estimate population genetic structure; including both nuclear and mitochondrial DNA (mtDNA) among animals. Both mtDNA and bi-parentally inherited nuclear markers, such as polymorphic allozymes and microsatellites (repeat motif nuclear DNA), can estimate intraspecific gene flow (Avise 1994); whereas, analyses of the maternally inherited mitochondrial genome generate insight into the historical relationships of an organism (Avise 2000). Gene flow and historical events, as influenced by underlying ecological and environmental factors, often shape a population's genetic structure, often yielding geographically correlated genetic structure (Avise 2000).

In chiropterans, geographically correlated genetic structure has been attributed to large expanses of water (e.g., *Myotis myotis*, Castella et al. 2000; *Rhinolophus ferrumequinum*, Rossiter et al. 2000) and mountain ranges (e.g., *M. myotis*, Castella et al. 2001). Without geographic boundaries, behavior, such as migratory or non-migratory, may influence whether genetic structure is observed in a chiropteran species (see Burland and Worthington-Wilmer 2001). Considerable genetic structure has been observed in many non-migratory bat species via nuclear markers (Burland and Worthington-Wilmer

2001; Hisheh et al. 2004). On the other hand, in many migratory chiropterans substantial gene flow among populations has been inferred from nuclear markers (Burland and Worthington-Wilmer 2001; Hisheh et al. 2004; Rivers et al. 2005); even when mtDNA infers some geographically correlated genetic structure (Bilgin 2006; Petit and Mayer 1999; Ruedi and Castella 2003). This observation has been attributed to female philopatry with male-mediated dispersal.

As with any generalities, empirical observations report exceptions which make it difficult to infer expectations of a chiropteran's genetic structure. Genetic structure, inferred from nuclear markers, may be observed in a migratory species displaying philopatric behavior (e.g., *Miniopterus schreibersii natalensis*, Miller-Butterworth et al. 2003). In contrast, non-migratory, philopatric species may also exhibit a lack of nuclear genetic structure (e.g., *Corynorhinus townsendii ingens*, Weyandt et al. 2005). Furthermore, mtDNA may not yield geographically correlated genetic structure in a non-migratory group if high dispersal of migratory individuals is reported in a species documented to contain geographically structured migratory and non-migratory subspecies (e.g., North American subspecies of *Tadarida brasiliensis*, Russell and McCracken 2006; Russell et al. 2005a).

Population-level relationships are often difficult to determine because of the nocturnal volant nature of chiropterans, as well as low recovery rates in banding studies (Cockrum 1969; Hayward 1970). Therefore, genetic analyses are a useful tool to estimate not only intraspecific genetic variation (Burland and Worthington-Wilmer 2001) but also population boundaries (e.g., *Leptonycteris curasoae*, Wilkinson and Fleming 1996; *M. s. natalensis*, Miller-Butterworth et al. 2003), and demographic analyses (e.g., *T.*

brasiliensis, Russell 2003; Russell and McCracken 2006). Prior to this study, allozymes were used to study the genetic variability of *Myotis velifer* (Straney et al. 1976); no significant structure was observed among Texas Panhandle and Arizona populations (Burland et al. 1999). Unfortunately, many historical roosts are now abandoned, culminating in a species of concern designation (Texas Parks and Wildlife Department 2005). This has increased the need for a more detailed genetic evaluation of *M. velifer*.

The current taxonomic subdivisions of *M. velifer* are geographically distributed, yet the number of historically recognized taxonomic subdivisions varies from three (Fitch et al. 1981; Hayward 1970) to four (Hall 1981). The inconsistency in the recognition of the number of subspecies is between those who recognize Vaughan's (1954) subspecific splitting, such as Hall (1981), or those who recognize Hayward's (1970) subsequent subspecific merging. Vaughan (1954) recognized a significant difference in morphological variation of *M. v. velifer* and *M. v brevis* specimens. Hayward (1970), unlike Vaughan (1954), found no significant differences among several hundred specimens of *M. v. brevis* and *M. v. velifer*. The lack of morphological differentiation, attributed to the two groups representing opposite ends of a cline, caused Hayward (1970) to group the two subspecies into the nominotypic subspecies, *M. v. velifer*. Hayward (1970) proposed the three taxonomic subdivisions were potentially influenced by barriers to gene flow. Later, the taxonomy of *M. velifer* was again modified when no significant differences were found among *Myotis magnamolaris* and specimens of *M. v. grandis*. Dalquest and Stangl (1984) referred specimens of *M. v. grandis* to *M. v. magnamolaris*. The most recent taxonomic evaluation of *M. velifer* was conducted by Hayward (1970); therefore I deem it most appropriate to use his taxonomy and refer to the northernmost

subspecies as *M. v. magnamolaris* as discussed by Dalquest and Stangl (1984). Hence, in this study, three taxonomic subdivisions of *M. velifer* will be discussed, *M. v. magnamolaris*, *M. v. incautus*, and *M. v velifer*.

The distribution of *M. v. incautus* ranges from west Texas, south to northeastern Mexico; *M. v. magnamolaris* is found from the Texas Panhandle northward to south-central Kansas; and *M. v. velifer* occurs from southeastern California south to Central America (Hayward 1970). Roost estimates of the number of individuals for this gregarious species are highly variable. Twente (1955a) estimated 5,000 individuals per roost during hibernation in Oklahoma and Kansas. During summer, 15,000+ individuals were observed in roosts in Texas, (Pekins 2006, 2007a), Arizona (Hayward 1970), and Oklahoma and Kansas (Twente 1955a), although Hayward (1970) reported estimates of less than 5,000 individuals in Arizona were more frequent. Fitch et al. (1981) reported roost estimates of 2,000 to 5,000 individuals occupied roosts throughout much of the range; however, sources of these estimates were not cited. *Myotis velifer* displays weak roost fidelity by not always returning to the same roosting site (Barbour and Davis 1969; Tinkle and Patterson 1965; Twente 1955b); yet they appear to remain within a home range of approximately 932 to 1,619 km^2 (Hayward 1970). Behavioral variation was reported among *M. velifer* subspecies. *Myotis v. magnamolaris* populations have been noted as permanent residents (Texas Panhandle: Tinkle and Patterson 1965; Kansas and Oklahoma: Twente 1955c), while other subspecies were thought to migrate due to their absence in caves during winter (Arizona: Hayward 1970; central Texas: Pekins 2006).

The variable migratory behavior displayed among subspecies, the proposed barriers to gene flow among morphologically evaluated taxonomic subdivisions, as well

as the abandonment of historical roosting sites, makes *M. velifer* an interesting candidate for genetic exploration. Considering the often observed genetic structure of non-migratory species, could the dispersal capability of the migratory subspecies result in a lack of genetic structure among the subspecies? Furthermore, will the proposed barriers of gene flow, based on morphological taxonomic evaluations (Hayward 1970), result in observable mtDNA or nuclear DNA structure? And lastly, could the abandonment of historical roosts (Texas Parks and Wildlife Department 2005) have been occurring longer than documented, resulting in a history of demographic contraction? With these questions in mind, the objectives of my study were to: 1) evaluate whether differences exist among the behaviorally differing *M. velifer* designations via analysis of mtDNA and nuclear DNA variation; 2) evaluate the validity of genetic barriers among mtDNA and nuclear DNA resulting in the taxonomic subdivision proposed by Hayward (1970); and 3) evaluate whether the demographic history of *M. velifer* populations in Texas indicate a demographic contraction has been occurring; thus, whether the population has been declining for longer than documented roost abandonment.

CHAPTER II

MATERIALS AND METHODS

Samples.—Genetic samples from as many as 20 individuals were collected at each of the 20 roosts (Burland and Worthington-Wilmer 2001; Table 1; Fig. 1) distributed among the eastern and western extremities of the species range within the United States. Of the 20 roosts, four were located at the California-Arizona border, representing the taxonomic subdivision *M. v. velifer* ($n = 34$). The other 16 roosts were located throughout Texas, three of which were located in the Texas Panhandle representing *M. v magnamolaris* ($n = 56$) and the other 13 representing *M. v. incautus* ($n = 236$). The sampling sites within Texas were selected from a compiled list provided by Bat Conservation International (J. Kennedy, pers. comm.). Four other Texas roosts were visited during the 2006 field season; unfortunately, two gypsum caves in the Panhandle had collapsed, one limestone cave in west Texas was overgrown with brush, and one limestone cave in central Texas was found vacant on two separate occasions. No samples were obtained from these four locations. Individuals were captured via harp trap or hand net and temporarily detained in cotton bags secured with draw-strings (Kunz and Kurta 1988) until processing was completed. They were immediately released at their capture location.

To assist in proper identification, measurements of forearm length (mm) and weight (g) were taken using calipers and a Pesola spring scale (Forestry Suppliers

Incorporated), respectively. Individuals were aged (Anthony 1988) as well as sexed with reproductive status determined (Racey 1988). A 2 mm^2 biopsy punch was taken from each wing membrane (Worthington-Wilmer and Barratt 1996) and stored in 95% ethanol. The biopsy punch was flame sterilized to avoid cross contamination of samples (Worthington-Wilmer and Barratt 1996). Wing tissues were collected from 319 specimens representing three taxonomic subdivisions of *M velifer* (Hayward 1970; Table 1; Fig. 1). Liver tissue was provided by The Museum at Texas Tech University for a specimen from Santa Ana, El Salvador (*M. v. velifer*; Museum No. TK34862; hereafter referred to as ELS).

Mitochondrial Genetic Techniques.—Extraction of DNA from tissues was performed using the QIAGEN DNeasy Blood & Tissue Kit following the 07/2006 handbook (QIAGEN, Inc., Valencia, CA, U.S.A.). Amplified fragments were produced using the polymerase chain reaction (PCR) and carried out in a GeneAmp® PCR System 9700 (Applied Biosystems, Foster City, CA, USA). The complete mitochondrial *Cytochrome b* (*Cyt b*) gene was amplified and sequenced with the addition of the sequencing primers NEWGLUMAMF (5'-CCAYGACTAATGACATGAAAAATCACCGTTG-3'), THRMYOR (5'-CCAGTATAATTARTATACTACATAGAC-3'), INTCYTBMYOF (5'-GTAATTACTAATTTACTCTCTGCAATCCC-3'), INTCYTBMYOR1 (5'-TGGAAAGCAAAGAATCGGGTTAAGG-3'), and INTCYTBMYOR2 (5'-GGAATTGATCGTAAAATTGCGTATG-3'). Primers used to bracket the *Cyt b* gene were NEWGLUMAMF (5' primer) and THRMYOR (3' primer). PCR products were obtained using 0.5 μL to 2.0 μL of genomic DNA with the thermocycling profile of denaturation at 94° C for 30 s, annealing of primers at 55° C for 1 min, elongation of

sequence at 72° C for 1 min for 40 cycles, with a final elongation of 72° C for 5 min. Excess primers and deoxynucleotides (dNTPS) were removed from amplified mitochondrial product via AGENCOURT AMPURE PCR purification following protocol 000601v024. Cycle sequencing of PCR products was carried out using DTCS Quick Start Mix (Beckman Coulter, Fullerton, CA, U.S.A.) by the 09/04 protocol and excess primers, dNTPs, and di-deoxynucleotides (ddNTPS) were removed using AGENCOURT CLEANSEQ Dye-Terminator Removal for CEQ DTCS following protocol 000402v001. Final mitochondrial products were bi-directionally read to minimize base calling errors and ambiguities using CEQ™ 8800 Genetic Analysis Systems (Beckman Coulter, Fullerton, CA, U.S.A.).

Microsatellite Genetic Techniques.—Thirty-four primer pairs cited in the literature were acquired to determine their utility in amplifying *M. velifer* microsatellite loci (Burland et al. 1998; Castella and Ruedi 2000; Miller-Butterworth et al. 2002; Racey et al. 2007; Russell et al. 2005b; Vonhof et al. 2002). Forward primers for microsatellite loci found to be suitably polymorphic were labeled at the 5' end with WellRED dyes. PCR products were obtained using 0.5 μL to 2.0 μL of genomic DNA with the thermocycling profile of denaturation at 94° C for 30 s, annealing of primers for 1 mın, elongation of sequence at 72° C for 1 min for 40 cycles, with a final elongation of 72° C for 5 min. Amplification parameters of microsatellite loci are available in Table 2. Microsatellite alleles were analyzed with CEQ™ 8800 Genetic Analysis Systems (Beckman Coulter, Fullerton, CA, U.S.A.) using CEQ™ 400 Size Standard Kit (Beckman Coulter, Fullerton, CA, U.S.A.). Sequence verification of microsatellite alleles was performed by removing excess primers and ddNTPS from amplified microsatellite products of homozygous individuals via

PROMEGA Wizard® SV gel and PCR Clean-up System following the 01/05 handbook. Cycle sequencing of microsatellite PCR products was carried out using DTCS Quick Start Mix (Beckman Coulter, Fullerton, CA, U.S.A.) by the 09/04 protocol and excess primers, dNTPs, and ddNTPS were removed using AGENCOURT CLEANSEQ Dye-Terminator Removal for CEQ DTCS following protocol 000402v001. Final microsatellite products were bi-directionally read to minimize base calling errors and ambiguities using CEQ™ 8800 Genetic Analysis Systems (Beckman Coulter, Fullerton, CA, U.S.A.).

Sequence Analyses.—Sequences of the *Cyt b* gene were edited and aligned using SEQUENCHER 4.7 (Gene Code Corp.). Sequence data were checked for stop codons by translating to amino acids using MACCLADE 4.05 (Madison and Madison 2002) and chromatograms were verified by eye. Haplotype networks were generated to illustrate intraspecific divergence using the median-joining method implemented in NETWORK version 4.5.0.0 (MJN, Bandelt et al. 1999) and the statistical parsimony method implemented in TCS version 1.21 (TCS, Clement et al. 2000). A single most parsimonious network is generated by the MJN method as minimum spanning trees are located with consensus sequences representing extinct or unsampled haplotypes inserted among sampled haplotypes (Bandelt et al. 1999). A statistically parsimonious haplotype network is generated by TCS by calculating pairwise differences among haplotypes and assembling haplotypes into networks via minimum pairwise distance until a parsimony probability of 95% is exceeded (Clement et al. 2000). Both haplotype networks have been presented in this study because, while TCS is one of the most commonly reported

haplotype networks, MJN has been noted to outperform TCS in instances of missing node haplotype data (Cassens et al. 2005).

Uncorrected pairwise distances were calculated for haplotypes using PAUP* 4.0b10 (Swofford 2002). These uncorrected pairwise distances were converted into percentages. To test the hypothesis of ingroup monophyly, outgroup(s) were incorporated within phylogenetic analyses. Multiple outgroups may be evaluated in phylogenetic methods to reduce the potential of a single outgroup's influence on the relationships among ingroup specimens. Therefore, phylogenetic analyses included previously sequenced individuals from GenBank. Of these previously sequenced individuals, *Myotis yumanensis* (GenBank Accession No. AF376875), *Myotis thysanodes* (GenBank Accession No. AF376869), and *Pipistrellus subflavus* (GenBank Accession No. AJ504449), were outgroups representing the sister taxa, clade and subfamily respectively (Hoofer and Van den Bussche 2003; Ruedi and Mayer 2001; Stadelmann et al. 2007). Also, a *M. velifer* from Sonora, Mexico (GenBank Accession No. AF376870; hereafter referred to as SMX) was evaluated as an ingroup. I used phylogenetic methods to analyze sequence data (Neighbor-Joining [NJ], Maximum Parsimony [MP], Maximum Likelihood [ML] and Bayesian methods) using PAUP* 4.0b10 (Swofford 2002). Heuristic searches were applied for MP and ML methods. For bootstrapped analyses, 2,000 heuristic pseudoreplicates were executed for the MP phylogenies, 2,000 NJ pseudoreplicates adhering to the estimated models of evolution (see results) for NJ phylogenies, and 100 heuristic pseudoreplicates for ML phylogenies. Addition sequences were random and as-is for MP and ML methods, respectively. Tree-bisection-reconnection (TBR) branch swapping algorithm was used for bootstrapped MP and ML

methods. Bayesian analyses were conducted with 10,000,000 generations, a sampling frequency of 1,000, one cold and three heated chains and random starting trees. Majority rule consensus phylogenies were generated from Bayesian analyses with a burn-in of 1,000. *Myotis velifer* sequences were analyzed with MODELTEST 3.1.1 (Posada and Crandall 1998) and MRMODELTEST 2.2 (Nylander 2004). These programs estimate the best model(s) of evolution for NJ, ML and Bayesian analyses with two tests, the hierarchical likelihood ratio test (hLRT) and the Akaike Information Criterion (AIC). Computationally intense analyses were submitted to the Cyberinfrastructure for Phylogenetic Research (CIPRES) portal (http://www.phylo.org/) and evaluated with the ML-based inference program Genetic Algorithm for Rapid Likelihood Inference (GARLI). The assumed model for GARLI evaluated data sets is the general time reversible (GTR) model of evolution; therefore parameters similar to the model(s) of evolution estimated by MODELTEST or MRMODELTEST were applied.

Microsatellite Analyses.—In an infinitely large, randomly mating population, with no migration, mutation, or natural selection, conformities to Hardy-Weinberg equilibrium (HWE) typically occur. Tests for significant departures from HWE locus by locus within each roost (hereafter referred to as population) were executed with ARLEQUIN version 3.01 using an approximation of Fisher's exact tests and a Markov-chain algorithm (10,000 dememorization, 100,000 expectation-maximization [EM] steps, Excoffier et al. 2005; Guo and Thompson 1992) as well as with GENEPOP version 1.2 (Raymond and Rousset 1995). To reduce the potential of making a type-I error, statistical significance levels ($\alpha = 0.05$) were adjusted with a sequential Bonferroni correction (Rice 1989).

Next, tests for linkage disequilibrium were executed with ARLEQUIN using the EM algorithm and likelihood ratio tests to determine if the genotypes are significantly nonrandomly distributed ($\alpha = 0.05$, 10,000 initial conditions for EM, 100,000 permutations, Excoffier et al. 2005; Slatkin and Excoffier 1996) as significant levels of linkage disequilibrium may indicate inbreeding due to reduced recombination, genetic drift in small populations, natural selection due to association of certain alleles, or gene flow between previously separated populations (Ardlie et al. 2002; Hartl and Clark 2007).

To determine why populations may not be conforming to HWE, GENEPOP (Raymond and Rousset 1995) was used to determine if populations were deficient, or had an excess of, heterozygotes. Populations deficient in heterozygotes were tested for the presence of null alleles population by population and locus by locus using MICRO-CHECKER (van Oosterhout et al. 2004). When loci within populations were suggested to contain null alleles via MICRO-CHECKER (van Oosterhout et al. 2004), PCR products for individuals initially scored as homozygotes within the population were re-amplified using 1.0 μL of genomic DNA. The re-amplified PCR microsatellite product was analyzed to verify congruence with the individual's previous allelic score(s). If individuals were found to differ from their previous score(s), the final microsatellite data set was updated to include the new score(s). The final microsatellite data set was reanalyzed for conformities to HWE. Lastly, null allele frequencies were calculated with CERVUS 3.0 (Marshall et al. 1998).

Population Genetic Analyses.—To evaluate the population genetic structure of *M. velifer*, both mitochondrial and nuclear data were analyzed to infer whether structure exists and whether such structure conforms to the behavioral or subspecific designations of

Hayward (1970). Only specimens collected within the United States were used for analyses of population genetic structure since significant sample sizes were obtained among regions. Evidence indicates that the mutation process of most microsatellite loci follow a stepwise mutation model (Ellegren 2000; Renwick et al. 2001; Xu et al. 2000). Because of the stepwise mutation model and high mutation rate of microsatellite loci, a fixation index (R_{ST}) considered more appropriate than the traditional (F_{ST}) was derived for estimating population differentiation (Rousset 1996; Slatkin 1995). However, it is still debatable which fixation index is better to report as the amount of gene flow has been shown to influence these statistics (Balloux and Goudet 2002; Lugon-Moulin et al. 1999). Furthermore, loci found to contain null alleles do not follow the stepwise mutation model of evolution. Therefore both F_{ST} and R_{ST} were estimated and reported with preference given to the more applicable fixation index with respect to gene flow and null alleles (Balloux and Goudet 2002).

An analysis of molecular variance (AMOVA) assessed, if present, intraspecific differentiation for both mitochondrial and microsatellite data using ARLEQUIN (Excoffier et al. 2005). Subspecific, as well as behavioral, designations were assigned *a priori* according to Hayward's (1970) classification. Next, to discern the greatest genetic partitioning of the sampled populations, I used a spatial analysis of molecular variance (SAMOVA) for both mitochondrial and microsatellite data from Texas and California populations only (Dupanloup et al. 2002). Specimens SMX and ELS were not included in SAMOVA analyses due to a lack of GPS coordinates. The partitioning method of SAMOVA indicates possible genetic barriers to gene flow (Dupanloup et al. 2002). As the smallest partitioning of data for SAMOVA is two hypothetical populations,

ARLEQUIN was used to estimate Φ_{ST} and compared with the values estimated by SAMOVA to evaluate whether the best partitioning was none. Lastly, STRUCTURE 2.2 was used to partition individuals into designations based solely on microsatellite data (Falush et al. 2007). STRUCTURE assigns individuals to their most probable grouping using a Bayesian clustering algorithm (Falush et al. 2007). Parameters for the STRUCTURE program are as follows: burnin length = 10,000; simulation length = 50,000; ancestry model = admixture; linkage model = none; prior information model = yes, assignment of individuals to roosts where collected; allele frequency model = correlated. In cases of subtle population structure, the admixture ancestry model and correlated allele frequencies were the most sensitive (Falush et al. 2003). Only individuals with no missing genotypic data were evaluated in STRUCTURE. I compared the data generated from SAMOVA and STRUCTURE with *a priori* designations used in AMOVA to examine if behavioral, taxonomic, or geographic consistencies existed.

Reevaluation of Hayward's (1970) Morphological Data.—Subspecies represent observable intraspecific variation partitioned among geographic regions. Recognition of diversity within a species can be especially important when geographic regions are impacted differently by environmental or anthropogenic effects. A morphological evaluation is required to appropriately evaluate whether any change to the currently recognized taxonomic subdivisions of *M velifer* is warranted; therefore, I reevaluated the morphological data reported by Hayward (1970). The measurements provided by Hayward (1970) were used because only non-consumptive sampling was practiced during my field season.

