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Evolutionary history of Caribbean species of *Myotis*, with evidence of a third Lesser Antillean endemic

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Abstract

Currently, four species of *Myotis* are known from the islands of the Caribbean (*Myotis dominicensis*, *M. martiniquensis*, *M. nesopolus*, and *M. nigricans*). *Myotis dominicensis* and *M. martiniquensis* are endemic to the Lesser Antilles, whereas *M. nesopolus* and *M. nigricans* are considered conspecific with mainland populations. Recent phylogenetic and phylogeographic studies provided hypotheses regarding the origin and diversification of *M. dominicensis* and *M. martiniquensis*. However, these studies focused primarily on convergent morphology or distribution patterns of this genus and not on the evolutionary history of Caribbean *Myotis*. Here, we explore variation across multiple datasets generated from Caribbean *Myotis*. We present morphologic and genetic (mitochondrial and nuclear) data from an extensive sample of Caribbean *Myotis* species, including the previously unsampled taxa *M. martiniquensis nyctor* and *M. nesopolus*. Our data indicate that the historically recognized subspecies *M. m. nyctor* is genetically and morphologically distinct from *M. martiniquensis*, warranting recognition of a third Caribbean endemic—*Myotis nyctor*. Moreover, we provide evidence of unrecognized species-level variation in Caribbean and northern South American populations of *Myotis*.

Keywords: *Myotis dominicensis*, *Myotis martiniquensis*, *Myotis nesopolus*, *Myotis nyctor*, AFLPs, Lesser Antilles

Introduction

Of the thirty-eight species of *Myotis* distributed throughout the Neotropics (Simmons 2005), four are from Caribbean islands—*M. dominicensis* Miller 1902, *M. martiniquensis* LaVal, 1973, *M. nesopolus* Miller, 1900, and *M. nigricans* (Schinz, 1821). *Myotis dominicensis* (Dominica, Guadeloupe) and *M. martiniquensis*, with subspecific taxa *M. m. martiniquensis* (Martinique) and *M. m. nyctor* (Barbados), are endemic to the Lesser Antilles (see Figure 1), whereas Caribbean populations of *M. nesopolus* and *M. nigricans* are considered conspecific with South American mainland populations (Koopman 1968; LaVal 1973; Genoways et al. 1998). *Myotis nesopolus* is distributed on the continental islands off the northern coast of South America (Bonaire and Curacao; Genoways and Williams 1979; Petit et al. 2006) and *M. nigricans* is reported from Grenada, Trinidad, and Tobago (Jones 1951; Goodwin and Greenhall 1961; Koopman 1968; Genoways et al. 1998; see Figure 1 for collection localities).

Although extant populations of Caribbean *Myotis* are hypothesized to be a result of one (Baker and Genoways 1978; Koopman 1968) or two invasions from northern South America (LaVal and Schwartz 1974; Stadelmann et al. 2007), no analysis has tested these hypotheses with data from all known Caribbean taxa.

Most detailed analyses of Caribbean *Myotis* are morphologically based (Koopman 1968; LaVal 1973; LaVal and Schwartz 1974; Genoways and Williams 1979; Genoways et al. 1998, 2001), whereas DNA sequences have been analyzed from only a few individuals or Lesser Antillean endemics (Ruedi and Mayer 2001; Hoofer and Van Den Bussche 2003; Stadelmann et al. 2004a,b, 2007; Lack et al. 2010). Stadelmann et al. (2007) hypothesized that the most recent common ancestor for *M. dominicensis* and *M. martiniquensis* existed during the Pliocene epoch (2.6–5.3 million years ago [mya]). Although this hypothesis is compatible with the geological history of the Caribbean (Graham 2003), it provides a limited view of the evolutionary history of Caribbean *Myotis* and closely related mainland species.

Figure 1. Map of the Lesser Antilles and northern South America (A), and the islands where Lesser Antillean *Myotis* are distributed (B; shaded in black). Numbers 1–8 indicate the sampling localities: 1 – Dominica, 2 – Martinique, 3 – Barbados, 4 – Grenada, 5 – Tobago, 6 – Bonaire, 7 – Venezuela, and 8 – Suriname.

Given the paucity of genetic data from Caribbean populations of *Myotis*, and potential for discovering unrecognized species, a closer examination of diversification, origin, and relationships among Caribbean *Myotis* is justified. We study intraspecific and interspecific relationships of *Myotis* by examining genetic data from the largest sample of Caribbean and northern South American taxa to date. We present phylogenetic analyses of both mitochondrial (cytochrome-*b*) and nuclear (recombination activating gene 2 [RAG 2]) genes, and explored genome-wide variation using Amplified Fragment Length Polymorphisms [AFLPs] (Vos et al. 1995). Our sample included *Myotis* collected from the islands of Barbados, Bonaire, Dominica, Grenada, Martinique, and Tobago, as well as from mainland Venezuela and Suriname, to test hypotheses regarding the taxonomy and evolution of Caribbean taxa (Koopman 1968; LaVal 1973; Baker and Genoways 1978; Stadelmann et al. 2007). Furthermore, to estimate the timescale of diversification of Caribbean *Myotis*, we perform relaxed molecular clock analyses using Bayesian statistics and secondary calibration points from Stadelmann et al. (2007).

Material and methods

Molecular methods

Whole genomic DNA was extracted from liver, muscle, or wing punches following standard methods (Longmire et al. 1997), or using the DNeasy Blood and Tissue Kit (Qiagen Inc., Chatsworth, California). Whole genomic DNA was extracted from a wing clip (2 mm²) of a museum specimen of *M. nigricans* collected from Grenada (CM 83427) in 1986. The Promega pGEM-T Vector System II kit (Promega Corporation, Madison, Wisconsin) was used to clone fragments of the cytochrome-*b* gene (~500 base pairs [bps]) in this individual.

Primers used to amplify and sequence the cytochrome-*b* gene are listed in Table 1. PCR methods of Larsen et al. (2007) were followed with slight modification. The highest quality and quantity PCR products were obtained by heating the reactants at 94 °C for 2 min, with 34–38 cycles of denaturation at 94 °C for 45 s,

Table 1. External and internal primers used in amplification and sequencing of the cytochrome-*b* gene in Caribbean and South American *Myotis*. Letters F and L refer to forward direction and R and H refer to reverse direction.

