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Phylogeography of *Marmosa robinsoni*: insights into the biogeography of dry forests in northern South America

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Robinson's mouse opossum (*Marmosa robinsoni*) typically inhabits xeric shrublands, savannas, and deciduous forests from Panama through Colombia and Venezuela, to the islands of Trinidad, Tobago, and Grenada. We assessed its phylogeographic structure in the 1st such study based on dense geographic sampling of any vertebrate from dry habitats in this region. We sequenced the cytochrome-*b* gene and the X-linked intron O-linked N-acetylglucosamine transferase, largely from dried skins and residual tissue on osteological material of museum specimens. Phylogenetic analyses revealed the existence of 2 well-supported phylogroups primarily distributed to the east and west of the Cordillera de Mérida. The estimated time since divergence between these phylogroups postdates the Miocene; therefore, Andean uplifts, changes in the course of the Río Orinoco, and marine transgressions of that epoch cannot be implicated as causal vicariant agents. Instead, expansion of humid forest or marine transgressions, or both, during the Pliocene and Pleistocene more likely led to this differentiation. We encountered little structure among populations east of the Cordillera de Mérida, suggesting recent range expansion in this region. Surprisingly, isolated populations from the Península de Paraguaná (northwestern Venezuela) are not closely related to geographically proximate mainland populations, but rather to more distant populations to the west in Colombia and Panama. By contrast, populations from central and eastern Venezuela are closely related to those on the islands of Trinidad and Tobago. This genetic similarity among currently disjunct populations of *M. robinsoni* might have resulted from Holocene fragmentation of a more extensive Pleistocene distribution on coastal plains that were exposed during the last glacial maximum.

Key words: Andes, Didelphidae, dry habitats, glaciations, land bridges, marine transgressions, *Marmosa*, Paraguaná, Trinidad and Tobago, Venezuela

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In contrast to the biota of Neotropical rain forests, the fauna and flora distributed in seasonally dry habitats—both forests and savannas—of northern South America have received little attention from biogeographers and phylogeographers. In South

America, seasonally dry (deciduous) forests occur where rainfall is less than 1,600 mm/year, with 5 or more months receiving less than 100 mm (Gentry 1995; Graham and Dilcher 1995), whereas savannas are found under similar or slightly wetter conditions, but on poorer soils (Sarmiento 1992; Pennington et al. 2000). The largest areas with seasonally dry vegetation in South America include the Arid Diagonal (Caatinga, Cerrado, and Chaco—Vanzolini 1974; Prado and Gibbs 1993), and a less familiar series of semiarid landscapes in northern South America that includes the Venezuelan and Colombian Llanos, the inter-Andean valleys of Colombia, and parts of the Caribbean coast of both countries (Pennington et al. 2000). Similar vegetation also occurs on adjacent Caribbean islands, such as Aruba, Bonaire, and Isla Margarita.

Whereas the biota of the Arid Diagonal has been the focus of several biogeographic studies (e.g., Mares et al. 1985; Almeida et al. 2007; Caramaschi et al. 2011; Nascimento et al. 2011; Turchetto-Zolet et al. 2013), similar attention has not been given to the biota inhabiting seasonally dry habitats of northern South America. A few studies focused on organisms widely distributed in the Neotropics have included samples from these habitats, but most lack sufficiently dense geographic representation to identify historically interesting patterns of genetic or phenotypic character variation within this region (e.g., Salazar-Bravo et al. 2001; Wüster et al. 2005; Bradley et al. 2008). In 1 exception, based on geographic patterns of morphological variation in rodents and lagomorphs, Voss (1991) hypothesized the historical importance of the moist-forested northern Andean cordilleras (e.g., the Cordillera de Mérida and the Cordillera de la Costa) as potential barriers to gene flow for small mammals that inhabit the dry forests and savannas of this region.

More extensive phylogeographic focus on northern South America is warranted because several major events of biogeographic interest occurred in this region, including the uplift of northern Andean cordilleras, changes in the course of the Río Orinoco, expansion and contraction of vegetation types, and sea-level changes with concomitant marine introgressions and appearances and disappearances of land bridges between mainland South America and certain Caribbean islands (Webb and Bartlein 1992; Hoorn et al. 1995; Rasänen et al. 1995; Díaz de Gamero 1996; Paxton et al. 1996; Lovejoy et al. 1998; Turchetto-Zolet et al. 2013). In particular, recent systematic and biogeographic research has highlighted the biogeographic uniqueness of the small-mammal fauna of the Península de Paraguaná (Anderson 2003; Gutiérrez and Molinari 2008; Anderson et al. 2012). This peninsula was an island during marine introgressions (such as the Last Interglacial), and it is currently connected to mainland Venezuela by a narrow and sparsely vegetated isthmus (Istmo de los Médanos—Lara and González 2007; Anderson et al. 2012). However, broad connections previously existed between the Península de Paraguaná and the northern South American mainland during earlier periods of lower sea level, suggesting that the biogeographic history of the peninsula is complex.

Here we report the 1st phylogeographic analyses based on dense geographic sampling of a non-volant vertebrate distributed throughout most of the dry habitats of northern South America. Our focal species, *Marmosa robinsoni* (Robinson's mouse opossum) occurs in eastern Panama, the inter-Andean valley of the Río Magdalena in Colombia, the Río Chama valley in the Venezuelan Cordillera de Mérida, the Venezuelan Llanos, the Caribbean coastal lowlands of both countries, and the adjacent islands of Margarita, Trinidad, Tobago, and Grenada (Rossi et al. 2010; Gutiérrez et al. 2014). This species inhabits deciduous forests, gallery forests surrounded by savannas, and xeric shrublands at elevations from sea level to about 1,200 m. However, *M. robinsoni* also has been collected, possibly in anthropogenic clearings, at a few mesic localities between 1,200 and 2,000 m above sea level, and it inhabits mesic habitat on the isolated Cerro Santa Ana (Península de Paraguaná, Venezuela), where evergreen and cloud forests occur at atypically low elevations (350–700 m—Anderson et al. 2012). Furthermore, it inhabits lowland rain forest on Trinidad and Tobago (an insular niche shift previously observed in other small mammals that inhabit dry forest and savannas on the South American mainland [Voss 1991]). By assessing the phylogeographic structure and divergence times among populations of *M. robinsoni*, we aim to gain insights about the possible impact of geologic and climatic events on the evolution of the biota from dry habitats in northern South America, thus providing testable hypotheses for further comparative phylogeographic studies.