First, values for the four reported morphological characters provided by Hayward's (1970) evaluation were estimated to the nearest tenth of a mm (forearm length, greatest length of skull) or nearest hundredth of a mm (maxillary tooth row, cranial breadth; Table 3). These estimates were then calculated as proportions (0 to 1) of the largest value for that character and ranked, where one was given the highest ranking of 10. The ranked values were then averaged and the final values listed from least to greatest and again ranked with the smallest value given one. For visual association of the morphological differences, the final ranked averages were plotted on a map. This was deemed the most appropriate method to reevaluate the morphological data as no sample sizes were reported by Hayward (1970).

Demographic Analyses.—A loss of habitat (Texas Parks and Wildlife Department 2005) may influence a decline in population size; however, whether a historical demographic contraction among Texas populations of *M. velifer* is unknown. Therefore, estimates of population stability using molecular data may elucidate the severity of this decline. To determine if the effective population size of females (N_{ef}) matches expectations of historical demographic contraction, analyses implementing the neutral theory of evolution and coalescent theory were evaluated for the mitochondrial data of specimens collected within Texas.

First, tests for departures from the neutral model of evolution were executed in ARLEQUIN (Excoffier et al. 2005). Under the infinite-alleles model, with the assumption of no recombination for mitochondrial data, the Ewens-Watterson test for neutrality compares a known null distribution to the estimated haplotype homozygosity and determines whether the haplotype homozygosity varies significantly from the neutral

model (Ewens 1972; Watterson 1978). When mtDNA fits a neutral model of evolution, two other neutrality tests, Tajima's D and Fu's F_S, which assume no recombination under the infinite-sites model, may be evaluated as estimators of population growth. Population growth is inferred when calculations of Tajima's D and Fu's F_S yield negative values due to their sensitivity to an excess of rare alleles or singletons (Fu 1997; Tajima 1989). Tajima's D statistic is calculated from the number of pairwise distances and the number of segregating sites in a sample of nucleotide sequences (Tajima 1989). Similar to Tajima's D, Fu's F_S also utilizes the number of pairwise differences as well as evaluates the probability of observing a random number of alleles and comparing that to the observed number of alleles in a population (Fu 1997). Simulation studies have found Fu's F_S out-performs Tajima's D; however, both estimates are reported with preference given to Fu's F_S (Ramos-Onsins and Rozas 2002; Ray et al. 2003). To further support that Fu's F_S is inferring population growth and not genetic hitchhiking, Fu and Li's (1993) D^* and F^* were calculated using DNASP 4.0 (Rozas and Rozas 1997). If Fu and Li's (1993) D^* and F^* are significant and Fu's F_S is not, then genetic hitchhiking is inferred for the negative Fu's F_S (Fu 1997). However, if the reverse is true, then population expansion is inferred (Fu 1997).

Next, I evaluated two coalescent-based approaches. Mismatch distributions, or the observed distribution of pairwise nucleotide differences among haplotypes within each lineage, were compared to expectations of historical demographic expansion and equilibrium (Rogers 1995; Rogers and Harpending 1992; Slatkin and Hudson 1991). Sum of squared deviation probability (SSD) and Harpending's raggedness index (Harpending et al. 1993; HRI) were calculated with ARLEQUIN (Excoffier et al. 2005). When

population growth is low or negative, these methods may be less effective than the methods implemented in the program FLUCTUATE (Kuhner et al. 1998). FLUCTUATE differs from the mismatch distribution analyses in that it implements a Metropolis-Hastings sampling of genealogies to evaluate the posterior probabilities of the observed data generating maximum likelihood estimates of Θ and exponential growth rate (g) (Kuhner et al. 1998). Parameters set in FLUCTUATE included utilizing Watterson's estimate of Θ, a random starting tree, a transition/transversion ratio of 11.143 for the sequence data (see results), empirical base frequencies, a run of 10 short chains with 1,000 steps and sampling increments every 20^{th} step, 5 long chains with 20,000 steps and sampling increments every 100^{th} step. In an attempt to report the most conservative estimate, analyses were executed five times with the smallest growth rate (g) reported. Estimates of population growth result in a positive g, and estimates of population decline result in a negative g (Kuhner et al. 1998).

CHAPTER III

RESULTS

Phylogenetic Analyses.—From the *Cyt b* gene, 982 base pairs (bp) of the total 1,140 nucleotide length were verified and evaluated. The final dataset contained no insertions or deletions and a total of 53 haplotypes from 103 sequenced individuals (GenBank Accession Nos. EU680196-EU680298, EU680300; Table 4). Inclusion of SMX increased this total to 54 haplotypes. Of the 53 haplotypes, 39 were singletons and distributed throughout the taxonomic and geographic divisions without any obvious clustering. Observed haplotypic diversity was moderate to high ($h \geq 0.700$), with low nucleotide diversity ($\pi \leq 0.24$; Table 4). The two intraspecific network algorithms produced similar networks, with the MP network recovering three haplogroups (Figs. 2-3). Individuals sampled from Texas populations were found among both haplogroups A and B, whereas ELS and individuals from California were found only within haplogroup B (Fig. 3). The SMX individual was represented within its own haplogroup, haplogroup C (data not shown).

The taxonomic and geographic distribution of haplotypes is presented in Table 5. Percent uncorrected pairwise distance among all *M. velifer* ranged from 0.01% to 3.36%. For specimens collected within the United States, representing haplogroups A and B (see Fig. 3), the percent uncorrected pairwise distances ranged from 0.01% to 0.60% within

haplogroup A, 0.10% to 1.42% within haplogroup B, and 1.63% to 2.65% among the two groups. Percent uncorrected pairwise distances were greatest when the haplotypes of SMX and ELS were included: 2.34% to 2.85% (haplogroup A – ELS); 2.85% to 3.36% (haplogroup A – SMX); 1.32% to 1.94% (haplogroup B – ELS); 2.14% to 2.65% (haplogroup B – SMX); and 2.96% (ELS – SMX). Compared to *Myotis yumanensis* (GenBank Accession No. AF376875), percent uncorrected pairwise sequence divergence ranged from 5.80% to 6.31% among haplogroup A, from 4.99% to 5.50% among haplogroup B, and was at 5.91% with haplogroup C.

The best models of evolution estimated by MODELTEST were HKY + G for the hLRT and TrN + I for the AIC. Parameters estimated for *Cyt b* as evaluated by the MODELTEST hLRT model of evolution were: base frequencies of A = 0.2979, C = 0.2682, G = 0.1395, and T = 0.2943; transition to transversion ratio = 11.1434; proportion of invariable sites = 0 and gamma distribution shape parameter = 0.0102. For the estimated MODELTEST AIC model of evolution, parameters were: base frequencies of A = 0.2943, C = 0.2721, G = 0.1354, and T = 0.2981; the rate matrix substitution model estimated the stochastic rate of change for all transversions equal 1.000, purine (A⇔G) transitions equal 30.9641 and pyrimidine (C⇔T) transitions equal 18.6472; the proportion of invariable sites = 0.8339; and the proportion of variable sites as equal. The best models of evolution estimated by MRMODELTEST were HKY + G for the hLRT and HKY + I for the AIC. Parameters for 982bp evaluated by the MRMODELTEST hLRT model of evolution were: base frequencies of A = 0.2979, C = 0.2682, G = 0.1395, and T = 0.2943; transition to transversion ratio = 11.1435; proportion of invariable sites = 0 and gamma distribution shape parameter = 0.0146. For the MRMODELTEST AIC model of evolution, parameters

were estimated as: base frequencies of A = 0.2975, C = 0.2684, G = 0.1393, and T = 0.2948; transition to transversion ratio = 11.3438; and proportion of invariable sites = 0.8367. Due to the similarities among the hLRT models estimated by the two tests, the estimates of MRMODELTEST were applied for all hLRT analyses. The models of evolution estimated by MRMODELTEST for the codon partitioned Bayesian analysis were the same for both the hLRT and AIC. The parameters for the codon partitioned analyses were: 1st: K80; equal base frequencies; transition to transversion ratio = 5.5159; proportion of invariable sites = 0; rates = equal; 2nd: F81; base frequencies: A = 0.2075, C = 0.2589, G = 0.1411, T = 0.3925; equal transition to transversion ratio; proportion of invariable sites = 0; rates = equal; 3rd: GTR + G; base frequencies: A = 0.3974, C = 0.3131, G = 0.0430, T = 0.2465; rate matrix substitution model: A⇔C = 0.0813, A⇔G = 22.2223, A⇔T = 0.2315; C⇔G = 0; C⇔T = 6.0212; G⇔T = 1.0000; proportion of invariable sites = 0; gamma distribution shape parameter = 0.5009.

The estimated HKY + G models of evolution were very similar, except the gamma shape parameter estimated by MRMODELTEST was larger. As this site to site rate variation has been observed to cause bias in ML methods (Gaut and Lewis 1995; Kuhner and Felsenstein 1994), the smaller gamma shape parameter was incorporated in ML and Bayesian phylogenies in an attempt to minimize possible bias; however, due to excessive computation times, HKY + G models bootstrapped for ML methods were evaluated using the CIPRES portal GARLI option. Parameters evaluated with the GARLI option were: ratematrix = 2, statefrequencies = empirical, invariatesites = none.

The treelength of the best 85 trees for the MP phylogeny was 406 steps with 120 parsimony-informative characters. Other values calculated from the MP phylogeny

include a consistency index (CI) of 0.77, retention index (RI) of 0.84, and rescaled consistency index (RC) of 0.65. The minimum-evolution score of the NJ phylogeny was 0.38369. Scores (-ln-likelihood) of the ML phylogenies were 3228.03973, 3867.42270, and 3289.67790 for the HKY + G (hLRT), TrN + I (MODELTEST AIC), and HKY + I (MRMODELTEST AIC), respectively. Two phylogenies were found to have the best score for the HKY + G model of evolution, the other ML phylogenies generated only one best score phylogeny.

The phylogenies generated from the MP, NJ and codon partitioned Bayesian analyses indicated a monophyletic group for *M. velifer* with respect to *M. thysanodes*, *M. yumanensis* and *P. subflavus* (≥84%; Figs. 4-7). Monophyly was not supported in the ML or other Bayesian phylogenies (Figs. 8-11). Phylogenies generated from the MP, NJ, and the two of the Bayesian methods (HKY + G and codon partitioned) also indicated one strongly supported subclade (≥96%; Figs. 5-7, 11), or haplogroup A (Fig. 3), containing both behavioral and subspecific designations, *M. v. magnamolaris* and *M. v. incautus*. Haplogroup A was not supported in the ML (HKY + I) phylogeny (Fig. 8) but was weakly supported in three other evaluated phylogenies (70%-74%; Figs. 9-11). Many of the phylogenies indicate some mild structure within haplogroup B; however, this mild structure did not indicate behavioral, geographic, or taxonomic division (≤80%; Figs. 5-10). The subclades which were strongly supported within haplogroup B were not behaviorally, geographically, nor taxonomically structured (>90%; Figs. 7, 10-11) except for the B19-B20 subclade which represents individuals sampled from Real County, Texas (Figs. 7, 11). Unlike all other phylogenetic analyses, those which evaluated a single

model of evolution resulted in a polytomy as the *Myotis* outgroups nested within the ingroup without any disruption of the general structure of the topology (Figs. 8-11).

Evaluation of Microsatellite Loci.—Evaluation of the 34 loci yielded suitable cross-species amplification in *M. velifer* for five autosomal loci (E24, F19, D15, H29: Castella and Ruedi 2000; Vonhof et al. 2002) and one X-linked locus (PAUR03: Burland et al. 1998). Sequence verification of microsatellite loci (GenBank Accession Nos. EU680301-EU680306; Table 2) revealed that locus D15 (Castella and Ruedi 2000) is an interrupted microsatellite in *M. velifer*. Due to the problematic variation found in D15 (i.e., $((CA)_nG)_n$), caused by allele size homoplasy, this locus was excluded from analyses.

All loci were evaluated for conformation to HWE expectations among populations (Table 6). Ten populations were estimated to have null alleles, of which three, ED2 ($n = 3$), MEN ($n = 1$) and HAR ($n = 1$), displayed allelic dropout with locus E24 (see Table 1 for abbreviations). After amending the final microsatellite data set, populations found to be deficient of heterozygotes analyzed with the four autosomal loci were: BLA ($P = 0.0047$), CHI ($P = 0.0118$), COM ($P = 0.0029$), COR ($P = 0.0210$), HAR ($P = 0.0072$), HAY ($P = 0.0247$), MED ($P = 0.0018$), MEN ($P = 0.0003$), SNB ($P = 0.0010$), SAN ($P = 0.0025$), SUT ($P = 0.0265$) and UVA ($P = 0.0430$) (see Table 1 for abbreviations). Heterozygosity deficiency was detected in the loci E24, F19 and H29. No heterozygosity excess was detected with any of the loci or populations.

Linkage disequilibrium was found among five populations (Table 7). Two populations had the same linked pairs of loci (Table 7); however, due to lack of linkage across multiple populations, these loci were thought to be linked due to random chance alone and not adjacent to one another on a chromosome.

Population Genetic Analyses.—Population comparisons of pairwise differences (Φ_{ST}) were calculated for each population and indicated some significant differences between populations (Table 8). No significant differences were observed among populations of *M. v. velifer*, but were observed among populations of *M. v. incautus* and among populations of *M. v. magnamolaris* (Table 8). Most populations of *M v. velifer* significantly differed from populations of *M. v. magnamolaris* (6 of 9 comparisons; Table 8). Most significantly different pairwise comparisons between populations of *M. v. magnamolris* and *M. v. incautus* were due to one population (CHI) of *M. v. magnamolaris* (10 of 39 comparisons). This was in contrast to the three significantly different pairwise comparisons observed among the other two *M. v. magnamolaris* populations (HAR and WHE) and *M. v. incautus* populations (Table 8). No significant differences were between populations of *M. v. incautus* and one population of *M. v. velifer* (MOH) but were observed between the other two populations of *M. v. velifer* (RV1 and SNB; Table 8).

Pairwise differences calculated for microsatellite data (F_{ST}) indicated few significant differences between all populations (Table 9). Population comparisons among populations within subspecific designations indicated no significant differences but did indicate a significant lack of difference between populations (Table 9). Between populations of *M. v. magnamolaris* and *M. v. incautus*, 11 pairwise comparisons significantly differed (Table 9). In contrast to the mtDNA, one pairwise comparison significantly differed between populations of *M. v. magnamolaris* and *M. v velifer* (Table 9). Pairwise comparisons between populations of *M. v. incautus* and *M v. velifer* indicated six significantly different comparisons and three marginally ($0.10 < F_{ST} < 0.15$) different, but significant, comparisons (Table 9).

Populations assigned *a priori* into their behavioral designations and taxonomic subdivisions indicated a lack of substantial variation between these designations for both mitochondrial and microsatellite data (Tables 10-12). A majority of the genetic variation was distributed among populations (mtDNA) or individuals (microsatellite data) and not the *a priori* designations (Tables 10-12).

Partitioning of mitochondrial data by SAMOVA indicated that the best number of populations was two by separating one population located in the Texas Panhandle (CHI) from the remaining sampled populations (Table 13; Fig. 12). Partitioning of the mtDNA by SAMOVA does not reflect geographically isolated populations or regions (Fig. 12). The microsatellite data, evaluated with SAMOVA (Table 11) and STRUCTURE (Table 14; Fig. 13), indicated no barriers to gene flow among sampled individuals.

Reevaluation of Hayward's (1970) Morphological Data.—Reevaluation of the combined and ranked morphological data demonstrate that individuals in Kansas, Oklahoma and the Texas Panhandle were the largest (Table 3; Fig. 14). A group of the smallest individuals were found in California, Arizona, and Sonora, Mexico (Table 3; Fig. 14). The second largest individuals were found in Veracruz, Morelos, southern Texas, Guatemala, Tlaxcala and Michoacan (Table 3; Fig. 14). Individuals decreased in size northward and the smallest individuals were in the Chihuahuan and Sonoran desert regions (Table 3; Fig. 14).

Demographic Analyses.—No significant deviations from neutrality were detected using the Ewens-Watterson test when all Texas populations were pooled ($P = 0.99$). Furthermore, by excluding populations containing one haplotype and populations in which all haplotypes were different, I observed no significant deviations from neutrality

in any populations (P >0.61). While Tajima's D statistic was not significant (D = 0.07460; P = 0.604), Fu's F_S was significant (F_S = -14.00071; P = 0.006). Also, Fu and Li's D^* and F^* were not significant (D^* = -2.03138, P >0.05; F^* = -1.43594, P >0.10).

Graphing of the mismatch distributions recovered a multimodal distribution (Fig. 15). The null model of sudden population expansion among specimens of *M. velifer* cannot be rejected (SSD P = 0.25). Furthermore, the small value of HRI cannot be rejected as a good fit of the data to the model (HRI = 0.013, P = 0.25). Population growth among Texas samples is also supported by the growth parameter generated with the program FLUCTUATE (g = 369.62806 ± 57.30494).

CHAPTER IV

DISCUSSION

The genetic data of *Myotis velifer* is not partitioned according to the behavioral differences or subspecific designations (Tables 10-12). Instead, the three distinct haplogroups recovered from the mtDNA of *M. velifer* showed no geographically correlated structure. Of these haplogroups, haplogroup A, although distributed among both subspecies (Hayward 1970), was only found within Texas and haplogroup C was only represented in one region by one individual (SMX). The most prevalent haplogroup was haplogroup B (~73% of sequenced individuals) and was found among the extremities of the sampled range as well as among all the subspecies. Both haplogroups A and C appear to have some geographical conformity; although it is possible that these haplogroups have not yet been sampled in other parts of the species range. The levels of sequence divergence found among these haplogroups may be due to historical barriers separating groups of individuals as the haplogroups are not found to represent current barriers to gene flow (Figs. 1-3, 13).

Many migratory chiropterans exhibit a lack of genetic structure across large geographic distances (see Burland and Worthington-Wilmer 2001). Russell et al. (2005a) found similar results among behavioral, as well as taxonomic, designations of North American *Tadarida brasiliensis*; however, Weyandt and Van Den Bussche (2007) found

geographically structured haplogroups among *Antrozous pallidus*, a species thought to migrate in parts of its range. As the haplotypes themselves are found to occur within specific regions (Fig. 2), the geographic distribution of the haplogroups recovered among *M. velifer* may confound interpretations of mitochondrial structure (Figs. 3, 12). Furthermore, while haplotypes are specific to a region, some of the haplotypes among the regions are more closely related to haplotypes from other regions (Fig. 2). Thus, a recent common ancestor exists for the similar haplotypes found among the regions. While the origin of these ancestors is unknown, a lack of regional philopatry is inferred from descendants that did not return to their natal region (Fig. 2). Although the nuclear data indicate a panmictic population, the lack of shared mtDNA haplotypes among the regions prevents full recognition of a panmictic population structure (Figs. 2, 13). Assuming the haplogroups represent former historic barriers among groups of individuals, the lack of geographic isolation of the haplogroups indicates some female dispersal. The haplotypes which are unique to the regions (not roosts) indicate some regional and not roost-specific female philopatry (Figs. 2, 3). Therefore, combining the inferences of some female dispersal and some female philopatry leads to an inference of weak regional fidelity supported by the mitochondrial data.

Banding data and reproductive behavior, both with limitations to their inferences, are integral parts of understanding the gene flow among *M. velifer*. The lack of roost fidelity among *M. velifer* has been well documented (Barbour and Davis 1969; Tinkle and Patterson 1965; Twente 1955b). Hayward (1970) conducted an extensive banding project among *M. velifer* in Tucson, Arizona and, with a return sample size of approximately 8.7%, concluded that *M. velifer* return to the same locality every year even

though no individual was recovered every year (Hayward 1970). Low recoveries are observed in banding studies of migratory chiropterans (e.g., *T. brasiliensis*, Cockrum 1969); however, the lack of individuals being observed every year may be indicative of weak summer site fidelity or weak regional fidelity. The inference of weak regional fidelity is congruent with the mitochondrial data as all regions are genetically very similar (minimum of 1 base pair difference), yet haplotypes are found to only occur within a geographically sampled region (Figs. 1-2). Therefore, while haplotypes indicate that some females return to a specific region (Figs. 1-2), the mitochondrial gene flow found among regions indicates that this is not always so (Tables 8, 10-13; Fig. 12).

Like banding studies, inferences of reproductive behavior are unfortunately also not robust. Reproductive behavior has been inferred from changes in the size of the testes and epididymides as well as field observations of copulation (Hayward 1970; Kunz 1973). These observations have led to inferences of mating prior to migration during the fall, through wakeful periods during hibernation in winter (Hayward 1970), and even after hibernation in the spring (Kunz 1973). Males also exhibiting weak regional fidelity and mating in the spring after migrating from hibernacula, as well as mating in the winter in shared hibernacula, may be inferred from the panmictic structure among microsatellite data. Although copulation in the fall before migration to hibernacula can not be disputed, fertilization from August or September copulations is less likely if sperm storage does not exceed 200 days (Racey 1979) when females are impregnated in March or April, respectively (Kunz 1973). Therefore, mitochondrial data are congruent with the inference of weak regional fidelity among females; whereas the panmictic structure of the microsatellite data may be due to mating occurring in multiple seasons.

Taxonomy.— Myotis velifer is distinct both morphologically and genetically as a species (Miller 1897; Ruedi and Mayer 2001; Stadelmann et al. 2007). Although distinguishing among species of *Myotis* can be difficult, *M. velifer* is the largest extant *Myotis* in the United States and is often characterized by a sparsely furred intrascapular area (Miller 1897; Vaughan 1954). Previous investigations into the taxonomic subdivisions support three morphologically distinct subspecies (Hayward 1970). However, the subspecies tree, based on morphometric analyses (Hayward 1970), is not congruent with the mitochondrial gene tree generated for *M. velifer* in this study. Partitioning of the mitochondrial data supports two haplogroups among United States specimens which do not reflect geographic structuring (Figs. 1-7); therefore, propositions of subspecific boundaries based on the mitochondrial data in this study are inappropriate.