Primer	Sequence (5'–3')	Source
External		
glo7 L	CAY CGT TGT ATT TCA ACT RTA AGA AC	Hoffmann and Baker (2001)
glo6 H	CGG TGT AAT GRA TAT ACT ACA TRG	Hoffmann and Baker (2001)
L 14724	CGA AGC TTG ATA TGA AAA ACC ATC GTT G	Irwin et al. (1991)
H 15915	AAC TGC AGT CAT CTC CGG TTT ACA AGA C	Irwin et al. (1991)
Internal		
Myo250R	TAT RGA GGC TCC RTT TGC ATG TAR	This study
Myo450F	CTC TCT GCR ATY CCA TAY ATY GG	This study
Myo500R	AGG GTR GCY TTG TCA ACA GAR AAT	This study
Myo650F	CCY TTY CAY CCC TAY TAT ACA AT	This study
Myo1L (F)	RGG MCA AAT RTC YTT YTG AGG	This study
MVZ04 (R)	GCA GCC CCT CAG AAT GAT ATT TGT CCT C	Smith and Patton (1991)
G7LHK (F)	CGT TGT ATT TCA ACT RTA AGA	This study
EpH520 (R)	RAA KGG GAG TAG AAA GTG GAA GGC	Anwarali Khan et al. (2008)
Myo2F	CTC TCT GCM ATY CCW TAY ATT GG	This study

annealing at 47–49 °C for 1 min, extension at 72 °C for 1 min 15 s, followed by 72 °C for 10 min. Methods of Stadelmann et al. (2007) were followed to obtain RAG 2 amplifications. The thermal profile that produced the best amplicons included 3 min of denaturation at 94 °C, followed by 37–39 cycles at 94 °C for 45 s, 60–61 °C for 45 s and 72 °C for 1 min 30 s, with a final extension at 72 °C for 5 min. PCR products were purified using the QIAquick PCR Purification Kit (Qiagen Inc., Chatsworth, California) or ExoSAP-IT (Affymetrix, Inc., Santa Clara, California).

Sequencing for cytochrome-*b* and RAG 2 was performed using ABI Big Dye chemistry chain terminators (version 3.1) and fragments were electrophoresed on an ABI 3100-Avant Genetic Analyzer (PE Applied Biosystems, Foster City, California). Sequences were verified and assembled using Sequencher 4.10.1

Figure 2. Bayesian phylogram based on DNA sequence data of the cytochrome-*b* gene. Scores are Bayesian posterior probabilities (top score) and bootstrap support values (percentage of 1000 iterations) from maximum-parsimony (middle score) and maximum-likelihood (bottom score) analyses. *Myotis latirostris* and *M. alcahoie* were used as outgroups but are not shown. Asterisk identifies specimen collected from Grenada (see Species Account).

(Gene Codes Corporation, Ann Arbor, Michigan). Multiple sequence alignments were performed manually and verified in MacClade (version 4.08 OS X; Maddison and Maddison 2003). AFLPs were generated following protocols from Vos et al. (1995) and McDonough et al. (2008). A labeled (6FAM fluorophore; Applied Biosystems, Foster City, California) selective EcoRI primer and seven non-labeled selective primers were used to generate

AFLPs from 18 individuals. Labeled fragments were detected using an ABI 3100-Avant genetic analyzer, manually scored for presence/absence using GeneMapper version 4.0 (Applied Biosystems), and converted to a binary data matrix using GenAlEx version 6.1 (Peakall and Smouse 2006). Fragments (50–400 bp in length) with intensities larger than 100 RFUs (relative fluorescence units) were scored.

Phylogenetic analyses

Phylogenetic analyses were performed using MrBayes (version 3.1.2; Ronquist and Huelsenbeck 2003), MEGA (version

5.0; Tamura et al. 2011), and PAUP* (version 4.0b10; Swoford 2002). Maximum-likelihood, maximum-parsimony (unweighted), and Bayesian analyses were used to infer phylogenies. Bootstrap support values ($\geq 75\%$) and Bayesian posterior probabilities (≥ 0.95) measured statistical support. Genetic distance values for cytochrome-*b* were generated in MEGA using the Kimura 2-parameter model (Kimura 1980), which allowed for comparisons with studies of *Myotis* (Ruedi and Mayer 2001; Stadelmann et al. 2007) and other mammalian taxa (Bradley and Baker 2001). The outgroup for cytochrome-*b* analyses included *M. latirostris* and *M. alcahoie*. Nei genetic distances (Nei and Li 1979) were generated from the AFLP binary data using GenAlEx and a Principal Coordinates Analysis (PCoA) was used to visualize the AFLP dataset.

Levels of phylogenetic signal and saturation were explored in the cytochrome-*b* dataset using PAUP* and DAMBE version 5.2 (Xia and Xie 2001). Phylogenetic signal was examined via the *g*₁-statistic (Hillis and Huelsenbeck 1992) for 100,000 randomly drawn trees and saturation was examined using Xia's method (Xia et al. 2003) implemented in DAMBE. Follow-

ing Stadelmann et al. (2007), a partition-homogeneity test using PAUP* was performed to determine whether significantly different phylogenetic signals were present across our cytochrome-*b* and RAG 2 datasets. Datasets were concatenated and combined with sequences from Stadelmann et al. (2007; cytochrome-*b* and RAG 2). Separate RAG 2 analyses did not resolve currently recognized intraspecific or interspecific relationships in the sampled *Myotis* or provide adequate independent characters for use in phylogenetic analyses (see Section 'Results'). Therefore no independent RAG 2 phylogenies are reported. The RAG 2 dataset was prepared specifically to combine with cytochrome-*b* and provide a comparable dataset with Stadelmann et al. (2007). The sequences and diversification estimate parameters from Stadelmann et al. (2007) were used to compare our sequences, and to estimate diversification times with our new sequences in the context of their study.

A maximum-parsimony analysis was performed on each dataset (cytochrome-*b*, RAG 2) using heuristic searches, 25 replicates of the random taxon addition option, each with random starting trees, and tree-bisection-reconnection branch swapping. For bootstrap support values, 1000 replicates were conducted using the heuristic search criterion. Nucleotide substitution models were analyzed using MEGA software to determine the appropriate model of evolution for the cytochrome-*b* and RAG 2 genes. Based on the Bayesian Information Criterion, the HKY+G+I model was chosen for cytochrome-*b*, the HKY+G model for RAG 2, and the Tg2+G model for the combined dataset (the model for the concatenated dataset was used in the estimations of divergence time [see below] following the methods of Stadelmann et al. (2007)). Maximum-likelihood analyses were performed in MEGA for the cytochrome-*b* dataset, with bootstrap support values based on 1000 iterations. The Bayesian analysis was performed to obtain posterior probabilities and consisted of one run with 4 Markov chain Monte Carlo chains (1 heated and 3 cold) run for 2 million generations. Trees were sampled every 1000 generations with a burn-in value of 1000. Output files were examined in Tracer version 1.5 (Rambaut and Drummond 2007) to determine whether convergence was attained and to review the effective sample size (ESS) of the Bayesian runs.