Materials and Methods

Sources of material.—Our analyses are based on sequences of the mitochondrial cytochrome-*b* gene (*Cytb*; ~1.1 kilobases) and the X-linked intron O-linked N-acetylglucosamine transferase (*Ogt*; ~660 base pairs [bp]). We used a total of 39 *Cytb* sequences (38 from *M. robinsoni* and 1 from its sister taxon *M. xerophila*—Gutiérrez et al. 2010) and 9 *Ogt* sequences (8 *M. robinsoni* and 1 *M. xerophila*). We downloaded 6 *Cytb* sequences from GenBank and generated all of the remaining sequences (Table 1). Among the latter were 17 *Cytb* and 5 *Ogt* sequences from freshly preserved tissue samples; however, in order to increase the geographic sampling, we obtained 16 *Cytb* and 4 *Ogt* sequences from DNA extracted and amplified from dried tissue snipped from skins or scraped from skeletal material of museum specimens. We personally examined voucher material for every sequence to determine taxonomic identifications. All generated sequences, along with their voucher specimen numbers, have been deposited in GenBank (KM099919–KM099960). Voucher specimens and associated tissues from which sequences were obtained are housed in the following institutions: American Museum of Natural History (AMNH), New York; Museo de la Estación Biológica de Rancho Grande (EBRG), Maracay, Venezuela; Museum of Southwestern Biology (MSB), University of New Mexico, Albuquerque; United States National Museum of Natural History (USNM),

Table 1.—Sequenced specimens used to assess the phylogeographic structure of *Marmosa robinsoni*.

Taxon	Tissue or DNA no.[a]	Voucher[b]	Locality[c]	*Cytb* (bp)[d]	*Ogt* (bp)[d]
M. robinsoni	—	USNM 541860	Colombia: Huila (1)	192	—
M. robinsoni	—	USNM 280883	Colombia: La Guajira (2)	180	—
M. robinsoni	JRT 1198	AMNH 207766	Colombia: Tolima (3)	402	—
M. robinsoni	NK 101529	MSB 94363*	Panama: Los Santos (4)	1,146	668
M. robinsoni	NK 101606	MSB 94366*	Panama: Los Santos (4)	1,146	—
M. robinsoni	NK 101633	MSB 94368*	Panama: Veraguas (5)	1,146	—
M. robinsoni	NK 101634	MSB 94369*	Panama: Veraguas (5)	1,146	—
M. robinsoni	RSV 2450	AMNH 276741	Trinidad and Tobago: Tobago (6)	1,136	—
M. robinsoni	RSV 2451	AMNH 276742	Trinidad and Tobago: Tobago (6)	1,136	—
M. robinsoni	RSV 2453	AMNH 276744	Trinidad and Tobago: Tobago (6)	1,142	—
M. robinsoni	RSV 2455	AMNH 276746	Trinidad and Tobago: Tobago (6)	1,140	664
M. robinsoni	RSV 2457	AMNH 276748	Trinidad and Tobago: Tobago (6)	1,138	—
M. robinsoni	RSV 2458	AMNH 276749	Trinidad and Tobago: Tobago (6)	1,138	—
M. robinsoni	RSV 2461	AMNH 276752	Trinidad and Tobago: Tobago (6)	1,140	—
M. robinsoni	RSV 2464	AMNH 276755	Trinidad and Tobago: Tobago (6)	1,140	—
M. robinsoni	RSV 2465	AMNH 276756	Trinidad and Tobago: Tobago (6)	1,138	—
M. robinsoni	TRVL 4816	AMNH 206596	Trinidad and Tobago: Trinidad (7)	192	—
M. robinsoni	—	AMNH 169671	Trinidad and Tobago: Trinidad (8)	384	192
M. robinsoni	—	USNM 448524	Venezuela: Apure (9)	206	192
M. robinsoni	—	USNM 314171	Venezuela: Aragua (10)	352	—
M. robinsoni	—	USNM 517270	Venezuela: Aragua (11)	206	—
M. robinsoni	—	USNM 418532	Venezuela: Falcón (12)	206	—
M. robinsoni	RPA 199	EBRG 25349	Venezuela: Falcón (13)	726	—
M. robinsoni	RPA 208	AMNH 276533	Venezuela: Falcón (13)	1,146	—
M. robinsoni	JOG 4501	AMNH 276496	Venezuela: Falcón (14)	1,146	—
M. robinsoni	RPA 215	EBRG 25357	Venezuela: Falcón (14)	726	—
M. robinsoni	RPA 280	AMNH 276562	Venezuela: Falcón (14)	1,146	668
M. robinsoni	RPA 289	AMNH 276568	Venezuela: Falcón (14)	726	—
M. robinsoni	RPA 262	EBRG 25389*	Venezuela: Falcón (15)	1,146	664
M. robinsoni	—	USNM 443905	Venezuela: Guárico (16)	402	—
M. robinsoni	—	USNM 418519	Venezuela: Guárico (17)	367	—
M. robinsoni	—	USNM 443797	Venezuela: Guárico (17)	180	—
M. robinsoni	—	USNM 443913	Venezuela: Lara (18)	385	192
M. robinsoni	—	AMNH 24323	Venezuela: Mérida (19)	402	328
M. robinsoni	—	AMNH 33166	Venezuela: Mérida (19)	192	—
M. robinsoni	—	USNM 406951	Venezuela: Monagas (20)	389	—
M. robinsoni	—	USNM 388377	Venezuela: Sucre (21)	180	—
M. robinsoni	—	USNM 443803	Venezuela: Zulia (22)	402	—
M. xerophila	RPA 324	AMNH 276586*	Venezuela: Falcón (23)	1,146	665

[a] Alphanumeric identifiers (including various field collection numbers) used by institutional tissue collections (and to label terminals in Figs. 2–4); sequences amplified from skins or dried tissue lack tissue or DNA numbers.

[b] Catalog numbers of voucher specimens; see "Materials and Methods" for names of museum collections identified by abbreviations in this table. An asterisk (*) next to the voucher number indicates that sequences were downloaded from GenBank.

[c] Country and next-largest administrative unit (state, department, province, etc.); numbers in parentheses refer to Gazetteer (Appendix I) entries, which provide additional geographic information.

[d] Number of base pairs (bp) sequenced. *Cytb* = cytochrome-*b* gene; *Ogt* = X-linked intron O-linked N-acetylglucosamine transferase.

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Geographic sampling.—Our geographic sampling for the *Cytb* data set includes most of the known distribution of *M. robinsoni* (see Rossi et al. 2010; Gutiérrez et al. 2014). We used *Cytb* sequence data of samples collected from 22 localities of *M. robinsoni* (Table 1; Fig. 1). The only substantial gaps in our sampling correspond to the eastern portion of the Caribbean coast of Colombia, the eastern part of the Venezuelan Llanos, and the islands of Grenada and Margarita. Unfortunately, we were not able to obtain *Ogt* sequences from most dried-tissue samples; however, we generated at least 1 *Ogt* sequence from each major *Cytb* phylogroup. Although the geographic density of sampling for *Ogt* data was limited (8 localities [Table 1]), these nuclear sequences provide the basis for independent tests of at least some of the relationships found with the *Cytb* data.