Recognition of taxonomic subdivisions is necessary, especially for listed species, since subspecies represent various levels of geographically correlated diversity within that species. The importance of incorporating other factors into determining support of separate subspecies, despite a lack of reproductive isolation among genetic data, has been discussed (Crandall et al. 2000; Fraser and Bernatchez 2001). Therefore, although no evidence exists to support reproductive isolation among the taxonomic subdivisions of *M. velifer*, it is important to incorporate other factors, such as morphology and behavior, in determining whether multiple subspecies of *M. velifer* exist. Hayward's (1970) morphological data were reevaluated and combined with my genetic data, as well as reported behavioral data (Hayward 1970; Pekins 2006, 2007a; Tinkle and Patterson 1965; Twente 1955c), to evaluate whether any changes in the currently recognized taxonomic subdivisions were warranted.

First, my reevaluation of the morphological characters supports Hayward's (1970) assessment that the northernmost specimens were larger (Table 3, Fig. 14). While Hayward (1970) depicted disjunct populations of *M. v. incautus-magnamolaris*, this is no longer supported (Schmidley 2004). However, an examination of Hayward's (1970) data found the overall rank size of populations sampled within the Texas Panhandle almost double that of other populations within Texas (Table 3; Fig. 14). Aside from morphological characters, individuals found in the northernmost portion of the range are historically reported to exhibit non-migratory behavior as individuals are observed year-round (Tinkle and Patterson 1965; Twente 1955c). Morphology and behavior support the uniqueness of these individuals; therefore, I support the maintenance of this subspecies despite the lack of detected reproductive isolation or genetic differentiation (Crandall et al. 2000; Fraser and Bernatchez 2001).

The body size of *M. velifer* increases southward from central Texas into the tropic regions south of central Mexico and Central America, although none of the southern specimens attained the large body size ranks observed among *M. v magnamolaris* (Table 3, Fig. 14). The smaller individuals are found among the Chihuahuan and Sonoran deserts, with the smallest found in the Sonoran Desert consistent with the evaluation of Vaughan (1954; Table 3; Fig. 14). Both Hayward (1970) and Vaughan (1954) found the differentiation among *M. v. brevis-velifer* subspecies to be a result of clinal variation; however, the split was not supported by Hayward (1970) because he found the clinal variation was not significant. Little genetic and no behavioral differences were found among individuals sampled from California, central Texas, and west Texas (Hayward 1970; Pekins 2006). Also, morphological variation may represent ecological or clinal

variation (Fig. 14). Therefore, based on these data I am unable to support that differences exist among specimens from California, central Texas, and west Texas. Cautiously, I retain *M. v. velifer* as it was originally described from Jalisco, Mexico (Allen 1890) and because a unique haplogroup was recovered from Sonora, Mexico. Consequently, *M. v. velifer* is now restricted to the current distribution of the subspecies in Mexico and Central America. Therefore, in the United States, only two subspecies of *M. velifer* exist, *M. v. incautus* (Allen 1896) from central Texas westward and *M. v. magnamolaris* (Hayward 1970; Dalquest and Stangl 1984) from the Texas Panhandle northward. Additional data from specimens occurring in Mexico may also not detect reproductive isolation and, therefore, suggest no difference among *M. v. incautus-velifer*. A lack of difference among *M. v. incautus-velifer* would support the findings of Miller (1897), although his findings were based on a small sample size ($n \leq 5$) from each locality. If a lack of reproductive isolation is detected from a more thorough genetic analysis of specimens sampled from Mexico, Central American and *M. v. incautus*, then only two subspecies of *M. velifer* would be recognized, *M. v. velifer* and *M. v. magnamolaris* (Allen 1890, 1896). Examination of Mexican and Central American specimens should also consider the observation of a non-hibernating population in south-central Mexico to infer proper taxonomic subdivisions (Avila-Flores and Medellín 2004).

When considering taxonomic subdivisions, there are no clear rules to follow for designations; however, to base designations solely on genetic data is rash. Therefore, although genetic data does not support the taxonomic subdivisions proposed by Hayward (1970), both morphological and behavioral data do support maintaining *M. v. magnamolaris* as a separate adaptive evolutionary unit (Crandall et al. 2000; Fraser and

Bernatchez 2001). Furthermore, as similarities exist for both genetic and behavioral data, as well as ecological or clinal morphological variation, the two taxonomic subdivisions found in the southeastern United States (*M. v. incautus-velifer*) should be assigned to the subspecific designation *M. v. incautus* (Allen 1896, 1890). However, I cautiously retain *M. v. velifer* within the current distribution south of the United States until Mexico and Central America are sufficiently sampled for further evaluation of genetic and behavioral data.

Demographic History of Myotis velifer *in Texas.*—Expectations conforming to historical demographic expansion were recovered in the two more robust analyses (Fu 1997; Kuhner et al. 1998). The estimated demographic growth of *M. velifer* was within the range of that observed in other widely distributed chiropterans (*T. brasiliensis*: F_S = -13.13, g = 128.79 ± 7.87, Russell 2003; Sunda sampled *Cynopterus brachyotis*: F_S = -24.881, g = 482.072 ± 29.709, Campbell et al. 2007). An upward bias was demonstrated in simulation studies of FLUCTUATE's g (Kuhner et al. 1998); however, incorporation of Fu's F_S in my study allowed for a comparison of demographic estimates. Graphing of the mismatch distributions did not result in a unimodal distribution as expected in populations experiencing ancient demographic expansion (Rogers 1995; Rogers and Harpending 1992; Slatkin and Hudson 1991; Fig. 15). While multimodal mismatch distributions have been observed in species experiencing bottlenecks (e.g., *Tympanuchus cupido*, Johnson et al. 2007; *Arctocephalus townsendi*, Weber et al. 2004); they may also represent a panmictic population of constant size (Slatkin and Hudson 1991). Simulation studies indicated sensitivity to small migration rates in multimodal mismatch distributions (Ray et al. 2003); however, mitochondrial data only indicate low levels of

gene flow among *M. v. magnamolaris-velifer* populations. Based on estimates of Fu's F_S and FLUCTUATE's g, it may be more proper to assume that *M velifer* in Texas are recovering from a bottleneck rather than maintaining constant population size; however, this assumption has not been documented and is not consistent with the observed low HRI (HRI = 0.013) and empirical studies involving known bottlenecks (e.g., *Tympanuchus cupido*, Johnson et al. 2007; *Arctocephalus townsendi*, Weber et al. 2004). Further investigation into the observed multimodal mismatch distribution is needed to support the potential of a bottleneck among *M. velifer* and may be accomplished by comparing data of specimens prior to the observed roost abandonment (i.e., from the 1800s or early 1900s) with those analyzed in my study (e.g., *Arctocephalus townsendi*, Weber et al. 2004). These data indicate estimates of historical demographic expansion with no support of recovery from a population bottleneck; therefore, caution must be taken when interpreting the results inferred by the demographic analyses. Furthermore, as this study was not designed to conclude explanations for historical roost abandonment, populations of *M. velifer* should continue to be monitored.

Potential Explanations for the Observed Non-Monophyly in Likelihood Methods.— Multiple outgroups were utilized to test the hypothesis of ingroup monophyly. Unexpectedly, likelihood topologies evaluated with a single model of evolution (LSM) recovered a topology of non-monophyletic relationship among *M velifer* specimens. General relationships among the *M. velifer* haplotypes were maintained throughout all topologies; however, the LSM topologies recovered ingroups which were paraphyletic with respect to the assigned (monophyletic) outgroups. Assuming that the recovered LSM topologies are incorrect, an attempt to explain this assumption involved investigating the

influences of outgroup sensitivity, third codon position homoplasy, long-branch attraction (LBA), long-branch repulsion (LBR), and whether a single model of evolution is potentially misleading. Exclusion of one of the various outgroup taxa and third codon positions did not change the general structure of the topologies (data not shown). Investigating LBA, where long branches are "attracted" to one another, as a source of the incongruence yielded disruption of the ingroup by misplacement of the outgroup as a possible explanation (Holland et al. 2003). Holland et al. (2003), considering the evaluated four-state K2P model with sequence length of 100, observed that disruption of the ingroup occurred most often in MP methods; however, ML methods were also susceptible although less likely. Hence, the disruption of the ingroup, as a form of LBA, seems unlikely as this scenario is recovered in the LSM and not MP methods (Figs. 5, 8-10).

A characteristic of LBR, distinguishing it from other potential explanations, is that it performs better under MP than likelihood methods (Siddall 1998). In contrast to LBA, long branches are "repulsed" in LBR and this occurs not only in ML analyses but also among the long branches of sister, or closely related, taxa (Siddall 1998). With respect to mtDNA analyses, *M. velifer* and *M. yumanensis* are well-documented sister taxa (Hoofer and Van den Bussche 2003; Ruedi and Mayer 2001; Stadelmann et al. 2007); however, exclusion of *M. yumanensis* generally recovered topologies with disrupted ingroups. Lastly is the consideration of the single model of evolution as potentially misleading. The monophyly of *M. velifer* was not recovered in any LSM method; however, it was recovered in a more parameter-rich model in which models of evolution were evaluated among codon positions (Fig. 7). Other studies support codon

partitioning of protein coding genes as they provide a better fit of these data and a more accurate measurement of phylogenetic estimates (Goldman and Yang 1994). Furthermore, comparisons of model likelihood scores also support the codon partitioned model as a better fit to the final dataset (Brandley et al. 2005). As the above-mentioned explanations cannot be completely discredited, a more systematic approach must be developed to further investigate and determine if the disruption of the ingroup is due to an oversimplified model of evolution, or another, more likely, explanation.

Potential Explanations for the Observed Heterozygosity Deficiency.—In an infinitely large, randomly mating population, with no migration, mutation, or natural selection, conformities to HWE typically occur. When estimates do not meet the expectations of HWE, tests are executed to determine if the population has an excess of homozygotes or heterozygotes. In my study, an excess of homozygotes were observed (see results). Theories which may explain the observed heterozygote deficieny include selection, inbreeding, the Wahlund effect, and null alleles. First, selection against heterozygotes, possibly due to a decrease in individual fitness, would result in heterozygote deficiency. However, microsatellites, with the exception of sex-linked loci, are presumed to be neutral markers and not to be linked to non-neutral loci (Avise 1994) and therefore the observed heterozygosity deficiency is unlikely to be due to selection. Furthermore, heterozygosity deficiencies were not detected for any of the populations with the X-linked locus, PAUR03 (Burland et al. 1998), among females in this study (Table 6). Second, inbreeding, resulting in a loss of variation, will cause heterozygosity deficiency. While possible, is highly unlikely as a cause of the observed heterozygosity deficiency among *M. velifer* as heterozygosity deficiency is not observation across all loci. Third,

the Wahlund effect, occurs when the division of populations results in a loss of heterozygotes (Hartl and Clark 2007). As STRUCTURE and SAMOVA analyses failed to detect cryptic substructuring among nuclear data (Tables 13, 14; Fig. 13), this would imply that the population of *M. velifer* is larger than that sampled in this study. Therefore, while the Wahlund effect may be an explanation for the observed heterozygosity deficiency, evidence was recovered for the last potential explanation, null alleles.

Null alleles, due to sequence polymorphism in PCR-binding sites, have previously been observed at high frequencies in cross-species amplified loci (Hedgecock et al. 2004). High frequencies of null alleles (>0.05) were observed in the cross-species amplified microsatellite loci used in this study (Table 2). Partial nulls, a type of null allele, occur when smaller alleles are preferably amplified in comparison to larger alleles (Dakin and Avise 2004). The only observation of partial nulls occurred when individuals were re-amplified for the E24 locus, resulting in five of 43 (~12%) heterozygotes that were initially scored as homozygotes. The individuals reamplified for other loci did not result in previously unobserved heterozygotes. Therefore, with evidence for the presence of null alleles, it is the most likely explanation for the observed heterozygosity deficiency among *M. velifer*.

The presence of null alleles could seriously effect parentage analyses (Dakin and Avise 2004); however, population genetic analyses estimates of F_{ST} are unbiased when population genetic structure is absent and gene flow is high (Chapuis and Estoup 2007). Regardless, further testing of artifacts due to null alleles was conducted to determine any nuclear bias in this study. I tested for the influence of null alleles on analyses by executing simulations in which all loci with null allele frequencies >0.05 were excluded.

Simulations were executed with only females which allowed inclusion of the X-linked locus in analyses and as many loci as possible. Simulations excluding high null allele frequencies continued to best represent a near-panmictic population (data not shown). Coltman et al. (2007) analyzed a greater number of loci and also observed similar results when loci with high null allele frequencies were excluded. Therefore, I concluded that inclusion of more microsatellite loci, even those which are species-specific and lack null alleles, would most likely continue to support the near-panmictic structure of *M. velifer* observed among the microsatellite data in this study.

Considerations for Management.—Most of the demographic analyses conform to expectations of historical demographic expansion; however, the mismatch distribution was not unimodal failing to support this conclusion. Furthermore, as no published data confirm a bottleneck has occurred among *M. velifer* within Texas, I am unable to refute the interpretation of historic population equilibrium from the multimodal mismatch distribution (Slatkin and Hudson 1991). Until future studies confirm and support declines of historical roost abandonment, no conclusions should be drawn based on my study to change the species of concern designation of *M. velifer*.

Morphological and behavioral data, unlike genetic data, support maintaining two taxonomic subdivisions within the United States. Management considerations must also acknowledge the vagility of *M. velifer* as temporary colony shifts to more hospitable environments during the maternity season were observed (Pekins 2007b). Therefore, management within the United States should focus on two separate adaptive evolutionary units, *M. v. magnamolaris* and *M. v. incautus*, among broad areas comprised of multiple roosts across variable habitats.

Care should also be given when evaluating various populations as one sampling excursion resulted in the collection of *M thysanodes* alongside specimens of *M. velifer*. Although it is common to observe multiple species in one cave, inclusion of *M. thysanodes* would result in erroneous interpretations of gene flow and population size. The analyses described above along with photo verification distinguished the two species, resulting in exclusion of inappropriate data. As many species of *Myotis* are difficult to distinguish morphologically, precautious measures must be taken to allot a "security net" to reduce errors when non-consumptive sampling is practiced. Such occurrences are necessary to note for future management studies so that novices can review and ensure data integrity.

Conclusion.—Specimens collected in the United States indicated two mitochondrial haplogroups among *Myotis velifer*, but these do not conform to geographic, behavioral or previous taxonomic designations. The one haplogroup only found in one area, haplogroup C, was only represented by one individual from Sonora, Mexico. Pairwise comparisons of mitochondrial data indicate weak site fidelity and microsatellite data indicate homogenizing levels of male-mediated gene flow. Data do not support historical demographic contraction but do support historical demographic expansion. Potential explanations for the observed non-monophyly of *M. velifer* and heterozygote deficiency were discussed. Incorporation of morphological and behavioral data, unlike genetic data, supports the maintenance of the northernmost, non-migratory subspecies, *M. v. magnamolaris*, and the assignment of specimens of *M. v. velifer* within the United States to *M. v. incautus*. In the absence of data from additional Mexican specimens, *M. v. velifer* is retained only south of the United States border with Mexico until genetic analyses of

those southern populations are completed. Future analyses are required to evaluate if these assignments are appropriate, particularly for the Mexican and Central American populations.

TABLES AND FIGURES

TABLE 1. *Myotis velifer* roosts. Roosts are denoted in by the first three letters of the county name from which they were located, unless multiple roosts were sampled in one county as seen in Edwards, Texas (ED1, ED2) and Riverside, California (RV1, RV2) or if otherwise the same abbreviation would represent a roost as seen in San Saba, Texas (SAN) and San Bernardino, California (SNB). Individuals collected outside of the United States were provided by The Museum at Texas Tech University and are represented by location, not actual roosts. Samples from El Salvador were collected from the department (referenced as State) of Santa Ana.

TAXONOMIC SUBDIVISION	ROOST	COUNTY	STATE	COUNTRY	N
Myotis velifer magnamolaris	CHI	Childress	Texas	USA	17
	HAR	Hardeman	Texas	USA	19
	WHE	Wheeler	Texas	USA	20
M. v. incautus	BLA	Blanco	Texas	USA	19
	BRE	Brewster	Texas	USA	10
	COM	Comal	Texas	USA	21
	COR	Coryell	Texas	USA	13
	ED1	Edwards	Texas	USA	20
	ED2	Edwards	Texas	USA	20
	HAY	Hays	Texas	USA	22
	MED	Medina	Texas	USA	20
	MEN	Menard	Texas	USA	20
	REA	Real	Texas	USA	20
	SAN	San Saba	Texas	USA	12
	SUT	Sutton	Texas	USA	21
	UVA	Uvalde	Texas	USA	11
M. v. velifer	MOH	Mohave	Arizona	USA	6
	RV1	Riverside	California	USA	4
	RV2	Riverside	California	USA	5
	SNB	San Bernardino	California	USA	19
	ELS	N/A	Santa Ana	El Salvador	1

TABLE 2. All 34 loci tested for cross-species amplification in *Myotis velifer*. Suitably polymorphic loci which were evaluated in this study are listed first. The annealing temperature (T_A), salt concentration of the buffer (mM $MgCl_2$), number of alleles observed per gene, and source of the primers are reported. Null allele frequencies were calculated in CERVUS (Marshall et al. 1998). Sequences of suitably polymorphic and problematic loci were deposited on GenBank and accession numbers are given.

LOCUS	N	T_A (°C)	MM $MgCl_2$	ALLELES	ALLELE SIZE RANGE	NULL ALLELE FREQUENCY	GENBANK ACCESSION NUMBER	SOURCE
E24	192	59	1 5	25	195-249	0 0499	EU680303	(Castella and Ruedı 2000)
F19	192	59	1 5	5	194-218	0.2681	EU680305	(Castella and Ruedi 2000)
H29	192	52	1 5	13	153-191	0.0794	EU680302	(Castella and Ruedı 2000)
EF6	192	45	2.0	23	178-238	0.0305	EU680304	(Vonhof et al 2002)
PAUR03	167	57	2.5	21	225-263	-0.0004	EU680306	(Burland et al 1998)
D15	N/A	57	2.0	N/A	N/A	N/A	EU680301	(Castella and Ruedı 2000)
TABRA10	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(Russell et al. 2005b)
TABRA30	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(Russell et al 2005b)
TABRD10	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(Russell et al. 2005b)
TABRD15	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(Russell et al. 2005b)
TABRE9	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(Russell et al. 2005b)
TABRH2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(Russell et al. 2005b)
TABRH3	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(Russell et al. 2005b)
TABRH6	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(Russell et al 2005b)
TABRH12	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(Russell et al 2005b)
PAUR01	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(Burland et al. 1998)
PAUR02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(Burland et al. 1998)
PAUR05	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(Burland et al 1998)
B22	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(Castella and Ruedı 2000)
C113	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(Castella and Ruedı 2000)
D9	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(Castella and Ruedı 2000)
G9	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(Castella and Ruedı 2000)
G30	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(Castella and Ruedi 2000)
EF4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(Vonhof et al 2002)
EF5	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(Vonhof et al. 2002)
MSCHREIB2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(Mıller-Butterworth et al. 2002)
MSCHREIB3	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(Mıller-Butterworth et al 2002)
MSCHREIB5	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(Miller-Butterworth et al 2002)

TABLE 2 continued.

PPIP01	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(Racey et al. 2007)
PPIP02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(Racey et al. 2007)
PPIP03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(Racey et al 2007)
PPIP04	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(Racey et al. 2007)
PPIP05	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(Racey et al. 2007)
PPIP06	N/A	N/A	N/A	N/A	N/A	N/A	N/A	(Racey et al. 2007)

TABLE 3. Estimates of lengths (mm) of the four morphological features reported by Hayward (1970) among evaluated *Myotis velifer*. Abbreviations are as follows: forearm length (FA), greatest length of skull (GLS), maxillary tooth row (MTR), and cranial breadth (CB). Lengths were ranked as proportions to largest measurement. The average of the ranks were then calculated and ranked again. For geographic distribution of morphological character ranks please refer to Figure 13.

SAMPLING LOCALITY	FA	FA RANK	GLS	GLS RANK	MTR	MTR RANK	CB	CB RANK	AVERAGE OF RANKS	OVERALL RANK
Kansas	44.5	8	16.8	10	6.54	10	8.40	10	9.50	11
Oklahoma	44.5	8	16.6	9	6.46	9	8.30	9	8.75	9
Texas Panhandle	45.2	10	16.7	9	6.50	9	8.30	9	9.25	10
West Texas	42.5	4	16.0	5	6.30	5	8.00	5	5.00	4
Central Texas	42.4	4	16.1	6	6.26	6	7.95	5	5.25	5
South Texas	42.8	5	16.1	6	6.36	6	7.95	5	5.75	7
Coahuila	42	3	16.0	5	6.29	5	7.90	4	4.50	3
California	42.1	3	15.9	5	6.30	5	7.85	3	4.25	2
Arizona	41.9	3	15.9	5	6.30	5	7.80	3	4.25	2
Sonora	41.9	3	15.8	4	6.20	4	7.70	2	3.50	1
Sinaloa	43.3	6	16.0	5	6.30	5	7.90	4	5.25	5
Veracruz	43.9	7	16.1	6	6.45	6	7.92	4	6.50	8
Tlaxcala	43.1	5	15.9	5	6.39	5	7.91	4	5.50	6
Michoacan	43.4	6	16.0	5	6.32	5	7.91	4	5.50	6
Morelos	43.1	5	16.4	8	6.40	8	7.95	5	6.50	8
Guatemala	44.2	8	15.9	5	6.29	5	7.92	4	5.75	7

TABLE 4. Molecular diversity indices among *Myotis velifer*. The first 982 base pairs of the mitochondrial *Cytochrome b* gene were sequenced. Number of individuals, number of haplotypes, haplotype diversity (h), number of polymorphic sites and nucleotide diversity (π) for each subpopulation and the total population. For roost abbreviations refer to Table 1. Calculations were executed with ARLEQUIN 3.01.

