Phylogeography of Dominican Republic bats and implications for systematic relationships in the Neotropics

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The majority (90%) of native terrestrial mammal species living in the Dominican Republic are bats, and two-thirds of these species are endemic to the Caribbean. However, recent molecular studies using DNA barcoding of the mitochondrial cytochrome c oxidase subunit 1 gene have suggested at least a 25% underestimation of biodiversity in bats throughout the world. A recent survey of bats in the Dominican Republic documented 15 of the 18 known species on the island of Hispaniola. Phylogenetic analysis of 132 individuals resulted in well-supported monophyletic species-level clades (maximal bootstrap values) with intraspecific variation ranging from 0% to 4.7% and interspecific variation ranging from 14.1% to 32.5%. A phylogeographic pattern separating the northern and southern Dominican Republic was recovered in 3 species of bats (*Macrotus waterhousii*, *Pteronotus parnellii*, and *Pteronotus quadridens*). The inclusion of broader geographic sampling across the Neotropics indicated that 3 widely distributed species (*Eptesicus fuscus*, *Molossus molossus*, and *Monophyllus redmani*) have high sequence divergence among insular or between insular and continental populations. Further systematic study is needed to identify morphologically cryptic species and their implications for conservation priorities in the Caribbean.

La mayoría (90%) de los mamíferos terrestres nativos que viven en la República Dominicana son murciélagos, y dos tercios de estas especies son endémicas en el Caribe. Sin embargo, estudios moleculares recientes que utilizan código de barras de ADN del gen mitocondrial citocromo c oxidasa subunidad 1, han sugerido que existe al menos una subestimación del 25% de la biodiversidad en los murciélagos de todo el mundo. Un reciente estudio de los murciélagos en la República Dominicana documentó 15 de las 18 especies conocidas en la isla de La Hispaniola. El análisis filogenético de 132 individuos resultó soportando clados monofiléticos a nivel de especie (valores máximos con el método Bootstrap) con variación intraespecífica de 0–4.7% y variación interespecífica de 14.1–32.5%. Un patrón filogeográfico que separa el norte y el sur de la República Dominicana fue recuperado en 3 especies de murciélagos (*Macrotus waterhousii*, *Pteronotus parnellii*, y *Pteronotus quadridens*). La inclusión de un muestreo geográfico más amplio a través del Neotrópico indicó que 3 especies de amplia distribución (*Eptesicus fuscus*, *Molossus molossus*, y *Monophyllus redmani*) tienen alta divergencia de secuencia entre las poblaciones insulares o entre poblaciones insulares y las continentales. Se necesitan más estudios sistemáticos para identificar morfológicamente especies crípticas y sus implicaciones para las prioridades de conservación en el Caribe.

Key words: Caribbean, Chiroptera, cryptic species, DNA barcoding, Hispaniola

In the Greater Antilles of the western Caribbean, bats account for most (74%) of the 58 species of nonextinct indigenous land mammals and a majority of these (72%) are endemic (Dávalos and Turvey 2012). The Dominican Republic and Haiti comprise the island of Hispaniola, the 2nd largest in the region; however, its bat fauna is not well known relative to neighboring countries (Cuba—Silva Taboada 1979; Puerto Rico—Gannon et al. 2005, and Jamaica—Genoways et al. 2005, although a species checklist is provided by Dávalos and Turvey 2012). Furthermore, a recent molecular study of mammals in the Neotropics suggested

that the species diversity is underestimated by at least 25% (Lim 2012), which is a dynamic exacerbated by undersampling of taxa in the Caribbean region for the DNA barcoding reference database based upon the mitochondrial gene cytochrome oxidase c subunit 1 (COI). A better understanding of the extent of biodiversity in the Caribbean will have important implications to the conservation of this hotspot of endemism. Of the 31 species of bats found only in the Greater Antilles, 13 are on the International Union for the Conservation of Nature's Red List of Threatened Species. Although the extinction rate is not as high as that for nonvolant mammals, 12 species of bats have become extinct during the Late Quaternary, which represents 22% of total known diversity in this region (Dávalos and Turvey 2012).

