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Molecular Systematics of Mouse Opossums (Didelphidae: *Marmosa*): Assessing Species Limits using Mitochondrial DNA Sequences, with Comments on Phylogenetic Relationships and Biogeography

ELIÉCER E. GUTIÉRREZ,[1,2] SHARON A. JANSA,[3] AND ROBERT S. VOSS[4]

ABSTRACT

The genus *Marmosa* contains 15 currently recognized species, of which nine are referred to the subgenus *Marmosa*, and six to the subgenus *Micoureus*. Recent revisionary research based on morphological data, however, suggests that the subgenus *Marmosa* is more diverse than the currently accepted taxonomy indicates. Herein we report phylogenetic analyses of sequence data from the mitochondrial cytochrome-*b* gene representing 12 of the 14 morphologically defined taxa recently treated as valid species of *Marmosa* (*Marmosa*) in the aforementioned revisionary work. These data provide a basis for testing the monophyly of morphologically defined taxa in the subgenus *Marmosa*, and they afford the first opportunity to assess phylogenetic relationships among the majority of species currently referred to the genus. Ten of 11 species of *Marmosa* (*Marmosa*) represented by multiple sequences in our analyses were recovered as monophyletic. In contrast, our samples of *M. mexicana* were recovered as two deeply divergent haplogroups that were not consistently associated as sister taxa. Among other results, our analyses support the recognition of *M. isthmica* and *M. simonsi* as species distinct from *M. robinsoni*, and the recognition of *M. macrotarsus* and *M. waterhousei* as species distinct from *M. murina*. The validity of three other species long recognized as distinct (*M. rubra*, *M. tyleriana*, and *M. xerophila*) is also clearly supported by our results. Although cytochrome-*b* sequence data are not consistently informative about interspecific relationships in this study, we found

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strong support for several clades, including (1) the subgenus *Micoureus*; (2) a group comprised of *Marmosa macrotarsus*, *M. murina*, *M. tyleriana*, and *M. waterhousei*; (3) a group comprised of *M. robinsoni* and *M. xerophila*; and (4) a group comprising all of the species in the subgenus *Marmosa* that occur north and west of the Andes (*M. isthmica*, *M. mexicana*, *M. robinsoni*, *M. simonsi*, *M. xerophila*, and *M. zeledoni*). Our discovery of the latter clade suggests that the Andes may have played a major role in the early diversification of this speciose radiation of small Neotropical marsupials.

INTRODUCTION

Species of the didelphid marsupial genus *Marmosa* inhabit tropical and subtropical vegetation from Mexico to northern Argentina, including such diverse habitats as xerophytic thorn scrub, savannas, lowland rain forests, and humid-montane ("cloud") forests from sea level to about 3000 meters (Creighton and Gardner, 2008). As currently understood (Voss and Jansa, 2009), the genus contains 15 species, of which nine are referred to the paraphyletic subgenus *Marmosa* Gray, 1821, and six to the monophyletic subgenus *Micoureus* Lesson, 1842. By virtue of its wide ecogeographic range, the genus is of exceptional biogeographic interest, but effective analysis of distributional patterns is prevented by a host of taxonomic problems, not the least of which concerns species delimitation.

Tate (1933) recognized 10 species referable to the subgenus *Marmosa* (sensu Voss and Jansa, 2009), which he organized into "sections" based on subjectively inferred relationships (table 1). Subsequently, Hershkovitz (1951) synonymized all of the taxa in Tate's Mitis Section (for which the oldest available name is *robinsoni*; Cabrera, 1958), and new species were later described by Pine (1972) and Handley and Gordon (1979). As a result, recent taxonomic synopses (Gardner, 2005; Creighton and Gardner, 2008; Voss and Jansa, 2009) have recognized nine species: *M. andersoni*, *M. lepida*, *M. mexicana*, *M. murina*, *M. quichua*, *M. robinsoni*, *M. rubra*, *M. tyleriana*, and *M. xerophila*. Despite such consensus, several of these species have improbably wide geographic distributions (e.g., *M. mexicana*, *M. murina*, and *M. robinsoni*), and previously published analyses of mitochondrial gene sequences suggest that at least some include genetically divergent forms (Steiner and Catzeflis, 2003, 2004; Patton and Costa, 2003).

In a recent revisionary study, Rossi (2005) recognized 14 valid species in the nominotypical subgenus of *Marmosa*. Based on his examination of approximately 2500 specimens (including most of the relevant type material), he resurrected five species that had previously been treated as junior synonyms or subspecies: *M. simonsi* and *M. isthmica* (formerly synonymized with *M. robinsoni*); *M. zeledoni* (formerly synonymized with *M. mexicana*); and *M. tobagi* and *M. waterhousei* (formerly synonymized with *M. murina*).[5] Although Rossi's unpublished results (summarized, in part, by Rossi et al., 2010) are compellingly supported by morphometric analyses and by qualitative characters of the integument, skull, and dentition, his proposed taxonomy (table 1) remains to be tested with molecular data.

Herein we report phylogenetic analyses of DNA sequences from the mitochondrial cytochrome-*b* gene representing most of the species recognized by Rossi (2005) in the subgenus *Marmosa* as well as several species of the subgenus *Micoureus*. These data provide a basis for testing the monophyly of Rossi's morphologically defined species, and they afford an opportunity to infer phylogenetic relationships among the majority of species currently referred to the genus. Although our results include novel insights concerning biogeography and subgeneric classification, we defer formal treatment of these topics to future reports that will incorporate additional sequence data from other genes.

MATERIALS AND METHODS

Source of Material: Except as noted, all voucher specimens and associated tissues are preserved in the following collections (listed alphabetically by institutional abbreviation): AMNH, American Museum of Natural

[5] Rossi (2005) additionally suggested that *macrotarsus* Wagner, 1842, is the oldest available name for the species formerly known as *quichua* Thomas, 1899. Contra Creighton and Gardner (2008), *macrotarsus* Wagner, 1842, is not preoccupied by *macrotarsos* Schreber, 1777 (a primate).

TABLE 1
Species of *Marmosa* (subgenus *Marmosa*) Recognized as Valid by Authors[a]

Tate (1933)[b]	Gardner (2005)[c]	Rossi (2005)
Murina Section	*M. andersoni*[d]	*M. mexicana*
M. murina	*M. lepida*	*M. zeledoni*[g]
M. rubra	*M. mexicana*	*M. isthmica*[h]
M. tyleriana	*M. murina*	*M. robinsoni*
M. quichua	*M. quichua*	*M. simonsi*[h]
Mitis Section	*M. robinsoni*[e]	*M. xerophila*
M. mitis	*M. rubra*	*M. rubra*
M. chapmani	*M. tyleriana*	*M. andersoni*
M. simonsi	*M. xerophila*[f]	*M. tyleriana*
M. ruatanica		*M. lepida*
Mexicana Section		*M. murina*
M. mexicana		*M. macrotarsus*[i, j]
Lepida Section		*M. waterhousei*[i]
M. lepida		*M. tobagi*[i]

[a]Only taxa referable to the nominotypical subgenus (as recognized by Voss and Jansa, 2009) are listed. Taxa are listed in the same order as in the cited works.

[b]Note that species were organized by ''sections'' within Tate's (1933) system.

[c]Also the taxonomy followed by Creighton and Gardner (2008) and Voss and Jansa (2009). Names are used in the same sense as by Tate (1933) except as noted otherwise.

[d]Described by Pine (1972).

[e]Senior synonym of *mitis*. Includes *chapmani*, *simonsi*, and *ruatanica* (after Hershkovitz, 1951).

[f]Described by Handley and Gordon (1979).

[g]Formerly included in *M. mexicana*.

[h]Formerly included in *M. robinsoni* (sensu Gardner, 2005).

[i]Formerly included in *M. murina*.

[j]Includes *quichua*.

History (New York); BMNH, Natural History Museum (London); CM, Carnegie Museum of Natural History (Pittsburg); EBRG, Museo de la Estación Biológica de Rancho Grande (Maracay); FMNH, Field Museum of Natural History (Chicago); INPA, Instituto Nacional de Pesquizas da Amazônia (Manaus); ISEM, Institut des Sciences de l'Evolution de Montpellier (Montpellier); LSUMZ, Louisiana State University, Museum of Natural Science (Baton Rouge); MHNG, Muséum d'Histoire Naturelle de Genève (Geneva); MNK, Museo de Historia Natural Noel Kempff Mercado (Santa Cruz); MSB, Museum of Southwestern Biology, University of New Mexico (Albuquerque); MVZ, Museum of Vertebrate Zoology, University of California (Berkeley); ROM, Royal Ontario Museum (Toronto); T-, tissue collection of the Laboratoire de Paleontologie at the Institut des Sciences de l'Evolution de Montpellier (ISEM; Montpellier); TTU, Museum of Texas Tech University (Lubbock); UFMG, Universidade Federal de Minas Gerais (Belo Horizonte); UMSNH, Universidad Michoacana de San Nicolas de Hidalgo (Morelia); USNM, United States National Museum of Natural History (Washington); V-, voucher collection of Francois M. Catzeflis (currently at ISEM, these specimens will eventually be deposited either at the Muséum National d'Histoire Naturelle, Paris, or at MHNG; F.M. Catzeflis, in litt.).