TAXONOMIC SUBDIVISION	SAMPLED ROOST	NUMBER OF INDIVIDUALS SEQUENCED	NUMBER OF HAPLOTYPES	$h \pm$ SE	NUMBER OF POLYMORPHIC SITES	$\pi \pm$ SE
Myotis velifer magnamolaris	CHI	5	3	0.700 ± 0.218	3	0.001 ± 0.001
	HAR	7	6	0.952 ± 0.010	25	0.013 ± 0.008
	WHE	8	5	0.857 ± 0.108	24	0.012 ± 0.007
M. v. incautus	BLA	2	2	1.000 ± 0.500	1	0.001 ± 0.001
	BRE	1	1	1.000 ± 0.000	0	0.000 ± 0.000
	COM	2	2	1.000 ± 0.500	13	0.013 ± 0.013
	COR	2	2	1.000 ± 0.000	21	0.022 ± 0.022
	ED1	11	7	0.873 ± 0.089	27	0.010 ± 0.006
	ED2	15	12	0.962 ± 0.040	39	0.014 ± 0.008
	HAY	3	3	1.000 ± 0.272	22	0.015 ± 0.012
	MED	2	2	1.000 ± 0.500	23	0.024 ± 0.024
	MEN	7	7	1.000 ± 0.076	31	0.015 ± 0.009
	REA	9	9	1.000 ± 0.052	35	0.015 ± 0.008
	SAN	3	3	1.000 ± 0.272	12	0.008 ± 0.007
	SUT	11	6	0.854 ± 0.085	16	0.007 ± 0.004
	UVA	2	2	1.000 ± 0.500	20	0.021 ± 0.021
M. v. velifer	MOH	3	3	1.000 ± 0.272	8	0.005 ± 0.004
	RV1	3	2	0.667 ± 0.314	1	0.001 ± 0.001
	SNB	6	3	0.600 ± 0.215	9	0.004 ± 0.003
	ELS	1	1	1.000 ± 0.000	0	0.000 ± 0.000
N/A	ALL	103	53	0.965 ± 0.009	72	0.013 ± 0.006

TABLE 5. Haplotype distribution recovered from the mitochondrial *Cytochrome b* gene among 103 *Myotis velifer* sequenced in this study. Haplotypes represented by multiple individuals in the same roost are in parentheses. For abbreviations refer to Table 1. For haplogroup designations refer to Figure 11.

TAXONOMIC DESIGNATION	ROOST	HAPLOTYPES
Myotis velifer magnamolaris	CHI	B02 (3), B03, B04
	HAR	A02, A03, A06, B01 (2), B03, B05
	WHE	A01, A04, A05, B01 (3), B02 (2)
M. v. incautus	BLA	B14, B16
	BRE	B26
	COM	B07, B13
	COR	A07, B06
	ED1	A17, A18, B07, B09, B16 (4), B17 (2), B23
	ED2	A07 (2), A09, A12, A16, A18, A19, B07, B10, B16 (3), B18, B22, B25
	HAY	A07, A13, B07
	MED	A09, B16
	MEN	A13, A17, A20, B11, B16, B17, B26
	REA	A07, A08, A11, A15, B07, B15, B19, B20, B21
	SAN	B07, B12, B16
	SUT	B07 (4), B09, B16 (2), B17 (2), B22, B24
	UVA	A10, B27
M. v. velifer	MOH	B29, B30, B32
	RV1	B28, B29 (2)
	SNB	B29, B30 (4), B31
	ELS	B33

TABLE 6. Hardy-Weinberg equilibrium. Values reported are observed (H_{Obs}) and expected heterozygosity (H_{Exp}), and loci which did not significantly (NS) or did significantly (*P*) differ from Hardy-Weinberg expectations (HWE). Loci were tested against HWE using sequential Bonferroni analyses at 0.05 (*), 0.025(**), 0.0167 (***), 0.0125(****), and 0.01 (*****) significance levels with *P*-value in parentheses. Loci found to be monomorphic are indicated and without HWE analyses. Individuals (n) which contained no missing data were analyzed for autosomal loci and all amplifying females (♀) were analyzed for the X-linked locus (PAUR03). Loci among populations indicated to contain null alleles are noted (^{N}). Populations deficient of heterozygotes and with insufficient data to test for null alleles are noted (^{I}). ARLEQUIN 3.01 was used for all calculations, MICRO-CHECKER to detect null alleles. For abbreviations and total number of individuals (N) collected from each roost refer to Table 1. For sources of loci refer to Table 2.

POPULATION		E24	F19	H29	EF6	PAUR03
BLA	H_{Obs}	0.6471	0.2941	0.8235	0.9412	0.7778
n=17	H_{Exp}	0.8164	0.3832	0.8770	0.8574	0.9281
♀=9	*P*	NS (0.4060)	NS (0.5369)	NS (0.0610)	NS (0.5476)	NS (0.5532)
BRE^{I}	H_{Obs}	0.6667	monomorphic	1.0000	1.0000	1.0000
n=3	H_{Exp}	0.9333	-	0.9333	0.7333	0.9556
♀=5	*P*	NS (0.4770)	-	NS (1)	NS (1)	NS (1)
CHI	H_{Obs}	0.3000	0.1000	0.8000	0.6000	0.8000
n=10	H_{Exp}	0.5842	0.1947	0.8947	0.8158	0.8345
♀=15	*P*	NS (0.0539)	NS (1.000)	NS (0.3813)	* (0.0266)	NS (0.4888)
COM	H_{Obs}	0.5385	0.0769	0.5385	0.8462	0.8750
n=13	H_{Exp}	0.4708	0.2892	0.8769	0.9262	0.8667
♀=8	*P*	NS (1)	* (0.0402)	**** (0.0105)^{N}	NS (0.2408)	NS (0.8561)
COR^{I}	H_{Obs}	monomorphic	0.0000	0.6667	0.6667	0.8000
n=3	H_{Exp}	-	0.8000	0.9333	1.0000	0.9111
♀=5	*P*	-	NS (0.2011)	NS (0.5929)	NS (0.1840)	NS (0.8076)
ED1	H_{Obs}	0.4375	0.2500	0.8125	1.0000	0.9091
n=16	H_{Exp}	0.5706	0.2863	0.8690	0.8952	0.9134
♀=11	*P*	NS (0.1502)	NS (1)	NS (0.1460)	NS (0.5269)	NS (0.1391)
ED2	H_{Obs}	0.5000	0.2500	0.7500	0.6667	1.0000
n=12	H_{Exp}	0.4420	0.4348	0.3498	0.8587	0.8831
♀=11	*P*	NS (1)	NS (.0657)	NS (0.3498)	NS (0.4487)	NS (0.8261)

TABLE 6 continued.

HAR	H_{Obs}	0.4444	0.1111	0.6667	0.7778	0.7778
n=9	H_{Exp}	0.7059	0.2157	0.8954	0.8673	0.7778
♀=9	*P*	NS (0.1196)	NS (1)	NS (0.2327)	NS (0.3328)	NS (0.8759)
HAY	H_{Obs}	0.7000	0.0000	0.7000	0.7000	0.7000
n=10	H_{Exp}	0.6895	0.4316	0.8895	0.8263	0.8579
♀=10	*P*	NS (0.6373)	***** (0.0091)^{N}	NS (0.4579)	NS (0.1884)	NS (0.8553)
MED	H_{Obs}	0.7143	0.1429	0.7857	0.8571	0.7778
n=14	H_{Exp}	0.8360	0.3280	0.8677	0.8492	0.9346
♀=9	*P*	NS (0.6444)	NS (0.2119)	NS (0.4384)	NS (0.1581)	NS (0.3704)
MEN	H_{Obs}	0.3333	0.0000	0.6667	1.0000	1.0000
n=15	H_{Exp}	0.5609	0.1931	0.9058	0.8805	0.9341
♀=7	*P*	* (0.0389)^{N}	* (0.0339)	NS (0.0651)	NS (0.9824)	NS (1)
MOH^{I}	H_{Obs}	0.7500	monomorphic	0.7500	0.7500	0.7500
n=4	H_{Exp}	0.7500	-	0.7500	0.9286	0.9643
♀=4	*P*	NS (0.3192)	-	NS (1)	NS (0.4757)	NS (0.3094)
REA	H_{Obs}	0.7692	0.0769	0.6923	0.9231	1.0000
n=13	H_{Exp}	0.6923	0.3969	0.8154	0.8985	0.8877
♀=12	*P*	NS (0.7819)	* (0.0256)^{N}	NS (0.4248)	NS (0.5156)	NS (0.9859)
RV1^{I}	H_{Obs}	1.0000	monomorphic	1.0000	0.5000	1.0000
n=2	H_{Exp}	1.0000	-	1.0000	1.0000	0.9333
♀=3	*P*	NS (1)	-	NS (1.000)	NS (0.3421)	NS (1)
RV2^{I}	H_{Obs}	0.6667	monomorphic	1.0000	1.0000	-
n=3	H_{Exp}	1.0000	-	0.9333	0.8667	-
♀=0	*P*	NS (0.1927)	-	NS (0.9333)	NS (1)	N/A
SAN^{I}	H_{Obs}	0.7143	0.1429	0.2857	0.7143	0.8333
n=7	H_{Exp}	0.5934	0.2747	0.8242	0.9341	0.8939
♀=6	*P*	NS (1)	NS (1)	***** (0.0074)	NS (0.2612)	NS (0.7186)

TABLE 6 continued.

SNB	H_{Obs}	0.5000	0.1000	0.9000	0.4000	0.9167
n=10	H_{Exp}	0.7105	0.1947	0.8368	0.9263	0.8768
♀=12	*P*	NS (0.1950)	NS (1)	NS (0.7102)	***** (0.0002)[N]	NS (0.9281)
SUT	H_{Obs}	0.6667	0.3333	0.7333	0.9333	0.8667
n=15	H_{Exp}	0.7149	0.4920	0.8345	0.8966	0.8460
♀=15	*P*	NS (0.8279)	NS (0.2150)	NS (0.5251)	NS (0.3144)	NS (0.2994)
UVA[I]	H_{Obs}	0.6000	monomorphic	0.4000	0.8000	1.0000
n=5	H_{Exp}	0.5111	-	0.9778	0.8889	0.8939
♀=6	*P*	NS (1)	-	***** (0.0104)	NS (0.9056)	NS (1)
WHE	H_{Obs}	0.5455	monomorphic	0.7273	0.8182	0.7000
n=11	H_{Exp}	0.6883	-	0.8355	0.7836	0.6790
♀=10	*P*	NS (0.6529)	-	NS (0.6999)	NS (0.3709)	NS (0.2673)

TABLE 7. Tests of linkage disequilibrium among *Myotis velifer*. Tests were executed for among all loci and all populations. Loci found to have significant levels of linkage disequilibrium are indicated by population in the table. Calculations were executed in ARLEQUIN 3.01 ($\alpha = 0.05$, 10,000 initial conditions for EM, 100,000 permutations). For population abbreviations refer to Table 1. For sources of loci refer to Table 2. For number of individuals analyzed in each population refer to Table 6.

	E24	F19	H29	PAUR03
F19		-		
H29	WHE, SNB	SUT	-	
PAUR03		ED1		-
EF6	SUT			MEN

TABLE 8. Pairwise Φ_{ST}s of mitochondrial data for each roost sampled for *Myotis velifer*. Sequence data analyzed from 982 base pairs of the mitochondrial *Cytochrome b* gene. Significance of values were tested using sequential Bonferroni analyses with 110 permutations and are as follows: P <0.05 are in bold; P <0.025 are in italics; P <0.01 are noted with an (*). Comparisons between populations within the same subspecific grouping are shaded light grey. Specimens from El Salvador (The Museum at Texas Tech, TK34862) and Sonora (GenBank, AF376870) were not included in this analysis. Calculations were executed in ARLEQUIN 3.01. For abbreviations refer to Table 1. For number of individuals sequenced from each population refer to Table 4.

	M. v. magnamolaris			*M. v incautus*												*M. v. velifer*		
	CHI	HAR	WHE	BLA	BRE	COM	COR	ED1	ED2	HAY	MED	MEN	REA	SAN	SUT	UVA	MOH	RV1	SNB
CHI	-																		
HAR	**0.24**	-																	
WHE	0.19	-0.13	-																
BLA	**0.90**	0.35	0.37	-															
BRE	0.88	0.13	0.16	0.71	-														
COM	0.43	0.01	0.01	-0.77	-0.73	-													
COR	0.50	-0.30	-0.23	0.35	0.27	-0.17	-												
ED1	0.42*	**0.18**	0.18	-0.10	-0.32	-0.17	0.15	-											
ED2	0.38*	0.03	0.07	0.14	-0.07	0.02	-0.19	0.64	-										
HAY	*0.60*	-0.10	-0.03	0.47	0.19	0.15	-0.55	**0.27**	-0.08	-									
MED	**0.63**	-0.04	0.03	0.00	-0.70	-0.20	-0.52	-0.04	-0.29	-0.30	-								
MEN	0.41*	0.00	0.04	0.80	-0.22	-0.07	-0.21	-0.01	-0.10	-0.09	-0.33	-							
REA	0.42*	0.05	0.09	0.19	-0.07	0.03	-0.22	0.10	-0.06	-0.10	-0.27	-0.08	-						
SAN	**0.30**	0.02	0.00	0.34	0.00	-0.47	-0.05	0.01	0.10	0.23	0.07	0.04	0.11	-					
SUT	**0.34**	**0.23**	0.21*	0.15	-0.11	-0.27	0.28	0.03	**0.21**	0.43*	0.27	0.17	*0.23*	-0.16	-				
UVA	**0.62**	-0.14	-0.07	0.22	-0.54	-0.14	-0.64	0.04	-0.28	-0.41	-0.79	-0.31	-0.32	0.08	0.28	-			
MOH	**0.68**	0.21	0.21	0.36	-0.07	-0.07	0.23	0.02	0.13	0.37	0.10	0.08	0.12	0.13	0.07	0.06	-		
RV1	**0.90**	**0.39**	0.39*	0.80	0.85	0.33	0.49	0.14	**0.22**	0.54	0.30	0.20	**0.24**	0.48	**0.28**	0.37	0.25	-	
SNB	*0.67*	**0.30**	*0.30*	**0.57**	0.38	0.24	**0.43**	*0.20*	**0.24**	**0.51**	0.38	*0.23*	*0.25*	0.32	0.24*	0.32	-0.13	0.53	-

TABLE 9. Pairwise F_{ST}s and R_{ST}s of microsatellite data for each roost sampled for *Myotis velifer*. Individuals were analyzed at four autosomal loci (n = 192) F_{ST} (number of different alleles) below diagonal and R_{ST} (sum of squared size differences) above diagonal. Comparisons between populations within the same subspecific grouping are shaded light grey. Significance of values were tested using sequential Bonferroni analyses with 110 permutations and are as follows: P <0.05 are in bold; P <0.025 are in italics; P <0.01 are noted with an (*). Calculations were executed in ARLEQUIN 3.01. For abbreviations refer to Table 1. For sources of loci refer to Table 2. For number of individuals included from each population refer to Table 6.

	M. v. magnamolaris			*M. v incautus*												*M. v. velifer*			
	CHI	HAR	WHE	BLA	BRE	COM	COR	ED1	ED2	HAY	MED	MEN	REA	SAN	SUT	UVA	MOH	RV1	RV2	SNB
CHI	-	-0.04	-0.02	-0.01	-0.11	0.01	0.36	**0.09**	0.04	-0.02	-0.02	0.02	-0.01	-0.03	-0.01	0.04	-0.04	-0.12	0.08	-0.02
HAR	-0.03	-	0.00	0.01	-0.07	-0.03	0.32*	0.04	-0.02	-0.04	-0.03	-0.01	-0.04	-0.07	-0.02	-0.03	0.00	-0.09	0.09	-0.02
WHE	0.04	0.00	-	-0.07	0.05	0.02	*0.38*	0.04	-0.01	0.02	0.03	-0.01	-0.03	-0.06	-0.01	-0.02	0.04	-0.04	**0.17**	0.00
BLA	0.04*	0.02	0.05*	-	-0.04	0.07	*0.32*	**0.08**	**0.07**	0.01	-0.02	0.03	0.04	0.17	-0.01	0.09	-0.04	-0.11	**0.15**	0.03
BRE	0.00	-0.02	0.05	0.02	-	-0.03	0.35	0.04	0.00	-0.04	-0.07	-0.02	-0.06	-0.10	-0.06	0.01	-0.02	-0.16	0.08	-0.06
COM	0.02	0.00	0.02	0.02	0.06	-	0.37*	0.06	-0.03	-0.02	0.01	0.00	**-0.03**	-0.05	0.01	-0.03	0.05	-0.04	0.10	0.00
COR	0.07	0.06	**0.11**	0.05	0.17	0.05	-	0.06	*0.29*	**0.46**	0.21	**0.17**	0.40*	**0.28**	0.23*	0.26	**0.48**	0.31	0.53	*0.46*
ED1	**0.02**	0.00	0.03	0.01	*0.06*	-0.01	0.05	-	0.02	0.08	0.02	-0.01	**0.09**	0.02	0.03	-0.01	**0.12**	0.06	0.24*	0.13*
ED2	0.03	0.01	**0.04**	0.03*	**0.07**	0.00	0.05	-0.01	-	0.01	0.00	-0.02	0.00	-0.04	0.00	-0.05	0.09	0.00	**0.17**	0.04
HAY	0.00	-0.01	0.01	0.02	0.03	0.01	0.03	0.01	0.00	-	-0.01	0.00	-0.04	-0.04	-0.01	0.05	-0.03	-0.11	0.13	-0.04
MED	0.05*	0.03	**0.03**	0.00	0.04	**0.03**	0.08	0.02	**0.03**	0.03	-	-0.02	0.01	-0.03	-0.03	0.00	-0.02	-0.09	0.12	0.02
MEN	-0.01	-0.02	0.02	0.01	0.02	-0.01	0.05	0.00	0.01	-0.01	0.03*	-	0.01	-0.03	0.00	-0.03	0.02	-0.05	**0.14**	0.04
REA	*0.04*	0.01	**0.04**	-0.01	0.03	0.00	0.06	0.00	0.01	0.01	-0.01	0.01	-	-0.04	0.01	0.00	0.00	-0.09	0.06	-0.03
SAN	**0.05**	0.01	0.04	0.02	0.08	0.02	0.10	0.03	0.05	0.03	0.05*	0.01	0.02	-	-0.02	-0.06	0.01	-0.09	0.09	-0.02
SUT	0.03	0.01	**0.04**	0.00	0.04	0.00	0.04	0.00	0.00	0.00	0.02	0.01	0.00	0.01	-	0.01	-0.02	-0.09	**0.12**	0.01
UVA	-0.02	-0.02	-0.02	0.04	0.03	-0.01	0.08	0.00	0.03	0.00	0.03	-0.02	0.02	0.03	0.02	-	**0.19**	0.05	**0.22**	0.07
MOH	0.03	-0.01	0.03	0.00	0.03	0.00	**0.12**	-0.01	-0.02	-0.01	0.00	-0.01	0.00	0.01	-0.02	**0.19**	-	-0.23	0.09	-0.05
RV1	0.00	-0.03	0.01	0.00	-0.05	0.02	0.15	0.02	0.06	-0.01	0.00	0.00	-0.01	-0.09	-0.09	0.05	-0.01	-	-0.11	-0.14
RV2	0.06	0.02	**0.06**	0.00	-0.01	0.03	0.15	0.02	0.06	0.04	-0.01	0.03	-0.01	0.09	**0.12**	**0.22**	-0.02	-0.03	-	0.01
SNB	0.01	-0.02	0.02	**0.03**	0.02	0.00	0.11	0.02	0.03	0.02	0.05*	0.00	0.01	-0.02	0.01	0.07	-0.01	-0.01	0.01	-

TABLE 10. Fixation indices for mitochondrial and microsatellite data. Mitochondrial analysis evaluates sequenced samples of the *Cytochrome b* gene (982 base pairs; 102 specimens collected within the United States). Nuclear analyses evaluate 192 individuals amplified at four autosomal loci with no missing data. Individuals were designated behaviorally (non-migratory versus migratory) or subspecifically *a priori* (see Hayward 1970). Subscripts refer to I for individual, S for roost or population, R for the *a priori* designation, and T for total population. Fixation index values are reported for their respective hierarchy with significance indicated (*P* value). Calculations were executed in ARLEQUIN 3.01. For sources of loci refer to Table 2.

DESIGNATION	DATA	HIERARCHY	FIXATION INDEX VALUE	*P* VALUE
BEHAVIORAL	MITOCHONDRIAL	Φ_{SR}	0.09	<0.001
		Φ_{ST}	0.20	<0.001
		Φ_{RT}	0.13	= 0.003
	NUCLEAR	F_{IS}	0.13	<0.001
		F_{IT}	0.004	<0.001
		F_{SR}	0.01	= 0.035
		F_{RT}	0.14	= 0.010
	NUCLEAR	R_{IS}	0.15	= 0.002
		R_{IT}	0.02	<0.001
		R_{SR}	-0.01	= 0.093
		R_{RT}	0.16	=0.601
TAXONOMIC	MITOCHONDRIAL	Φ_{SR}	0.05	<0.001
		Φ_{ST}	0.19	= 0.012
		Φ_{RT}	0.15	<0.001
	NUCLEAR	F_{IS}	0.13	<0.001
		F_{IT}	0.14	<0.001
		F_{SR}	0.003	= 0.053
		F_{RT}	0.01	= 0.002
	NUCLEAR	R_{IS}	0.15	<0.001
		R_{IT}	0.01	<0.001
		R_{SR}	0.02	= 0.218
		R_{RT}	0.17	= 0.027

TABLE 11. Tests of differentiation among behavioral designations. AMOVA for mitochondrial and microsatellite data. Mitochondrial analysis evaluates sequenced samples of the *Cytochrome b* gene (982 base pairs; 102 specimens collected in the United States). Nuclear analyses evaluate 192 individuals amplified at four autosomal loci with no missing data. Individuals were behaviorally grouped *a priori*. For abbreviations refer to Table 1. For sources of loci refer to Table 2. Calculations were executed in ARLEQUIN 3.01.

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	VARIANCE COMPONENTS	PERCENT OF VARIATION
MITOCHONDRIAL (Φ_{ST})				
Among Groups	1	36.306	0.8525	12.83
Among Populations within Groups	17	133.352	0.5003	7.53
Within Populations	83	439.313	5.2929	79.64
NUCLEAR (F_{ST})				
Among Groups	1	3.364	0.0178	1.36
Among Populations within Groups	18	27.942	0.0053	0.40
Among Individuals within Populations	172	249.988	0.1668	12.74
Within Individuals	192	215.000	1.1198	85.50
NUCLEAR (R_{ST})				
Among Groups	1	235.296	-1.6907	-0.64
Among populations within groups	18	7133.435	5.1219	1.94
Among individuals within populations	172	51560.216	38.6083	14.59
Within Individuals	192	42730.000	222.5521	84.11

TABLE 12. Tests of differentiation among taxonomic designations. AMOVA for mitochondrial and microsatellite data. Mitochondrial analysis evaluates sequenced samples of the *Cytochrome b* gene (982 base pairs; 102 specimens collected within the United States). Nuclear analyses evaluate 192 individuals amplified at four autosomal loci with no missing data. Individuals were taxonomically grouped *a priori* (see Hayward 1970). For abbreviations refer to Table 1. For sources of loci refer to Table 2. Calculations were executed in ARLEQUIN 3.01.

