

A MOLECULAR PERSPECTIVE ON THE DIVERSIFICATION OF SHORT-TAILED OPOSSUMS (*Monodelphis*: DIDELPHIDAE)

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ABSTRACT: As currently understood *Monodelphis* includes more than 22 species and is the most diverse genus of opossums (Didelphimorphia). No complete evaluation of the systematic relationships of its species has been attempted, despite the fact that several species groups and even genus-level groups have been proposed based on morphology, and that some of them are limited to recognized biogeographic regions. Here, genealogic relationships among 17 species were assessed based on phylogenetic analyses of 60 individual sequences (801 base pairs of the mitochondrial cytochrome *b* gene). The analyses cast doubts on the monophyly of *Monodelphis*, but species were consistently discriminated into eight species groups: (a) *brevicaudata* group, with five species, (b) *adusta* group, four species, (c) *dimidiata* group, two species, (d) *theresa* group, two species, (e) *emiliae* group, (f) *kunsi* group, (g) *americana* group, and (h) 'species C' group; the last four being monotypic. Genetic divergence among species within species groups ranges from 0.48 to 12.27%, and among species groups it goes from 15.61 to 20.15%. Further analyses in some species groups reveal congruence between genetic divergence, morphological traits, and geographic distribution, providing additional support for recognition of species limits. Although the cytochrome *b* gene may diverge too fast to evaluate relationships among the older lineages of the genus, the use of a broad taxon sampling allows for independent tests of hypotheses on species limits and relationships based on non-molecular characters. Congruent patterns offer a starting point for developing a sound taxonomy for *Monodelphis* and more robust hypotheses in regard to its diversification over many diverse Neotropical habitats.

RESUMEN: Una perspectiva molecular sobre la diversificación de las comadreas colicortas (*Monodelphis*: Didelphidae). La definición actual del género *Monodelphis* incluye más de 22 especies, siendo el género más diverso de marsupiales (Didelphimorphia). Una evaluación de las relaciones sistemáticas entre especies no ha sido intentada aún, a pesar de haberse propuesto, en base a morfología, varios grupos de especies e incluso nombres del nivel de género, y que algunos de ellos parecen limitados a regiones biogeográficas reconocidas. Las relaciones genealógicas entre 17 especies fueron evaluadas en base a análisis filogenéticos de 60 secuencias individuales (801 pares de bases del gen mitocondrial Citocromo *b*). Aunque los análisis no corroboran la monofilia de *Monodelphis*, las especies fueron discriminadas consistentemente en ocho grupos de especies: (a) grupo *brevicaudata*, con cinco especies, (b) grupo *adusta*, cuatro especies, (c) grupo *dimidiata*, dos especies, (d) grupo *theresa*, dos especies, (e) grupo *emiliae*, (f) grupo *kunsi*, (g) grupo *americana*, y (h) grupo 'especie C'; siendo los cuatro últimos monotípicos. La divergencia genética entre especies del mismo grupo varía entre 0.48 y 12.27%, y entre grupos varía de 15.61 a 20.15%. Análisis más detallados en algunos grupos muestran congruencia entre patrones de variación molecular, características morfológicas y distribución geográfica, brindando

soporte adicional a las relaciones encontradas. Aunque el gen citocromo *b* puede divergir demasiado rápido para evaluar relaciones entre grupos antiguos en este género, un muestreo taxonómico amplio y denso permite una prueba independiente de hipótesis basadas en caracteres no moleculares sobre límites entre especies y sus relaciones. Estos patrones congruentes ofrecen un punto de partida para el desarrollo de una taxonomía válida en *Monodelphis* e hipótesis más robustas respecto a su diversificación a través de los diversos hábitats del Neotrópico.

Key words. Didelphimorphia. Phylogenetics. Species limits. Taxonomy.

Palabras claves. Didelphimorphia. Filogenética. Límites entre especies. Taxonomía.

INTRODUCTION

The genus *Monodelphis* includes small to medium-sized short-tailed opossums found throughout various habitats from southeastern Panama to northeastern Argentina, ranging from near sea level up into montane forests (Solari, 2007). Although it is the most diverse genus of living didelphids, only five other genus-level names have been proposed to group species, but none is currently treated as a valid subgenus (Gardner, 2005; Pine and Handley, 2008). A number of species groups have been recognized in the literature (e.g., Matschie, 1916; Gilmore, 1941; Pine, 1976; Gomes, 1991), and sometimes allocated to the genus-level names, but their definition and limits have been inconsistent. Most of the groups are based on a few conspicuous external characters, the most obvious being fur coloration of the back and sides. Additional details concerning skulls and teeth appear in a few diagnoses, while occasionally species are aggregated based on geographic grounds (e.g., Solari, 2004).