TAXON SAMPLING: Our taxonomic sample (table 2) includes 71 individuals representing 12 of the 14 species of *Marmosa* (*Marmosa*) recognized by Rossi (2005) together with four of the six currently recognized species of the subgenus *Micoureus*. We were unable to obtain samples of *Marmosa* (*M.*) *andersoni*, *M.* (*M.*) *tobagi*, *M.* (*Mi.*) *alstoni*, or *M.* (*Mi.*) *phaea* for this study. Among other didelphid genera, *Tlacuatzin* and *Monodelphis* have been identified as phylogenetically closest to *Marmosa* (e.g., by Voss and Jansa, 2009, and references cited therein); therefore, we used sequences from two individuals of *Tlacuatzin canescens* and one of *Monodelphis brevicaudata* as outgroups to root our trees.

Within each recognized species of *Marmosa* (*Marmosa*), we chose individuals to represent as many nominal taxa (subspecies or subjective synonyms) and regions of vertebrate endemism (Müller, 1973; Cracraft, 1985) as available tissue resources would allow (fig. 1). For the majority of our samples (60 out of 71), we extracted high-molecular-weight DNA from field-preserved tissues. We extracted relatively poor-quality DNA from museum skins of five individuals (two of *M. tyleriana*, and one each of *M. rubra*, *M. zeledoni*, and *M. xerophila*), and we obtained six additional sequences from GenBank: three of *M. murina* (AJ486984, AJ486990, AJ486995), two of *M. demerarae* (AJ487005, AJ487006), and one of *M. mexicana* (AJ606454). After removing

TABLE 2
Sequenced Specimens of Ingroup and Outgroup Taxa

Taxon	Tissue/DNA#[a]	Voucher[b]	Locality[c]	bp[d]
Ingroup				
M. (Marmosa) isthmica	TK 135686	TTU 102969	Ecuador: Esmeraldas (17)	1145
M. (Marmosa) isthmica	FMG 2716	USNM 575395[e]	Panama: Bocas del Toro (37)	1140
M. (Marmosa) isthmica	FMG 2736	USNM 575397[e]	Panama: Bocas del Toro (37)	1146
M. (Marmosa) isthmica	TK 22555	TTU 39118[e]	Panama: Darién (39)	1146
M. (Marmosa) lepida	F 38809	ROM 107034[e]	Guyana: Potaro-Siparuni (30)	1146
M. (Marmosa) lepida	JLP 7844	MVZ 155245[e]	Peru: Amazonas (42)	1146
M. (Marmosa) lepida	DWF 717	AMNH 273186[e]	Peru: Loreto (46)	1146
M. (Marmosa) macrotarsus	LHE 1516	USNM 584462[e]	Bolivia: Santa Cruz (3)	797
M. (Marmosa) macrotarsus	LHE 1548	MNK [uncataloged]	Bolivia: Santa Cruz (3)	1146
M. (Marmosa) macrotarsus	JRM 202	MVZ 191187[f]	Brazil: Amazonas (5)	1146
M. (Marmosa) macrotarsus	MNFS 746	INPA 2912[f]	Brazil: Amazonas (6)	1087
M. (Marmosa) macrotarsus	JRM 450	INPA 2911[f]	Brazil: Amazonas (9)	1146
M. (Marmosa) macrotarsus	RSV 2303	AMNH 272816[e]	Peru: Loreto (46)	1146
M. (Marmosa) macrotarsus	RSV 2413	AMNH 272870[e]	Peru: Loreto (46)	860
M. (Marmosa) mexicana A	MHNG 1812007	MHNG 1812007	Belize: Corozal (1)	800[i]
M. (Marmosa) mexicana A	FN 32277	ROM 99608[e]	Guatemala: El Petén (26)	1146
M. (Marmosa) mexicana A	FN 34135	ROM 99776[e]	Guatemala: El Progreso (27)	1146
M. (Marmosa) mexicana A	FN 30771	ROM 96968[e]	Mexico: Campeche (31)	1146
M. (Marmosa) mexicana A	FN 30134	ROM 96318[e]	Mexico: Campeche (32)	1145
M. (Marmosa) mexicana A	FN 29881	ROM 96090[e]	Mexico: Campeche (34)	1144
M. (Marmosa) mexicana A	FN 29586	ROM 95795[e]	Mexico: Campeche (33)	1146
M. (Marmosa) mexicana B	JOM 7269	USNM 569858[e]	Guatemala: Alta Verapaz (24)	1087
M. (Marmosa) mexicana B	FN 31448	ROM 98459[e]	Guatemala: Baja Verapaz (25)	1146
M. (Marmosa) mexicana B	WB 8515	USNM 570071[e]	Guatemala: Zacapa (28)	1146
M. (Marmosa) murina	LPC 436	MVZ 197421[f]	Brazil: Mato Grosso (11)	1146
M. (Marmosa) murina	JLP 16986	UFMG 2599[f]	Brazil: Mato Grosso do Sul (10)	1146
M. (Marmosa) murina	LHE 503	USNM 549291[e]	Brazil: Pará (12)	1146
M. (Marmosa) murina	LHE 582	USNM 549292[e]	Brazil: Pará (12)	1146
M. (Marmosa) murina	LPC 715	MVZ 197433[f]	Brazil: Tocantins (14)	1092
M. (Marmosa) murina	T 2704	MHNG 1885048	French Guiana: Cayenne (21)	820[i]
M. (Marmosa) murina	T 2084	V-909[g]	French Guiana: Cayenne (22)	820[i]
M. (Marmosa) murina	T 2471	V-1206[g]	French Guiana: Cayenne (23)	820[i]
M. (Marmosa) murina	F 50629	ROM 113649[e]	Guyana: Demerara-Mahaica (29)	1146
M. (Marmosa) murina	F 41351	ROM 114321[h]	Surinam: Brokopondo (47)	770
M. (Marmosa) murina	TK 17359	CM 68346[e]	Surinam: Para (49)	1146
M. (Marmosa) murina	TK 17387	CM 68353[e]	Surinam: Para (49)	1146
M. (Marmosa) robinsoni	NK 101529	MSB 94363[e]	Panama: Los Santos (40)	1146
M. (Marmosa) robinsoni	NK 101606	MSB 94366[e]	Panama: Los Santos (40)	1146
M. (Marmosa) robinsoni	NK 101633	MSB 94368[e]	Panama: Veraguas (41)	1146
M. (Marmosa) robinsoni	NK 101634	MSB 94369[e]	Panama: Veraguas (41)	1146
M. (Marmosa) robinsoni	RPA 262	EBRG 25389[e]	Venezuela: Falcón (52)	1146
M. (Marmosa) rubra	F 54196	ROM 118744[e]	Ecuador: Orellana (20)	1146
M. (Marmosa) rubra	—	FMNH 84253[e]	Peru: Cusco (43)	402
M. (Marmosa) simonsi	NK 37836	MSB 87086[e]	Ecuador: El Oro (16)	1146
M. (Marmosa) simonsi	NK 37837	MSB 87087[e]	Ecuador: El Oro (16)	1146
M. (Marmosa) simonsi	TK 134911	TTU 103308[e]	Ecuador: Guayas (18)	1146
M. (Marmosa) tyleriana	—	AMNH 130510[e]	Venezuela: Bolívar (50)	398
M. (Marmosa) tyleriana	—	AMNH 130511[e]	Venezuela: Bolívar (50)	399
M. (Marmosa) waterhousei	F 40140	ROM 105889[e]	Ecuador: Orellana (19)	1146
M. (Marmosa) waterhousei	F 37580	ROM 105257[e]	Ecuador: Orellana (20)	727

TABLE 2
(Continued)

Taxon	Tissue/DNA#[a]	Voucher[b]	Locality[c]	bp[d]
M. (Marmosa) waterhousei	JLP 7480	MVZ 154754[e]	Peru: Amazonas (42)	726
M. (Marmosa) waterhousei	TK 73294	TTU 98717[e]	Peru: Loreto (44)	1146
M. (Marmosa) waterhousei	TK 73276	TTU 100922[e]	Peru: Loreto (44)	1050
M. (Marmosa) waterhousei	JMC 88	LSU 28017[e]	Peru: Loreto (45)	1146
M. (Marmosa) xerophila	—	USNM 443814[e]	Colombia: La Guajira (15)	402
M. (Marmosa) xerophila	RPA 315	AMNH 276582[e]	Venezuela: Falcón (51)	1146
M. (Marmosa) xerophila	RPA 324	AMNH 276586	Venezuela: Falcón (51)	1146
M. (Marmosa) zeledoni	—	AMNH 269997[e]	Panama: Chiriquí (38)	402
M. (Micoureus) constantiae	NK 15501	MSB 59883[e]	Bolivia: Santa Cruz (2)	1146
M. (Micoureus) constantiae	NK 23272	AMNH 275466[e]	Bolivia: Santa Cruz (4)	1146
M. (Micoureus) demerarae	T 2006	V-972	French Guiana: Cayenne (22)	820[i]
M. (Micoureus) demerarae	T 2083	V-884[e]	French Guiana: Cayenne (22)	820[i]
M. (Micoureus) demerarae	RSV 2029	AMNH 272667[e]	Peru: Loreto (46)	1146
M. (Micoureus) demerarae	RSV 2085	MUSM 13294[e]	Peru: Loreto (46)	1146
M. (Micoureus) paraguayana	MAM 46	MVZ 182064[e]	Brazil: São Paulo (13)	1146
M. (Micoureus) paraguayana	MAM 47	MVZ 182065[e]	Brazil: São Paulo (13)	1146
M. (Micoureus) regina	JLP 15435	MVZ 190323[e]	Brazil: Amazonas (7)	1146
M. (Micoureus) regina	MNFS 1232	MVZ 190332[e]	Brazil: Amazonas (8)	402
Outgroups				
Monodelphis brevicaudata	TK 17069	CM 68359[e]	Surinam: Nickerie (48)	1146
Tlacuatzin canescens	TK 11826	TTU 37700[e]	Mexico: Jalisco (35)	1146
Tlacuatzin canescens	TK 45085	UMSNH 2993[e]	Mexico: Michoacán (36)	1146