MITOCHONDRIAL (Φ_{ST})				
SOURCE OF VARIATION	**DEGREES OF FREEDOM**	**SUM OF SQUARES**	**VARIANCE COMPONENTS**	**PERCENT OF VARIATION**
Among Groups	2	62.961	1.0084	15.35
Among Populations within Groups	16	106.696	0.2700	4.11
Within Populations	83	439.313	5.2929	80.55
NUCLEAR (F_{ST})				
Among Groups	2	5.505	0.0157	1.20
Among Populations within Groups	17	25.801	0.0034	0.26
Among Individuals within Populations	172	249.988	0.1668	12.78
Within Individuals	192	215.000	1.1198	85.76
NUCLEAR (R_{ST})				
Among Groups	2	1595.368	5.8337	2.17
Among populations within groups	17	5773.363	2.0891	0.78
Among individuals within populations	172	51560.216	38.6083	14.35
Within Individuals	192	42730.000	222.5521	82.71

TABLE 13. Results of SAMOVA analyses. Fixation indices and significance (*P*) generated by evaluation of mitochondrial and microsatellite data with SAMOVA for hypothesized population partitioning (K). Mitochondrial analysis evaluates 102 sequenced samples of the *Cytochrome b* gene (982 base pairs) obtained within the United States. Nuclear analyses evaluate 192 individuals amplified at four autosomal loci with no missing data. For sources of loci refer to Table 2. For number of individuals evaluated from each population refer to Table 4. For the mtDNA, K=1 was calculated with ARLEQUIN (Φ_{ST} = 0.07266; *P* <0.01).

K	MITOCHONDRIAL		NUCLEAR	
	Φ_{CT}	P	F_{CT}	P
2	0.21826	0.05865	0.31091	0.05572
3	0.22306	<0.01	0.22802	<0.01
4	0.23662	<0.01	0.17132	<0.01
5	0.25289	<0.01	0.12305	<0.01
6	0.2621	<0.01	0.10255	<0.01
7	0.27663	<0.01	0.08996	<0.01
8	0.2753	<0.01	0.08062	<0.01
9	0.28479	<0.01	0.07916	<0.01
10	0.30311	<0.01	0.08046	<0.01

TABLE 14. Results of STRUCTURE 2.2 analyses. Values are the averages of 10 simulations for the estimated Ln probability of the data (LN) and mean alpha value plus/minus standard deviation (SD). Analyses evaluate four autosomal loci for both sexes (n = 192) and among haplogroups (n = 66), as well as four autosomal loci and one X-linked locus for females only (n = 103). Only specimens obtained within the United States are included in these analyses. For sources of loci refer to Table 2.

K	BOTH SEXES		FEMALES ONLY	
	LN ± SD	ALPHA ± SD	LN ± SD	ALPHA ± SD
1	-2643.43 ± 0.20	N/A	-1828.69 ± 0.41	N/A
2	-2689.62 ± 21.55	4.95 ± 1.19	-1843.76 ± 5.14	6.46 ± 1.54
3	-2795.19 ± 61.31	4.04 ± 1.60	-1873.63 ± 19.79	4.53 ± 1.34
4	-3005.86 ± 153.18	3.33 ± 1.79	-1903.15 ± 46.57	4.95 ± 1.86
5	-3072.56 ± 161.20	1.99 ± 0.34	-1962.28 ± 55.12	3.73 ± 1.43
6	-3196.78 ± 281.26	1.74 ± 0.72	-2093.51 ± 107.77	2.44 ± 0.84
7	-3367.98 ± 237.80	1.08 ± 0.10	-2290.14 ± 284.89	2.58 ± 1.65
8	-3425.96 ± 192.14	0.79 ± 0.08	-2289.91 ± 139.76	1.01 ± 0.17
9	-3512.95 ± 250.83	0.62 ± 0.06	-2517.92 ± 310.32	1.03 ± 0.26
10	-3487.52 ± 149.49	0.53 ± 0.05	-2387.60 ± 163.62	0.73 ± 0.05

TABLE 15. Summary of objectives evaluated, expected results, genetic markers used, analytical methods, observed results, and reference table(s) or figure(s) for specimens of *Myotis velifer*. Abbreviation are as follows: MtDNA = mitochondrial DNA and nDNA = microsatellite loci. The programs used for the analytical methods are as follows: PAUP, NETWORK, TCS, STRUCTURE 2.2, SAMOVA, ARLEQUIN 3.01, and FLUCTUATE. Final datasets consisted of 982 base pairs of the mitochondrial *Cytochrome b* gene and four or five autosomal loci. For loci sources refer to Table 2. For number of individuals evaluated for mitochondrial data refer to Table 4.

OBJECTIVE EVALUATED	EXPECTED RESULTS	MARKER	ANALYSIS	OBSERVED RESULTS	TABLE(S) / FIGURE(S)
Behavioral Designations	Genetic Partitioning Among Behavioral Designations	MtDNA	Haplotype Network	Structure not correlated	Figs. 2-3
		MtDNA	Phylogenetic Methods	Structure not correlated	Figs. 4-11
		MtDNA & nDNA	SAMOVA	MtDNA structure not correlated, No nDNA structure	Table 13; Fig. 12
		nDNA	STRUCTURE 2.2	No structure	Table 14; Fig 13
		MtDNA & nDNA	Fixation Indices	Little variation among behavioral designations	Table 8, 9
		MtDNA & nDNA	AMOVA	Little variation among behavioral designations	Table 10, 11
Subspecific Designations	Genetic Partitioning Among Subspecific Designations	MtDNA	Haplotype Network	Structure not correlated	Figs. 2-3
		MtDNA	Phylogenetic Methods	Structure not correlated	Figs. 4-11
		MtDNA & nDNA	SAMOVA	MtDNA structure not correlated, No nDNA structure	Table 13; Fig. 12
		nDNA	STRUCTURE 2.2	No structure	Table 14; Fig. 13
		MtDNA & nDNA	Fixation Indices	Little variation among subspecific designations	Table 8, 9
		MtDNA & nDNA	AMOVA	Little variation among subspecific designations	Table 10, 12
Demographic Parameters	Historic Demographic Contraction	MtDNA	Tajima's D	Not significant	See Results
		MtDNA	Fu's F_S	Population growth	See Results
		MtDNA	Mismatch Distribution	Population equilibrium or bottleneck?	Fig. 15
		MtDNA	FLUCTUATE	Population growth	See Results

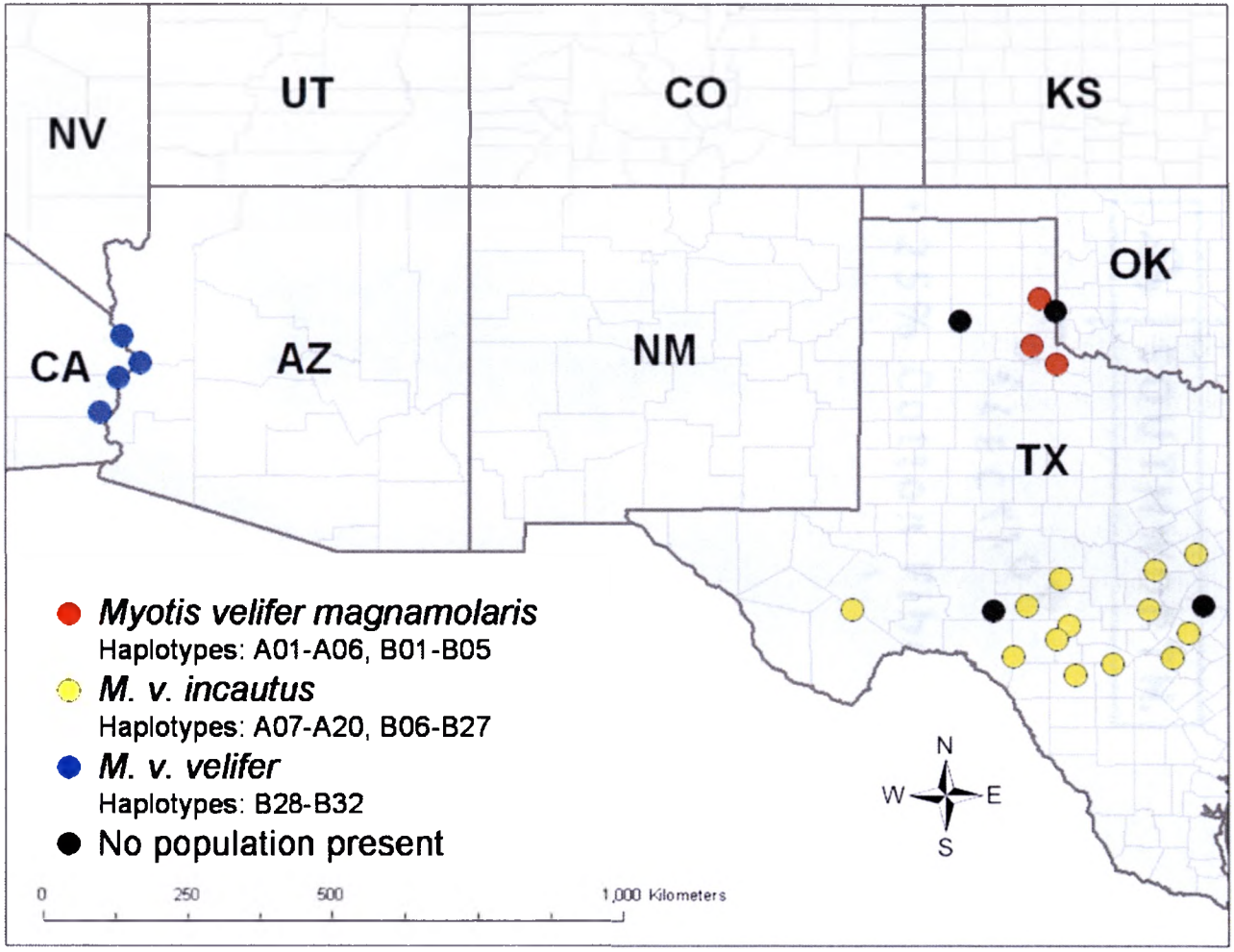


FIGURE 1. Roosts sampled (n = 24) for populations of *Myotis velifer* throughout the United States. Populations historically reported for a location but absent during the 2006 sampling season are indicated by "No population present." Haplotypes recovered from each subspecies are noted. State abbreviations are Arizona (AZ), California (CA), New Mexico (NM), Oklahoma (OK) and Texas (TX).

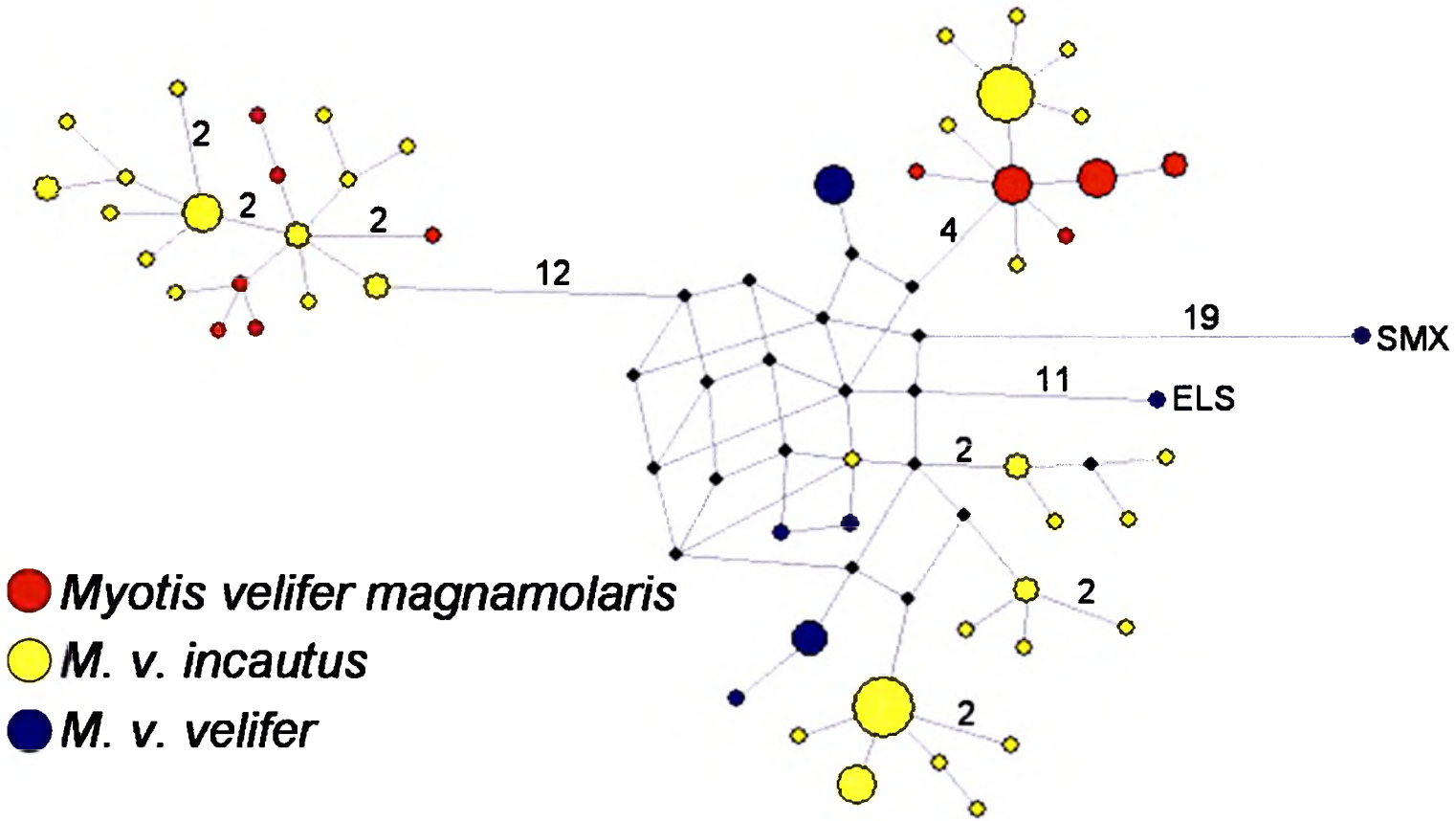


FIGURE 2. Median-joining haplotype network generated by NETWORK (Bandelt et al. 1999). Abbreviations represent individuals from Sonora, Mexico (SMX; GenBank Accession No. AF376870; Ruedi and Mayer 2001) and El Salvador (ELS; The Museum at Texas Tech University; TK34862). Black circles are hypothesized unsampled haplotypes. Circles are proportional to number of individuals (n = 104) which share that haplotype based on 982 base pairs of the mitochondrial *Cytochrome b* gene. All lines represent one mutation unless otherwise specified by a number above. For number of individuals sequenced from each taxonomic subdivision refer to Table 4.

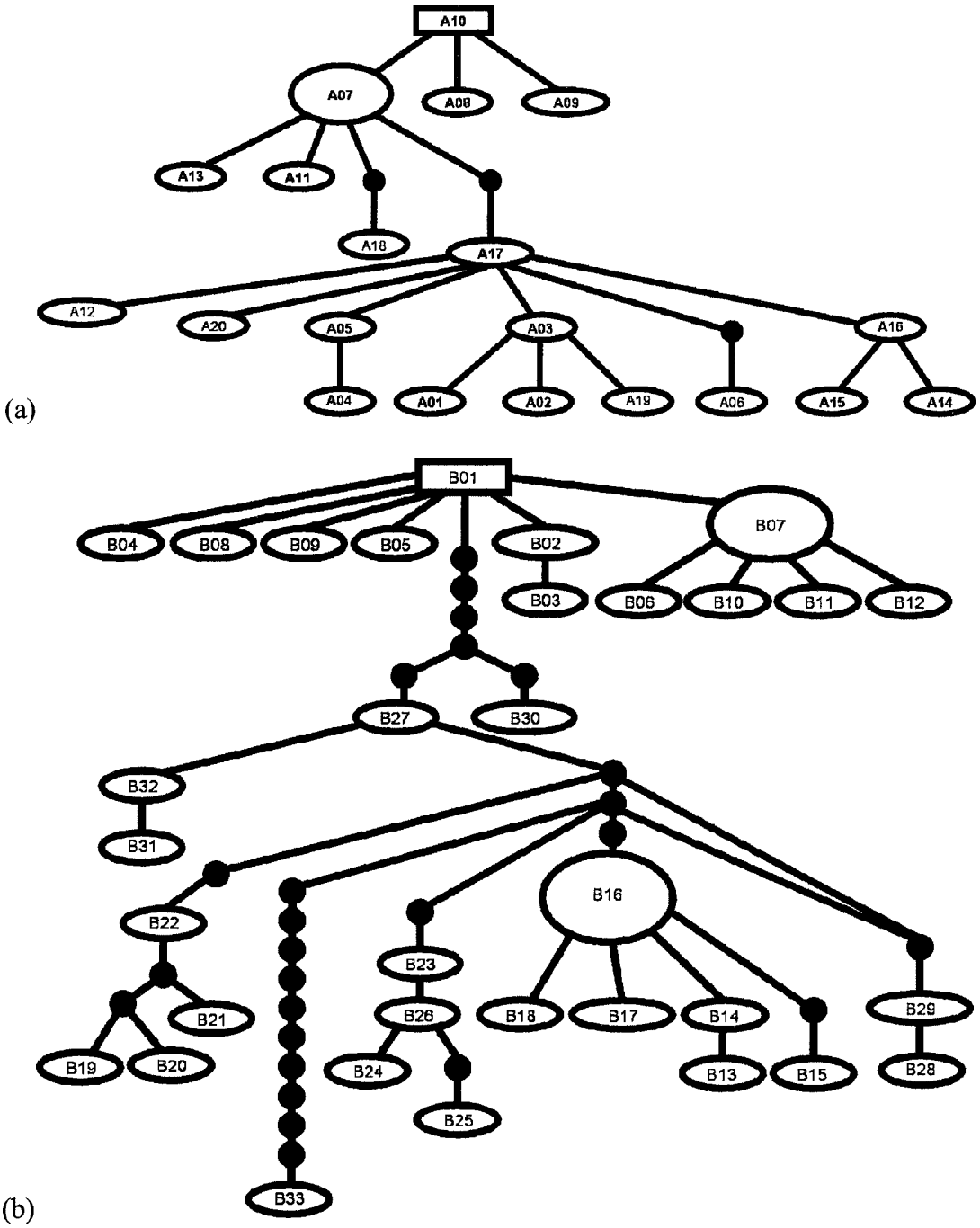


FIGURE 3. Maximum parsimony haplotype networks generated by TCS (Clement et al. 2000). Represented recovered haplogroups are haplogroup A (a) and haplogroup B (b). *Myotis velifer* from GenBank (AF376870) is the only individual representing haplogroup C (not shown). Each line represents a single base change. Solid black circles represent hypothesized unsampled haplotypes. Oval size corresponds with number of individuals represented by that haplotype. Rectangles indicate ancestral haplotype as estimated by TCS. Haplotypes are joined with 95% confidence and no more than 13 missing nodes. These haplogroups are recovered from an evaluated 982 base pairs of the mitochondrial *Cytochrome b* gene from 104 individuals of *Myotis velifer*.

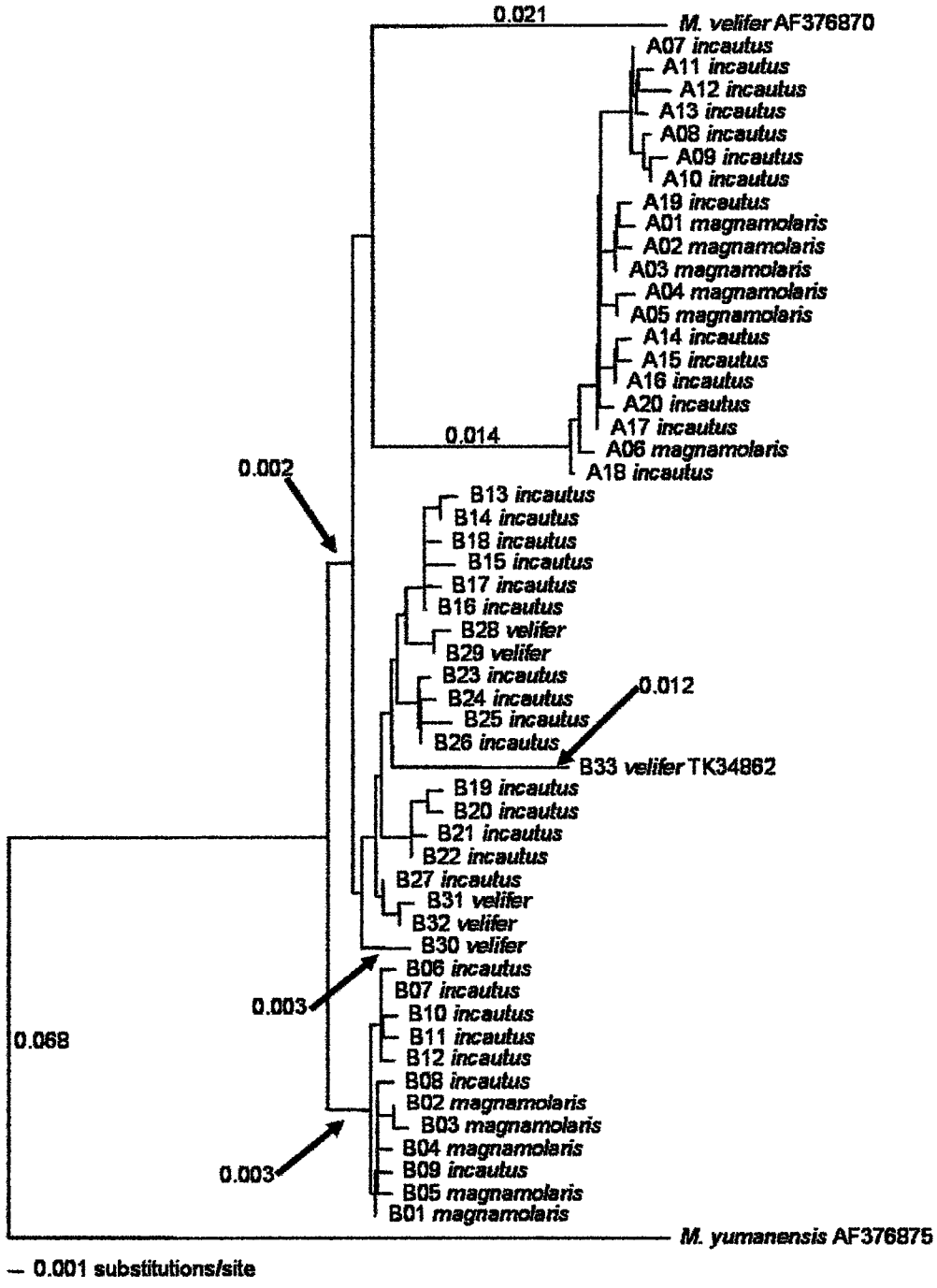


FIGURE 4. Neighbor-joining phylogram generated with the HKY model of evolution. Phylogram generated using PAUP from 982 characters of the mitochondrial *Cytochrome b* gene displaying distances (>0.002 on terminal branches). The outgroup obtained from GenBank is represented by the GenBank accession number after species name. Specimens obtained from The Museum at Texas Tech University are represented by haplotype reference, taxonomic subdivision and museum number. Abbreviation is *M.* for *Myotis*. Individuals collected by the author are represented by haplotype reference and taxonomic subdivision within *Myotis velifer* (*magnamolaris*, *incautus* or *velifer*).