A 1st step to fill this Caribbean geographic gap in DNA barcodes recently was undertaken for Jamaica (Lim and Arcila Hernandez 2016). They found phylogeographic patterns that included the identification of deep divergence (> 4%) among island and mainland populations within 3 species (*Glossophaga soricina*, *Molossus molossus*, and *Pteronotus parnellii*) widely occurring across the Neotropics. Herein, we extend the sampling coverage to the Dominican Republic where 18 species of bats have been documented, of which 12 are endemic to the Caribbean, including 2 that occur only in Hispaniola (Dávalos and Turvey 2012). The objectives of this study are to: 1) assess the genetic diversity of bats from across the Dominican Republic, 2) analyze the phylogeography of conspecific populations in the Caribbean, and 3) more broadly examine the systematic relationships of closely related species throughout the Neotropical region.

MATERIALS AND METHODS

Fieldwork was conducted from 20 January to 9 February 2015 to survey bat species diversity across the Dominican Republic.

Capture methods for bats included the use of 1 triple-high canopy mist net system, 1 harp trap, and up to 4 understory mist nets set in the vicinity of caves, forests, and buildings. Nets and traps were set at different localities during each of the 20 survey nights. This study conformed to the animal care and use guidelines of the American Society of Mammalogists (Sikes et al. 2011) and was approved by the Animal Use Committee of the Royal Ontario Museum (ROM), Toronto, Ontario, Canada. A total of 160 specimens representing 15 species of bats were collected during fieldwork with materials deposited at both the ROM and Museo Nacional de Historia Natural in Santo Domingo, Dominican Republic. DNA barcodes were successfully sequenced from 132 specimens (Table 1) and deposited in Genbank under accession numbers KX354998–KX355129.

In chronological order, the general localities within a 1-km radius (see Supplementary Data SD1), and secondary localities if present, include description, latitude, longitude, and elevation (Garmin GPSmap 76CSx with WGS84 datum): 1) Santo Domingo, Jardín Botánico, 18.49399, −69.95606, 50 m; 2a) 9 km NE of Pedernales, 18.08317, −71.67085, 380 m; 2b) 15 km N of Pedernales, 18.16949, −71.76516, 351 m; 2c) 1 km SE of Aguas Negras, 18.16282, −71.69053, 884 m; 3) Cueva de Marazate, 14 km SE of Pedernales, 17.96624, −71.63553, 12 m; 4) Cueva de los Patos, 17.96002, −71.18325, 13 m; 5) Cueva del Pomier, 18.46698, −70.1358; 6a) Parque Nacional Armando Bermúdez, headquarters, 19.06727, −70.86323, 1113 m; 6b) Parque Nacional Armando Bermúdez, 1 km S of Los Tablones trail, 19.0632, −70.86419, 1134 m; 7) Cueva Honda de Julián, 4 km E of Plantanal, 19.13234, −70.0796, 68 m; 8) Cueva la Chepa, 14 km NE of Bayaguana, 18.86914, −69.5763, 187 m; 9a) Miches, Hotel Coco Loco, 18.98384, −69.03708, 0 m; 9b) 7 km SE of Miches, 18.9254, −69.00776, 55 m; 9c) 10 km NE of Miches, 19.02935, −68.94881, 0 m; 10a) Laguna Bávaro, 18.64782, −68.36404, 0 m; 10b) Cueva

Table 1.—Sample size of specimens sequenced for cytochrome c oxidase subunit 1 in 15 species of bats from the Dominican Republic. Localities are 1) Santo Domingo, Jardín Botánico; 2a) 9 km NE of Pedernales; 2b) 15 km N of Pedernales; 2c) 1 km SE of Aguas Negras; 3) Cueva de Marazate; 4) Cueva de los Patos; 5) Cueva del Pomier; 6a) Parque Nacional Armando Bermúdez, headquarters; 6b) Parque Nacional Armando Bermúdez, 1 km S of Los Tablones trail; 7) Cueva Honda de Julián; 8) Cueva la Chepa; 9a) Miches, Hotel Coco Loco; 9b) 7 km SE of Miches; 9c) 10 km NE of Miches; 10a) Laguna Bávaro; 10b) Cueva Taina; 10c) Barceló Bávaro Resort Golf Course; 10d) Barceló Bávaro Resort Golf Course Road; 11a) Laguna Yauya, Reserva Ecológica Ojos Indígenas, Punta Cana; and 11b) Punta Cana Ecological Foundation.