Divergence time estimates

Molecular clock analyses were performed to elucidate time of origin and diversification of Caribbean *Myotis*. Analyses generally followed those of Stadelmann et al. (2007) in that relaxed molecular clock methods were performed using a bi-locus dataset consisting of cytochrome-*b* and RAG 2 gene sequence data. Molecular clock analyses were performed using BEAST version 1.6.1 (Drummond and Rambaut 2007). Diversification dates proposed by Stadelmann et al. (2007) were used as secondary calibration points with normal distribution priors (Ho 2007) in all analyses. Two calibration points were used from Stadelmann et al. (2007), time to the most recent common ancestor (tMRCA) for Neotropical, Nearctic (*M. austroriparius*, *M. grisescens*, *M. velifer*, *M. vivesi*, *M. yumanensis*), and Palearctic *Myotis* = 8.6mya (± 1.6 mya) and tMRCA between *M. levis* and *M. nigricans* = 1.2 mya (± 0.5 mya). MEGA was used to generate a maximum-likelihood tree of the concatenated dataset, which was used as a starting tree in BEAST analyses. Node dates were examined using a relaxed uncorrelated lognormal molecular clock and a Yule species prior. Preliminary analyses consisted of four runs at 10,000,000 generations (10% burn-in), and final time estimates were calculated based on the combination of log and tree files produced from two runs at 20,000,000 generations. The program Tracer was used to examine convergence and ESS from the BEAST analyses.

Morphological methods

Abbreviations and acronyms for institutions or individual collections are in Appendix A. Following the definitions and methods of Hall (1946), six external and seven cranial measurements were recorded from 30 adult specimens. Measurements were taken from museum specimens using digital calipers (in millimeters to the nearest 0.1 mm). Measurements included: TL = length from tip of nose to tip of tail, TV = length of caudal vertebrae, FA = forearm length, HF = hind foot length (including claw), EL = ear length, and TIB = length from ankle to proximal end of tibia, TFA = tibia length/forearm length, GLS = greatest length of skull, CBL = condylobasal length, ZB = zygomatic breadth, POC = postorbital constriction, MB = mastoid breadth, MTR = maxillary tooth row length, and MM = breadth across upper molars.

A 1-way MANOVA was used to test for secondary sexual dimorphism among all individuals. *t*-Tests were used to determine the significance between pairs of species. Descriptive statistics were calculated for taxonomic groups. Principal Components Analysis (PCA) was used to explore variation among the 13 measured characters (excluding the ratio TFA) in individuals with measurements for all characters (12 *M. m. nyctor*, 1 *M. m. nyctor* Grenada, 3 *M. sp.* Suriname, 2 *M. martiniquensis*, 5 *M. dominicensis*), using log transformed characters. The PCA included 23 individuals of Lesser Antillean *Myotis* and individuals from other closely related island or mainland taxa (as determined by genetic analyses). PCA scores were used to visualize the tentative species groups. PCA loadings for the first two principal components were used to describe total variance. Statistical analyses were performed in R software 2.12.0 (R Development Core Team 2010).

Results

Specimens examined are listed in Appendix A, including information associated with tissues and museum vouchers. Twenty-four sequences of cytochrome-*b* and 9 sequences of RAG 2 were generated (GenBank Accession numbers JN020554–JN020586) along with AFLPs from 18 individuals. Forty-four sequences (23 cytochrome-*b* and 21 RAG 2; including outgroup taxa) from GenBank were included in phylogenetic analyses (Appendix A).

Phylogenetic analyses

Sequence alignment of cytochrome-*b* and RAG 2 genes was unequivocal and without stop codons. Three hundred twenty-six sites were parsimony-informative in the cytochrome-*b* gene with 60 at codon position 1, 15 at 2, and 251 at 3. Of the 1063 bps of RAG 2, 68 sites were polymorphic with 31 sites being parsimony-informative. Results of the partition-homogeneity test indicated no significant conflict between the cytochrome-*b* and RAG 2 datasets. The concatenated dataset consisted of 2203 bps from 21 species of *Myotis* most closely related to Caribbean *Myotis* (as determined by phylogenetic analyses reported above).

Parsimony analysis of the cytochrome-*b* sequences generated 6 most parsimonious trees of 1295 steps (retention index = 0.56, consistency index = 0.35). Topology of the strict consensus of the 6 equally parsimonious trees was similar to trees generated in all phylogenetic analyses. Maximum-likelihood analysis resulted in a single optimal tree ($-\ln L = 6785.08$; Figure 2) with a proportion of invariable sites of 58.35% and a gamma distribution parameter of 0.55. Based on the Bayesian analysis and use of the Tracer program, convergence was reached and the ESS was greater than 200 (with low autocorrelation) for the cytochrome-*b* dataset. Level of phylogenetic signal in the cytochrome-*b* dataset was significant ($P < 0.01$) and results of Xia's test for substitution saturation in-

licated low levels of saturation in cytochrome-*b* data. Stadelmann et al. (2007) did identify saturation of the cytochrome-*b* gene among old and new world *Myotis*, but only at patristic distances beyond 15%. Caribbean species of *Myotis* are beneath this threshold (range from 6.7 to 7.3% in pairwise patristic distances) and are relatively young as they share a most recent common ancestor within the last 5 million years (see below). Nonetheless, our results indicate some homoplasy in the sequence data, as several intermediate nodes remain unresolved (Figure 2).

Kimura 2-parameter distances averaged 9.0% among Lesser Antillean *Myotis* (Table 2). Average genetic distance between specimens of *M. martiniquensis* and *M. m. nyctor* was 9.0%. The single specimen from Grenada shared a close genetic relationship (0.4%) to individuals of *M. m. nyctor* from Barbados (Figure

2). When compared to the most similar haplotype of *M. m. nyctor* from Barbados, the cytochrome-*b* haplotype of the Grenada specimen (500 bps) varied by two transitions (both third position C-T). Specimens from Grenada and Barbados form a statistically supported sister relationship to specimens collected from northern Suriname (see Figure 2). Suriname specimens were originally identified as *M. nigricans*, yet are distinguished by a genetic distance of 12.1% from *M. nigricans* from Brazil (AF376864).

