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Original Investigation

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*.

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 Fig. 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 Fig. 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

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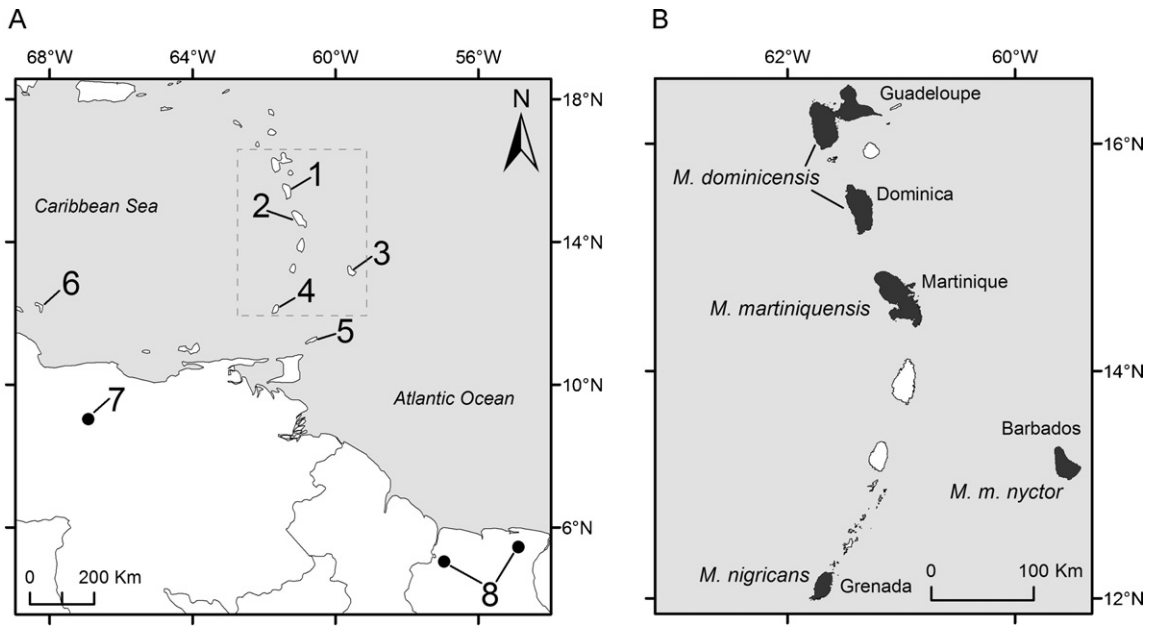

Fig. 1. Map of the Lesser Antilles and northern South America (A), and the islands where Lesser Antillean *Myotis* are distributed (B; shaded in black). Numbers 1–8 indicate the sampling localities: 1 – Dominica, 2 – Martinique, 3 – Barbados, 4 – Grenada, 5 – Tobago, 6 – Bonaire, 7 – Venezuela, and 8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

view of the evolutionary history of Caribbean *Myotis* and closely related mainland species.

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).

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

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 mm^2) 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, 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 (Gene Codes