Species	1	2a	2b	2c	3	4	5	6a	6b	7	8	9a	9b	9c	10a	10b	10c	10d	11a	11b
Artibeus jamaicensis	1	2	2	2		1				1	1	2		2	2	3	2	3	2	
Brachyphylla pumila						3														
Eptesicus fuscus			1					1												
Erophylla bombifrons			1		3	3				3					1				2	1
Lasiurus borealis			3				1													
Macrotus waterhousii		1		2	3						3					3				
Molossus molossus	4											4								
Monophyllus redmani				3			2	2		3	3	1								1
Mormoops blainvillei				3						3	3									
Natalus major											1									
Noctilio leporinus														3						
Phyllonycteris poeyi																	2			
Phyllops falcatus	2		1				1	1							1	1		3	3	1
Pteronotus parnellii			1							3	3									
Pteronotus quadridens		1								3						3		1	3	1

Taina, 3 km S of Macao, 18.76605, −68.54824, 10 m; 10c) Barceló Bávaro Resort Golf Course, 18.65971, −8.39421, 0 m; 10d) Barceló Bávaro Resort Golf Course Road, 18.65277, −68.40011, 0 m; 11a) Laguna Yauya, Reserva Ecológica Ojos Indígenas, Punta Cana, 18.50922, −68.37421, 6 m; and 11b) Punta Cana Ecological Foundation, 18.52531, −68.3808, 10 m.

Molecular analyses were done in the Laboratory of Molecular Systematics at the ROM on the mitochondrial DNA COI gene, with DNA extraction, COI amplification, and nucleotide sequencing following the DNA barcoding protocol outlined for Neotropical bats (Clare et al. 2007). An additional 1,271 sequences of conspecific and closely related species were downloaded from Genbank for comparative study (see Supplementary Data SD2).

Analyses of genetic variation among sequences of bats from the Dominican Republic and other species from across the Neotropics were conducted with MEGA6 (Tamura et al. 2013). To enable comparison to the mitochondrial cytochrome b gene, which has a similar rate of evolution and performance in recovering phylogenies as COI (Zardoya and Meyer 1996; Pfunder et al. 2004; Baker and Bradley 2006), the Kimura 2-parameter model of nucleotide substitution was used to calculate percent sequence divergence between clades. The neighbor-joining and maximum likelihood methods with midpoint rooting using 500 bootstrap replications were used to assess phylogenetic relationships. Neighbor-joining trees were constructed using the Kimura 2-parameter model, as is typically done for DNA barcoding (e.g., Clare et al., 2007). Maximum likelihood trees were based on the best-fit model as calculated in MEGA6.

RESULTS

Our 20-day survey of bats in the Dominican Republic recorded 15 of the 18 known species (Table 1). Cueva Honda de Julian had the highest number of species at 7 and the clearings near Hotel Coco Loco in Miches documented only 1 species. Up

to 3 specimens per species from each site were prepared as part of the representation collection, except for 4 specimens of *M. molossus* from 2 sites. Other individual bats caught above this operational limit were identified and released in the vicinity. However, we were not marking the bats and some caves had large populations that did not make it practical for comparing sites. Similarly, it was difficult to compare sampling methods as harp traps and mist nets set near caves typically had higher numbers of captures that were not conducive for meaningful comparisons.

Phylogenetic analyses of COI based on neighbor-joining and maximum likelihood methods recovered 15 monophyletic clades that were well supported (bootstrap values of 100%) and assignable to recognized species in the Dominican Republic (see Supplementary Data SD3). Both reconstruction methods gave similar tree topologies in all analyses for branches with > 50% bootstrap support except that neighbor-joining trees had greater support for sister species relationship of *Pteronotus gymnonotus* and *P. fulvus*, and monophyly of *Monophyllus redmani*.