In AFLP analyses, seven primer pairs used for selective amplification produced 326 scorable bands from 18 individuals. Each island taxon (*M. m. nyctor*, *M. dominicensis*, *M. martiniquensis*, and *M. sp.* Tobago) showed a distinct number of private bands ranging from 13 to 24 (average 21; Figure 3). The PCoA of AFLP eigenvalues showed visual separation of each species cluster

Table 2. Average Kimura 2-parameter distances (percent values) within (boldface, along diagonal) and among (below diagonal) *Myotis* taxa based on cytochrome-*b* gene sequences. Asterisk indicates sequence from Grenada specimen was included in *M. m. nyctor*.

Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
(1) <i>M. latirostris</i> (n=1)	—																			
(2) <i>M. alcahoie</i> (n = 1)	18.5	—																		
(3) <i>M. albesens</i> (n = 1)	17.0	15.8	—																	
(4) <i>M. atacamensis</i> (n = 1)	18.2	18.1	11.1	—																
(5) <i>M. austroriparius</i> (n = 1)	18.1	18.2	13.1	12.0	—															
(6) <i>M. californicus</i> (n = 1)	16.2	17.0	13.8	14.4	15.9	—														
(7) <i>M. chiloensis</i> (n = 1)	17.1	15.7	10.6	8.8	11.4	14.1	—													
(8) <i>M. dominicensis</i> (n = 3)	19.8	16.2	11.8	8.8	12.6	11.1	8.6	0.3												
(9) <i>M. grisescens</i> (n = 1)	17.9	17.2	10.9	9.3	9.4	12.4	9.1	8.1	—											
(10) <i>M. keaysi</i> (n = 1)	16.7	16.9	12.5	13.0	14.5	17.3	13.0	13.6	13.9	—										
(11) <i>M. martiniquensis</i> (n = 6)	16.6	15.5	10.6	7.7	9.9	13.2	9.4	8.2	7.4	12.2	0.3									
(12) <i>M. m. nyctor</i> (n = 6) *	20.1	17.9	14.9	10.4	12.1	15.9	10.6	9.8	12.4	13.2	9.0	0.4								
(13) <i>M. nesopolus</i> (n = 3)	18.7	15.5	12.0	12.4	13.4	16.0	14.0	11.1	13.3	15.1	11.9	15.1	—							
(14) <i>M. sp. Venezuela</i> (n = 2)	18.4	17.1	11.3	10.8	13.2	15.1	11.0	10.2	11.5	14.3	10.1	14.0	4.1	0.6						
(15) <i>M. nigricans</i> (n = 1)	16.5	17.6	10.2	12.5	13.1	12.2	10.7	10.4	11.4	12.5	9.8	13.8	12.8	12.4	—					
(16) <i>M. oxyotus</i> (n = 1)	17.1	16.4	9.6	12.8	12.6	11.9	10.7	11.8	11.4	13.1	10.1	13.6	13.1	11.8	4.0	—				
(17) <i>M. sp. Suriname</i> (n = 3)	19.1	15.3	12.6	9.1	12.4	15.5	10.7	8.2	10.2	10.1	9.0	8.6	12.6	12.2	12.1	12.9	0.6			
(18) <i>M. sp. Tobago</i> (n = 2)	15.9	15.8	12.2	8.4	11.2	14.7	10.3	8.4	9.8	12.5	8.1	10.2	8.7	7.2	11.1	12.2	9.7	0.0		
(19) <i>M. velifer</i> (n = 1)	16.5	15.2	10.6	11.2	12.8	13.3	9.8	8.5	10.6	13.3	9.2	11.8	13.7	12.1	10.9	11.5	9.4	11.1	—	
(20) <i>M. yumanensis</i> (n = 1)	16.5	15.5	11.7	9.6	11.8	11.7	9.9	7.0	11.2	12.8	8.1	10.7	11.5	9.7	10.9	10.9	9.8	8.1	5.9	—

Table 3. Summary statistics (mean and standard deviation followed by range and sample size) of measurements taken from Caribbean and South America *Myotis*. Specimens examined are in Appendix A.

Character	<i>M. m. nyctor</i> (Barbados)	<i>M. m. nyctor</i> (Grenada)	<i>M. martiniquensis</i> (Martinique)	<i>M. dominicensis</i> (Dominica)	<i>M. sp.</i> (Suriname)
TL	85.6±1.82 81.0–88.0 (16)	84.0 —	78.2±3.70 75.0–84.0 (5)	76.0±1.61 74.0–78.0 (5)	77.5±3.11 75.0–81.0 (3)
TV	38.8±1.22 37.0–41.0 (16)	36.0 —	36.2±1.64 34.0–38.0 (5)	32.2±1.53 29.9–34.0 (5)	31.7±2.00 30.2–34.0 (3)
FA	36.0±0.44 35.4–36.7 (16)	33.4 —	37.0±1.41 34.9–38.1 (5)	35.0±0.85 34.0–36.0 (5)	34.6±0.35 34.2–34.9 (3)
HF	7.6±0.62 7.0–9.0 (16)	7.0 —	7.2±0.45 7.0–8.0 (5)	7.0±0.88 6.0–8.0 (5)	6.9±0.79 6.0–7.5 (3)
EL	13.3±1.30 12.0–16.0 (16)	13.0 —	12.2±0.45 12.0–13.0 (5)	10.5±0.67 10.0–11.4 (5)	11.5±1.32 10.5–13.0 (3)
TIB	17.0±0.45 16.3–18.1 (16)	14.1 —	15.4±0.30 15.1–15.8 (5)	14.2±0.49 13.6–14.9 (5)	14.8±0.31 14.5–15.1 (3)
TFA	0.47±0.01 0.46–0.51 (16)	0.42 —	0.42±0.02 0.39–0.45 (5)	0.41±0.03 0.38–0.44 (5)	0.43±0.01 0.42–0.44 (3)
GLS	14.4±0.05 14.1–14.8 (16)	13.5 —	14.5±0.35 14.1–14.8 (3)	13.3±0.26 13.0–13.7 (5)	13.6±0.51 13.2–14.2 (3)
CBL	13.5 ±0.23 13.1–13.9 (16)	12.4 —	13.4±0.33 13.1–13.8 (3)	12.2±0.19 12.0–12.5 (5)	12.6±0.20 12.4–12.8 (3)
ZB	8.7±0.30 8.0–9.0 (12)	8.3 —	8.9±0.11 8.8–9.0 (2)	7.7±0.15 7.6–7.9 (5)	8.3±0.12 8.2–8.4 (3)
POC	3.35±0.09 3.2–3.5 (16)	3.4 —	3.5±0.11 3.3–3.6 (4)	3.3±0.07 3.2–3.4 (5)	3.6±0.12 3.5–3.7 (3)
MB	7.2±0.11 7.0–7.4 (16)	6.8 —	7.4±0.16 7.2–7.5 (3)	6.6±0.05 6.6–6.7 (5)	6.9±0.23 6.8–7.2 (3)
MTR	5.5±0.08 5.4–5.7 (16)	4.9 —	5.6±0.12 5.4–5.7 (4)	5.0±0.13 4.8–5.2 (5)	5.1±0.10 5.0–5.2 (3)
MM	5.4±0.16 5.0–5.6 (16)	5.2 —	5.6±0.07 5.5–5.7 (4)	5.1±0.11 4.9–5.2 (5)	5.5±0.10 5.4–5.6 (3)