[a]Alphanumeric identifiers used by institutional tissue collections (and to label terminals in accompanying trees; figs. 2, 3). Sequences amplified from morphological specimens lack tissue/DNA numbers.
[b]See Materials and Methods for names of museum collections identified by abbreviations in this table.
[c]Country and next-largest administrative unit (state, department, province, etc). Numbers in parentheses refer to gazetteer entries (appendix), which provide additional geographic information.
[d]Number of base pairs sequenced. All sequences were obtained by us except as indicated otherwise.
[e]Examined by the authors.
[f]Examined by Rossi (2005).
[g]Examined by Steiner and Catzeflis (2003).
[h]Examined by Lim et al. (2005).
[i]From GenBank.

identical haplotypes, our phylogenetic analyses were based on a matrix that included cytochrome-*b* sequences from 66 individuals.

Although many sequences identified as *Marmosa murina* are available in GenBank, most of them are from localities on the Guiana Shield, where there is very little genetic variation and apparently no phylogeographic structure (Steiner and Catzeflis, 2003, 2004); therefore, we included only three (all from French Guiana) in our study. Two other GenBank sequences reported as *M. murina* in previous studies are from Peru and correspond to *M. macrotarsus* (sensu Rossi, 2005). We resequenced the tissues from which these published sequences were obtained and found several discrepancies. For example, our sequence of AMNH 272816 should be identical to GenBank accession number AJ487003, but these differ in an A/C mutation at position 783 (as numbered from the start codon). Additionally, our sequence of AMNH 272870 was generated from the same specimen as GenBank accession AJ487002, but the two differ at four sites (59 A/G, 246 C/T, 813 G/A, 819 T/C). Our sequences, which were generated from at least two strands, are unambiguous at these sites. Any number of reasons, including error incorporated by Taq polymerase, could explain such differences. However,

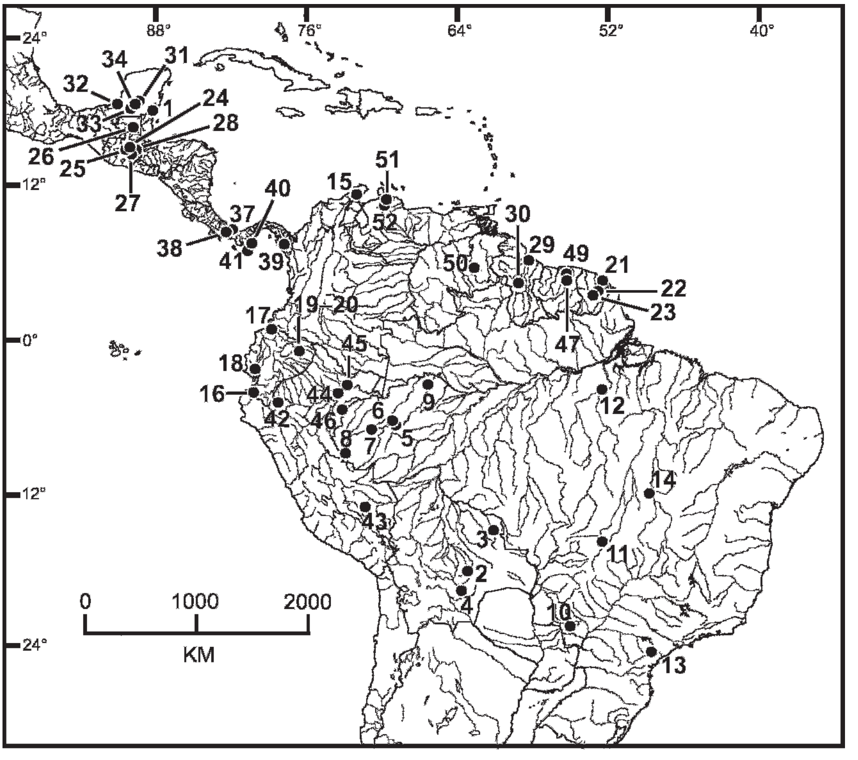

Fig. 1. Provenance of sequenced specimens of *Marmosa* (localities of sequenced outgroup specimens are not shown). Numbers refer to entries in the Gazetteer (appendix).

because we do not have the original chromatograms from the GenBank reports, we used the sequences generated in our lab from these specimens.

Laboratory Methods: Genomic DNA was extracted from all samples using DNeasy extraction kits (Qiagen, Inc.). Whenever possible, we amplified the entire cytochrome-*b* gene using primers CYTB-F1 and CYTB-R1 (table 3) located in the flanking tRNAs. To generate fragments of a suitable size for sequencing, we used this PCR product in two separate reamplification reactions, one using primer CYTB-F1 paired with CYTB-730R and one using either CYTB-540F or CYTB-650F paired with CYTB-R1. In cases where we extracted poor-quality DNA from skin samples (two samples of *Marmosa tyleriana* and one each of *M. xerophila*, *M. zeledoni*, and *M. rubra*), we generated a short (~400 bp) PCR product using CYTB-F1 paired with CYTB-420R and sequenced it directly using amplification primers.

Initial PCR amplifications using genomic DNA as a template were performed in 20 μL reactions using GoTaq DNA polymerase (Promega Corp.) and recommended concentrations of primers, unincorporated nucleotides, buffer, and $MgCl_2$. These reactions were performed in a four-stage touchdown protocol. The first stage consisted of 5 cycles of denaturation at 95°C for 20 seconds, annealing at 59°C for 20 seconds, and extension at 72°C for 30 seconds. The second and third stages were identical to the first except for lowered annealing temperatures of 57°C and 55°C, respectively. The final stage consisted of 25 cycles with an annealing temperature of 52°C. Subsequent reamplification reactions using this product as a template consisted of a single stage of 25 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute. All reactions were preceded by an initial denaturation at 95°C for 2 minutes and followed by a 7 minute extension at 72°C.

TABLE 3
Name and DNA Sequence of Primers Used for DNA Amplification and Sequencing

Primer name	Primer sequence
CYTB-F1-Didelphidae	5′ ATAACCTATGGCATGAAAAACCATTGTTG
CYTB-R1-Didelphidae	5′ CCTTCATTGCTGGCTTACAAGGC
CYTB-420R-Didelphidae	5′ GCTCCTCAGAAGGATATTTGTCCTCA
CYTB-730R-*Marmosa*	5′ TCWCCTAATARRTCWGGTGARAATATTGC
CYTB-540F-*Marmosa*	5′ GAGGAGGMTTYTCHGTTGATAAAGC
CYTB-650F-*Marmosa*	5′ CTATTCCTTCACGAAACAGGCTC
CYTB-217R-*Marmosa*	5′ TCTGTAGCCCAYATYTGYCGWGAYG
CYTB-70F-*Marmosa*	5′ CCMTCAAATATTTCAGCCTGATG

Gene fragments were sequenced in both directions using amplification primers and ABI BigDye version 3.1 terminator chemistry (Applied Biosystems, Inc.). Reactions were run on either an ABI 3130xl or ABI 3730xl capillary sequencer. Sequences were edited and compiled using Sequencher version 4.8 (Gene Codes Corporation, 2007). All sequences, along with their specimen voucher numbers, have been deposited in GenBank with accession numbers HM106338–HM106402.

Analytical Methods: We performed multiple sequence alignment in Clustal X version 2.0 (Larkin et al., 2007) and adjusted the resulting alignment with reference to translated amino-acid sequences. We used maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI) to analyze the resulting data matrix; missing bases were coded as unknown for all phylogenetic analyses. To assess nodal support, we used nonparametric bootstrapping (Felsenstein, 1985) for the MP and ML analyses and nodal posterior probability estimates for the BI analysis (Ronquist and Huelsenbeck, 2003). Parsimony analyses were performed in PAUP* 4.0b10 (Swofford, 2002) using equal weighting and the heuristic search option with 1000 replicate searches, 10 random-addition replicates, and tree bisection-reconnection (TBR) branch swapping. Maximum-parsimony bootstrap analyses were performed in PAUP* using 1000 pseudodoreplicated data matrices, each with 5 random-addition sequences and TBR branch-swapping. To determine the appropriate model of evolution for ML and BI analyses, we considered both hierarchical likelihood-ratio tests (hLRT) and the Akaike information criterion (AIC) as implemented in ModelTest v. 3.7 (Posada and Crandall, 1998) and PAUP* (Swofford, 2002). For ML analyses, we performed 20 independent searches in GARLI 0.96 beta (Zwickl, 2006) using the default settings. Maximum-likelihood bootstrap analyses were performed in GARLI 0.96 beta using 100 pseudoreplicated data matrices, with 10 searches performed on each. Bayesian analyses were performed using the Markov Chain Monte Carlo (MCMC) sampling approach in MrBayes v. 3.1.2 (Huelsenbeck and Ronquist, 2001; Altekar et al., 2004; Ronquist et al., 2005) through the Computational Biology Service Unit from Cornell University (http://cbsuapps.tc.cornell.edu/mrbayes.aspx). The search started with a random tree, and consisted of one cold chain and three heated chains (temperature = 0.2) and default priors. The Markov chains were run for 1×10^6 generations, and trees were sampled every 1000 generations. Default values were kept for the ''relburnin'' and ''burninfrac'' options in MrBayes (i.e., relburnin = yes; burninfrac = 0.25); therefore, the first 250,000 generations (250 trees) were discarded as burn-in, and posterior probability estimates of all model parameters were based on the remaining (750) trees.