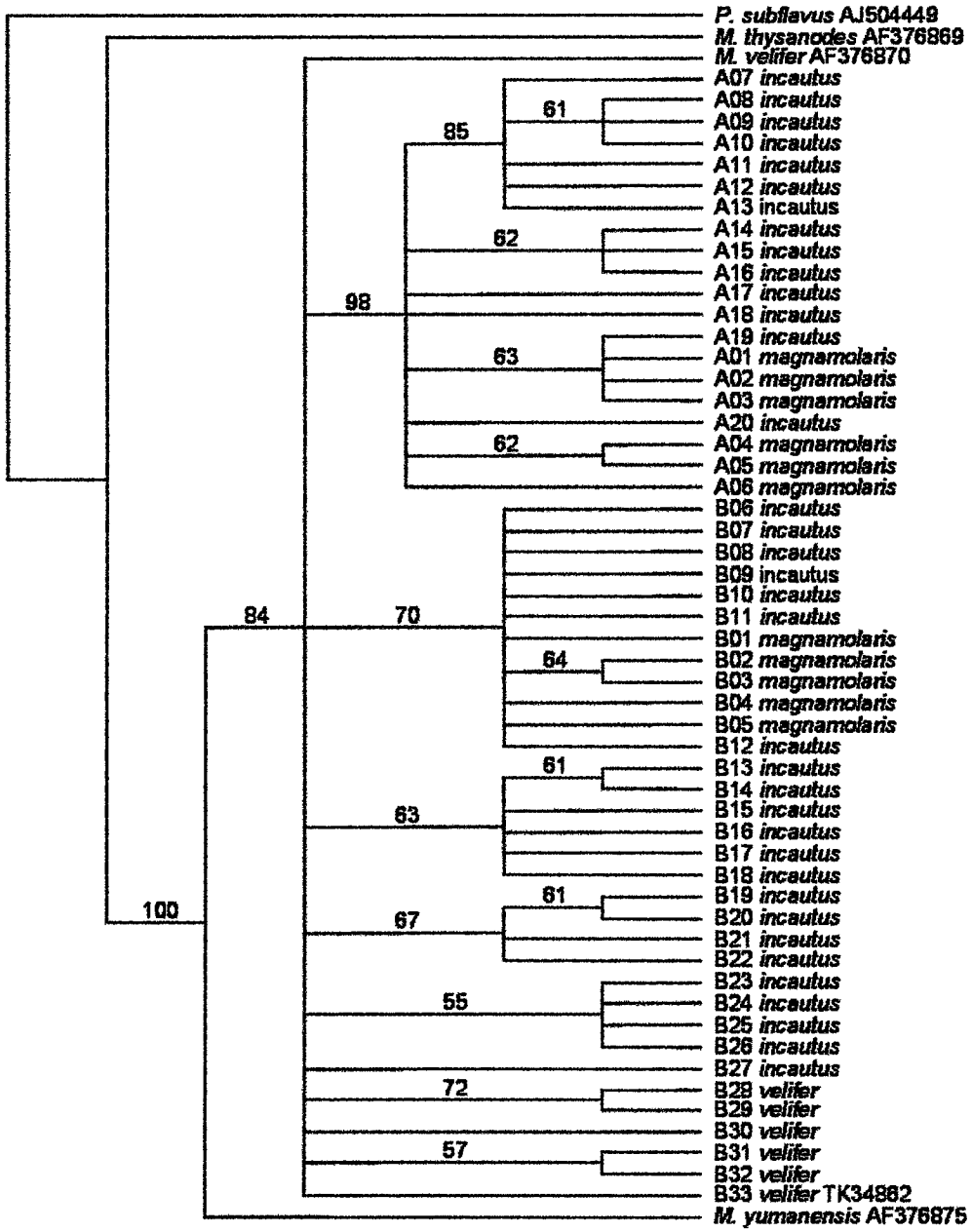


FIGURE 5. Maximum parsimony phylogeny. Phylogeny (treelength = 406) generated using PAUP from 982 characters of the mitochondrial *Cytochrome b* gene, of which 120 were parsimony informative. Values above lines represent bootstrap values from 2,000 heuristic search replicates. Outgroups obtained from GenBank are represented by GenBank accession number after species name. Specimens obtained from The Museum at Texas Tech University are represented by haplotype reference, taxonomic subdivision and museum number. Abbreviations are *P.* for *Pipistrellus* and *M.* for *Myotis*. Individuals collected by the author are represented by haplotype reference and taxonomic subdivision within *Myotis velifer* (*magnamolaris*, *incautus* or *velifer*).

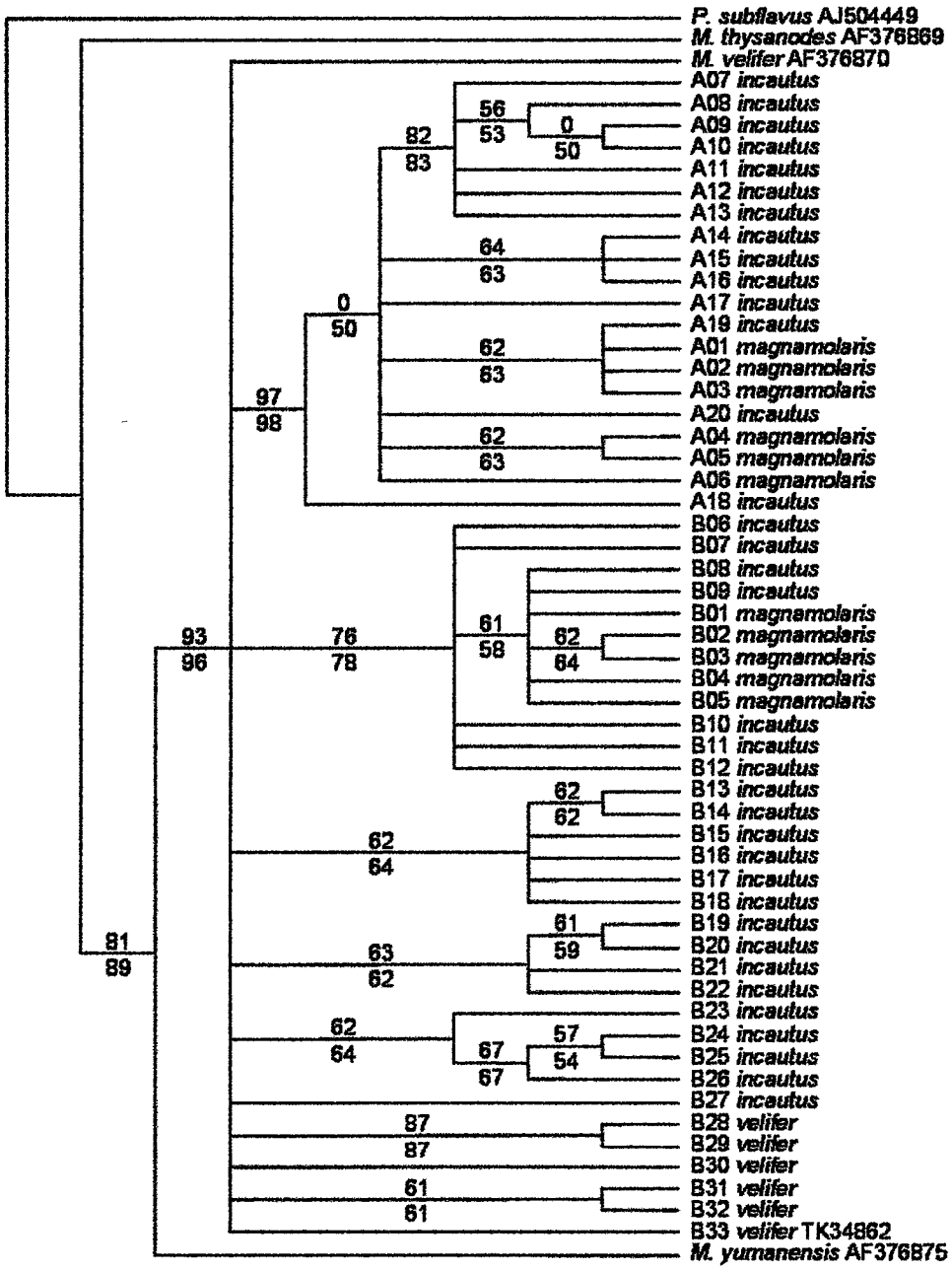


FIGURE 6. Bootstrapped neighbor-joining phylogeny. Phylogeny generated using PAUP from 982 characters of the mitochondrial *Cytochrome b* gene. Bootstrap values are obtained from 2,000 neighbor-joining replicates. Values above and below the lines represent the HKY + I (AIC; MRMODELTEST) and TrN + I (AIC; Modeltest) models of evolution, respectively. Outgroups obtained from GenBank are represented by GenBank accession number after species name. Specimens obtained from The Museum at Texas Tech University are represented by haplotype reference, taxonomic subdivision and museum number. Abbreviations are *P.* for *Pipistrellus* and *M.* for *Myotis*. Individuals collected by the author are represented by haplotype reference and taxonomic subdivision within *Myotis velifer* (*magnamolaris*, *incautus* or *velifer*).

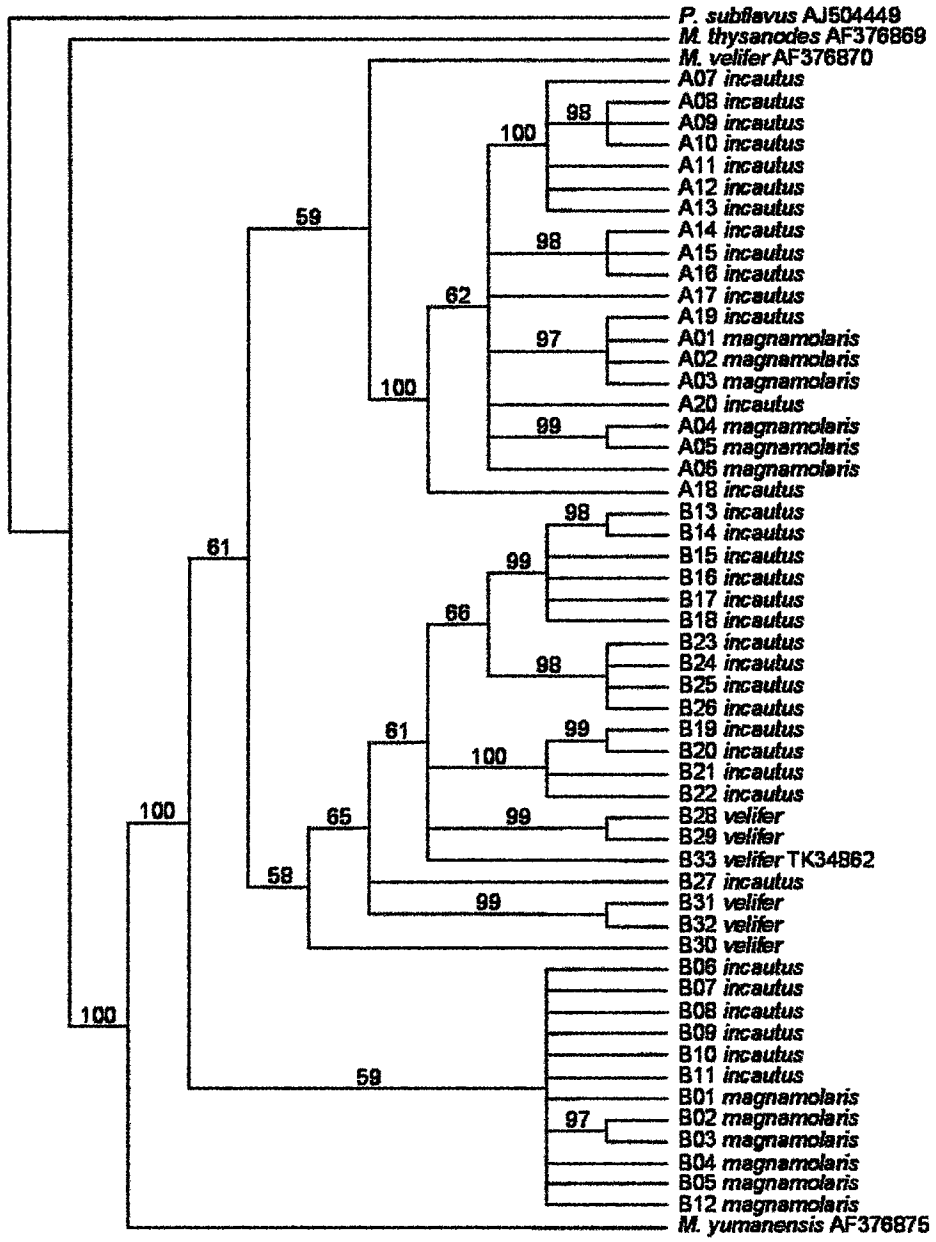


FIGURE 7. Majority rule consensus of codon partitioned Bayesian analysis. Phylogeny generated using MRBAYES from 982 base pairs of the mitochondrial *Cytochrome b* gene. Values above lines are majority rule consensus values. Codon position models of evolution were estimated by using MRMODELTEST and were the same for both hLRT and AIC. Models for codon positions are: 1st: K80; 2nd: F81; 3rd: GTR + G. Outgroups obtained from GenBank are represented by GenBank accession number after species name. Specimens obtained from The Museum at Texas Tech University are represented by haplotype reference, taxonomic subdivision and museum number. Abbreviations are *P.* for *Pipistrellus* and *M.* for *Myotis*. Individuals collected by the author are represented by haplotype reference and taxonomic subdivision within *Myotis velifer* (*magnamolaris*, *incautus* or *velifer*).

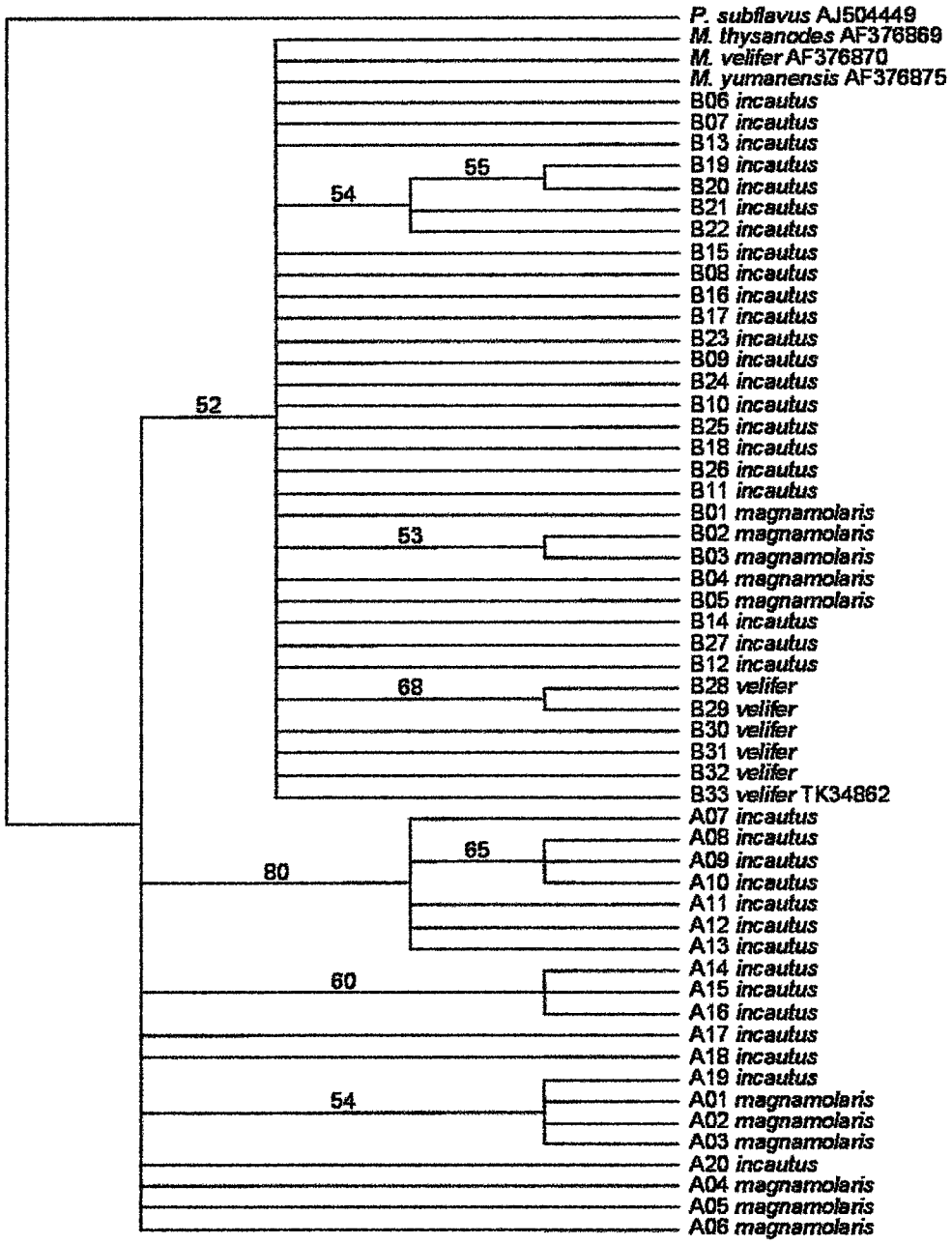


FIGURE 8. Bootstrapped maximum likelihood phylogeny generated with the HKY + I model of evolution. Phylogeny (score = -ln 3289.67790) evaluated 982 characters of the mitochondrial *Cytochrome b* gene and generated using PAUP. Bootstrap values are from 100 heuristic replicates utilizing HKY + I (AIC) model of evolution estimated by MRMODELTEST. Outgroups obtained from GenBank are represented by GenBank accession numbers after species names. Specimens obtained from The Museum at Texas Tech University are represented by their taxonomic subdivision and museum number. Abbreviations are *P.* for *Pipistrellus* and *M.* for *Myotis*. Individuals collected by the author are represented by taxonomic subdivision within *Myotis velifer* (*magnamolaris*, *incautus* or *velifer*).

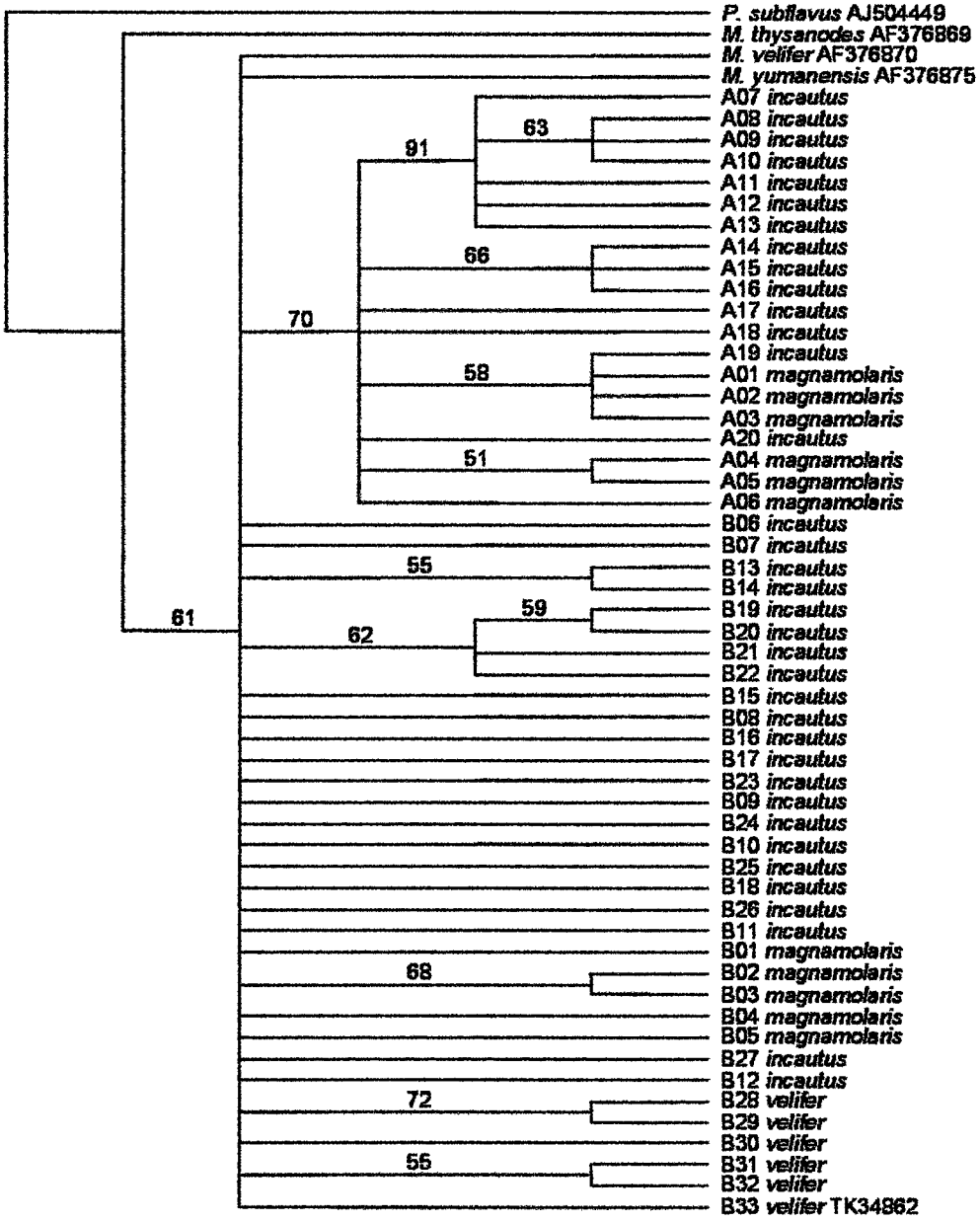


FIGURE 9. Bootstrapped maximum likelihood phylogeny generated using the TrN + I model of evolution. Phylogeny (score = -ln 3867.42270) evaluated 982 characters of the mitochondrial *Cytochrome b* gene and generated using PAUP. Bootstrap values are from 100 heuristic replicates utilizing TrN + I (AIC) model of evolution estimated by MODELTEST. Outgroups obtained from GenBank are represented by GenBank accession numbers after species names. Specimens obtained from The Museum at Texas Tech University are represented by their taxonomic subdivision and museum number. Abbreviations are *P.* for *Pipistrellus* and *M.* for *Myotis*. Individuals collected by the author are represented by taxonomic subdivision within *Myotis velifer* (*magnamolaris*, *incautus* or *velifer*).