Figure 3. Principal Coordinates Analysis of the binary AFLP matrix, based on 326 scored bands. A three-dimensional view from above the X-axis (A), and from below the X-axis (B) is shown. Note placement of *M. m. nyctor* and *M. martiniquensis* above and below the X-axis, respectively. The number of unique bands for each cluster are as follow: *M. dominicensis* = 24, *M. m. nyctor* = 24, *M. sp. Tobago* = 13, *M. martiniquensis* = 22, *M. sp. Suriname* = 9, and *M. sp. Venezuela* = 13.

(Figure 3). The interspecific Nei Li genetic distance values averaged 3.5% and ranged from 1.8 to 5.1% among Lesser Antillean *Myotis*. Intraspecific Nei Li genetic distance values averaged 0.9% (range 0.5–1.9%).

Relaxed molecular clock analyses

Divergence times for 21 species of Neotropical, Nearctic, and Palearctic *Myotis* used in this study were within the time estimates reported by Stadelmann et al. (2007). The tMRCA for the clade comprised of Caribbean *Myotis* was estimated at 4.15 mya (95% highest posterior density [HPD]: 3.07–5.32). Diversification events for Caribbean *Myotis*, and closely related mainland taxa, were during the early to mid-Pliocene (Figure 4). The tMRCA between *M. martiniquensis nyctor* and its sister lineage from Suriname was estimated at 2.31 mya (HPD: 1.47–3.23; Figure 4).

Table 4. PCA loadings of the first two principal components. CBL, MB, and MTR (in bold) have the largest magnitudes on PC1, and POC, MM, and TV (in bold) on PC2.

Character	PC1 loading	PC2 loading
TL	-0.287	-0.212
TV	-0.301	-0.288
FA	-0.223	-0.161
HF	-0.181	-0.287
EL	-0.255	-0.143
TIB	-0.296	-0.161
GLS	-0.311	0.000
CBL	-0.332	0.000
ZB	-0.311	0.166
POC	-0.109	0.708
MB	-0.321	0.152
MTR	-0.321	0.000
MM	-0.263	0.399

Morphological analyses

Secondary sexual dimorphism was not significant among our sample of *Myotis*, hence sexes were pooled for statistical analyses. The *t*-test of group means between *M. m. nyctor* and *M. martiniquensis* indicated 7 measurements were significant at the $P \leq 0.05$ level. Variables included three cranial measurements (POC, MB, and MM), four external measurements (TL, TV, FA, and TIB), and the ratio of tibia length to forearm length (TFA; Table 3). The PCA revealed no overlap among Caribbean taxa (Figure 5). The first principal component accounted for 67.13% of the variation, whereas the second principal component accounted for 11.32%. PCA loadings with the largest magnitudes on PC 1 were CBL, MB and MTR, whereas those on PC 2 were POC, MM, and TV (Table 4).

Discussion

Our results indicate that *Myotis* species richness is underestimated in the Caribbean and northern South America. Analyses of cytochrome-*b*, RAG 2, and AFLP data indicate three Lesser Antillean *Myotis* species-level lineages and three closely related species-level lineages from northern South America and Tobago (Figures 2 & 3). The third Lesser Antillean species-level lineage described herein is composed of specimens collected from Barbados, and thus is referable to the historically recognized subspecies *M. martiniquensis nyctor* (LaVal 1973). The three unrecognized species-level lineages most closely related to Caribbean taxa (*M. dominicensis*, *M. martiniquensis*, *M. m. nyctor*, and *M. nesopolus*) are comprised of specimens collected from Suriname, Tobago, and Venezuela (Figure 2). We discuss the systematics and taxonomy of these lineages below.

The traditionally recognized subspecies *M. martiniquensis nyctor* is paraphyletic with respect to *M. martiniquensis* and sister to a currently unrecognized species collected from Suriname (Figure 2). Our analyses of cytochrome-*b* sequence data indicate that the genetic distance separating *nyctor* from *martiniquensis* is approximately 9.0%, a value typical of (or greater than) species-level genetic distances within *Myotis* (Table 2). Additionally, AFLP data indicate that *M. m. nyctor* forms a cluster separate from *M. martiniquensis* (Figure 3). Structure in the nuclear AFLP data when coupled with structure in mitochondrial and morphological datasets (Figures 2 & 5) indicate that *M. m. nyctor* is on a separate and unique evolutionary trajectory from *M. martiniquensis*. Based on these results, we recognize *nyctor* as a species distinct from *M. martiniquensis*.

Species account

Myotis nyctor LaVal and Schwartz, 1974

Myotis martiniquensis LaVal, 1973

Myotis martiniquensis nyctor LaVal and Schwartz, 1974

Figure 4. Chronogram of Caribbean, Nearctic, and Neotropical *Myotis* based on cytochrome-*b* and RAG 2 sequence data. Horizontal gray bars represent 95% highest posterior density intervals for divergence estimates. Vertical gray bars identify Miocene, Pliocene, and Pleistocene epochs. Arrow identifies node that is statistically supported by a Bayesian posterior probability of 1 and a bootstrap support value of 83% in maximum-likelihood analyses. Statistical support for this node indicates the potential for multiple colonization events of the Lesser Antilles by *Myotis* and/or reverse colonization of mainland South America (see Section 'Discussion').

Holotype

KU 109473, adult male, skin and skull, collected 16 February 1961 by D. C. Leber and A. Schwartz (original number 5139 of Albert Schwartz collection).