To estimate genetic divergence, we calculated average uncorrected (p) distance within each species and average pairwise p distances among species. In addition, we report K2P-corrected distances for interspecific comparisons. These model-corrected statistics are the traditional metric for genetic divergence in the didelphid literature (e.g., Patton et al., 2000; Patton and Costa, 2003), so we computed them to allow comparisons with values reported in previous studies. All distances were calculated using MEGA version 4 (Tamura et al., 2007).

RESULTS

There are five pairs of identical haplotypes among our 71 sequences: two specimens of *Marmosa simonsi* (NK37836 and NK37837) from Ecuador, two specimens of *M. robinsoni* (NK101606 and NK101633) from Panama, two specimens of *M. murina* (TK17359 and TK17387) from Surinam, two specimens of *M. murina* (T2471 and T2704) from French Guiana, and two specimens of *M. paraguayana* (MAM46 and MAM47) from Brazil. In each of these cases, we excluded the sequence corresponding to the second-listed specimen from all subsequent phylogenetic analyses, resulting in a final data matrix comprising 42 complete cytochrome-*b* sequences (each with 1146 bp) and 24 partial sequences (ranging in length from 398 to 1145 bp; table 2). As expected of mitochondrial sequences, average base composition across this dataset is relatively poor in guanine (30.7% A, 22.9% C, 12.5% G, 33.9% T), but there is no significant departure from base-compositional stationarity among taxa ($\chi^2 = 121.83$, df = 186, $p = 0.99$; see Saccone et al., 1989). All sequences translate to open reading frame.

Our dataset contains 508 variable characters, 465 of which are parsimony informative. Maximum-parsimony analysis recovered 96 minimum-length trees, the strict consensus of which is shown in figure 2. For the model-based analyses (ML and BI), the hierarchical likelihood-ratio test (hLRT) selected the most complex model (GTR+I+Γ), whereas the simpler HKY+I+Γ model was preferred using the AIC. To test for possible effects of model selection on our phylogenetic analyses, we performed ML analyses specifying each of these models and obtained identical topologies; therefore, we report only the results obtained under the more complex GTR+I+Γ model (table 4; fig. 3).

Species Limits: We were able to test the monophyly of just 11 of the 14 morphologically defined species in the subgenus *Marmosa* recognized by Rossi (2005) because we lacked samples of two taxa (*Marmosa andersoni* and *M. tobagi*), and we had only a single representative sample of *M. zeledoni*. For 10 of these 11 cases, morphologically defined species were recovered as monophyletic groups, usually with moderate to very strong support in both the MP and the model-based analyses (figs. 2, 3). The only noteworthy exception concerns *M. mexicana*, samples of which form two deeply divergent haplogroups (hereafter referred to as "*M. mexicana* A" and "*M. mexicana* B") that were not consistently recovered as sister taxa. Although the model-based analyses recovered these two haplogroups as a clade, the MP analysis placed *M. zeledoni* as the sister taxon to *M. mexicana* A and *M. isthmica* as sister to *M. mexicana* B; as might be expected, both of these alternatives are weakly supported.

Mean uncorrected sequence divergence within species (provisionally including *M. mexicana* A and *M. mexicana* B, see below; table 5) ranges from 0.2 to 4.2%. However, sequence divergence across the basal split within some species is considerably higher than these average within-group values. In particular, Panamanian sequences of *M. robinsoni* differ from the single available Venezuelan sequence by 6.2%, Bolivian sequences of *M. macrotarsus* differ from Brazilian and Peruvian sequences by 6.5%, and Peruvian sequences of *M. demerarae* differ from French Guianan sequences by 5.7%. By contrast, average interspecific divergence values within three consistently recovered sister-species pairs (*M. constantiae* + *M. regina*, *M. demerarae* + *M. paraguayana*, and *M. robinsoni* + *M. xerophila*) range from 9.5% to 18.6%.

Phylogenetic Relationships: Whereas cytochrome-*b* sequences are clearly useful for testing the monophyly of morphologically defined species and for assessing intraspecific genetic divergence, they are less consistently informative about phylogenetic relationships among species. Approximately half of the interspecific nodes resolved in our trees were recovered with strong support in both MP and model-based analyses. Among these well-supported nodes are the sister-species pairs *Marmosa robinsoni* + *M. xerophila*, *M. constantiae* + *M. regina*, and *M. demerarae* + *M. paraguayana*. At deeper levels, all of our

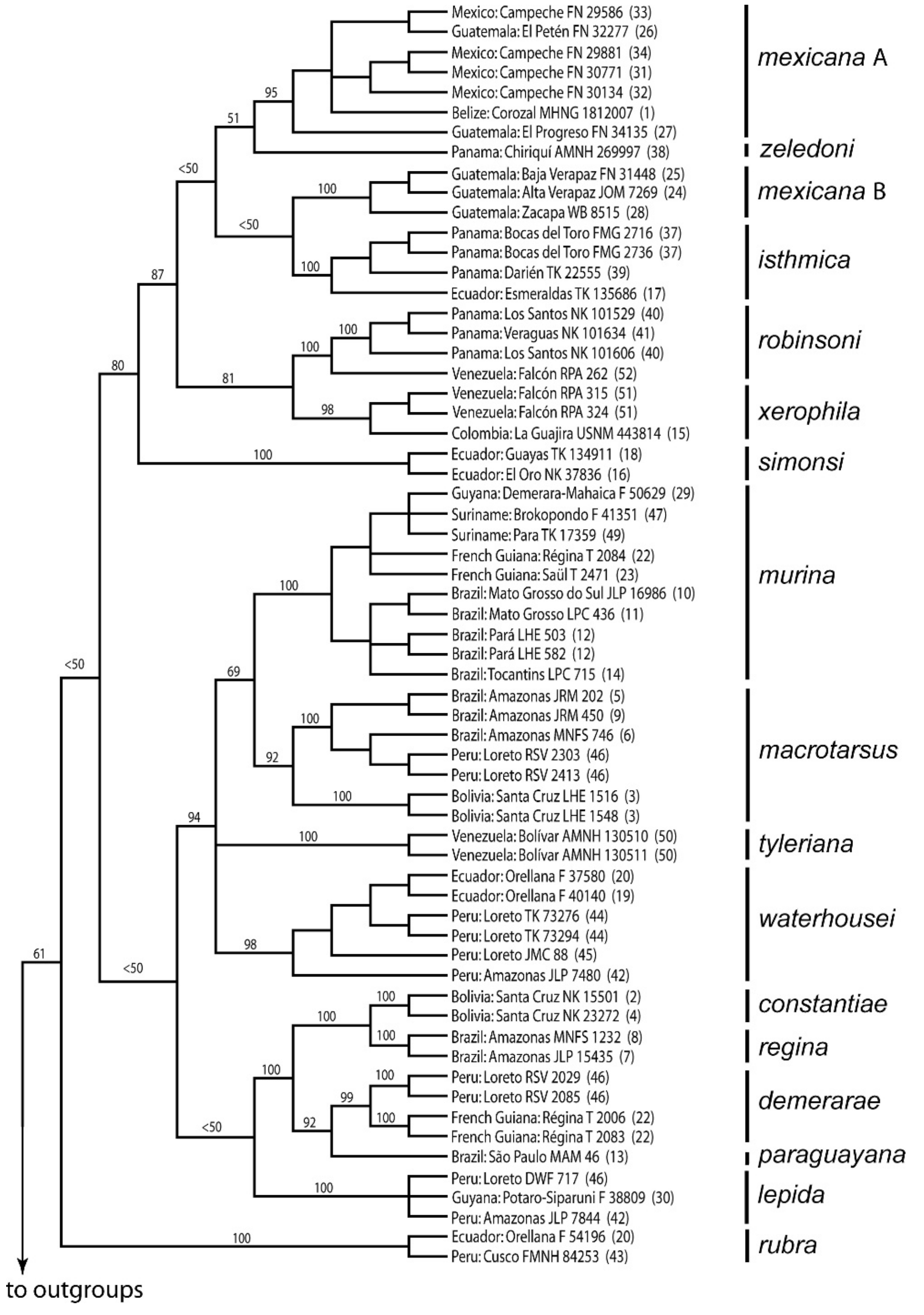

Fig. 2. Strict consensus of 96 equally most-parsimonious trees (L = 2198; CI = 0.36; RI = 0.80). Bootstrap support values are indicated above branches subtending species and conspecific haplogroups discussed in the text. For each terminal, country of origin, next-largest political unit (state, department, province, etc.), and an alphanumeric specimen identifier (from table 2) are provided. Numbers in parentheses refer to localities mapped in figure 1 and listed in the Gazetteer (appendix).

phylogenetic analyses supported the monophyly of the genus *Marmosa* sensu Voss and Jansa (2009). Also, all analyses recovered a well-supported group comprising *M. robinsoni*, *M. xerophila*, *M. isthmica*, *M. mexicana*, *M. zeledoni*, and *M. simonsi* (hereafter the "*mexicana-robinsoni* clade"); within this group, *M. simonsi* was consistently recovered as the sister taxon to the remaining species with moderate to strong support. *Marmosa murina* and three other species (*M. tyleriana*, *M. waterhousei*, and *M. macrotarsus*) formed another consistently well-supported clade, and the subgenus *Micoureus* (represented by *M. constantiae*, *M. regina*, *M. demerarae*, and *M. paraguayana*) was also recovered as monophyletic in all of our analyses.