Aside from the conspicuously short tail common to all species, other evidence suggesting a monophyletic *Monodelphis* is a diploid number of $2N=18$, unique among marsupials (Reig et al., 1977). Preliminary and sparse data on chromosomes (Reig et al., 1977; Langguth and Lima, 1988; Palma and Yates, 1996; Carvalho et al., 2002) show some diversity worthy of further study, but no one has proposed relationships based on that dataset. Currently, based on morphological and genetic data, the genus is considered monophyletic (Voss and

Jansa, 2003, 2009). Hypotheses concerning relationships have been advanced for some species, based on morphometric (Ventura et al., 1998) or genetic data such as mitochondrial (Patton and Costa, 2003; Solari, 2007) and nuclear genes (Voss and Jansa, 2003, 2009). The most extensive (but unpublished) review of the genus is that of Gomes (1991), with emphasis on species from Brazil; however, that taxonomy disagrees with the one used by most current authors (e.g., Gardner, 2005; Pine and Handley, 2008). The taxonomy of the genus and species has been updated by Pine and Handley (2008), summarizing their previous findings, as well as those by Ventura et al. (1998), Voss et al. (2001), and Solari (2007). These studies have provided valuable insight into the systematics of the genus, but the clear existence of species complexes raises questions about the number of species and their actual relationships.

This study examines the diversity and phylogenetic relationships among several species of *Monodelphis*. Given our limited knowledge of the relationships within *Monodelphis*, and that so far no study has included a representative subset of species, wide taxonomic and geographic sampling is imperative; as such, emphasis is given to obtaining a broad representation of taxa and collection localities. This dataset includes 14 currently recognized species plus three recognized as distinct but unnamed forms (species B, species C, and species D; see Pine and Handley, 2008). Mitochondrial sequences (cytochrome *b* gene) are used to provide a genealogical framework for assessing species limits and to address the

existence of species groups within the genus, and if any of these correspond to groups previously identified.

MATERIALS AND METHODS

Samples. Forty one tissues samples representing 15 *Monodelphis* species, one tissue sample of *Tlacuatzin* (part of the outgroup), plus 25 cytochrome *b* sequences (including five *Monodelphis* species and five of the outgroup species) directly obtained from GenBank (**Appendix**) were used in this research. Collecting localities and museum catalog [or collector's field] numbers are given for vouchers and tissues, or at least for whichever one was available at the time of analysis (**Appendix**); the localities are mapped in **Fig. 1**. Voucher specimens are housed in the following institutions: American Museum of Natural History (AMNH, New York), Colección Boliviana de Fauna (CBF, La Paz, Bolivia), Laboratorio de Evolución, Universidad de la República (EV, Montevideo, Uruguay), Field Museum (FMNH, Chicago), University of Kansas Museum of Natural History (KU, Lawrence), Museum of Southwestern Biology (MSB, Albuquerque), Museo de Historia Natural, Universidad Nacional Mayor de San Marcos (MUSM, Lima, Peru), Museum of Vertebrate Zoology (MVZ, Berkeley), Natural Sciences Research Lab, The Museum, Texas Tech University (TTU, Lubbock), and National Museum of Natural History (USNM, Washington, D.C.). Some Brazilian specimens sequenced by J. L. Patton and collaborators are deposited in the following institutions: Instituto Nacional de Pesquisas da Amazonia (INPA, Manaus), Museu Paraense Emilio Goeldi (MPEG, Belém), Museu de Zoologia, Universidade de São Paulo (MZUSP, São Paulo), and Universidade Federal de Minas Gerais (UFMG, Belo Horizonte).