Type locality

Barbados: Cole's Cave, St. Thomas Parish.

Distribution

Lesser Antillean island of Barbados (Figure 1). *Myotis nyctor* or a related genetic lineage occurs on the island of Grenada (see Remarks).

Specimens Examined

(9♂, 8♀ examined morphologically; cytochrome-*b* = *, RAG2 = §, AFLP = †; GenBank accession numbers included). Grenada: St. David Parish: 1.25 km S, 0.75 km W Beaton [12° 01.5' N, 61° 42' W], 1♂ (CM 83427, TK 125846), JNo20562 *. Barbados: St. George Parish: Gun Hill, 153 m [13° 08' 33.7" N, 59° 33' 24.0" W], 1♂ (TTU 109224, TK 161093), JNo20566 *. St. Joseph Parish: Flower Forest, 0.15 km S, 0.6 km W Melvin Hill, 233 m [13° 12' 12.3" N, 59° 34' 01.1" W], 1♂ (TTU 109265, TK 161060 †). Horse Hill, 251 m [13° 12' 02.5" N, 59° 32' 14.9" W], 1♂ 1♀ (TTU 110061); (TTU 109229, TK 161049 †), JNo20565*. Joe's River, 1.2 km W Bathsbeba, 80 m [13°

12' 40.7" N, 59° 32' 25.6" W], 3♂ 1♀ (TTU 110052); (TTU 110054); (TTU 110059); (TTU 109228, TK 161028 †). St. Thomas Parish: Friendship Terrace, 54 m [13° 08' 24.4" N, 59° 36' 07.1" W], 1♂ 1♀ (TTU 110040); (TTU 109230, TK 161125 †), JNo20567 *. Welchman Hall Gully, 0.5 km N Welchman Hall, 290 m [13° 11' 40.1" N, 59° 34' 35.2" W], (TTU 109226, TK 151419), JNo20564 *, JNo20584 §; 1♂ 5♀ (TTU 109225, TK 151413), JNo20563 *, JNo20583 §; (TTU 109263, TK 151415 †); (TTU 109264, TK 151418 †); (TTU 110034); (TTU 110035); (TTU 110036).

Measurements

Measurements of *Myotis nyctor* and *M. martiniquensis* overlap in 12 of 14 characters, the exceptions being length of tibia and ratio of tibia to forearm length, for which there was no overlap in the range of measurements (Table 3). *Myotis nyctor* has a longer tibia than *M. martiniquensis* and the TFA ratio is larger in *M. nyctor* (Table 3). As discussed by LaVal and Schwartz (1974) and confirmed by our data, these two taxa show little difference in overall size when comparing ranges for each character. However, comparison of mean values for characters indicates *M. martiniquensis* has larger average values for six of seven cranial measurements (GLS, ZB, POC, MB, MTR, MM), whereas *M. nyctor* has larger average values for five of six external measurements (TL, TV, HF, EL, TIB).

Figure 5. Scatter plots of the first and second principal component scores of Caribbean *Myotis*. *Myotis* (Δ : *m. nycor*; $\star$: *m. nycor* [Grenada]; $\square$: sp. Suriname; $\square$: *martiniquensis*; $\square$: *dominicensis*).

and the ratio of tibia length to forearm length (Table 3). Characters that are statistically significant ($P \leq 0.05$) between these two taxa include four external measurements (TL, TV, TIB larger and FA smaller in *M. nycor*) and three cranial measurements (POC, MB, MM larger in *M. martiniquensis*). LaVal and Schwartz (1974) also observed that FA and MM was significantly different between *M. martiniquensis* and *M. nycor* (Table 3).

Revised diagnosis

Myotis nycor is almost uniformly larger externally and smaller cranially than *M. martiniquensis*. Our results showed *M. nycor* to be significantly larger than *M. martiniquensis* in TL, TV, and TIB and significantly smaller than *M. martiniquensis* in POC, MB, MM, and one external measurement (FA). These results match those of LaVal and Schwartz (1974) for length of forearm and breadth across the upper molars. *Myotis nycor* and *M. dominicensis* do not overlap in nine of 13 cranial and external measurements (Table 3), with *M. dominicensis* being smaller in all nine measurements. They overlap in range of measurements in FA, HF, POC, and MM (Table 3). *Myotis dominicensis* does not overlap the range of the TFA ratio in *M. nycor*. However, *M. dominicensis* has smaller average values for all 13 measurements and the TFA ratio (Table 3).

Remarks

Myotis nycor is the only known species of *Myotis* on Barbados. Koopman (1968) and Genoways et al. (1998) discuss the presence of *Myotis nigricans* on the neighboring island of Grenada (~240 km southwest of Barbados). We extracted DNA and amplified a portion of the cytochrome-*b* gene from a voucher specimen (CM83427) collected from Grenada in 1986. Our results indicate a close genetic relationship between this specimen and *M. nycor* from Barbados (0.4%; Figure 2). However,

measurements of this specimen are below the range of *M. nycor* from Barbados (Table 3), and it appears as an outlier in the PCA (Figure 5). Two additional specimens of *M. nigricans* from Grenada are at the National Museum of Natural History (NMNH 254717, fluid; NMNH 252600, skin/skull). Based on Genoways et al. (1998), cranial and external measurements of NMNH 252600 are also below the range of *M. nycor*. Additional voucher specimens with genetic data are needed to test for a close genetic relationship between *Myotis* from Barbados and Grenada. If deemed conspecific, then the distribution of *M. nycor* would include Grenada; however, the morphological distinctness of the Grenada population likely would warrant subspecific recognition.

Evolutionary history of Lesser Antillean *Myotis*

Ancestors of Lesser Antillean species of *Myotis* are hypothesized to have colonized the Caribbean by overwater dispersal from the South American mainland (Koopman 1968; LaVal and Schwartz 1974; Baker and Genoways 1978; Stadelmann et al. 2007). This hypothesis is based largely on the close geographic proximity of South America to the Lesser Antilles (Figure 1) and on studies identifying morphological similarities between Lesser Antillean and South American species (Miller and Allen 1928; Koopman 1968; LaVal 1973; LaVal and Schwartz 1974). DNA sequence data presented herein and in Stadelmann et al. (2007) reveal close genetic relationships between Lesser Antillean and South American *Myotis* (e.g. *M. atacamensis*; see Figures 2 & 4); however there is a lack of concordance and statistical support for these relationships (Figures 2 & 4, see Stadelmann et al. 2007). Although a North or Central American origin for Lesser Antillean species of *Myotis* cannot be discounted with the available molecular data, this seems unlikely because *Myotis* is absent from the extant and fossil Greater Antillean bat fauna (Silva Taboada 1979; Griffiths and Klingener 1988; MacFarlane et al. 2002; Gannon et al. 2005).