By contrast, our MP and model-based analyses were notably inconsistent in their placement of *Marmosa lepida* and *M. rubra*. Whereas model-based analyses recovered *M. lepida* as sister to the *murina* cluster + *Micoureus* (with weak ML bootstrap but strong Bayesian support), the parsimony analysis recovered *M. lepida* as the sister taxon to *Micoureus* (with negligible bootstrap support). In the model-based analyses, *M. rubra* was recovered as the sister taxon to the *mexicana-robinsoni* clade (again with weak ML bootstrap but impressive Bayesian support), whereas *M. rubra* was recovered as the sister taxon to all other analyzed congeners in the parsimony tree (with <50% bootstrap support).

The remaining interspecific nodes either agree or differ between the MP and model-based analyses, but all have uniformly weak support values. Within the *mexicana-robinsoni* clade, for example, the ML analysis recovered the two haplogroups of *M. mexicana* (A and B) as a clade, with *M. zeledoni* and *M. isthmica* as sequentially less closely related sister taxa (fig. 3), whereas the MP analysis placed *M. zeledoni* as sister to *M. mexicana* A and *M. isthmica* as sister to *M. mexicana* B (fig. 2); neither alternative received strong Bayesian or bootstrap support. Although both ML and MP analyses recovered the more inclusive clade comprised of *M. mexicana*, *M. isthmica*, and *M. zeledoni*, Bayesian and bootstrap support for this relationship is negligible.

TABLE 4
Parameter Estimates from the Best-Fit Model of Nucleotide Substitution for Cytochrome *b*

-ln L	10807.817
Base frequencies	
πA	0.349
πC	0.251
πG	0.059
πT	0.341
Rate matrix R	
rA-C	1.320
rA-G	13.495
rA-T	1.026
rC-G	1.500
rC-T	13.189
rG-T	1.000
Proportion of invariant sites	0.515
Shape parameter for the Γ distribution (α)	1.139

Patterns of interspecific relationships within the robustly supported *murina* cluster are similarly equivocal. Whereas the ML analysis recovered the sister-species pair *M. tyleriana* + *M. waterhousei*, with *M. murina* and *M. macrotarsus* as sequentially more distantly related sister taxa (fig. 3), the MP analysis placed *M. murina* and *M. macrotarsus* as sister taxa and left the positions of *M. tyleriana* and *M. waterhousei* unresolved (fig. 2); neither of these alternatives received compelling support. A clade comprising the four species of *Micoureus* was recovered as the sister taxon to the *murina* clade in both of our model-based analyses, but always with low support.

DISCUSSION

Despite ongoing debate about species concepts in the systematic literature (reviewed by de Queiroz, 1998; Mayden, 1999; Coyne and Orr, 2004; Baker and Bradley, 2006), most researchers agree that genetically independent lineages are fundamentally important units of evolutionary diversification. We therefore adopt a lineage-based concept of species (after de Queiroz, 1998), for which we use mtDNA haplotype monophyly (as recovered by this study) and morphological diagnosability (as documented by Rossi, 2005; Rossi et al., 2010) as operational criteria for species recognition. Whereas mtDNA sequences provide crucial

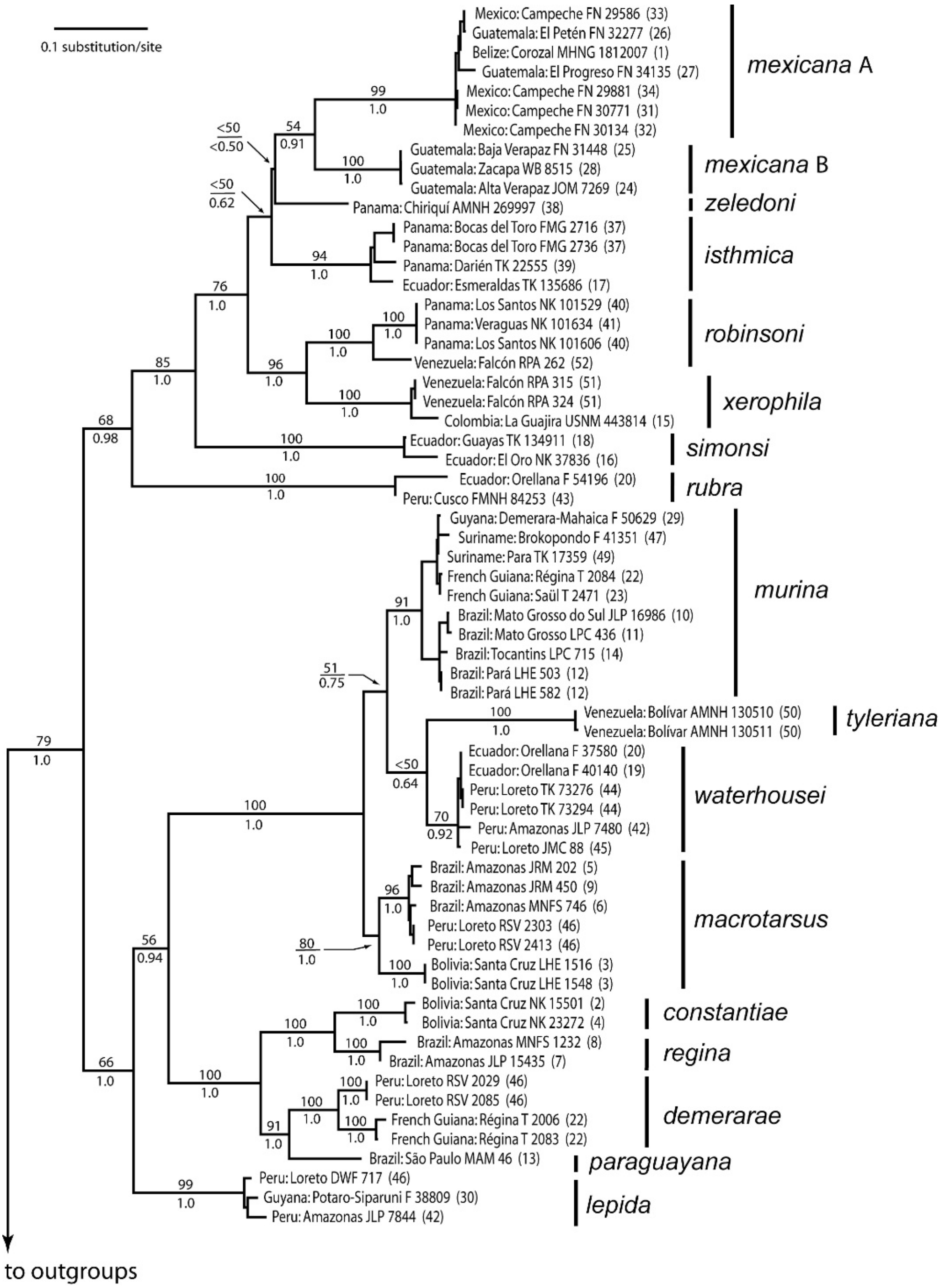

Fig. 3. The maximum-likelihood tree inferred from the best-fit model of nucleotide substitution (table 4). ML bootstrap support values and Bayesian posterior probabilities are indicated above and below branches, respectively. Branch and terminal labels follow the same conventions explained in the caption to figure 2.