DNA sequence acquisition. Total genomic DNA was extracted following available protocols based on phenol-chloroform, proteinase K-ribonuclease (Longmire et al., 1997; Sambrook et al., 1989), or manufacturer's protocol of the DNeasy tissue kit (Qiagen Inc., Valencia, CA). Double-stranded amplification of the entire cytochrome *b* gene was performed via polymerase chain reaction (PCR), using primers MVZ05 and MVZ14 (Patton et al., 1996). Conditions for these reactions were: 35 cycles of 94°C for 50 seconds, 47°C for 1 minute, and 72°C for 1.5 minutes; the cycle was then held at 72°C for 30 minutes to allow for extension of DNA. PCR products were then purified using a

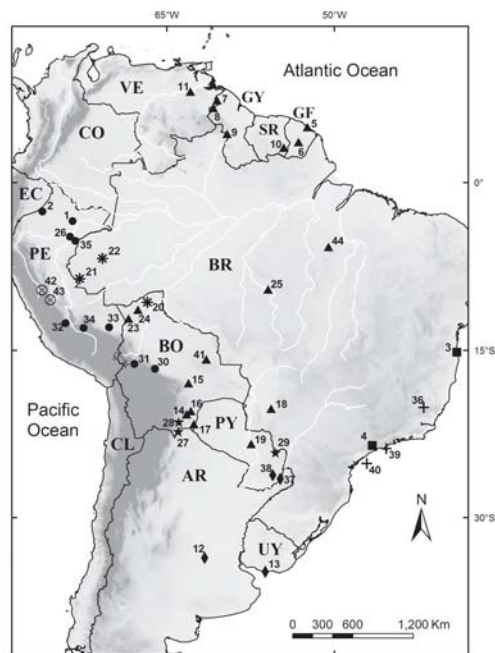


Fig. 1. Collection localities of the specimens included in the present analysis. To improve resolution, samples are arranged according to the resulting groups (see included species in text). Locality records correspond to *brevicaudata* group [triangles], *adusta* group [circles], *dimidiata* group [diamonds], *theresa* group [crosses], *emiliae* complex [asterisks], *kunsi* complex [stars], *americana* complex [squares], and 'species C' complex [encircled dots]. Numbers beside the symbols make reference to the localities as detailed in Appendix 1. Country names as follows: AR=Argentina, BO=Bolivia, BR=Brazil, CL=Chile, CO=Colombia, EC=Ecuador, GF=French Guiana, GY=Guyana, PE=Peru, PY=Paraguay, SR=Suriname, UY=Uruguay, VE=Venezuela.

Qiagen PCR purification kit (Qiagen Inc.) following the instructions of the manufacturer. Amplicons were sequenced using ABI Big Dye Terminator Ready Reaction Mix, following manufacturer's instructions. Eight primers aimed to obtain the whole gene were used in the sequencing protocol: two (MVZ05 and MVZ14) used in the amplification, three (MVZ04, MVZ11, and MVZ16) used by Patton et al. (1996) and Patton and Costa (2003), and three (mono700H1, mono480L, and mono860L) specifically designed for *Monodelphis* (Solari, 2007). DNA was sequenced in both directions to ensure accuracy of generated sequences in

an ABI Prism 377 Genetic Analyzer (Perkin Elmer, Applied Biosystems, Foster City, CA).

Phylogenetic analyses. Sequence alignment was performed using Vector NTI Suite 6.0 (InforMax Inc., Bethesda, MD), specifically ContigExpress (for DNA sequence assembly), and Align X (for multiple alignment of sequences) components. Three different analyses: maximum parsimony, maximum likelihood, and Bayesian were performed using PAUP* 4.0b 10 (Swofford, 2002) and MrBayes 2.01 (Huelsenbeck and Ronquist, 2001). Topologies were rooted using an outgroup formed by six species: *Glironia venusta*, *Didelphis marsupialis*, *Philander opossum*, *Tlacuatzin canescens*, *Marmosa (Marmosa) murina*, and *M. (Micoureus) regina*. Genera *Tlacuatzin* and *Marmosa*, along with *Monodelphis*, have been included in the tribe Marmosini Hershkovitz 1992 by Voss and Jansa (2009). For Maximum Parsimony (MP) analysis, nucleotide positions were treated as discrete, unordered, and equally weighted characters with four character states (A, C, G, and T). Trees were found by heuristic search with 100 random sequence addition, and tree-bisection-reconnection (TBR) branch swapping. When multiple minimum-length trees were found, strict consensus trees were obtained. Maximum Likelihood (ML) trees were calculated based on the selection of the most appropriate model of DNA sequence evolution as determined by Modeltest (Posada and Crandall, 1998). The GTR + Γ + I model generated significantly better likelihood scores and therefore was used in all likelihood analyses. This model included a proportion of invariable sites and a gamma shape parameter. Analyses employed empirical base compositional biases, 50 random input orders, and TBR branch swapping. Reliability of nodes was evaluated through bootstrap analyses (Felsenstein, 1985) with 200 iterations in the full-heuristic mode (5 random-additions) for the MP topology, and 1000 iterations in fast mode for the ML topology.