Laboratory methods.—All genomic DNA extractions were carried out using DNeasy extraction kits (Qiagen, Inc., Valencia, California). When the source of DNA was dried tissues, we avoided contamination with foreign DNA by extensively washing each tissue using decreasing concentrations of ethanol (Giarla et al. 2010). That procedure and subsequent polymerase chain reactions using DNA obtained from dried tissue were conducted in an ultraviolet-

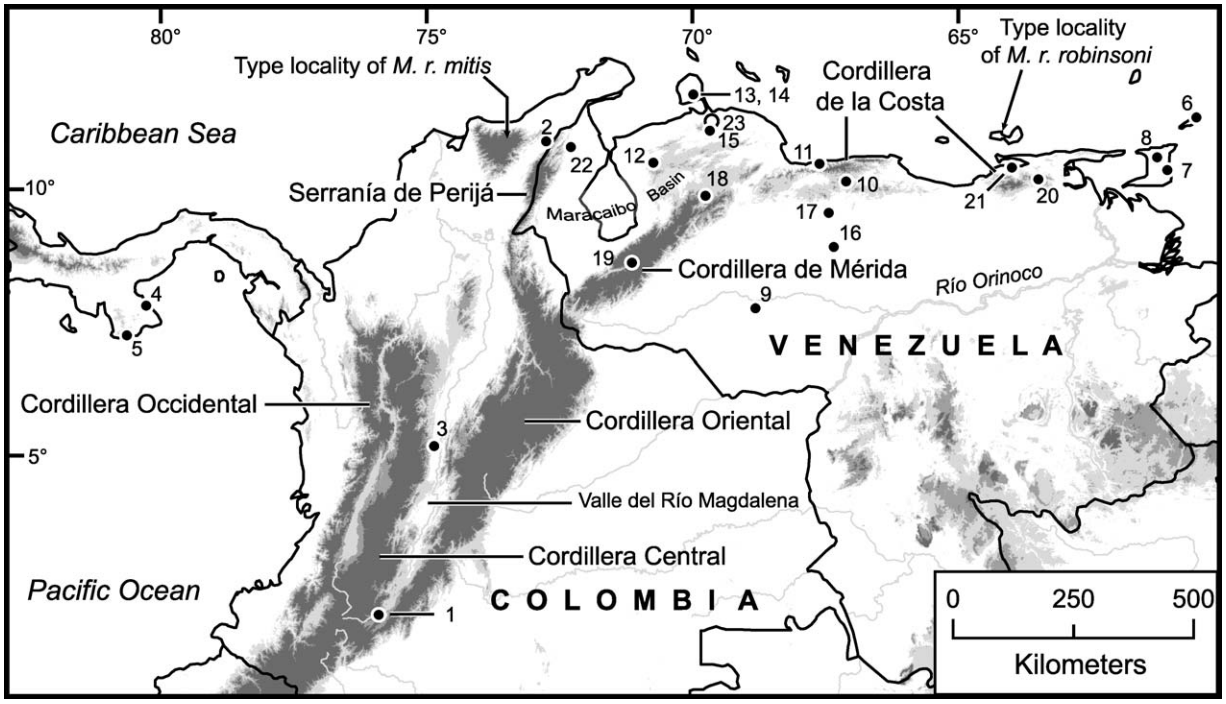

Fig. 1.—Provenance of sequenced specimens of *Marmosa robinsoni* (solid circles) and *M. xerophila* (open circle). Numbers refer to entries in the Gazetteer (Appendix I). Regions shown in increasingly darker tones of gray shading correspond to those with elevations $\geq$ 500 m, $\geq$ 1,000 m, and $\geq$ 1,500 m, respectively.

sterilized hood located in a laboratory where mammalian DNA had not previously been extracted and amplified.

Amplification of *Cytb* and *Ogt* from frozen or ethanol-preserved tissue required 2 steps: 1 to amplify the whole gene, followed by a 2nd round of amplification using internal primers, which yielded fragments of suitable length for sequencing. When amplifying highly degraded DNA extracted from dried tissue, we used various combinations of primers to obtain shorter fragments. Polymerase chain reaction protocols followed Gutiérrez et al. 2010 (see Table 2 for primer sequences and annealing temperatures). We used Exonuclease I and Shrimp Alkaline Phosphatase (Hanke and Wink 1994) to

Table 2.—Name and DNA sequences of pairs of primers used for amplification and sequencing of the cytochrome-*b* gene (*Cytb*; primer label CYTB) and X-linked intron O-linked N-acetylglucosamine transferase (*Ogt*; primer label OGT). Touchdown polymerase chain reactions are labeled as "TD" and their ranges of annealing temperatures are indicated (°C); otherwise polymerase chain reactions were conducted using a single annealing temperature (see "Materials and Methods").

Primer name	Primer sequence	Annealing temperature
CYTB-F1-Didelphidae	5′ ATAACCTATGGCATGAAAAACCATTGTTG	TD 59–52
CYTB-R1-Didelphidae	5′ CCTTCATTGCTGGCTTACAAGGC	
CYTB-F1-Didelphidae	5′ ATAACCTATGGCATGAAAAACCATTGTTG	52
CYTB-217R-*mexicana*	5′ CRTCWCGRCARATRTGGGCTACAGA	
CYTB-F1-Didelphidae	5′ ATAACCTATGGCATGAAAAACCATTGTTG	52
CYTB-420R-*Marmosa*	5′ GCTCCTCAGAAGGATATTTGTCCTCA	
CYTB-F1-Didelphidae	5′ ATAACCTATGGCATGAAAAACCATTGTTG	55
CYTB-730R-*Marmosa*	5′ TCWCCTAATARRTCWGGTGARAATATTGC	
CYTB-540F-*Marmosa*	5′ GAGGAGGMTTYTCHGTTGATAAAGC	55
CYTB-R1-Didelphidae	5′ CCTTCATTGCTGGCTTACAAGGC	
CYTB-650F-*Marmosa*	5′ CTATTCCTTCACGAAACAGGCTC	55
CYTB-R1-Didelphidae	5′ CCTTCATTGCTGGCTTACAAGGC	
OGT-F1-Didelphidae	5′ AAATCATTTCATCGACCTTTCTCAG	TD 55–52
OGT-R1-Didelphidae	5′ GCTGCTTTTCCATTACAGGGAAT	
OGT-F1-Didelphidae	5′ AAATCATTTCATCGACCTTTCTCAG	TD 55–52
OGT-360R-Didelphidae	5′ CATCCCYGCTTGGCCCAACCACA	
OGT-300F-Didelphidae	5′ GTGATTTTGACTTTTCTCCTGGCCT	TD 55–52
OGT-R1-Didelphidae	5′ GCTGCTTTTCCATTACAGGGAAT	
OGT-120F-Didelphidae	5′ GGACATGGAAGAATTTGCTTTTGG	TD 55–52
OGT-540R-Didelphidae	5′ GCTCTGAATTCACAGCATCACCA	

prepare polymerase chain reaction products for sequencing. All polymerase chain reaction products were sequenced in both directions using amplification primers and dye-terminator chemistry (BigDye version 3.1 Cycle Sequencing Kit; Applied Biosystems Inc., Foster City, California). All sequences that we generated were compiled and edited with Sequencher 4.7 (Gene-Codes Inc., Ann Arbor, Michigan).