For Dominican Republic samples, interspecific variation ranged from 32.5% between *Erophylla bombifrons* and *Lasiurus minor* to 14.1% between *Artibeus jamaicensis* and *Phyllops falcatus* (Table 2). Intraspecific variation ranged from 4.7% within *Macrotus waterhousii* to 0% within *Noctilio leporinus* and *Phyllonycteris poeyi* (*Natalus major* had only 1 sample). In the phylogenetic trees, terminal taxon clades show relative sample size by vertical height and depth of divergence by horizontal distance. The "barcode gap" between and within species is 3×, i.e., the highest average intraspecific variation is 3 times greater than the lowest average interspecific variation within the Dominican Republic.

Phylogeographic analyses within individual species using samples from the Dominican Republic then were compared to the variation in DNA barcodes of other populations from across the Neotropics. There were 9 species that did not exhibit any

Table 2.—Average pairwise nucleotide substitutions based on Kimura 2-parameter model within species (diagonal) and between species (lower matrix) of bats from the Dominican Republic. Species abbreviations are: Aj = *Artibeus jamaicensis*; Bp = *Brachyphylla pumila*; Ef = *Eptesicus fuscus*; Eb = *Erophylla bombifrons*; Lm = *Lasiurus minor*; Mw = *Macrotus waterhousii*; Mm = *Molossus molossus*; Mr = *Monophyllus redmani*; Mb = *Mormoops blainvillei*; Nm = *Natalus major*; Nl = *Noctilio leporinus*; Ppo = *Phyllonycteris poeyi*; Pf = *Phyllops falcatus*; Ppu = *Pteronotus pusillus*; Pq = *Pteronotus quadridens*.

	Aj	Bp	Ef	Eb	Lm	Mw	Mm	Mr	Mb	Nm	Nl	Ppo	Pf	Ppu	Pq
Aj	0.0001														
Bp	0.190	0.006													
Ef	0.234	0.271	0.006												
Eb	0.255	0.247	0.304	0.005											
Lm	0.268	0.277	0.250	0.325	0.004										
Mw	0.212	0.220	0.245	0.283	0.237	0.047									
Mm	0.232	0.223	0.231	0.281	0.223	0.230	0.001								
Mr	0.228	0.202	0.277	0.264	0.249	0.240	0.250	0.008							
Mb	0.223	0.236	0.270	0.270	0.286	0.256	0.262	0.244	0.004						
Nm	0.252	0.250	0.265	0.309	0.244	0.233	0.199	0.264	0.263						
Nl	0.236	0.226	0.285	0.263	0.252	0.233	0.238	0.259	0.240	0.243	0				
Ppo	0.233	0.239	0.298	0.149	0.327	0.276	0.255	0.270	0.293	0.291	0.271	0			
Pf	0.141	0.193	0.269	0.249	0.262	0.225	0.244	0.251	0.231	0.243	0.229	0.241	0.007		
Ppu	0.217	0.225	0.239	0.272	0.254	0.229	0.214	0.240	0.218	0.253	0.217	0.279	0.237	0.010	
Pq	0.256	0.238	0.274	0.271	0.277	0.241	0.237	0.249	0.232	0.254	0.234	0.283	0.249	0.195	0.002

obvious geographic structuring and had low sequence divergence among individuals (< 2%). However, 6 species with distinctive phylogeographic patterns and intraspecific sequence divergence > 2% warranted further systematic scrutiny. *Eptesicus fuscus* from the Dominican Republic forms a well-supported monophyletic clade with an average of 8% sequence divergence from conspecific populations in continental North America (see Supplementary Data SD4). *M. molossus* from the Dominican Republic forms a well-supported monophyletic clade with at least 3.7% sequence divergence from other conspecific populations, including Jamaica, and closely related species throughout the Neotropics (Fig. 1A). The Caribbean endemic *M. redmani*, from the Dominican Republic, forms a well-supported monophyletic clade with an average of 8.2% sequence divergence from conspecific individuals in Jamaica (Fig. 1B). However, these 2 lineages form a trichotomy with *Monophyllus plethodon* in the maximum likelihood tree, although *M. redmani* is monophyletic and well supported in the neighbor-joining tree. Individuals of *M. waterhousii* from the northern and southern Dominican Republic are sister clades that

average 7.4% sequence divergence between each other, but we did not have other conspecific samples from Central America and Mexico (Fig. 2A). Similarly, this biogeographic break in Hispaniola also is recovered in 2 species of *Pteronotus*, including *P. pusillus* with 5% sequence divergence between poorly supported non-sister clades of northern and southern populations (Fig. 2A) and *P. quadridens* with 2% sequence divergence between poorly supported non-sister clades of northern and southern populations.