Baker and Genoways (1978) hypothesized a single invasion by *Myotis* into the Lesser Antilles followed by subsequent diversification; alternatively, LaVal and Schwartz (1974) hypothesized two invasions from South America. Testing hypotheses of one versus multiple invasions requires statistically supported phylogenies. We were unable to recover statistical resolution for intermediate relationships using sequence data from the mitochondrial cytochrome-*b* gene alone (Figure 2). Difficulty in resolving phylogenetic relationships among *Myotis* is not surprising, as several authors have encountered this same problem (Ruedi and Mayer 2001; Rodriguez and Ammerman 2004; Dewey 2006; Stadelmann et al. 2007). However, our analyses of the concatenated cytochrome-*b* and RAG 2 data resulted in nodal support for a sister relationship between a clade comprised of *M. dominicensis*, *M. nyctor*, and *M. sp.* (Suriname) and another comprised of specimens from Tobago and Venezuela (see Figure 4). Notably, *M. martiniquensis* is basal to these lineages (Figure 4). This topology suggests at least two separate invasions by *Myotis* into the Lesser Antilles (depending on the phylogenetic position of *M. atacamensis*) and/or reverse colonization by Caribbean *Myotis* to South America (*M. sp.* Suriname). Reverse colonization has been documented in other Caribbean bat lineages (Natalidae: Davalos 2005; Genoways et al. 2005; Mormoopidae: Davalos 2006; Phyllostomidae: Genoways et al. 2005; Davalos 2010), as well as non-volant Caribbean vertebrates (*Anolis* lizards: Nicholson et al. 2005). These examples are a departure from traditional island biogeography (i.e. MacArthur and Wilson 1967) providing evidence that taxa, which evolved on oceanic islands, could contribute to continental biodiversity (Bellemain and Ricklefs 2008).

Molecular clock analyses indicate that *M. dominicensis* and *M. martiniquensis* evolved during the Pliocene epoch (Figure 4; see also Stadelmann et al. 2007; Lack et al. 2010), whereas *M. nyctor* likely originated during the late Pliocene to mid-Pleistocene. The geological history of the islands that *M. dominicensis* and *M. martiniquensis* currently inhabit do not conflict with a Pliocene origin, as each island was available for colonization at least 11 million years ago (Graham 2003). However, Barbados was below sea level completely or partially until 1 mya and perhaps as recent as 700,000 years ago (Speed and Keller 1993; Lovette et al. 1999), thus it is likely that *M. nyctor* is a recent (<1 mya) arrival. Moreover, Grenada may have served as a source for the Barbados population as the island rose above sea level approximately 20 mya (Graham 2003). This does not conflict with the late Pliocene origin for *M. nyctor* (Figure 4). Recent fieldwork (2005–2009) failed to document the presence of *Myotis* on St. Lucia, St. Vincent, and the Grenadines, therefore *Myotis* has either gone extinct on these intervening islands or remains to be documented.

Species diversity in northern South America

Although South American *Myotis* have undergone several genetic investigations (Ruedi and Mayer 2001; Stadelmann et al. 2007; Aires 2008), none of these studies have included extensive geographic sampling of each species complex in their experimental design. Such lack of genetic information provided little resolution of species boundaries and numbers of species-level lineages in South America. The most recent taxonomic assessments (Simmons 2005; Wilson 2008) recognize 12 species of *Myotis* in South America, with 4 taxa having expansive distributions (*M. albescentis*, *M. keaysi*, *M. nigricans*, and *M. riparius*). Our experimental design included specimens (identified in the field as *M. nigricans*) collected from North and Central America, as well as several localities in South America. This approach was successful in identifying three lineages that are sister or closely related to Caribbean *Myotis*, but available genetic data do not provide support for the taxonomic names given to these specimens (Figures 2 & 4 and Table 2). Of these, the specimens with the

smallest genetic divergences to Caribbean *Myotis* were restricted to northern South America. A review of the potential taxonomic classification of each of these lineages is beyond the scope of this study and will require genetic data from or near type localities of several species of *Myotis*. However, since our molecular sampling included specimens of *M. nesopolus* from Bonaire (near the species' type locality of Curaçao; Miller 1900), we are able to provide a brief assessment of the Venezuelan lineage that is sister to *M. nesopolus* (Figures 2 & 4).

Two specimens from northern Venezuela have a close genetic relationship (4.1%, Table 2) to *M. nesopolus nesopolus* and likely represent individuals of the traditionally recognized taxon *M. nesopolus larensis*. Measurements and external characteristics of one of these individuals (TTU 48161 from Calabozo, Venezuela) are within the range of measurements provided by Genoways and Williams (1979) and LaVal (1973) for *M. n. larensis*. Moreover, our specimen matched the external morphology for *M. n. larensis*, as well as the ratios of tibia and skull length to forearm length (characteristics identified by LaVal (1973)). LaVal (1973) also noted that the high ratios of tibia and skull to forearm readily distinguish *M. n. larensis* from *nigricans*, *albescentis*, and *keaysi*, as do the absolute length of forearm and tibia. With the given morphological descriptors and the genetically based relationship suggested, the individual from Calabozo, Venezuela, extends the range of *M. n. larensis* further southeast in Venezuela (previously known from the states of Falcón, Lara, and Zulia; LaVal 1973; Wilson 2008). The genetic distance value separating *M. n. larensis* from *M. n. nesopolus* (4.1%) is greater than would typically be expected of subspecific variation within mammalian taxa (Bradley and Baker 2001). Thus, further analyses on the taxonomic status of *M. n. larensis* is merited.

Minimally, our data indicate the geographic boundaries of species complexes, like *M. nigricans*, are not as expansive as currently thought. *Myotis nigricans* is hypothesized to occur in the Caribbean, but the type locality of *M. nigricans* is in southeast Brazil and no molecular sequence data from Caribbean *Myotis* are similar to the sequence of *M. nigricans* collected from Brazil. In fact, the purported Neotropical-wide distribution (Simmons 2005; Wilson 2008) of the small brown bat that has been repeatedly referred to as *M. nigricans* is not supported by the available DNA sequence data. Our genetic data indicate that *M. nigricans* is not present on Grenada and that specimens referable to *M. nigricans* from Tobago and Suriname are distantly related to Brazilian *M. nigricans* (Figure 2 and Table 2). These observations provide evidence that the widespread *M. nigricans* is likely comprised of several morphologically similar or indistinguishable (LaVal 1973; i.e. cryptic species, see Bickford et al. 2007), but genetically distinct species (Baker and Bradley 2006; Aires 2008; this study). Our data clearly demonstrate that an extensive review of the genetic diversity within Neotropical *Myotis* is warranted.