Selection of models of nucleotide substitution.—We aligned sequences of each gene using Clustal X version 2.0 (Larkin et al. 2007) using default values of alignment parameters. Prior to phylogenetic analyses, identical sequences with the same geographic origin were removed from the matrices of each molecular marker. Identical *Cytb* sequences and their locality numbers (indicated within parentheses [see Fig. 1 and Appendix I]) are as follows: RPA 208 (13) = JOG 4501 (14) = RPA 280 (14); NK 101529 (4) = NK 101606 (4) = NK 101633 (5) = NK 101634 (5); RSV 2453 (6) = RSV 2455 (6) = RSV 2464 (6). All nucleotide-substitution models were selected based on the corrected Akaike information criterion (AIC_C) as implemented in jModeltest version 2.1 (Darriba et al. 2012). Two characteristics of the *Cytb* matrix complicated the selection of its best-fit model of nucleotide substitution. First, this matrix contained a large percentage of missing data (entries coded as unknown; see below), due primarily to short sequences obtained from museum skins. Although the existence of missing data entries might not be problematic for phylogenetic reconstructions (Wiens 2006; Wiens and Moen 2008), the effect of missing data entries on model selection remains poorly understood (see Waddell 2005; Roure et al. 2013). Second, and perhaps more importantly, the *Cytb* matrix contained few guanine residues and no guanine–thymine transversions. This situation is known to occur in data sets of mitochondrial sequences of closely related organisms, and it can produce spurious parameter estimations under complex models (see Powell et al. [2013] for a similar case). In fact, when we attempted to assess model fit on the *Cytb* matrix, the General Time Reversible (GTR) model was selected with unrealistically high estimated rates for guanine–thymine tranversions. To select a more appropriate model for this matrix, we therefore trimmed it to include only the first 200 bp, so the percentage of missing data was only 2%. We then applied the resulting best-fitting model to all analyses based on the untrimmed matrix. It was not necessary to trim the *Ogt* matrix prior to model selection because the parameters estimated for the selected model were not unrealistic.

Phylogenetic analyses and genetic distances.—We analyzed each gene matrix using maximum likelihood (ML) and Bayesian inference (BI). Because a previous study based on *Cytb* data for the genus *Marmosa* recovered *M. robinsoni* and *M. xerophila* as sister species (Gutiérrez et al. 2010)—a finding later confirmed by analyses of unpublished data for multiple nuclear markers (by SAJ, RSV, and EEG)—we designated 1 complete sequence of *M. xerophila* (Table 1) as the outgroup for rooting our phylogenetic analyses. For the ML analysis, we conducted 20 independent searches using GARLI 0.96 beta (Zwickl 2006) with default settings. Bayesian analyses of each gene were conducted using 2 independent runs of 20 million generations each in BEAST 1.7.4 (Drummond et al. 2012). Trees and parameter values were sampled every 1,000 generations; the resulting parameter files were combined and assessed for stationarity and suitable effective sample size (ESS) values using Tracer 1.5 (Rambaut and Drummond 2007). The maximum clade-credibility tree was calculated from the post–burn-in trees using TreeAnnotator version 1.7.4. (a utility in BEAST, see above). For *Ogt*, all priors were kept as default, but we implemented priors for the *Cytb* data to allow simultaneous estimation of the phylogeographic tree and divergence times. After testing that a likelihood-ratio test could not reject a molecular clock for the *Cytb* data, we enforced a strict-clock prior using a substitution rate of 2% per million years (Brown et al. 1979, 1982; Kumar and Subramanian 2002) and a Yule process tree prior (Yule 1925). The latter assumes a constant speciation rate per lineage and is recommended for analyses based on sequences that were not collected from a panmictic population (see Drummond et al. 2007).

To assess nodal support, we used nonparametric bootstrapping (Felsenstein 1985) for the ML analysis and posterior probability estimates for the BI analysis (Drummond et al. 2012). The ML bootstrap analysis was performed using GARLI 0.96 beta (Zwickl 2006) with 10 searches performed on each of 100 pseudoreplicated data matrices. Bayesian posterior probabilities were calculated as described above. See Supporting Information S1 (DOI: 10.1644/14-MAMM-A-069.S1) for detailed methods employed for phylogenetic inferences. Bootstrap values were classified as follows: strong support, for bootstrap values $\geq$ 75%; moderate support, for bootstrap values $>$ 50% and $<$ 75%; and negligible support, for values $\leq$ 50%. For the BI analysis, we considered that nodes received strong (significant) support when their posterior probability was $\geq$ 0.95, and negligible (nonsignificant) support when their posterior probability values were $<$ 0.95.

To estimate genetic divergence, we calculated the mean uncorrected (p) distance among sequences within each haplogroup, and we calculated average pairwise p distances between haplogroups. In addition, we report Kimura 2-parameter (K2P)–corrected distances—the traditional metric for genetic divergence in the didelphid literature (e.g., Patton et al. 2000; Patton and Costa 2003)—for comparison with values reported in previous studies. All genetic distances were calculated using MEGA version 5.2.2 (Tamura et al. 2011).

Results

Models of nucleotide substitution.—After removing redundant sequences (i.e., identical sequences from the same locality), our *Cytb* matrix had a total of 36 sequences (35 *M. robinsoni* and 1 *M. xerophila*) and 41% missing data (matrix entries coded as unknown). The best-fit model of nucleotide substitution selected with this trimmed matrix was the

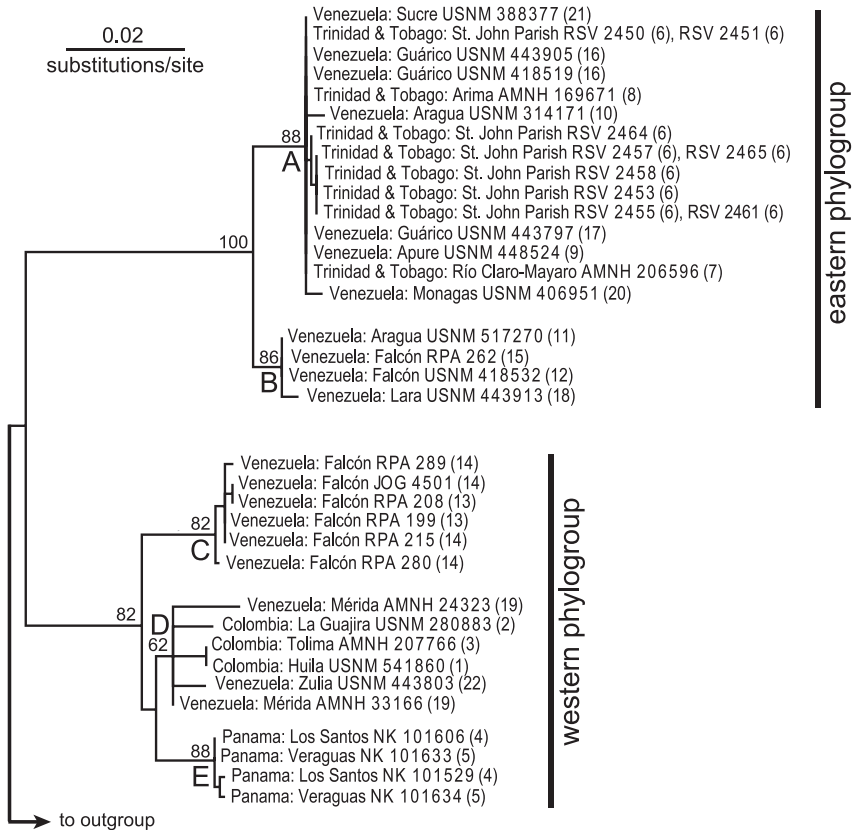

Fig. 2.—The maximum-likelihood tree (–lnL 2587.7231) resulting from analysis of the cytochrome-*b* sequence data to assess the phylogeographic structure of *Marmosa robinsoni*; *M. xerophila* was designated as outgroup. Bootstrap support is shown above branches for nodes of phylogeographic interest with values ≥ 50. Letters (A–E) identify lineages mentioned in the text. For each terminal, political unit (state, department, province, etc.), and an alphanumeric specimen identifier (from Table 1) are provided. Numbers in parentheses refer to localities mapped in Fig. 1 and listed in the Gazetteer (Appendix I). Identical haplotypes from the same locality are indicated on the label at the tip of the corresponding tree branch.