DISCUSSION

Our phylogenetic analyses of DNA barcodes used a fairly complete taxonomic coverage of bats (15 of 18 known species) in the Dominican Republic and relatively broad geographic sampling (20 localities) to identify 6 potential instances of morphologically cryptic species as compared to the taxonomy of Simmons (2005). However, the observed phylogeographic divergence patterns may be a result of limited sample size and localities sampled if the species is genetically diverse but less
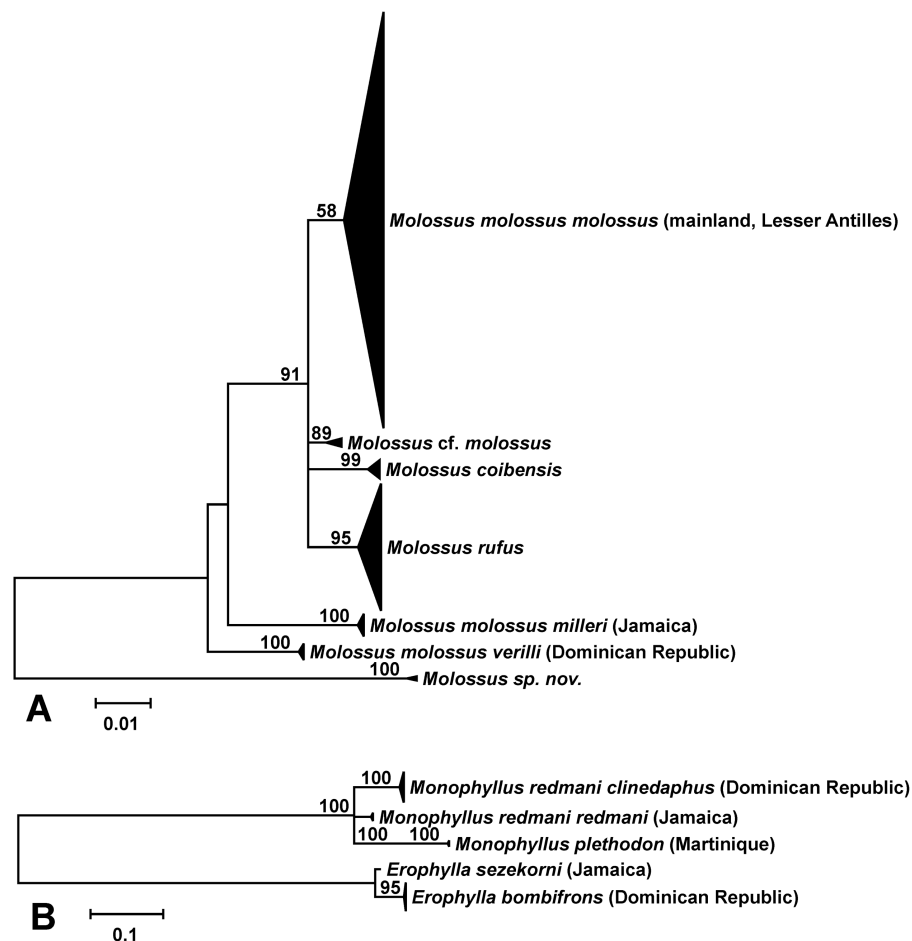
Fig. 1.—Taxonomic island endemism in the Caribbean. A) Maximum likelihood tree based on Hasegawa–Kishino–Yano substitution model with some invariable sites and bootstrap supports > 50% for DNA barcodes of the bat genus *Molossus*. Terminal taxa from the Caribbean are labeled with island localities that were sampled. *Molossus molossus* is paraphyletic in relation to *Molossus rufus* and *Molossus coibensis*. We retained a *Molossus sp. nov.