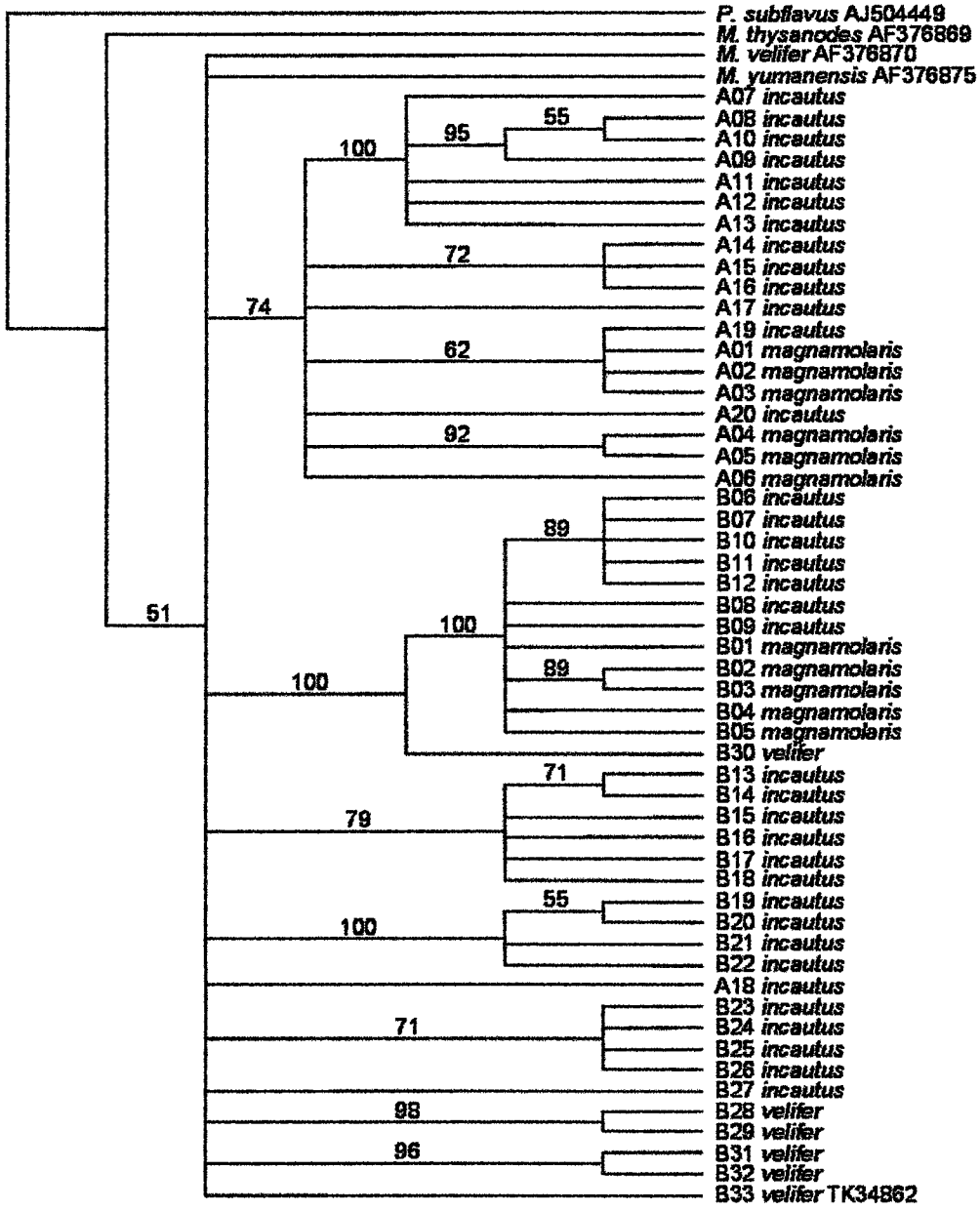


FIGURE 10. Majority rule consensus of 176 phylogenies generated from CIPRES using the ML GTR + G option with GARLI parameters similar to the HKY + G model of evolution. Parameters are as follows: ratematrix = 2, statefrequencies = empirical, and invariantsites = none. Phylogeny is the evaluation of 982 characters from the mitochondrial *Cytochrome b* gene. Sequences obtained from GenBank are represented by GenBank accession numbers after species names. Specimens obtained from The Museum at Texas Tech University are represented by their taxonomic subdivision and museum number. Abbreviations are *P.* for *Pipistrellus* and *M.* for *Myotis*. Individuals collected by the author are represented by taxonomic subdivision within *Myotis velifer* (*magnamolaris*, *incautus* or *velifer*).

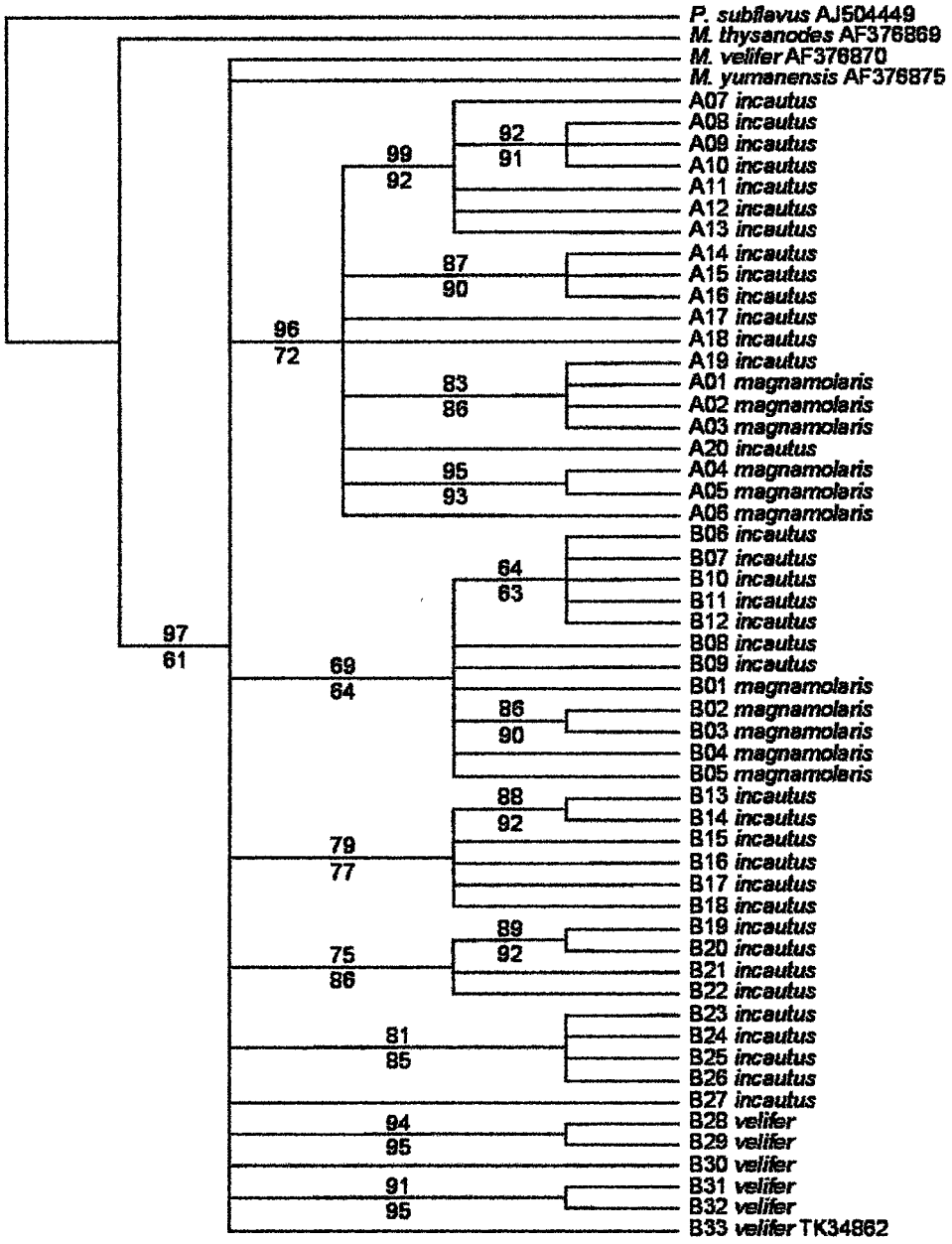


FIGURE 11. Majority rule consensus of Bayesian analysis generated with the HKY + G and HKY + I models of evolution. Phylogeny evaluated 982 base pairs of the mitochondrial *Cytochrome b* gene using MRBAYES. Values above and below lines are estimated from the HKY + G (hLRT) and HKY + I (AIC) models of evolution, respectively, estimated by MRMODELTEST. Outgroups obtained from GenBank are represented by GenBank accession number after species name. Specimens obtained from The Museum at Texas Tech University are represented by haplotype reference, taxonomic subdivision and museum number. Abbreviations are *P.* for *Pipistrellus* and *M.* for *Myotis*. Individuals collected by the author are represented by haplotype reference and taxonomic subdivision within *Myotis velifer* (*magnamolaris*, *incautus* or *velifer*).

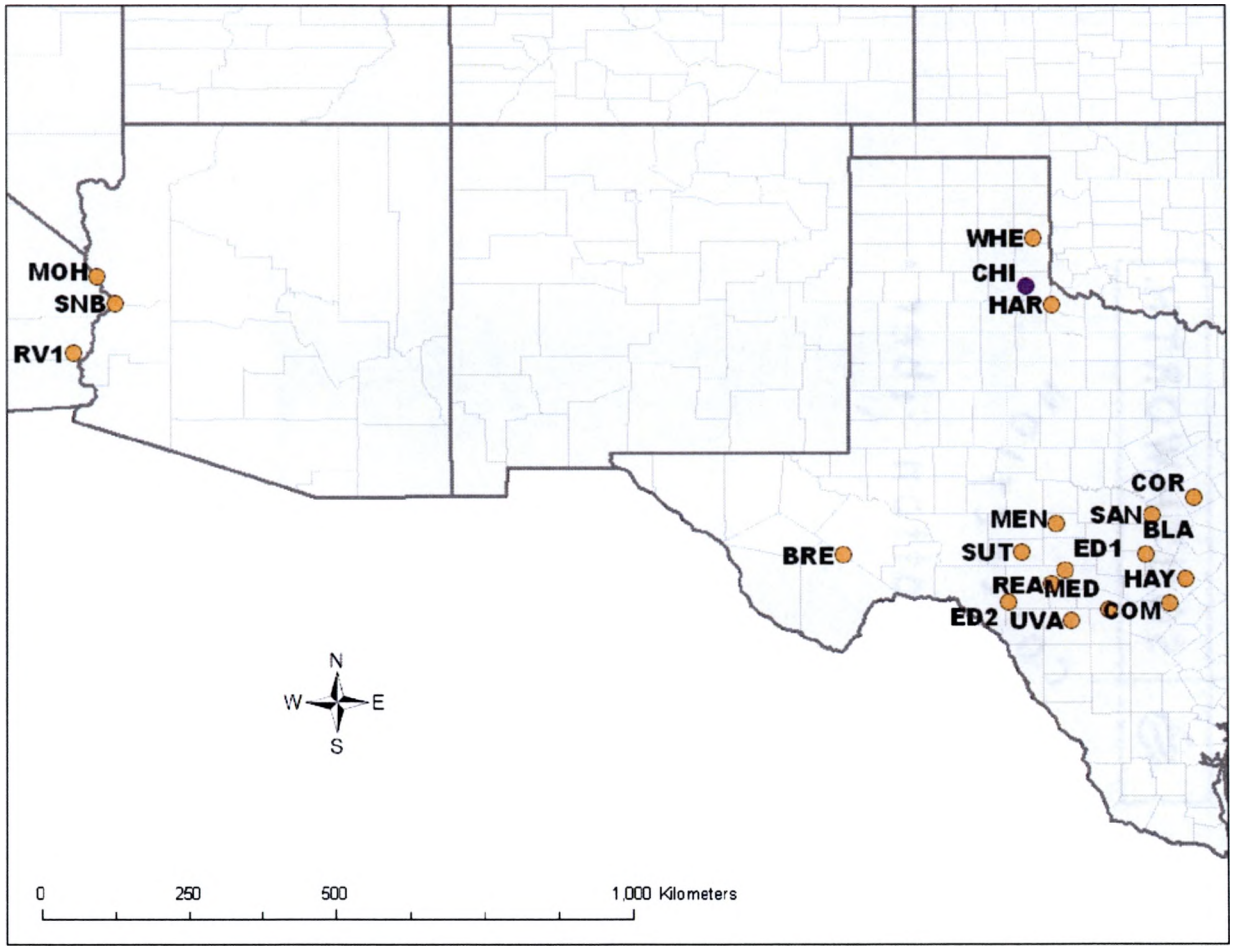
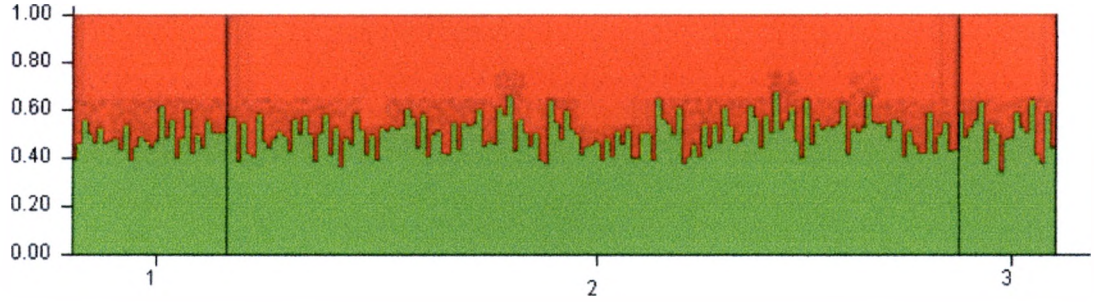


FIGURE 12. Map of SAMOVA results. Partitioning of mitochondrial data when the number of hypothesized populations (K) is two is represented by differing colors (purple and orange). A total of 102 individuals of *Myotis velifer* were evaluated with the first 982 bp of the *Cytochrome b* gene. For roost abbreviations refer to Table 1. For number of individuals sequenced from each population refer to Table 4.

(a)



(b)

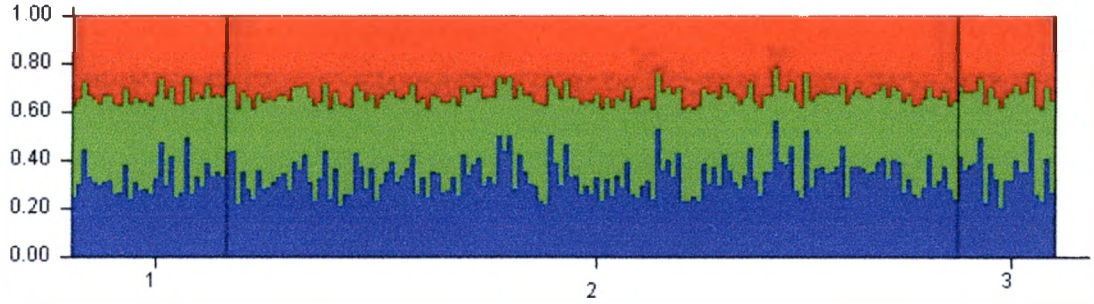


FIGURE 13. Barplots of STRUCTURE 2.2 results. Barplots are of (a) two and (b) three hypothetical populations and were generated from 192 individuals sampled within the United States using four autosomal loci with no missing data. Results of females only with five loci are similar (see Table 14). Numbers along the Y-axis refer to the probability of an individual being assigned to that population. Numbers along X-axis refer to taxonomic subdivisions among *Myotis velifer* according to Hayward (1970). Taxonomic subdivisions are: 1 = *M. v. magnamolaris*, 2 = *M. v. incautus*, and 3 = *M. v. velifer*.

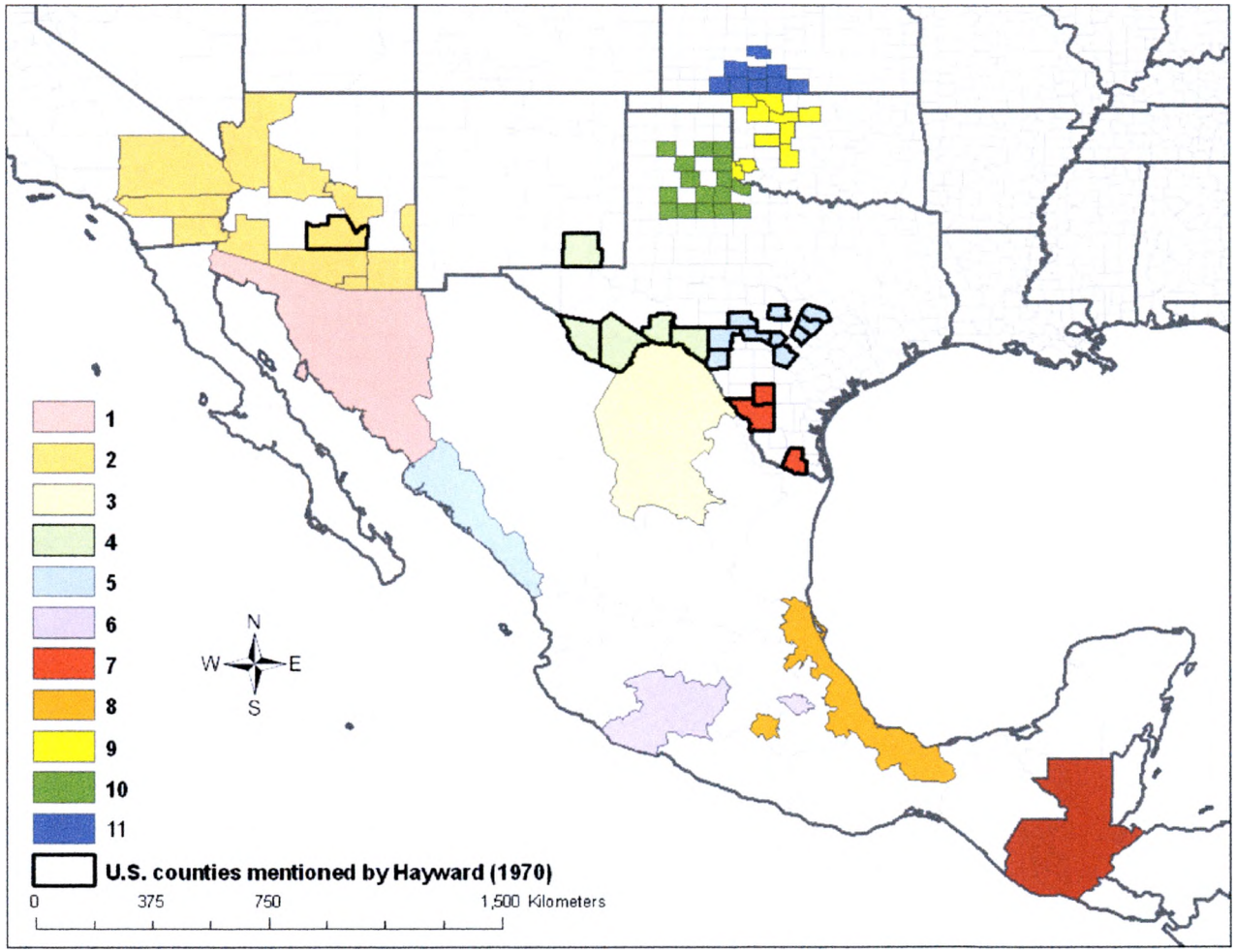


FIGURE 14. Distribution of the overall average of the ranked morphological characters. Morphological characters of *Myotis velifer* were evaluated by Hayward (1970). For calculation of ranked values, refer to Table 3. When sampling localities of United States counties were not mentioned by Hayward (1970), the online database MANIS (accessed December 14, 2007) was used to determine the current distribution for that region.