Hasegawa–Kishino–Yano model with gamma-distributed rate heterogeneity (HKY + Γ). After removing identical sequences, the *Ogt* matrix had 9 sequences (8 *M. robinsoni* and 1 *M. xerophila*) and 31% missing data. The best-fit model for this matrix was the HKY model.

Phylogeographic structure.—Both ML and BI analyses of the *Cytb* matrix revealed the existence of 2 strongly supported phylogroups of *M. robinsoni* (Figs. 2 and 3). The eastern phylogroup includes sequences from nonpeninsular parts of northern and central Venezuela (Apure, Aragua, Falcón, Guárico, Lara, and Monagas), northeastern Venezuela (Sucre), Trinidad, and Tobago. The western phylogroup includes haplotypes from Panama, Colombia, and northwestern mainland Venezuela (Zulia and Mérida), as well as the Península de Paraguaná (in Falcón). This split also was found in analyses of sequences from 3 localities reported by Gutiérrez et al. (2010). Both of these haplogroups have relatively low genetic variability (p distances within the eastern and western phylogroups were 0.5% and 1.4%, respectively [Table 3]), but genetic divergence between them is moderately high (p distance = 5.6%; K2P distance = 5.9%).

Within the eastern phylogroup, ML and BI analyses both recovered a strongly supported clade formed by haplotypes from the Llanos and north-central and northeastern Venezuela, as well as from the islands of Trinidad and Tobago (hereafter the Venezuela–Trinidad–Tobago lineage [Figs. 2 and 3, node A]). This lineage was recovered as sister to a haplogroup formed by 4 sequences from northern Venezuela (hereafter the eastern Maracaibo Basin lineage [Figs. 2 and 3, node B]), but this group only received strong support in the ML analysis.

Within the western phylogroup, ML and BI analyses consistently identified 3 lineages. The 1st includes all of the haplotypes from the Península de Paraguaná (hereafter the Paraguaná lineage [Figs. 2 and 3, node C]), the 2nd includes haplotypes from Colombia as well as from the Venezuelan states of Zulia and Mérida (hereafter the northwestern South America lineage; node D), and the 3rd includes haplotypes from Panama (hereafter the Panama lineage; node E). Both analyses recovered the northwestern South America lineage as sister to the Panama lineage, but in both cases with negligible support.

Topologies of the best trees resulting from ML and BI analyses of *Ogt* sequences were highly concordant, both analyses recovering the eastern and western phylogroups of *M. robinsoni* as reciprocally monophyletic with moderate-to-strong support (Fig. 4). Within the eastern phylogroup, 2

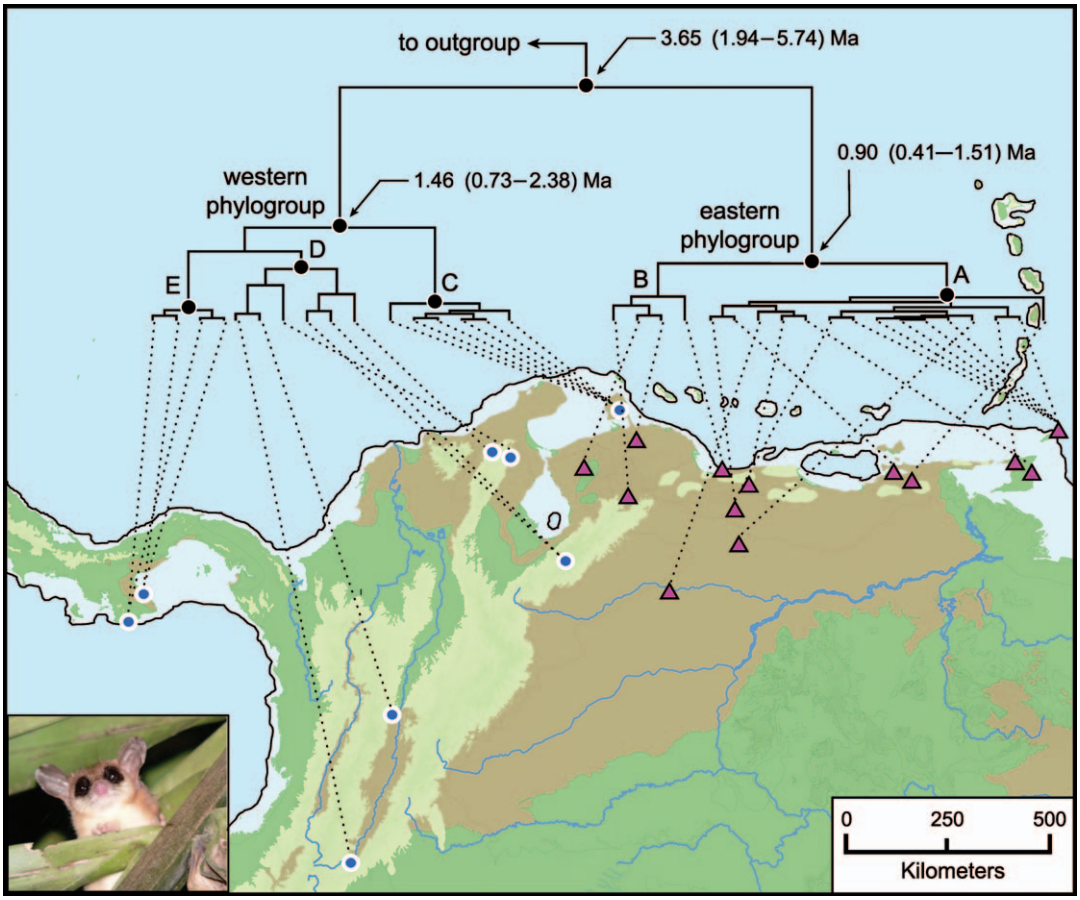

Fig. 3.—The Bayesian tree resulting from analysis of the cytochrome-*b* sequence data superimposed on a map showing habitat information as well as localities for the eastern (purple triangles) and western (blue circles) phylogroups of *Marmosa robinsoni*. Pie diagrams at internal nodes represent support for phylogroups of interest; filled circles correspond to nodes with strong or significant support (posterior probabilities ≥ 0.95). Median estimated time since divergence and the corresponding 95% highest posterior densities (within parentheses) are shown in mega-annum (Ma; 1×10^6 years) for the most basal splits within *M. robinsoni* and for within its eastern and western phylogroups. Letters (A–E) identify lineages mentioned in the text. Habitat information is adapted from the ecoregion designations published by Olson et al. (2001), and includes 3 broad categories: dry habitats, including savannas and dry (deciduous) forests (brown); humid lowland forest (dark green); and montane forest (light green). The estimated position of the coastline at the Last Glacial Maximum (LGM) is shown with a black line enclosing areas currently covered by the Caribbean Sea or Pacific Ocean but that were exposed during the LGM (pale gray-blue). This historical coastline was produced by the Paleoclimate Modelling Intercomparison Project Phase II and acquired via WorldClim (Hijmans et al. 2005). A color version of this figure is available online.

smaller phylogroups were recovered, one representing the Venezuela–Trinidad–Tobago lineage (node A) and another formed by sequences from the Venezuelan states of Lara and Falcón (node B); the former, however, received negligible support in both analyses. By contrast, neither analysis of *Ogt* sequences resolved area relationships within the western phylogroup.