* designation for a new species from Guyana and Ecuador that is being described based on molecular and morphological data. B) Maximum likelihood tree based on Kimura 2-parameter substitution model with some invariable sites and bootstrap supports > 50% for DNA barcodes of the endemic Caribbean bat genera *Monophyllus* and *Erophylla*. Both trees have divergent lineages of populations from Jamaica and the Dominican Republic.
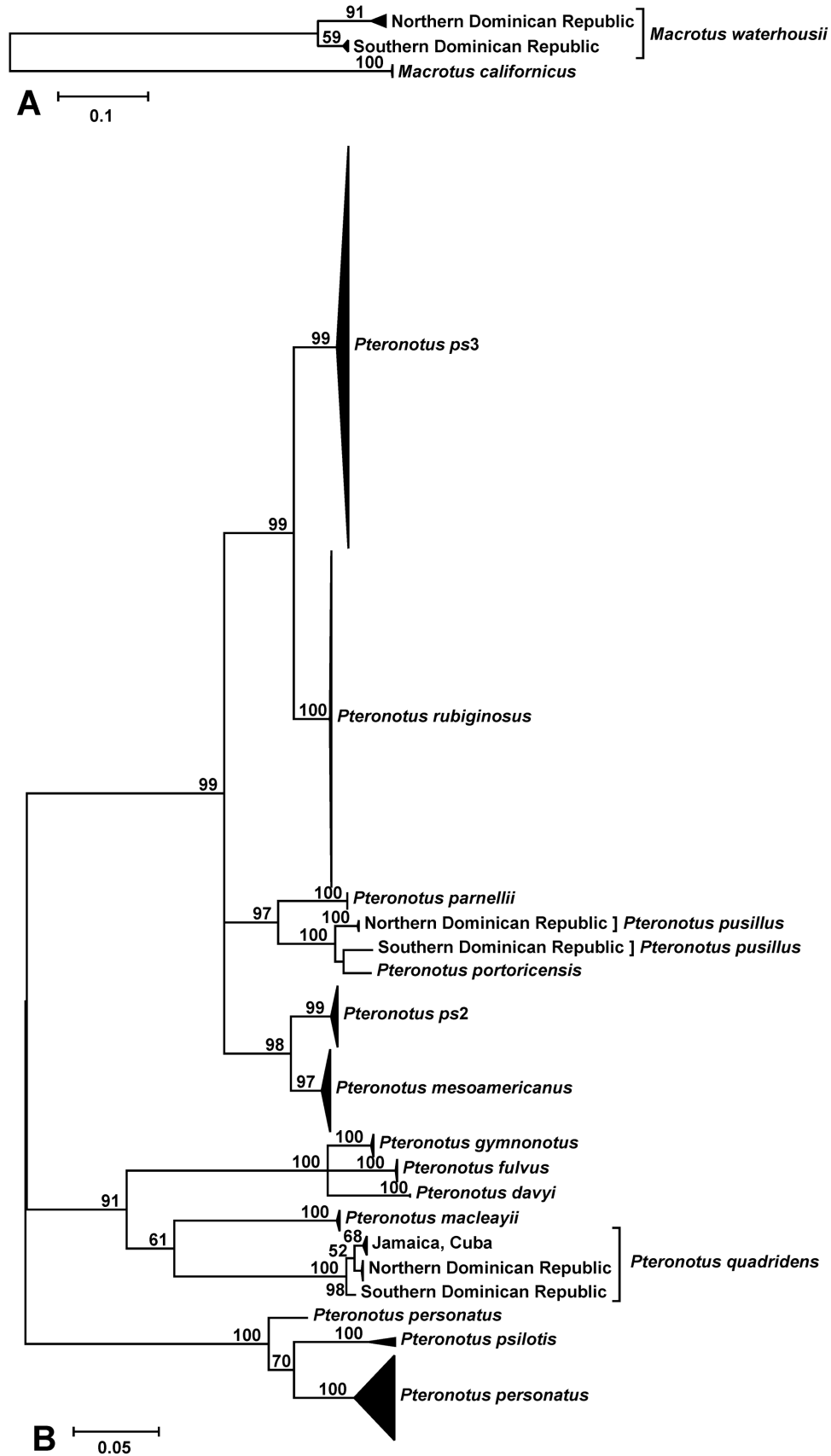
Fig. 2.—Phylogeographic splits between populations from the northern and southern Dominican Republic. A) Maximum likelihood tree based on Hasegawa–Kishino–Yano substitution model with Gamma distribution and bootstrap supports > 50% for DNA barcodes of the bat genus *Macrotus*. B) Maximum likelihood tree based on Tamura 3-parameter substitution model with some invariable sites and bootstrap supports > 50% for DNA barcodes of the bat genus *Pteronotus*. Species follow the names used by de Thoisy et al. (2014).