For Bayesian analyses, the GTR model of evolution, with a gamma parameter and a proportion of invariable sites, was used. Two runs were conducted simultaneously, each with four Markov chains with random starting trees ran for 1 000 000 generations; trees were sampled every 100th generation, and 'burnin' values determined by empirical evaluation of likelihood scores. A majority-rule consensus tree was calculated from the sample of stabilized trees in PAUP*, with branch lengths obtained via the 'sumt' option in MrBayes. Clade reliability was assessed via posterior probabilities, and values ≥ 0.95 regarded as significant.

Genetic divergence among species and clades was estimated from Kimura-2 parameter distances as implemented in PAUP*. This specific model of evolution allows direct comparison with previous studies involving short-tailed opossums (Patton and Costa, 2003; Steiner and Catzefflis, 2004; Solari, 2007), as well as to estimate minimum divergence between recognizable sister taxa (Avise, 2004; Baker and Bradley, 2006), thus helping to understand morphological and geographic diversification. It will allow testing the phylogenetic value of previous taxonomic arrangements, such as species groups or genus-level groups, as well as geographic hypotheses of diversification (e.g., Patton et al., 2000; Costa, 2003) in particularly well-sampled groups of species.

RESULTS

Partial sequences (ranging from 790 to 830 bp) were obtained from 17 species (60 individuals examined) of *Monodelphis*; 41 of these correspond to new sequences (GenBank accession numbers HM998558-998598). Analyses of the first 801 bp dataset, including the outgroup genera, resulted in 448 constant and 309 parsimony informative characters. Average nucleotide frequencies of informative characters were: adenine 0.33, cytosine 0.32, guanine 0.04, and thymine 0.31. Among these, 58 (19%) were 1st position changes, 14 (5%) were 2nd position, and 237 (76%) were 3rd position; this proportion is concordant with cytochrome *b* variation in opossums (Patton et al., 1996; Mustrangi and Patton, 1997). Likewise, saturation values indicate that third base positions reached saturation point for transitions at around 15% sequence divergence, with a possible loss of phylogenetic signal among more divergent taxa in the analysis.

The MP analysis generated 240 most parsimonious trees, each 1835 steps long, with a CI of 0.30 and a RI of 0.75; the consensus tree (not shown) recovered eight major lineages (thereafter, "species groups") with high nodal bootstrap support (**Table 1**); these groups are referred as to *adusta*, *americana*, *brevicaudata*, *dimidiata*, *emiliae*, *kunsi*, *theresa*, and one unnamed species group. Relationships among the species groups are unresolved. However, *Monodelphis* did not

appear as monophyletic because a clade with both species of *Marmosa* (subgenera *Marmosa* and *Micoureus*) is nested within these eight species groups.

The maximum likelihood analysis used the GTR + Γ + *I* model of evolution with gamma shape parameter = 1.2086, and proportion of invariable sites = 0.5383. The topology (Fig. 2; $-\ln L = 8592.478$) recovered the eight species groups of the MP analyses, all with BS \geq 90 (except for the *adusta* group; Table 1); however, the genus results polyphyletic by inclusion of both species of *Marmosa* as sister to the *theresa* species group, but this relationship had low nodal support (BS < 50). Likewise, deeper nodes and relationships among lineages had low support (BS < 65). Bayesian

analysis recovered a topology (not shown) similar to that of the ML, with minimal differences related to the position of some terminals within species.

Genetic divergence based on the Kimura 2-parameters model ranged from 0.4 to 20.6% among species of *Monodelphis*. Average internal divergence within species groups ranged from 0.48% (*dimidiata* species group) to 12.27% (*adusta* species group); pair-wise divergence had the highest value within the *brevicaudata* species group, with 13.9% between *brevicaudata* and *glirina*. Divergence between taxa in different species groups ranged from 15.8% (*americana* vs. species C) to 20.6% (*domestica* vs. *emiliae*). Table 2 lists genetic divergence values within and among

Table 1

Support values for specific nodes in each of the three phylogenetic analyses (bootstrap values [BS] are provided for MP and ML trees and posterior probabilities [PP] for Bayesian analyses) of different species of short-tailed opossums of the genus *Monodelphis*. Nodes are identified numerically as in the topology of Fig. 2.