Divergence-time estimation.—Most estimated divergence times correspond to the Pliocene or Pleistocene (we follow the United States Geological Survey Geologic Names

Table 3.—Matrix of genetic distances (percent sequence divergence) within and among lineages of *Marmosa robinsoni* based on cytochrome-*b* gene sequences. Average uncorrected (p) distances among haplotypes of same lineage are arrayed along the diagonal in boldface type, interlineage p distances are below the diagonal, and Kimura 2-parameter (K2P) distances are above the diagonal ("node" denotes those lineages in Figs. 2 and 3).

Lineage	Node	A	B	C	D	E
Venezuela–Trinidad–Tobago	A	**0.1**	1.3	6.4	5.9	6.3
Eastern Maracaibo Basin	B	1.2	**0.2**	5.0	4.5	4.8
Paraguaná	C	5.9	5.3	**0.2**	1.7	2.5
Northwestern South America	D	5.5	4.7	1.7	**0.9**	1.5
Panama	E	5.9	5.1	2.5	1.5	**0.1**

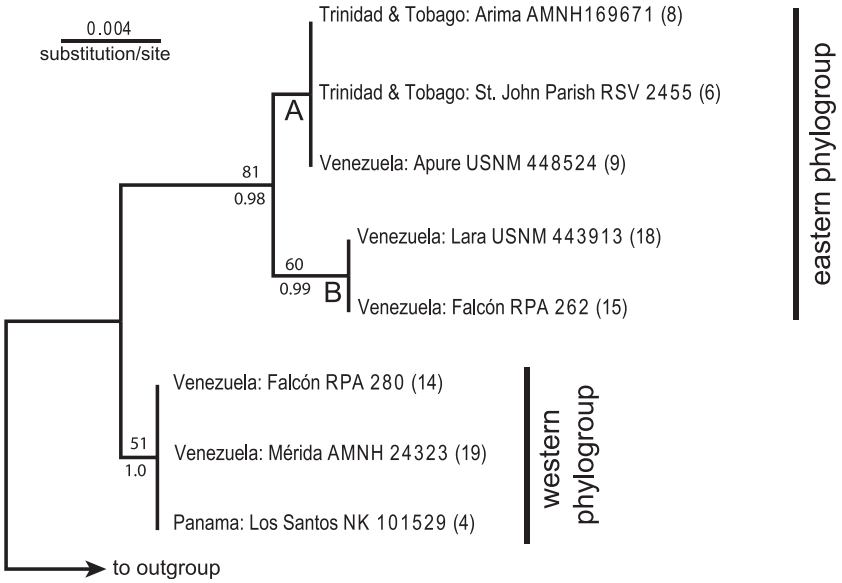

Fig. 4.—The maximum-likelihood tree (–lnL 1016.5415) resulting from analysis of the nuclear intron (X-linked intron O-linked N-acetylglucosamine transferase [*Ogt*]) sequence data confirming the existence of the eastern and western phylogroups of *Marmosa robinsoni*. Maximum-likelihood bootstrap support is shown above branches for nodes with values ≥ 50, and Bayesian posterior probability support ≥ 0.95 is shown below branches. Letters (A, B) identify lineages mentioned in the text. For each terminal, political unit (state, department, province, etc.), and an alphanumeric specimen identifier (from Table 1) are provided. Numbers in parentheses refer to localities mapped in Fig. 1 and listed in the Gazetteer (Appendix I).

Committee [2010] for divisions of geologic time). The eastern and western phylogroups of *M. robinsoni* were estimated to have diverged from each other at about 3.65 million years ago (mya), but this estimate has a wide credibility interval that encompasses the late Miocene to the early Pleistocene (Fig. 3). Estimates of both time since most recent common ancestor for all identified lineages (Table 4) and time since the earliest phylogeographic splits within the eastern and western phylogroups date to the Pleistocene (Fig. 3), but again these estimates have wide credibility intervals.

Discussion

Our results suggest the need for additional research to assess the possibility that 2 cryptic taxa are represented among the populations of *M. robinsoni* sampled by this study. Although Rossi et al. (2010) examined large series of specimens from areas occupied by the eastern and western phylogroups, those authors did not report any corresponding pattern of morphological variation, and we have yet to find morphological traits that consistently distinguish the eastern phylogroup from the western one. In the apparent absence of phenotypic differentiation, and lacking evidence that mitochondrial and nuclear phylogroups remain associated with one another in sympatry, we are reluctant to suggest that these are fully differentiated species.

Nevertheless, names can be associated with the eastern and western phylogroups of *M. robinsoni* on the basis of geography. The type locality of *robinsoni* (Bangs, 1898a) is Isla Margarita, a continental-shelf island adjacent to mainland Venezuelan sites occupied by the eastern phylogroup (e.g., our locality 21 [Fig. 1]). Therefore, *robinsoni* would presumably apply to this lineage if the name were to be used in a more restricted sense than it is now. The oldest name that seems to be available for the western phylogroup is *mitis* (Bangs, 1898b), the type locality of which is Pueblo Viejo (10°59′N, 73°27′W—Rossi et al. 2010) on the Caribbean coast of Colombia not far (about 80 km) from our locality 2. Although

Table 4.—Time to the most recent common ancestor (TMRCA) of analyzed haplotypes in phylogroups and lineages of *Marmosa robinsoni* identified in phylogenetic analyses (Figs. 2–4). Estimates are in mega-annum (Ma; 1 Ma equals 1×10^6 years).

Phylogroup or lineage	Node	TMRCA (median)	95% highest posterior densities
Eastern phylogroup	—	0.84	0.41–1.50
Western phylogroup	—	1.37	0.74–2.40
Venezuela–Trinidad–Tobago lineage	A	0.34	0.12–0.71
Eastern Maracaibo Basin lineage	B	0.35	0.05–0.83
Paraguaná lineage	C	0.25	0.09–0.52
Northwestern South America lineage	D	0.76	0.31–1.45
Panama lineage	E	0.16	0.04–0.35

trinomial nomenclature has fallen somewhat out of favor in recent years, names facilitate communication, and "*Marmosa robinsoni mitis*" is certainly more concise than "the western phylogroup of *Marmosa robinsoni*." Additionally, subspecies have recognized status as units of conservation concern in many countries and in the regulations enforced by international conservation treaties. Although neither the eastern nor the western phylogroups of *M. robinsoni* seem to be of any conservation concern at present, the trinomials *M. r. robinsoni* and *M. r. mitis* are available should they be needed for this or other reasons.