geographically structured. Dense population-level sampling, as is often done for faunal surveys, is a complimentary approach to typical molecular systematic studies that focus on species-level diversity. For example, earlier systematic research on the family Mormoopidae suggested that populations of *P. parnellii* from the Caribbean were distinct from mainland populations (Lewis-Oritt et al. 2001). However, more comprehensive sampling in Guyana of what was considered 1 species of *P. parnellii* actually found 3 distinct COI lineages (Clare et al. 2011) subsequently identified as *P. rubiginosus* and 2 undescribed species in northern South America (de Thoisy et al. 2014). Similarly, our survey across the Dominican Republic found distinct lineages for northern and southern populations of *P. pusillus*, itself a close relative of the *P. parnellii* complex. This north–south phylogeographic pattern also was recovered for *P. quadridens* and *M. waterhousii*.

The northern and southern palaeo-islands that formed Hispaniola as a result of tectonic activity during the mid-Miocene (~ 10 million years ago) may underlie current phylogeographic structures in bats and other animals (Iturralde-Vinent and MacPhee 1999; Graham 2003). Combined with fluctuations in sea levels, the intervening Neiba Valley (also referred to as the Enriquillo Basin) presently has saline lakes and areas below sea level that periodically are inundated by marine incursions, the last of which occurred in the early Holocene (Taylor et al. 1985). Molecular phylogenetic structuring coinciding with this biogeographic barrier was found in several groups of vertebrates at different taxonomic levels. For example, sister species of chat tanagers in the genus *Calyptophilus* were hypothesized to have arisen in allopatry prior to the fusion of the palaeo-islands in the mid-Miocene (Townsend et al. 2007). Two other species of birds, *Todus angustirostris* and *Microligea palustris*, have a Pleistocene divergence between northern and southern populations, suggesting a salt water barrier to dispersal (Sly et al. 2011). Populations of lowland teiid lizards (*Ameiva chrysolaema*) correspond to the southern, central, and northern Dominican Republic that were separated by the 2 palaeo-island mountain ranges during the Pliocene–Pleistocene approximately 1.6 mya (Gifford et al. 2004). Additionally, the Hispaniolan hutia (*Plagiodontia aedium*) has distinct genetic populations in the north and south of the island that were estimated to have diverged about 0.6 mya (Brace et al. 2012), with the southeastern population now described as a new subspecies *P. a. bondi* (Turvey et al. 2015).

Our report herein of north–south phylogeographic structuring in Hispaniolan bats broadens support for the hypothesis that the geological history of palaeo-islands has influenced the current biodiversity of many resident vertebrate groups, but it also highlights the need for more detailed analyses of population genetic structure. The wide range of sequence divergences (2–7%) within *P. pusillus*, *P. quadridens*, and *M. waterhousii* suggests that they did not all differentiate at the same time and in response to the same geological events. A further study of molecular dating would address this issue of divergence times and help to resolve the taxonomic status of potential cryptic species.

On a broader geographic scale, *M. redmani* has deep divergence (8.2%) between populations from Dominican Republic and Jamaica. This level of divergence is similar to that expected for sister species based on another mitochondrial protein-coding gene cytochrome b (Baker and Bradley 2006). Jamaican populations also are larger in cranial measurements than populations from Dominican Republic (Schwartz and Jones 1967). The species type locality is in Jamaica and the subspecies *M. r. clinedaphus* Miller 1900 occurs in Cuba, Hispaniola, and the Bahamas (Schwartz and Jones 1967). Although that subspecies type locality is unknown, it was restricted to Baracoa, Cuba, by Schwartz and Jones (1967), and has priority over the synonym *M. cubanus* Miller 1902 from the same locality. Another synonym originally described as the subspecies *M. cubanus ferreus* Miller 1918 has its type locality located in southwestern Haiti. However, populations from Cuba, Hispaniola, and the Bahamas are similar in size (Schwartz and Jones 1967; Buden 1975). Additional DNA barcodes of a 3rd subspecies *M. r. portoricensis* from Puerto Rico would greatly clarify the genetic variation in this species as members of this subspecies are the morphologically smallest of the species (Schwartz and Jones 1967).