TABLE 5

Matrix of Genetic Distances (percent sequence divergence) Within and Among Species of *Marmosa*[a]

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1. *M.* (*M.*) *isthmica*	**3.3**	18.8	21.5	17.0	13.7	20.7	15.0	18.9	18.0	18.4	20.5	14.2	11.1	21.8	18.4	19.9	22.1
2. *M.* (*M.*) *lepida*	16.4	**2.4**	17.5	21.0	20.0	17.9	19.9	18.5	19.5	18.6	17.2	18.7	18.2	17.8	17.0	17.2	16.7
3. *M.* (*M.*) *macrotarsus*	18.5	15.5	**3.7**	22.1	19.5	9.6	20.4	22.0	18.8	14.8	10.1	22.3	20.3	20.1	19.4	20.3	21.8
4. *M.* (*M.*) *mexicana* A	14.8	18.0	18.9	**1.1**	14.8	21.8	18.2	20.1	20.9	21.5	21.4	16.1	12.1	21.7	22.1	23.1	21.9
5. *M.* (*M.*) *mexicana* B	12.3	17.3	17.0	13.1	**0.2**	20.6	15.1	19.3	16.8	21.0	21.1	15.1	12.5	21.3	20.2	20.6	22.2
6. *M.* (*M.*) *murina*	17.9	15.8	8.9	18.6	17.8	**2.5**	20.5	21.2	18.4	11.8	9.3	21.0	16.7	20.7	18.3	19.8	20.8
7. *M.* (*M.*) *robinsoni*	13.4	17.2	17.7	15.7	13.5	17.7	**3.2**	19.6	16.8	19.8	19.8	12.3	12.4	22.0	21.3	21.8	22.3
8. *M.* (*M.*) *rubra*	16.5	16.3	19.0	17.4	16.8	18.3	17.0	**4.2**	22.0	20.1	19.8	20.2	19.8	20.8	21.4	19.8	22.1
9. *M.* (*M.*) *simonsi*	15.8	16.9	16.5	17.8	14.8	16.2	14.9	18.9	**3.1**	18.2	18.3	18.9	16.8	23.1	21.6	21.2	23.4
10. *M.* (*M.*) *tyleriana*	16.2	16.2	13.1	18.5	18.1	10.7	17.3	17.6	16.1	**0.3**	9.6	21.4	20.3	20.5	19.9	19.7	22.7
11. *M.* (*M.*) *waterhousei*	17.7	15.2	9.3	18.4	18.1	8.6	17.2	17.3	16.1	9.0	**0.7**	20.0	17.8	19.4	18.8	19.6	20.5
12. *M.* (*M.*) *xerophila*	12.6	16.3	19.0	14.2	13.4	18.1	11.1	17.4	16.4	18.4	17.4	**1.7**	11.7	20.6	20.5	19.6	21.3
13. *M.* (*M.*) *zeledoni*	10.1	15.9	17.6	11.0	11.2	14.9	11.2	17.2	14.8	17.6	15.8	10.6	—	19.6	18.9	18.4	21.6
14. *M.* (*Mi.*) *constantiae*	18.7	15.6	17.4	18.6	18.3	17.9	18.9	18.1	19.7	17.8	16.9	17.9	17.2	**1.2**	16.3	14.3	8.9
15. *M.* (*Mi.*) *demerarae*	16.1	15.0	16.9	18.8	17.5	16.0	18.3	18.4	18.5	17.3	16.4	17.7	16.6	14.3	**4.0**	10.3	15.2
16. *M.* (*Mi.*) *paraguayana*	17.3	15.2	17.6	19.5	17.8	17.2	18.7	17.3	18.2	17.2	17.0	17.0	16.2	12.9	9.5	—	12.5
17. *M.* (*Mi.*) *regina*	18.9	14.8	18.6	18.7	18.9	17.9	19.1	18.9	19.8	19.3	17.6	18.3	18.5	8.3	13.5	11.3	**2.2**

[a]Average uncorrected (p) distances among conspecific sequences are arrayed along the diagonal, interspecific p distances are below the diagonal, and Kimura two-parameter (K2P) distances are above the diagonal.

information about lineage membership based on maternally inherited genes, morphological diagnosability is important (1) as a proxy measure of evolutionary divergence at biparentally inherited nuclear loci, (2) because it enables mitochondrial clades to be associated with name-bearing types for which sequence data are not available, and (3) because it allows other unsequenced specimens to be used for mapping geographic ranges and for niche-based distributional modeling (Graham et al., 2004; Phillips et al., 2006). Although a high degree of sequence divergence is neither necessary nor sufficient for species recognition (Ferguson, 2002; Baker and Bradley, 2006), pairwise distances provide a heuristically useful basis for comparisons of genetic variation within and among lineages, whether or not the latter are formally recognized as taxa.

In general, our analyses of mitochondrial sequence data from the subgenus *Marmosa* corroborate the morphology-based taxonomy proposed by Rossi (2005), most of whose species (table 1) were recovered as well-supported monophyletic groups. Among the noteworthy taxonomic changes proposed by Rossi (2005) and by Rossi et al. (2010) that are unambiguously supported by our results are the recognition of *M. isthmica* and *M. simonsi* as species distinct from *M. robinsoni*, and the recognition of *M. macrotarsus* and *M. waterhousei* as species distinct from *M. murina*. Indeed, our failure to recover *Marmosa murina* (sensu lato: including *macrotarsus* and *waterhousei*) and *M. robinsoni* (sensu lato: including *isthmica* and *simonsi*) as clades convincingly refutes hoary taxonomic concepts dating back to the middle of the last century (Tate, 1933; Hershkovitz, 1951). The validity of three other species long recognized as distinct (*M. rubra*, *M. tyleriana*, and *M. xerophila*) is also clearly supported by our sequencing results.

The only exception in this context is *Marmosa mexicana* (sensu Rossi, 2005; Rossi et al., 2010), sequenced exemplars of which were not consistently recovered as a clade, and which exhibit very high sequence divergence (>13%) between two well-supported haplogroups. One haplogroup (*M. mexicana* A) is represented by samples from seven lowland localities (<300 m above sea level) in Belize, Guatemala, and southeastern Mexico, whereas the other haplogroup (*M. mexicana* B) is represented by samples from three localities in the Guatemalan highlands (>1500 m; fig. 4). Although examined voucher material of both haplogroups fits the morphological diagnosis of *M. mexicana* (sensu Rossi [2005] and Rossi et al. [2010]), noteworthy phenotypic variation does exist among our tissue vouchers. Among other differences, skins of *mexicana* A are distinctly paler than those of *mexicana* B, and skulls of *mexicana* A are visibly broader in proportion to their length than like-aged skulls of *mexicana* B. Additionally, small postorbital processes of the frontals are present in most examined adult specimens of *mexicana* A, whereas no examined adult specimen of *mexicana* B has any trace of a postorbital process. Although these differences are not taxonomically compelling due to small sample sizes, they do suggest the likelihood that more than one species is represented in our material.

Several names that are currently regarded as synonyms or subspecies of *Marmosa mexicana* might apply to these haplogroups, but we lack sequence data from samples adjacent to any of the relevant type localities: Juquila (Mexico, Oaxaca; type locality of *mexicana* Merriam, 1897), Isla de Roatán (Honduras, Islas de la Bahía; type locality of *ruatanica* Goldman, 1911), Izamal (Mexico, Yucatán; type locality of *mayensis* Osgood, 1913), and Boquerón (Panama, Chiriquí; type locality of *savannarum* Goldman, 1917). In the absence of relevant genetic data, we note that the best phenotypic and ecogeographic match for haplogroup A is *mayensis*, a pale-furred form from the same dry Yucatecan forest biome where at least some of our voucher material was collected. By contrast, the darker pelage and montane provenance of haplogroup B more closely resembles the phenotypic and ecogeographic attributes of the nominotypical form (*mexicana*). Obviously, future studies based on denser geographic sampling and more extensive sequencing within the *mexicana* complex will be necessary to test these conjectures.

Although other species of *Marmosa* were consistently recovered as monophyletic groups, unusual levels of sequence variation that we observed in some of them merit

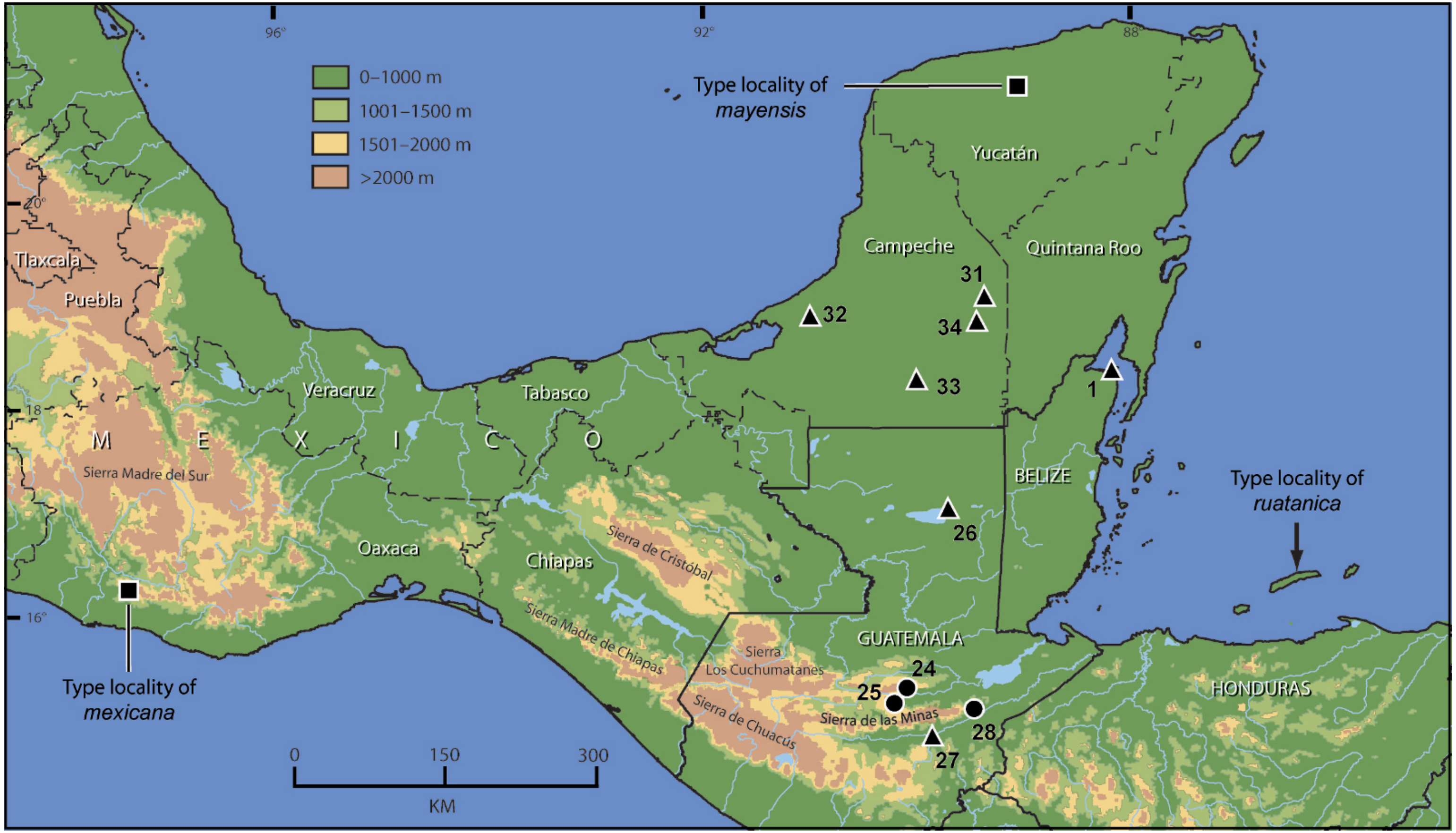

Fig. 4. Provenance of sequenced specimens of *Marmosa mexicana* A (▲) and *M. mexicana* B (●). Numbers refer to entries in the Gazetteer (appendix).