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Appendix A. Supplementary data — Supplementary data associated with this article can be found following the References.

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Appendix A.

Specimens examined for morphological comparisons and/or genetic analyses (cytochrome-*b* = *, Rag 2 = §, AFLP = †, and ♂ or ♀ for morphological analyses). GenBank Accession numbers are listed and indicate the gene sequence (cytochrome-*b*, Rag 2). Previously published GenBank specimens appeared in: Ruedi and Mayer (2001), Kawai et al. (2003), and Stadelmann et al. (2004a, 2007). Abbreviations and acronyms for institutions or individual collections are as follows: American Museum of Natural History (AMNH); Carnegie Museum of Natural History (CM); C. M. Francis (CMF); François M. Catzefflis, Université de Montpellier: Laboratoire de Paléontologie, France (Catzefflis-Montpellier-V-); Field Museum of Natural History (FMNH); Jean-François Maillard (JFM DIREN); the University of Kansas Natural History Museum (KU); Natural History Museum of Los Angeles County Museum (LACM); Museum National Histoire Naturelle, Paris, France (MNHN); Museum of Vertebrate Zoology (MVZ); Manuel Ruedi (MR); Smithsonian Institution National Museum of Natural History (NMNH); Rijksmuseum van Natuurlijke Historie (RMNH); Royal Ontario Museum (ROM); T. H. Kunz (THK); Museum of Texas Tech University (TTU = voucher specimen, TK = tissue); Universidad Autonoma Metropolitana-Iztapalapa (UAMI); University of Nebraska State Museum (UNSM); and United States National Museum (USNM), now NMNH.

Myotis albescens.—BOLIVIA: Tarija (FMNH 162543), AF376839 *, AM265661 §.

M. alcathoe.—SWITZERLAND: Vaud (MHNG 1828.73), AJ841955 *.

M. atacamensis.—PERU: Olmos (MVZ 168933), AM261882 *, AM265640 §.

M. austroriparius.—USA: Tennessee (THK 002), AM261885 *, AM265642 §.

M. brandtii.—RUSSIA: North West (NMP PB916), AM261886 *, AM265647 §.

M. californicus.—MEXICO: Durango (CDR 3276), AM261887 *, AM265649 §.

M. chiloensis.—CHILE: Santiago (THK 1), AM261888 *, AM265651 §.

M. dominicensis.—DOMINICA: St. Joseph's Parish (TK 15613), AF376848 *, AM265654 §; (TTU 31508, TK 15620), JNo20556 *, (TTU 31510, TK 15622 †); 1 ♂ 4 ♀ (TTU 31504); (TTU 31489, TK 15629 †); (TTU 31507, TK 15619 †), JNo20555 *, JNo20582 §; (TTU 31513, TK 15625 †); (TTU 31519, TK 15577 †), JNo20554 *.

M. elegans.—COSTA RICA: La Selva (MR 3011), AM261891 *, AM265655 §.

M. gracilis.—JAPAN: Hokkaido (KK 005), AB106609 *, AM265660 §.

M. grisescens.—USA: Tennessee (THK 11500), AM261892 *, AM265664 §.

M. keaysi.—MEXICO: Yucatan (TK 13532), AF376852 *, AM265668 §.

M. latirostris.—TAIWAN: Moi-Li Co (MR-608), AM262330 *.

M. levis.—BRAZIL: Sao Paulo (FMNH 141600), AF376853 *, AM265672 §.

M. martiniquensis.—MARTINIQUE: (JFM DIREN 2), AM262332 *, AM265676 §; Le Morne-Rouge (MNHN: 2005-895), JNo20557 *, JNo20578 §; (MNHN: 2005-896 †), JNo20558 *, JNo20579 §. Grand'Rivière (Catzefflis-Montpellier-V-2246 †); (Catzefflis-Montpellier-V-2352), JNo20559 *, (MNHN: 2008-974), JNo20560 *, (Catzefflis-Montpellier-V-2354), JNo20561 *. La Trinité 2 ♂ 3 ♀ (AMNH 214067); (AMNH 214068); (AMNH 214064); (AMNH 214065); (AMNH 214066).

M. nesopolus nesopolus.—NETHERLANDS, DUTCH CARIBBEAN: Bonaire (TK 128151), JNo20575 *; (TK 128152), JNo20576 *; (TK 128153), JNo20577 *.

M. sp.—VENEZUELA: Barinas (CM 78645, TK 19538 †). Guárico: Calabozo (TK 15035), JNo20568 *, 1 ♀ (TTU 48161, TK 15074 †), JNo20569 *, JNo20585 §.

M. nigricans.—BRAZIL: Paraiba (MVZ AD50), AF376864 *, (MVZ 185681), AM265682 §.

M. oxyotus.—PERU: Lima (FMNH 129208), AF376865 *, AM265684 §.

M. riparius.—BRAZIL: Pernambuco (MVZ AD119), AF376866 *, (MVZ 185996), AM265687 §.

M. ruber.—BRAZIL: Salesopolis (MVZ AD472), AF376867 *, (MVZ 185999), AM265688 §.

M. simus.—BRAZIL: (THK Mys-ET3), AM262336 *, AM265691 §.

M. sp.—SURINAME: Nickerie: Apoera 1 ♂ (CM 63933, TK 10123), JNo20570 *. Para: Zanderij 1 ♂ 1 ♀ (CM 69053, TK 17272 †), JNo20571 *, JNo20586 §; (CM 77699, TK 21050), JNo20572 *.

M. sp.—TOBAGO: St. George Parish (UNSM ZM-29470, TK 157625 †), JNo20573 *, JNo20580 §; (UNSM ZM-29483, TK 157638 †), JNo20574 *, JNo20581 §.

M. velifer.—MEXICO: Sonora (MVZ 146766), AF376870 *, AM265695 §.

M. vivesi.—MEXICO: Baja California, AJ504407 *, AM265696 §.

M. yumanensis.—USA: California (MVZ 155853), AF376875 *, AM265700 §.