Another example of cryptic island endemism is found in the species complex of *M. molossus*. It is widely distributed across the Neotropics with a complicated taxonomic history (Dolan 1989; Eger 2008). In relation to *M. coibensis* and *M. rufus*, *M. molossus* is polyphyletic with populations from the Greater Antilles more distantly related from other species of *Molossus* than to putative conspecifics (Fig. 1A). The type locality of *M. molossus* was restricted to Martinique (Husson 1962) and an individual from this island of the Lesser Antilles nests within the continental mainland clade of the COI barcoding tree of our analysis. The populations from the Dominican Republic and Jamaica are minimally 3.7% divergent from other conspecifics, which is the lower range of sister species for cytochrome b (Baker and Bradley 2006). More comprehensive analyses incorporating nuclear genes and morphological characters are needed, but available taxonomic names for Greater Antillean forms include *M. verrilli* Allen 1908 for the Dominican Republic and *M. milleri* Johnson 1952 for Jamaica.

The average genetic divergence (8%) within the widely distributed *E. fuscus* is also substantial, but the COI sampling is incomplete both geographically (it is widespread in North America) and taxonomically (not all subspecies sampled). There is a subspecies name assignable to each of the 4 divergent barcoding lineages (see Supplementary Data SD4): *E. f. fuscus* Palisot de Beauvois 1796 from Ontario; *E. f. bernardinus* Rhoads 1902 from British Columbia; *E. f. pallidus* Young 1908 from Arizona; and *E. f. hispaniolae* Miller 1918 from Dominican Republic. However, there are 7 other subspecies (Kurta and Baker 1990) that do not have DNA barcodes and geographic sampling for molecular data needs to be improved before taxonomic conclusions can be made.

Of the 15 currently recognized species of bats from the Dominican Republic that were DNA barcoded, 6 have phylogeographic structuring warranting more detailed taxonomic scrutiny. There is a biogeographic split between the northern and southern palaeo-islands of Hispaniola for populations in *M. waterhousii*, *P. pusillus*, and *P. quadridens*. *M. molossus* and *M. redmani* each have high sequence divergence between the Dominican Republic and Jamaica suggesting an increase in cryptic island endemism in the Caribbean. *E. f. hispaniolae*

has affinities to North America but there is deep divergence and poor representation of COI sequences for the other subspecies that is hindering any substantive taxonomic statements at this time. Overall underestimation of species diversity for ≤ 40% of the species sampled (6 of 15) highlights the fact that further systematic study will require the integration of nuclear genetic data, more thorough phylogenetic analyses, and increased geographic sampling in order to better understand Hispaniolan biodiversity and its implications to Caribbean conservation.

Supplementary Data

Supplementary Data SD1.—Map of the Dominican Republic with provincial boundaries and 11 general collecting localities surveyed for bats from 20 January to 9 February 2015. Black line represents the Neiba Valley separating the Late Pleistocene northern and southern palaeo-islands of Hispaniola. See "Materials and Methods" for full descriptions of localities: 1) Santo Domingo, 2) Pedernales, 3) Cueva de Marazates, 4) Cueva de los Patos, 5) Cueva del Pomier, 6) Parque Nacional Armando Bermúdez, 7) Cueva Honda de Julián, 8) Cueva la Chepa, 9) Miches, 10) Bávaro, and 11) Punta Cana.

Supplementary Data SD2.—Specimens used in the DNA barcoding analysis of bats from the Dominican Republic with sequences deposited in Genbank.

Supplementary Data SD3.—Maximum likelihood tree based on General Time Reversible substitution model with Gamma distribution and some invariable sites of COI DNA barcodes of Dominican Republic bats (bootstrap supports > 50% annotated on branches).

Supplementary Data SD4.— Maximum likelihood tree based on Tamura 3-parameter substitution model with some invariable sites and bootstrap supports > 50% for DNA barcodes of the bat genus *Eptesicus* from the New World.

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