The Dry Forest Refugia Hypothesis, originally established on the basis of floristic affinities among disjunct dry-forest regions of South America, suggests that present-day dry forests are remnants of a more widespread biome that existed during the cool, dry conditions of the Last Glacial Maximum, but contracted during the last interglacial (Prado and Gibbs 1993; Pennington et al. 2000; but see Mayle 2004). If true, this hypothesis predicts that the 3 disjunct dry-forest remnants currently inhabited by *M. robinsoni* (i.e., the Caribbean Coast of Venezuela and Colombia, the Magdalena Valley, and western Panama [Fig. 3]) should form 3 independent phylogeographic units that originated during the Pleistocene. However, this is not the pattern that we observe for *M. robinsoni*. Although western Panamanian populations and populations from the Magdalena Valley each form monophyletic groups, those from the dry-forests of northern Venezuela and Colombia do not form a single phylogeographic unit (Fig. 3). Rather, the principal phylogeographic division for *M. robinsoni* occurs within this Caribbean coastal dry-forest remnant, and defines eastern and western phylogroups as occurring roughly on either side of the Lago de Maracaibo.

Nevertheless, our observed phylogeographic pattern is not without precedent. One of the 1st studies to examine vertebrate biogeographic patterns in this region focused on the cricetid rodent genus *Zygodontomys* (Voss 1991). This study used variation in morphological traits to infer intraspecific population structure that was then examined in a geographic context. Notably, 2 diagnosable, geographically distinct sets of populations of *Z. brevicauda* emerged: one (referred to as *Z. b. cherriei*) that occurred in dry habitats north and west of the Andes (including the orogenic arc formed by the Cordillera Oriental of Colombia, the Cordillera de Mérida, and the Cordillera de la Costa of Venezuela) and another (*Z. b. brevicauda*) that occurred southeast of these cordilleras. A similar biogeographic pattern is seen in cottontail rabbits (*Sylvilagus floridanus*—Müller 1973; Voss 1991): 1 morphotype occurs in dry habitats of the Llanos and eastern Caribbean coast, whereas a 2nd occurs in the Maracaibo Basin, including the Península de Paraguaná and the offshore islands of Aruba and Curaçao.

As with *Zygodontomys brevicaudata cherriei*, our western phylogroup includes samples from disjunct dry habitats from Panama through the western flank of the Cordillera de Mérida, and our eastern phylogroup includes individuals from dry habitats east of the Lago de Maracaibo (Fig. 3). The discrepancies concern individuals of *M. robinsoni* from the eastern Maracaibo Basin and those from the Península de Paraguaná. Whereas the northwest–southeast break in *Z. brevicauda* was clearly defined by the mountain arc that extends from the Cordillera de Mérida to Cordillera de la Costa (Voss 1991:50, figure 24)—a pattern similar to that found by Wüster et al. (2005) in rattlesnakes—samples of *M. robinsoni* from the eastern Maracaibo Basin (north of the mountains) belong to our eastern phylogroup (Fig. 3). *Zygodontomys brevicauda* is unknown from the Península de Paraguaná (Linares 1998; Anderson et al. 2012), but individuals of *M. robinsoni* from this peninsula belong to our western phylogroup. This latter pattern is similar to that seen in cottontails, where specimens from the Península de Paraguaná and nearby offshore islands are morphologically allied with individuals from the western Maracaibo Basin (Voss 1991:99, figure 40).

These 3 independent but broadly coincident patterns of population subdivision in small mammals reinforce the hypothesis (Voss 1991) that dry habitats were once contiguous across northern South America and were subsequently separated into northwestern and southwestern regions along the montane arc formed by the Cordillera de Mérida and Cordillera de la Costa. Examination of molecular data from *M. robinsoni* refines this hypothesis by providing an estimated date for this phylogeographic split and evidence for additional phylogeographic structure (or lack thereof) in the 2 main phylogroups. In particular, based on the inferred date of divergence between the eastern and western phylogroups (between 1.94 and 5.74 mya), it seems unlikely that uplift of the Cordillera de Mérida—which had high exhumation rates from about 10 mya until to 4 mya (Bermúdez et al. 2011)—or Miocene marine transgressions (Lovejoy et al. 2006) initiated vicariance between these 2 phylogroups. More likely, sea-level and vegetational changes during glacial and interglacial periods played an important role in the differentiation between these 2 phylogroups (in the Pliocene—see Webb and Bartlein 1992; Dwyer and Chandler 2009) and among lineages within the western phylogroup (in the Pleistocene—Augustin et al. 2004).

It is perhaps surprising that biogeographic affinities of small mammals from the Península de Paraguaná lie with western dry-forest habitats rather than with those from the neighboring Venezuelan mainland. However, it seems likely that broad land connections and expansion of dry-forest habitats during glacial maxima could have facilitated dispersal of dry-forest–associated mammals throughout northern South America and across the Panamanian Isthmus (Fig. 3). Current sea levels in the Gulf of Venezuela are remarkably shallow, with most depths less than 45 m, and a maximum depth of approximately 75 m (Zeigler 1964; Defense Mapping Agency Hydrographic/Topographic Center 1981). Therefore, during glacial periods, broad land connections must have existed between the present-day Península de Paraguaná and northeastern Colombia, including intervening areas currently covered by the Caribbean Sea (Fig. 3). As climates warmed during interglacials, humid forest would have expanded across lowland northern South

America and into inter-Andean valleys, isolating dry-forest habitats and creating the phylogeographic structure that we observe in the western phylogroup of *M. robinsoni*. Although phylogeographic structure within the eastern phylogroup is not as strong, the initial split in this lineage (between phylogroups A and B [Fig. 3]) is defined by the same mountain arc that splits populations of *Z. brevicauda*. This pattern reinforces the idea (Voss 1991) that Pleistocene expansion of humid forests into intermontane gaps effectively blocked gene flow of dry-habitat–adapted mammals across the low passes of the cordilleras.

Finally, 2 mutually exclusive scenarios have been invoked to explain the presence and degree of distinctiveness of various mainland taxa on Trinidad and Tobago (Boos and Ratcliffe 1985; Camargo et al. 2009; Manzanilla et al. 2009; Jowers et al. 2011). The first implies that species dispersed to these areas during the Miocene, when these present-day islands were connected to continental Venezuela (Liddle 1946; Comeau 1991). Insular populations either remained isolated or became connected to mainland populations during the Pleistocene glacial cycles (Augustin et al. 2004), when lowered sea levels exposed dry-land connections between Trinidad and mainland Venezuela, and between Trinidad and Tobago (Comeau 1991; Murphy 1997). The other scenario suggests that these islands were not colonized (or recolonized) by taxa currently occupying them until the Pleistocene glacial maxima (with the accompanying drop in sea level) connected them to the mainland. Our results for *M. robinsoni* unambiguously reject a Miocene origin for the populations of this species on Trinidad and Tobago (Figs. 2 and 3; Table 4), suggesting much more recent gene flow between the island and mainland populations.

Future biogeographic studies of other dry-habitat organisms in northern South America are necessary to test the generality of our results, especially the importance of mesic forests as historical barriers to gene flow. Indeed, comparative phylogeographic analyses, ideally based on geographically dense sampling and multilocus approaches, coupled with ecological niche modeling analyses (see Kozak et al. 2008; Carnaval et al. 2009; Peterson et al. 2011; Alvarado-Serrano and Knowles 2014) represent the most promising approach to gain insights on the poorly studied, but biogeographically fascinating dry habitats of northern South America.