comment. In the case of *M. robinsoni*, moderately high divergence (ca. 6%) between Venezuelan and Panamanian sequences provides the first genetic evidence that this species, even in the restricted sense that it is now understood (Rossi, 2005; Rossi et al., 2010), might be geographically variable. Although the data at hand are too few to sustain taxonomic interpretation, we note that *M. robinsoni* is widely distributed and still includes several subjective synonyms representing insular and continental populations alleged to differ in size and pelage coloration (*casta*, *chapmani*, *fulviventer*, *grenadae*, *luridavolta*, *mitis*, *nesaea*, and *pallidiventris*; Rossi, 2005; Rossi et al., 2010). Therefore, assessing the significance of mtDNA divergence between our Panamanian and Venezuelan samples will require much broader geographic sampling. Future studies with this objective should include sequence data from as many nominal taxa as possible, including the typical form *robinsoni* Bangs, 1898 (from Isla de Margarita, Venezuela).

Another noteworthy example of intraspecific sequence variation concerns *Marmosa macrotarsus*, Bolivian samples of which differ by about 6% from Peruvian and western Brazilian material. Interestingly, both Bolivian samples come from the same region in northeastern Santa Cruz from which new cricetid rodent species have recently been described (Emmons and Patton, 2005; Carleton et al., 2009). Morphological exemplars of both haplogroups were examined by Rossi (2005), who referred the Peruvian and Brazilian material to *M. macrotarsus* but did not make a definitive taxonomic determination of the Bolivian material (which he referred to "*Marmosa* cf. *macrotarsus*"). Our examination of Bolivian voucher material, which we compared side-by-side with sequenced specimens from Peru, did not reveal any consistent differences in characters of the skin, skull, or dentition.

The last example of unusual intraspecific sequence variation in our study involves *Marmosa demerarae*, a member of the subgenus *Micoureus*. Consistent with the results of Patton and Costa's (2003) analysis of a 630 bp fragment of cytochrome *b* from 19 geographic populations referred to this species, our French Guianan sequences (representing their "northeastern" clade) differ from Peruvian sequences (representing their "southwestern" clade) by almost 6%. As documented elsewhere (Patton et al., 2000; Costa and Patton, 2006), the *demerarae* complex of *Micoureus* involves several additional phylogroups with equally divergent mtDNA sequences, the taxonomic interpretation of which is beyond the scope of this study.

Other geographically widespread species represented by multiple samples in our study (*Marmosa isthmica*, *M. lepida*, *M. murina*, and *M. waterhousei*) exhibit only modest sequence variation. Although phylogeographic structure is apparent in some cases (e.g., the discrete Guianan versus Brazilian clusters of *M. murina*), there are no clear indications in these results to challenge Rossi's (2005) interpretation that each of these taxa represents a single valid species. Indeed, the low level of sequence variation observed in *M. lepida*—a tiny species represented in our study by specimens from distant Guyanese and Peruvian localities—is at least as remarkable as the high levels of sequence variation that we discovered in other taxa.

Phylogenetic Relationships

Because the strength of the phylogenetic signal provided by the cytochrome-*b* gene typically declines with evolutionary depth (Meyer, 1994; Yoder et al., 1996; Zardoya and Meyer, 1996; Gissi et al., 2000; Springer et al., 2001), it is not surprising that few of the deeper nodes in our trees are well supported. Among those interspecific relationships with strong nodal support are (1) monophyly of the subgenus *Micoureus*; (2) monophyly of a group comprised of *M. macrotarsus*, *M. murina*, *M. tyleriana*, and *M. waterhousei*; (3) a sister-group relationship between *M. robinsoni* and *M. xerophila*; and (4) monophyly of a group comprised of *M. robinsoni*, *M. xerophila*, *M. isthmica*, *M. mexicana*, *M. zeledoni* and *M. simonsi*. Whereas some of these relationships have previously been recovered by authors, others are unique to this report.

The monophyly of the subgenus *Micoureus*—represented in our study by the species *M. constantiae*, *M. demerarae*, *M. paraguayana*, and *M. regina*—is a noncontroversial result previously reported by other sequence-based phylogenetic analyses (e.g., Patton et al., 1996; Voss and Jansa, 2003, 2009; Jansa and Voss, 2005; Jansa et al., 2006). Although *M. alstoni* and *M. phaea* are the only currently recognized species of *Micoureus* that are absent from our analyses, we caution that the subgenus has not been revised for many years and that several nominal taxa now considered to be synonyms or subspecies of *M. demerarae* and *M. regina* were treated as valid species by Tate (1933). Because no substantive analyses of character data have ever been published to support currently accepted synonymies in this group, it is possible that additional species of *Micoureus* will be recognized as valid by future taxonomic researchers. If so, then our taxon sampling in *Micoureus* may be far from complete and our recovered support for subgeneric monophyly correspondingly less compelling.

Strong support for a group that includes *Marmosa murina*, *M. macrotarsus*, *M. tyleriana*, and *M. waterhousei* has not previously been reported in the literature. Although this clade approximates the membership of Tate's "Murina Section" (table 1), it differs from Tate's concept[6] by excluding *M. rubra*, which might either be a basal lineage in the genus (fig. 2) or the sister taxon to the *mexicana-robinsoni* clade (fig. 3). Of these alternatives, the latter is strongly supported by recent analyses of concatenated nuclear-gene sequence data (Voss and Jansa, 2009). Whereas some previous analyses of mtDNA sequence data with much sparser taxonomic sampling (Steiner et al., 2005) have recovered *M. lepida* and *M. murina* as sister species, the relationships of *lepida* were not consistently resolved in our results. However, analyses of concatenated nuclear-gene sequence data (Voss and Jansa, 2009) suggest that *lepida* is sister to a group comprised of *Micoureus* and the *murina* cluster, as recovered by our model-based analyses (fig. 3).

A close relationship between *Marmosa robinsoni* and *M. xerophila* was implied by Handley and Gordon (1979), but our results provide the first phylogenetic evidence to support this notion. Although the data at hand suggest that these are reciprocally monophyletic sister taxa, we note that the range of *xerophila* is entirely contained within that of *robinsoni* (see Rossi et al., 2010: figs. 25, 26), and that the latter species includes numerous nominal taxa currently treated as synonyms. Because our geographic sampling of *robinsoni* haplotypes is sparse, the possibility exists that *xerophila* is a divergent peripheral isolate of a widespread and possibly paraphyletic complex of morphologically similar forms currently lumped together in *robinsoni*. Any future study focused on scenarios of speciation in the genus should include many more sequences from geographically representative populations of the latter taxon.

The discovery of a well-supported clade that includes *Marmosa isthmica*, *M. mexicana*, *M. robinsoni*, *M. simonsi*, *M. xerophila*, and *M. zeledoni* is a novel result of this study. This clade does not coincide in membership with any of Tate's "sections" (table 1), nor had its member taxa been explicitly associated with one another until the revisionary work by Rossi et al. (2010). To be sure, nuclear-gene datasets have consistently clustered *mexicana* with *isthmica* (previously reported as "*robinsoni*" by Voss and Jansa, 2003, 2009; Jansa and Voss, 2005; Jansa et al., 2006; Gruber et al., 2007), but no phylogenetic analysis of morphological or molecular data has hitherto included representative material of *robinsoni* (sensu stricto), *simonsi*, *xerophila*, or *zeledoni*. To our knowledge, no morphological character is uniquely shared by all of these forms to the exclusion of other species of *Marmosa*. Instead, their unifying characteristic seems to consist in a biogeographic criterion that has emerged in recent years as a fundamental dichotomy within several groups of codistributed Neotropical organisms.

Biogeographic Implications

The Andes are a formidable barrier to dispersal of lowland and lower-montane organisms that occur on opposite sides of the main cordilleras. Following Haffer (1967), we refer to the lowlands west and north of the Andes as trans-Andean, and those east and south of the Andes as cis-Andean. Examples

[6]Note that Tate (1933) considered *waterhousei* a subspecies of *murina* and used the name *quichua* for the taxon herein referred to as *macrotarsus*.

of trans-Andean landscapes include those in Central America, the contiguous Pacific lowlands of western Ecuador and Colombia, and the Caribbean lowlands of northern Colombia and northwestern Venezuela. Cis-Andean regions include most of the remainder of tropical and subtropical South America, including Amazonia and the Atlantic Forest of southeastern Brazil.