Node	Taxon / Clade	BS - MP	BS - ML	PP
1	BO and PA <i>domestica</i>	92	—	0.94
2	Other <i>domestica</i>	100	—	1.00
3	<i>M. domestica</i>	100	98	1.00
4	BO <i>glirina</i>	< 80	100	1.00
5	BR <i>glirina</i>	< 80	100	1.00
6	<i>M. glirina</i>	96	98	1.00
7	<i>M. glirina</i> + 'Species B'	81	87	1.00
8	<i>M. domestica</i> + <i>M. glirina</i> + 'Species B'	< 70	93	< 0.65
9	VE and GU <i>brevicaudata</i>	99	92	1.00
10	FG and SU <i>brevicaudata</i> (incl. 'Species D')	100	85	1.00
11	<i>M. brevicaudata</i> (incl. 'Species D')	99	< 80	1.00
12	<i>M. brevicaudata</i> species group	99	90	1.00
13	<i>M. americana</i> species group	98	93	1.00
14	'Species C' species group	100	100	1.00
15	<i>M. peruviana</i>	< 70	< 70	< 0.75
16	<i>M. handleyi</i>	100	100	1.00
17	<i>M. osgoodi</i>	< 75	< 75	0.97
18	<i>M. osgoodi</i> + <i>M. handleyi</i>	< 60	< 75	1.00
19	<i>M. peruviana</i> + <i>M. osgoodi</i> + <i>M. handleyi</i>	91	< 60	< 0.85
20	<i>M. adusta</i>	100	99	1.00
21	<i>M. adusta</i> species group	84	< 70	< 0.80
22	<i>M. theresa</i>	< 65	< 80	< 0.80
23	<i>M. theresa</i> species group	100	92	0.95
24	<i>Marmosa</i> (subgen. <i>Marmosa</i> + <i>Micoureus</i>)	100	100	1.00
25	<i>M. kunsii</i> species group	100	100	1.00
26	<i>M. emiliae</i> species group	100	99	1.00
27	<i>M. dimidiata</i> species group	100	100	1.00