Resumen

Marmosa robinsoni típicamente habita arbustales secos, sabanas, y bosques deciduos de Panamá, Colombia, y Venezuela, y se encuentra presente también en las islas de Trinidad, Tobago, y Granada. En este estudio evaluamos la estructura filogeográfica de *M. robinsoni* en lo que, hasta donde sabemos, representa el primer estudio de este tipo basado en un muestreo geográfico denso de un vertebrado de los hábitats secos del norte de Suramérica. Secuenciamos el gen mitocondrial citocromo b y el intron nuclear O-N-acetilglucosamina transferasa (ubicado en el cromosoma X). Para ello, obtuvimos ADN principalmente a partir de pieles de estudio y tejido residual tomado de material osteológico de ejemplares de museos. Los análisis filogenéticos revelaron (con buen apoyo) la existencia de dos filogrupos predominantemente distribuidos al Este y Oeste de la Cordillera de Mérida. La estimación del tiempo transcurrido desde que estos dos filogrupos divergieron claramente indica que tal divergencia ocurrió luego del Mioceno; por lo tanto, el levantamiento de los Andes, los cambios de curso del Río Orinoco, y las transgresiones marinas de esa época no pueden ser consideradas como posibles agentes vicariantes relevantes entre estos grupos. En cambio, expansiones de bosques húmedos y/o transgresiones marinas durante el Plioceno y Pleistoceno podrían haber causado esta divergencia. Encontramos poca estructura filogeográfica entre las poblaciones al Este de la Cordillera de Mérida, lo cual sugiere una expansión reciente a esta región. Sorprendentemente, poblaciones aisladas en la Península de Paraguaná (noroeste de Venezuela) no están cercanamente relacionadas con las poblaciones geográficamente más próximas (continentales y adyacentes a la península), sino a las poblaciones más distantes al Oeste en Colombia y Panamá. En cambio, las poblaciones del centro y del oriente de Venezuela están cercanamente relacionadas a poblaciones presentes en las islas Trinidad y Tobago. Esta similitud genética entre poblaciones disyuntas podría haberse originado en el Holoceno, posiblemente por la fragmentación de una distribución más amplia de *M. robinsoni* durante el fin del Pleistoceno, cuando la especie podría haber ocupado planicies costeras expuestas por el último máximo glacial.

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Supporting Information

Supporting Information S1.—Phylogenetic analyses conducted in BEAST.

Found at DOI: 10.1644/14-MAMM-A-069.S1 (79KB DOCX)

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APPENDIX I

Gazetteer of analyzed specimens.—Abbreviated locality names for specimens from which sequences of *Marmosa robinsoni* (ingroup) and *M. xerophila* (outgroup) were analyzed for this study. Italicized place names are those of the largest political unit (state, department, province, etc.) within each country. Elevational data (if any) are reproduced verbatim from specimen tags in meters (m). Geographic coordinates and their cited source are provided in parentheses. See Gutiérrez et al. (2014) for a more complete and extended list of locality information of the focal species.

Marmosa robinsoni

COLOMBIA

1. *Huila*, Valle de Suaza (02°01′N, 75°51′W [National Geospatial-Intelligence Agency of the United States 2010]).
2. *La Guajira*, Las Marimondas (10°52′N, 72°43′W [Hershkovitz 1947]).
3. *Tolima*, Mariquita, approximately 535 m (05°12′N, 74°54′W [Paynter 1982]).

PANAMA

4. *Los Santos*, Los Cuernitos (07°51′N, 80°16′W [collector's label]).
5. *Veraguas*, Río Portabelo (07°14′N, 80°37′W [collector's label]).

TRINIDAD AND TOBAGO

6. *Tobago* [St. John Parish], near Charlotteville, 275 m (11°18.789′N, 60°32.918′W [collector's field notes]).
7. *Trinidad* [Río Claro-Mayaro], Bush Bush, approximately 0 m (10°24′N, 61°03′W [Anderson and Gutiérrez 2009]).
8. *Trinidad* [Arima], St. Patricks, approximately 30 m (10°38′N, 61°17′W [Anderson and Gutiérrez 2009]).

VENEZUELA

9. *Apure*, Hato El Frío, 30 km W (by road) El Samán (07°49′N, 68°54′W [Google Earth 2010]).
10. *Aragua*, Campamento Rangel, 800–1200 m (10°09′N, 67°09′W [Anderson and Gutiérrez 2009]).
11. *Aragua*, Ocumare de la Costa, 2 km NE, 1 m (10°28′N, 67°45′W [Dirección de Cartografía Nacional 1971]).
12. *Falcón*, 24 km S and 93 km E of Maracaibo (Cerro Socopo), 1258 m (10°30′N, 70°44′W [Handley 1976]).
13. *Falcón*, Península de Paraguaná; Cerro Santa Ana; approximately 3 km N Santa Ana, approximately 200 m (11°48.352′N, 69°56.667′W [collector's field notes]).
14. *Falcón*, Península de Paraguaná; Cerro Santa Ana; approximately 4 km N Santa Ana, approximately 520–570 m (11°49′N, 69°57′W [collector's label]).

15. *Falcón*, Serranía de San Luis; approximately 4 km S + 3 km W Cabure, approximately 425 m (11°06.672′N, 69°38.263′W [collector's field notes]).
16. *Guárico*, 7 km S and 5 km E of Calabozo (Estación Biológica de Los Llanos), 100 m (08°52′N, 67°23′W [Handley 1976]).
17. *Guárico*, Hato Las Palmitas, 35 km SSW San Juan de Los Morros [includes "34 km S and 12 km W of San Juan de Los Morros (Hato Las Palmitas)"], 181 m (09°36′N, 67°27′W [Handley 1976]).
18. *Lara*, El Tocuyo, 10 km N of El Tocuyo, caserío Boro, 528 m (09°53′N, 69°47′W [Dirección de Cartografía Nacional 1975; see Handley 1976]).
19. *Mérida*, Cafetos de Chama, approximately 1,600 m (08°36′N, 71°08′W [Paynter 1982]; coordinates correspond to the city of Mérida, where the Río Chama matches the indicated elevation).
20. *Monagas*, 2 km N and km W of Caripe [number of km W of Caripe not written on collector's label] (Nr. San Agustin) [= San Agustin, 5 km NW Caripe], 1150 m (10°12′N, 63°32′W [Handley 1976]).
21. *Sucre*, 16 km E Cumaná (Quetepe), 0 m (10°27′N, 64°02′W [Gardner 2007 {2008}; see Handley 1976]).
22. *Zulia*, 18 km N and 56 km W of Maracaibo (Nr. Hacienda El Rodeo) [= Nr. Cerro Azul, 40 km NW La Paz = Hacienda El Tigre], 80 m (10°48′N, 72°18′W [Google Earth 2008]).

Marmosa xerophila

VENEZUELA

23. *Falcón*, Serranía de San Luis; approximately La Chapa; approximately 15 km N Cabure, approximately 350–380 m (11°16.911′N, 69°36.370′W [collector's field notes]).