The *mexicana-robinsoni* clade includes all of the trans-Andean species currently assigned to the subgenus *Marmosa*. Of these, five species (*isthmica*, *mexicana*, *simonsi*, *xerophila*, and *zeledoni*) are trans-Andean endemics, and one (*robinsoni*) occurs on both sides of the Andes (see Rossi et al., 2010, for range maps of all of these taxa). Although at least two species of the subgenus *Micoureus* (not represented in this study) also occur west of the Andes, the results in hand suggest that these mountains may have played a significant role in constraining the early biogeographic radiation of *Marmosa*.

Phylogenetic evidence for separate cis- and trans-Andean radiations has recently been reported for a number of terrestrial and freshwater organisms (e.g., Harvey and Gutbertlet, 2000; Perdices, 2002; Ribas et al., 2005; Noonan and Wray, 2006), suggesting that Andean crossings are rare events in some clades. However, cis- and trans-Andean taxa are sometimes scattered throughout recovered phylogenies (Weksler, 2006), implying that such events may have occurred frequently in other groups. In some studies, clades on opposite sides of the Andes are represented by distinct genera (Harvey and Gutbertlet, 2000). In others, cis- versus trans-Andean distributions distinguish reciprocally monophyletic groups of congeneric species (Perdices et al., 2002; Ribas et al., 2005), whereas distinct cis- and trans-Andean phylogroups have been discovered within certain widespread "species" (e.g., the tree *Symphonia globulifera*; Dick et al., 2003).

The origin of cis- versus trans-Andean distributions has been attributed to a variety of historical scenarios, including Andean uplift, marine transgressions, and Pleistocene climatic fluctuations (reviewed by Cracraft and Prum, 1988; Brumfield and Capparella, 1996). Because some of these postulated tectonic and paleoclimatic events occurred at different times, molecular dates are potentially useful for assessing the relevance of competing historical explanations. Estimated dates for phylogenetic nodes that separate cis- versus trans-Andean clades of parrots (Ribas et al., 2005) and pimelodid fishes (Perdices et al., 2002), for example, are in the range of 6–8 million years, much too old to support a Pleistocene origin for this distributional pattern (contra Haffer, 1967). In this context, time-calibrating the present molecular phylogeny of *Marmosa* will contribute toward the causal analysis of a taxonomically widespread biogeographic phenomenon, a goal that we defer to a subsequent report pending the analysis of sequence data from additional loci.

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APPENDIX

Gazetteer of Sequenced Specimens

Below we list all of the localities from which specimens of *Marmosa* and outgroup taxa were sequenced for this report. 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) or feet (ft). Geographic coordinates and their cited source are provided in parentheses.

BELIZE

1. *Corozal*, Shipstern Nature Reserve (18°18′N, 88°09′W; Quang Minh, 2007).

BOLIVIA

2. *Santa Cruz*, 27 km SE Santa Cruz (17°58′S, 63°03′W; Anderson, 1997).
3. *Santa Cruz*, El Refugio (14°43′S, 61°02′W; Emmons and Patton, 2005).
4. *Santa Cruz*, Estancia Isibobo (19°31′S, 63°36′W; Anderson, 1997).

BRAZIL

5. *Amazonas*, Altamira (6°35′S, 68°54′W; Patton et al., 2000).
6. *Amazonas*, Barro Vermelho (6°28′S, 68°46′W; Patton et al., 2000).
7. *Amazonas*, Igarapé Nova Empresa, left bank Rio Juruá (6°48′S, 70°44′W; collector's label).

8. *Amazonas*, Igarapé Porongaba, right bank Rio Juruá, Acre (8°40′S, 72°47′W; collector's label).
9. *Amazonas*, Ilhazinha (3°17′S, 66°14′W; Patton et al., 2000).
10. *Mato Grosso do Sul*, Fazenda Cedro, 517 m (22°17′S, 54°54′W; Rossi, 2005).
11. *Mato Grosso*, Fazenda São Luís, 389 m (15°38′S, 52°21′W; Rossi, 2005).
12. *Pará*, E bank Rio Xingu (3°39′S, 52°22′W; Voss and Emmons, 1996: appendix 8).
13. *São Paulo*, Capão Bonito, Fazenda Intervales, 700 m (24°20′S, 48°25′W; collector's label).
14. *Tocantins*, Rio Santa Teresa, 205 m (11°51′S, 48°38′W; Rossi, 2005).

COLOMBIA

15. *La Guajira*, La Isla (11°41′N, 71°55′W; Gardner, 2008).

ECUADOR

16. *El Oro*, Río Puyango, 370 m (3°53′S, 80°07′W; collector's label).
17. *Esmeraldas*, Comuna San Francisco de Bogotá (1°06′N, 78°42′W; Porter et al., 2007).
18. *Guayas*, B.P. [Bosque Protector] Cerro Blanco (2°11′S, 80°01′W; collector's label).
19. *Orellana*, 35 km S Pompeya Sur (0°41′S, 76°28′W; GE, 2008).
20. *Orellana*, 38 km S Pompeya Sur (0°39′S, 76°28′W; Gardner, 2008).

FRENCH GUIANA

21. *Cayenne*, Cayenne (4°56′N, 52°20′W; Stephens and Traylor, 1985).
22. *Cayenne*, Nouragues (4°05′N, 52°40′W; Voss and Emmons, 1996: appendix 5).
23. *Cayenne*, Pic Matecho (3°45′N, 53°02′W; GE, 2008).

GUATEMALA

24. *Alta Verapaz*, Chelem Há (Yalijux Mtn.), 2090 m (15°22′N, 90°03′W; Renner, 2003).
25. *Baja Verapaz*, 5 km E Purulhá, 1550 m (15°14′N, 90°11′W; GE, 2008).
26. *El Petén*, Biotopo Cerro Cahui, El Remate, 120 m (17°00′N, 89°44′W; collector's label).
27. *El Progreso*, Río Uyús, 5 km E San Cristóbal, Acasaguascatlán, 240 m (14°51′N, 89°50′W; collector's label).
28. *Zacapa*, 9.5 km NW Gualán, 1973 m (15°11′N, 89°27′W; GE, 2008).

GUYANA

29. *Demerara-Mahaica*, Ceiba Biological Station (6°30′N, 58°13′W; Lim et al., 2008).
30. *Potaro-Siparuni*, Iwokrama Reserve, 42 km WNW Siparuni, Pakatau Mt. (4°45′N, 59°01′W; Lim and Engstrom, 2001).

MEXICO

31. *Campeche*, 10 km N El Refugio (18°58′N, 89°19′W; GE, 2008).
32. *Campeche*, 3.7 km SE Chekubul (18°48′N, 90°58′W; GE, 2008).
33. *Campeche*, 44 km S Constitución (18°15′N, 90°04′W; ROM collection database).
34. *Campeche*, Xpujil, 25 km N of Xpujil (18°44′N, 89°24′W; GE, 2008).
35. *Jalisco*, 6 km SE Chamela (19°30′N, 105°03′W; Ceballos, 1990).
36. *Michoacán*, 1 km E Playa Azul, 25 m (17°59′N, 102°20′W; collector's label).

PANAMA

37. *Bocas del Toro*, Ñuri (8°55′N, 81°49′W; NGA, 2009).
38. *Chiriquí*, Reserva Forestal Fortuna, 1100 m (8°44′N, 82°16′W; NGA, 2009).
39. *Darién*, Cana, 600 m (7°47′N, 77°42′W; NGA, 2009).
40. *Los Santos*, Los Cuernitos (7°51′N, 80°16′W; collector's label).
41. *Veraguas*, Río Portabelo (7°14′N, 80°37′W; collector's label).

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42. *Amazonas*, Río Cenepa, vicinity of Huampami, 700 ft (4°40′S, 78°12′W; collector's label).
43. *Cusco*, Hacienda Villa Carmen (12°50′S, 71°15′W; Stephens and Traylor, 1983)
44. *Loreto*, 25 km S Iquitos (3°58′S, 73°25′W; Hice et al., 2004).
45. *Loreto*, Quebrada Orán (3°25′S, 72°35′W; Capparella et al., 1997).
46. *Loreto*, Río Gálvez, Nuevo San Juan (5°15′S, 73°10′W; Simmons et al., 2002).

SURINAM

47. *Brokopondo*, Brownsberg Nature Park, Km 1.2 Mazaroni trail (4°56′N, 55°11′W; Lim et al., 2005).
48. *Nickerie*, Kayserberg Airstrip (3°06′N, 56°28′W; Lim et al., 2008).
49. *Para*, Zanderij (5°27′N, 55°12′W; collector's label).

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50. *Bolívar*, Auyantepui (5°55′N, 62°32′W; Gardner, 2008).
51. *Falcón*, Serranía de San Luis; ca. La Chapa; ca. 15 km N Cabure, ca. 350–380 m (11°17′N, 69°36′W; collector's label).
52. *Falcón*, Serranía de San Luis, ca. 4 km S and 3 km W Cabure, ca. 425 m (11°07′N, 69°38′W; collector's label).

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