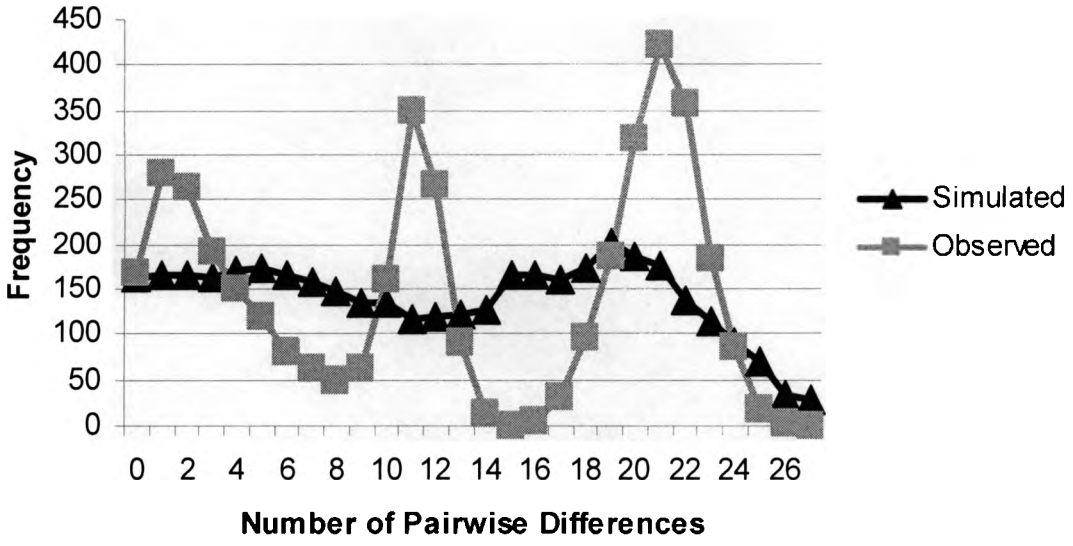


FIGURE 15. Multimodal mismatch distribution. Mismatch distribution generated from 982 base pairs of the mitochondrial *Cytochrome b* gene. Analyzed data represents all *Myotis velifer* specimens obtained within the Texas. Values of sum of squares (SSD $P = 0.25$) and the Harpending's raggedness index (HRI = 0.013, $P = 0.25$) were also calculated with ARLEQUIN 3.01.

LITERATURE CITED

ALLEN, J. A. 1890. Notes on collections of mammals made in central and southern Mexico by Dr. Audley C. Buller, with descriptions of new species of the genera *Vespertilio*, *Sciurus*, and *Lepus*. Bulletin American Museum of Natural History 3:175-194.

ALLEN, J. A. 1896. Descriptions of new North American mammals. Bulletin American Museum of Natural History 8:233-240.

ANTHONY, E. L. P. 1988. Age determination in bats. Pp. 47-58 in Ecological and Behavioral Methods for the Study of Bats (T. H. Kunz, ed.). Smithsonian Institute Press, Washington, D. C.

ARDLIE, K. G., L. KRUG;YAK, AND M. SEIELSTAD. 2002. Patterns of linkage disequilibrium in the human genome. Nature Reviews Genetics 3:299-309.

AVILA-FLORES, R., AND R. A. MEDELLÍN. 2004. Ecological, taxonomic, and physiological correlates of cave use by Mexican bats. Journal of Mammalogy 85:675-687.

AVISE, J. C. 1994. Molecular markers, natural history and evolution. Chapman and Hall, New York, New York.

AVISE, J. C. 2000. Phylogeography: the history and formation of species. Harvard University Press, Cambridge, Massachusetts.BALLOUX, F., AND J. GOUDET. 2002. Statistical properties of population differentiation estimators under stepwise mutation in a finite island model. Molecular Ecology 11:771-783.

BANDELT, H. J., P. FORSTER, AND A. ROHL. 1999. Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution 16:37-48.

BARBOUR, R. W., AND W. H. DAVIS. 1969. Bats of America. The University Press of Kentucky, Lexington, Kentucky.

BILGIN, I. R. 2006.The phylogeography and population genetics of three cave-dwelling bat species in southeastern Europe and Anatolia. Dissertation, Columbia University, New York, New York.

BRANDLEY, M. C., A. SCHMITZ, AND T. W. REEDER. 2005. Partitioned Bayesian analyses, partition choice, and the phylogenetic relationships of scincid lizards. Systematic Biology 54:373-390.

BURLAND, T. M., E. M. BARRATT, AND P. A. RACEY. 1998. Isolation and characterization of microsatellite loci in the brown long-eared bat, *Plecotus auritus*, and cross-species amplification within the family Vespertilionidae. Molecular Ecology 7:136-138.

BURLAND, T. M., E. M. BARRATT, M. A. BEAUMONT, AND P. A. RACEY. 1999. Population genetic structure and gene flow in a gleaning bat, *Plecotus auritus*. Proceedings of the Royal Society of London, B. Biological Sciences 266:975-980.

BURLAND, T. M., AND J. WORTHINGTON-WILMER. 2001. Seeing in the dark: molecular approaches to the study of bat populations. Biology Review 76:389-409.

CAMPBELL, P., C. J. SCHNEIDER, A. M. ADNAN, A. ZUBAID, AND T. H. KUNZ. 2007. Contrasting patterns of genetic differentiation between endemic and widespread species of fruit bats (Chiroptera: Pteropodidae) in Sulawesi, Indonesia. Molecular Phylogenetics and Evolution 44:474-482.

CASSENS, I., P. MARDULYN, AND M. C. MILINKOVITCH. 2005. Evaluating intraspecific "Network" construction methods using simulated sequence data: Do existing algorithms outperform the global maximum parsimony approach? Systematic Biology 54:363-372.

CASTELLA, V., AND M. RUEDI. 2000. Characterization of highly variable microsatellite loci in the bat *Myotis myotis* (Chiroptera: Vespertilionidae). Molecular Ecology 9:1000-1002.

CASTELLA, V., M. RUEDI, AND L. EXCOFFIER. 2001. Contrasted patterns of mitochondrial and nuclear structure among nursery colonies of the bat *Myotis myotis*. Journal of Evolutionary Biology 14:708-720.

CASTELLA, V., M. RUEDI, L. EXCOFFIER C. IBANEZ, R. ARLETTAZ, AND J. HAUSSER. 2000. Is the Gibraltar Strait a barrier to gene flow for the bat *Myotis myotis* (Chiroptera: Vespertilionidae)? Molecular Ecology 9:1761-1772.

CHAPUIS, M. P., AND A. ESTOUP. 2007. Microsatellite null alleles and estimation of population differentiation. Molecular Biology and Evolution 24:621-631.

CLEMENT, M., D. POSADA, AND K. A. CRANDALL. 2000. TCS: a computer program to estimate gene genealogies. Molecular Ecology 9:1657-1659.

COCKRUM, E. L. 1969. Migration in the guano bat, *Tadarida brasiliensis*. Miscellaneous Publications, Museum of Natural History, University of Kansas 51:303-336.

COLTMAN, D. W., G. STENSON, M. O. HAMMILL, T. HAUG, S. DAVIS, AND T. L. FULTON. 2007. Panmictic population structure in the hooded seal (*Cystophora cristata*). Molecular Ecology 16:1639-1648.

CRANDALL, K. A., O. R. P. BININDA-EMONDS, G. M. MACE, AND R. K. WAYNE. 2000. Considering evolutionary processes in conservation biology. Trends in Ecology and Evolution 15:290-295.

DAKIN, E. E., AND J. C. AVISE. 2004. Microsatellite null alleles in parentage analysis. Heredity 93:504-509.

DALQUEST, W. W., AND F. B. STANGL, JR. 1984. The taxonomic status of *Myotis magnamolaris*, Choate and Hall. Journal of Mammalogy 65:485-486.

DUPANLOUP, I., S. SCHNEIDER, AND L. EXCOFFIER. 2002. A simulated annealing approach to define the genetic structure of populations. Molecular Ecology 11:2571-2581.

ELLEGREN, H. 2000. Heterogenous mutation processes in human microsatellite DNA sequences. Nature Genetics 24:400-402.

EWENS, W. 1972. The sampling theory of selectively neutral alleles. Theoretical Population Biology 3:87-112.

EXCOFFIER, L., G. LAVAL, AND S. SCHNEIDER. 2005. ARLEQUIN version 3.01: An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online 1:47-50.

FALUSH, D., M. STEPHENS, AND J. K. PRITCHARD. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 164:1567-1587.

FALUSH, D., M. STEPHENS, AND J. K. PRITCHARD. 2007. Inference of population structure using multilocus genotype data: dominant markers and null alleles. Molecular Ecology Notes 7:574-578.

FITCH, J. H., K. A. SHUMP, JR., AND A. U. SHUMP. 1981. *Myotis velifer*. Mammalian Species. American Society of Mammalogy 149:1-5.

FRASER, D. J., AND L. BERNATCHEZ. 2001. Adaptive evolutionary conservation: towards a unified concept for defining conservation units. Molecular Ecology 10:2741-2752.

FU, Y. X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147:915-925.

FU, Y. X., AND W. H. LI. 1993. Statistical tests of neutrality of mutations. Genetics 133:693-709.

GAUT, B. S., AND P. O. LEWIS. 1995. Success of maximum likelihood phylogeny inference in the four-taxon case. Molecular Biology and Evolution 12:152-162.

GOLDMAN, N., AND Z. YANG. 1994. A codon-based model of nucleotide substitution for protein-coding DNA sequences. Molecular Biology and Evolution 11:725-736.

GUO, S. W., AND E. A. THOMPSON. 1992. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics 48:361-372.

HALL, E. R. 1981. The Mammals of North America. Vol. I, Second ed. John Wiley & Sons, New York, New York.

HARPENDING, H. C., S. T. SHERRY, A. R. ROGERS, AND M. STONEKING. 1993. The genetic structure of ancient human populations. Current Anthropology 34:483-496.

HARTL, D. L., AND A. G. CLARK. 2007. Principles of population genetics. Fourth ed. Sinauer Associates, Inc., Sunderland, Massachusetts.

HAYWARD, B. J. 1970. The natural history of the cave bat, *Myotis velifer*. Western New Mexico Research in Science 1:1-74.

HEDGECOCK, D., L. GANG, S. HUBERT, K. BUCKLIN, AND V. RIBES. 2004. Widespread null alleles and poor cross-species amplification of microsatellite DNA loci cloned from the Pacific oyster, *Crassostrea gigas*. Journal of Shellfish Research 23:379-385.

HISHEH, S., R. A. HOW, A. SUYANTO, AND L. H. SCHMITT. 2004. Implications of contrasting patterns of genetic variability in two vespertilionid bats from the Indonesian archipelago. Biological Journal of the Linnean Society 83:421-431.

HOLLAND, B. R., D. PENNY, AND M. D. HENDY. 2003. Outgroup misplacement and phylogenetic inaccuracy under a molecular clock - a simulation study. Systematic Biology 52:229-238.

HOOFER, S. R., AND R. A. VAN DEN BUSSCHE. 2003. Molecular phylogenetics of the chiropteran family Vespertilionidae. Acta Chiropterologica 5:1-59.

JOHNSON, J. A., P. O. DUNN, AND J. L. BOUZAT. 2007. Effects of recent population bottlenecks on reconstructing the demographic history of prairie-chickens. Molecular Ecology 16:2203-2222.

KUHNER, M. K., AND J. FELSENSTEIN. 1994. A simulation comparison of phylogeny algorithms under equal and unequal evolutionary rates. Molecular Biology and Evolution 11:459-468.

KUHNER, M. K., J. YAMATO, AND J. FELSENSTEIN. 1998. Maximum likelihood estimation of population growth rates based on the coalescent. Genetics 149:429-434.

KUNZ, T. H. 1973. Population studies of the cave bat (*Myotis velifer*): reproduction, growth, and development. Occasional Papers of the University of Kansas Museum of Natural History 15:1-43.

KUNZ, T. H., AND A. KURTA. 1988. Capture methods and holding devices. Pp. 1-29 in Ecological and Behavioral Methods for the Study of Bats (T. H. Kunz, ed.). Smithsonian Institute Press, Washington, D. C.

LUGON-MOULIN, N., H. BRUNNER, A. WYTTENBACH, J. HAUSSER, AND J. GOUDET. 1999. Hierarchical analyses of genetic differentiation in a hybrid zone of *Sorex araneus* (Insectivora: Soricidae). Molecular Ecology 8:419-431.

MADISON, D. R., AND W. P. MADISON. 2002. MacClade 4: analysis of phylogeny and character evolution. Sinauer Associates, Sunderland, Massachusetts.

MARSHALL, T. C., J. SLATE, L. E. B. KRUUK, AND J. M. PEMBERTON. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. Molecular Ecology 7:639-655.

MILLER, G. S., JR. 1897. Revision of the North American bats of the family Vespertilionidae. North American Fauna 13:1-140.

MILLER-BUTTERWORTH, C. M., D. S. JACOBS, AND E. H. HARLEY. 2003. Strong population substructure is correlated with morphology and ecology in a migratory bat. Nature 424:187-191.

MILLER-BUTTERWORTH, C. M., D. S. JACOBS, AND E. H. HARLEY. 2002. Isolation and characterization of highly polymorphic microsatellite loci in Schreibers' long-fingered bat, *Miniopterus schreibersii* (Chiroptera: Vespertilionidae). Molecular Ecology Notes 2:139-141.

NYLANDER, J. A. A. 2004. MrModeltest Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

PEKINS, C. E. 2006. Cave myotis bat population and cave microclimate monitoring at a maternity site on Fort Hood, Texas during 2005-2006 in Endangered Species Monitoring and Management at Fort Hood, Texas: 2006 Annual Report. The Nature Conservancy, Forth Hood, Texas.

PEKINS, C. E. 2007a. Cave myotis bat population and cave microclimate monitoring at a maternity site on Fort Hood, Texas during 2007 in Endangered Species Monitoring and Management at Fort Hood, Texas: 2007 Annual Report. The Nature Conservancy, Fort Hood, Texas.

PEKINS, C. E. 2007b. Cave myotis bat roost switch following prolonged torrential rainfall on Fort Hood, Texas in Endangered Species Monitoring and Management at Fort Hood, Texas: 2007 Annual Report. The Nature Conservancy, Fort Hood, Texas.

PETIT, E., AND F. MAYER. 1999. Male dispersal in the noctule bat (*Nyctalus noctula*): where are the limits? Proceedings of the Royal Society of London, B. Biological Sciences 266:1717-1722.

POSADA, D., AND K. A. CRANDALL. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14:817-818.

RACEY, P. A. 1979. The prolonged storage and survival of spermatozoa in Chiroptera. Journal of Reproduction and Fertility 56:391-401.

RACEY, P. A. 1988. Reproductive assessment in bats. Pp. 31-46 in Ecological and Behavioral Methods for the Study of Bats (T. H. Kunz, ed.). Smithsonian Institute Press, Washington, D. C.

RACEY, P. A., E. M. BARRATT, T. M. BURLAND, R. DEAVILLE, D. GOTELLI, AND S. B. PIERTNEY. 2007. Microsatellite DNA polymorphism confirms reproductive isolation and reveals differences in population genetic structure of cryptic pipistrelle bat species. Biological Journal of the Linnean Society 90:539-550.

RAMOS-ONSINS, S. E., AND J. ROZAS. 2002. Statistical properties of new neutrality tests against population growth. Molecular Biology and Evolution 19:2092-2100.

RAY, N., M. CURRAT, AND L. EXCOFFIER. 2003. Intra-deme molecular diversity in spatially expanding populations. Molecular Biology and Evolution 20:76-86.

RAYMOND, M., AND F. ROUSSET. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity 86:248-249.

RENWICK, A., L. DAVISON, H. SPRATT, J. P. KING, AND M. KIMMEL. 2001. DNA dinucleotide evolution in humans: fitting theory to facts. Genetics 159:737-747.

RICE, W. R. 1989. Analyzing tables of statistical tests. Evolution 43:223-225.

RIVERS, N. M., R. K. BUTLIN, AND J. D. ALTRINGHAM. 2005. Genetic population structure of Natterer's bats explained by mating at swarming sites and philopatry. Molecular Ecology 14:4299-4312.

ROGERS, A. R. 1995. Genetic evidence for a Pleistocene population explosion. Evolution 49:608-615.

ROGERS, A. R., AND H. HARPENDING. 1992. Population growth makes waves in the distribution of pairwise genetic differences. Molecular Biology and Evolution 9:552-569.

ROSSITER, S. J., G. JONES, R. D. RANSOME, AND E. M. BARRATT. 2000. Genetic variation and population structure in the endangered greater horseshoe bat *Rhinolophus ferrumequinum*. Molecular Ecology 9:1131-1135.

ROUSSET, F. 1996. Equilibrium values of measures of population subdivision for stepwise mutation processes. Genetics 142:1357-1362.

ROZAS, J., AND R. ROZAS. 1997. DnaSP version 2.0: A novel software package for extensive molecular population genetics analysis. Computer Applications in the Biosciences 13:307-311.

RUEDI, M., AND V. CASTELLA. 2003. Genetic consequences of the ice ages on nurseries of the bat *Myotis myotis*: a mitochondrial and nuclear survey. Molecular Ecology 12:1527-1540.

RUEDI, M., AND F. MAYER. 2001. Molecular systematics of bats of the genus *Myotis* (Vespertilionidae) suggests deterministic ecomorphological convergences. Molecular Phylogenetics and Evolution 21:436-448.

RUSSELL, A. L. 2003. Population genetics and effective population size of the Brazilian free-tailed bat, *Tadarida brasiliensis* (Chiroptera: Molossidae). Dissertation, University of Tennessee, Knoxville, Tennessee.

RUSSELL, A. L., AND G. F. MCCRACKEN. 2006. Population genetic structure of very large populations: the Brazilian free-tailed bat (*Tadarida brasiliensis*). Pp. 227-247 in Functional and Evolutionary Ecology of Bats (A. Akbar, G. F. McCracken and T. H. Kunz, eds.). Oxford University Press, New York, New York.

RUSSELL, A. L., R. A. MEDELLÍN, AND G. F. MCCRACKEN. 2005a. Genetic variation and migration in the Mexican free-tailed bat (*Tadarida brasiliensis mexicana*). Molecular Ecology 14:2207-2222.

RUSSELL, A. L., A. S. TURMELLE, V. A. BROWN, AND G. F. MCCRACKEN. 2005b. Extremely variable di- and tetranucleotide microsatellite loci in Brazilian free-tailed bats (*Tadarida brasiliensis*). Molecular Ecology Notes 5:669-671.

RUSSELL, P. J. 2006. iGenetics: a molecular approach. Second ed. Benjamin Cummings Publishing Company, San Francisco, California.

SCHMIDLEY, D. J. 2004. The mammals of Texas. Revised ed. University of Texas Press, Austin, Texas.

SIDDALL, M. E. 1998. Success of parsimony in the four-taxon case: long-branch repulsion by likelihood in the Farris Zone. Cladistics 14:209-220.

SLATKIN, M. 1995. A measure of population subdivision based on microsatellite allele frequencies. Genetics 139:457-462.

SLATKIN, M., AND L. EXCOFFIER. 1996. Testing for linkage disequilibrium in genotypic data using the Expectation-Maximization algorithm. Heredity 76:377-383.

SLATKIN, M., AND R. R. HUDSON. 1991. Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. Genetics 129:555-562.

STADELMANN, B., L. K. LIN, T. H. KUNZ, AND M. RUEDI. 2007. Molecular phylogeny of New World *Myotis* (Chiroptera, Vespertilionidae) inferred from mitochondrial and nuclear DNA genes. Molecular Phylogenetics and Evolution 43:32-48.

STRANEY, D. O., M. H. SMITH, R. J. BAKER, AND I. F. GREENBAUM. 1976. Biochemical variation and genetic similarity of *Myotis velifer* and *Macrotus californicus*. Comparative Biochemistry and Physiology 54B:243-248.

SWOFFORD, D. L. 2002. PAUP*. Phylogenetic analysis using parsimony (*and other methods). Sinauer Associates, Sunderland, Massachusetts.

TAJIMA, F. 1989. The effect of change in population size on DNA polymorphism. Genetics 123:597-601.

TEXAS PARKS AND WILDLIFE DEPARTMENT. 2005. Texas comprehensive wildlife strategy: 2005-2010. Texas Parks and Wildlife Department: 1131.

TINKLE, D. W., AND I. G. PATTERSON. 1965. A study of hibernating populations of *Myotis velifer* in northwestern Texas. Journal of Mammalogy 46:612-633.

TWENTE, J. W., JR. 1955a. Aspects of a population study of cavern-dwelling bats. Journal of Mammalogy 36:379-390.

TWENTE, J. W., JR. 1955b. Habitat selection of cavern-dwelling bats as illustrated by four vespertilionids. Dissertation, University of Michigan, Ann Arbor, Michigan.

TWENTE, J. W., JR. 1955c. Some aspects of habitat selection and other behavior of cavern-dwelling bats. Ecology 36:706-732.

VAN OOSTERHOUT, C., W. F. HUTCHINSON, D. P. M. WILLIS, AND P. SHIPLEY. 2004. Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4:535-538.

VAUGHAN, T. A. 1954. A new subspecies of bat (*Myotis velifer*) from Southeastern California and Arizona. The University of Kansas Publications, Museum of Natural History 7:507-512.

VONHOF, M. J., C. S. DAVIS, M. B. FENTON, AND C. STROBECK. 2002. Characterization of dinucleotide microsatellite loci in big brown bats (*Eptesicus fuscus*), and their use in other North American vespertilionid bats. Molecular Ecology Notes 2:167-169.

WATTERSON, G. 1978. The homozygosity test of neutrality. Genetics 88:405-417.

WEBER, D. S., B. S. STEWART, AND N. LEHMAN. 2004. Genetic consequences of a severe population bottleneck in the Guadalupe fur seal (*Arctocephalus townsendi*). Journal of Heredity 95:144-153.

WEYANDT, S. E., AND R. A. VAN DEN BUSSCHE. 2007. Phylogeographic structuring and volant mammals: the case of the pallid bat (*Antrozous pallidus*). Journal of Biogeography 34:1233-1245.

WEYANDT, S. E., R. A. VAN DEN BUSSCHE, M. J. HAMILTON, AND D. M. LESLIE, JR. 2005. Unraveling the effects of sex and dispersal: Ozark big-eared bat (*Corynorhinus townsendii ingens*) conservation genetics. Journal of Mammalogy 86:1136-1143.

WILKINSON, G. S., AND T. H. FLEMING. 1996. Migration and evolution of lesser long-nosed bats *Leptonycteris curasoae*, inferred from mitochondrial DNA. Molecular Ecology 5:329-339.

WORTHINGTON-WILMER, J., AND E. BARRATT. 1996. A non-lethal method of tissue sampling for genetic studies of chiropterans. Bat Research News 37:1-3.

XU, X., M. PENG, Z. FANG, AND X. XU. 2000. The direction of microsatellite mutations is dependent upon allele length. Nature Genetics 24:396-399.

VITA

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