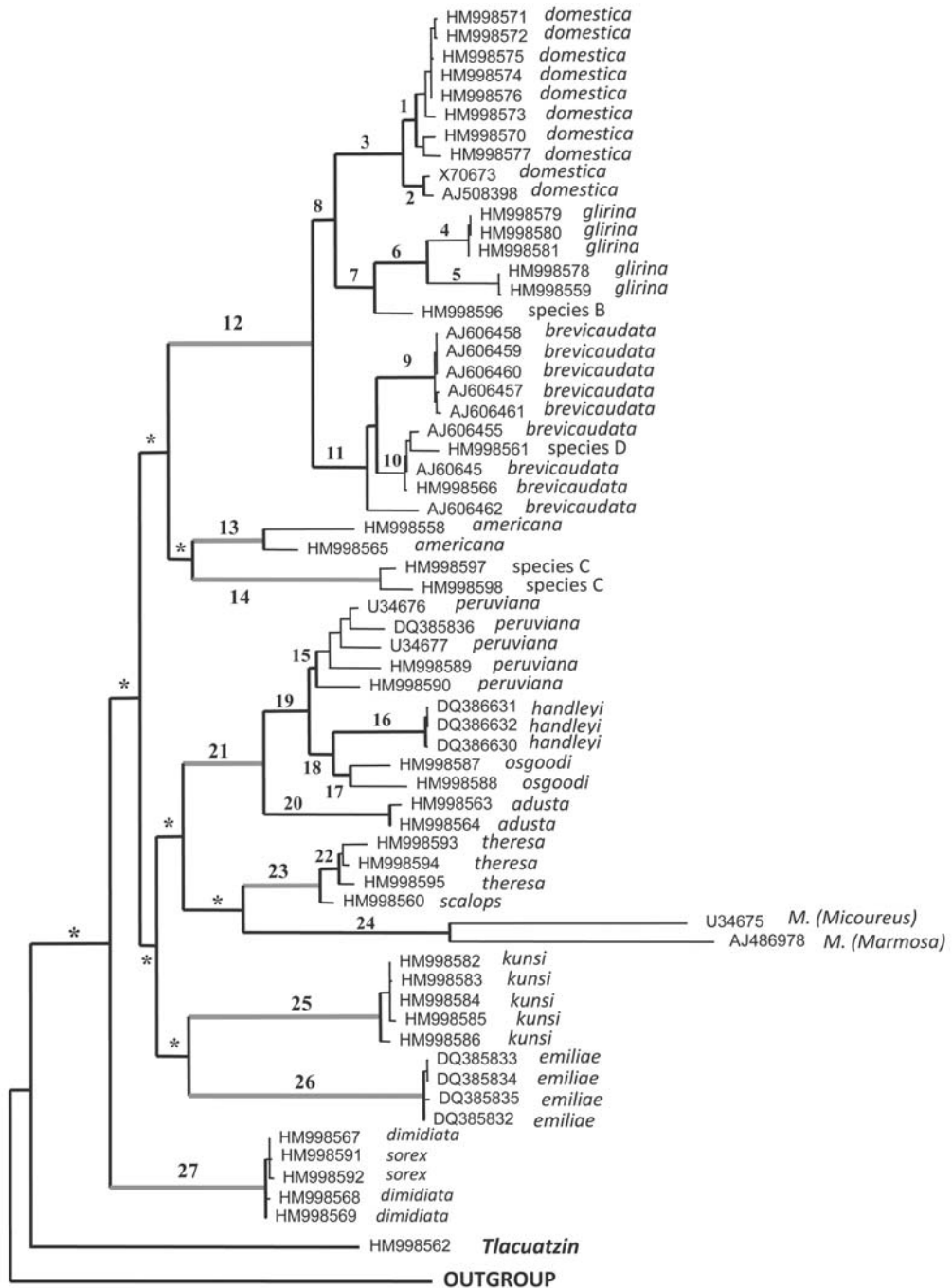


Fig. 2. Optimal tree obtained by Maximum Likelihood analysis (-ln L=8592.478) based on cytochrome *b* gene sequences of several species of *Monodelphis* using the GTR + Γ + I model of molecular evolution. Species groups are labeled as in text. See **Table 1** for bootstrap support values (form MP and ML trees), and Bayesian posterior probabilities corresponding to each labeled branches. Nodes marked with an asterisk (*) had low support values (BS < 50; PP < 0.7) in all the analyses.

Table 2

Genetic divergence (Kimura 2-parameter distance) within [along the diagonal] and among [below the diagonal] the eight species groups of *Monodelphis* species identified in the molecular analyses of cytochrome *b* gene sequences. *Tlacuatzin*, one of the genera used as outgroup, is included for comparisons.

	<i>Tlacuatzin</i>	<i>brevicaudata</i>	<i>adusta</i>	<i>dimidiata</i>	<i>theresa</i>	<i>emiliae</i>	<i>kunsi</i>	<i>americana</i>	Species C
<i>Tlacuatzin</i>	—								
<i>brevicaudata</i>	20.82	9.82							
<i>adusta</i>	18.90	18.76	12.27						
<i>dimidiata</i>	19.49	18.16	17.48	0.48					
<i>theresa</i>	18.44	17.60	15.61	15.76	3.00				
<i>emiliae</i>	20.73	20.15	19.56	19.00	18.84	0.49			
<i>kunsi</i>	19.13	19.34	16.50	17.78	15.92	16.74	0.81		
<i>americana</i>	20.24	16.95	16.00	15.63	16.10	17.61	17.54	8.11	
Species C	20.53	18.30	17.97	18.39	19.24	18.71	19.63	15.81	3.48

these eight species groups and with *Tlacuatzin*, the closest outgroup genus that stayed outside *Monodelphis*.

DISCUSSION

Results of maximum parsimony, maximum likelihood, and Bayesian analyses produced trees with slightly different topologies, although all depicting eight distinctive, consistent and well supported species groups (Table 1); four correspond to lineages composed by two or more species and the other four are mono-specific. Due to this overall similarity, the maximum likelihood tree (Fig. 2) was chosen for the following discussion. Relationships among species groups did not receive enough support to allow further discussion of deeper nodes.

Monophyly of the genus *Monodelphis*

None of these analyses recovered a monophyletic *Monodelphis*. Even with a large taxonomic sampling of the genus (17 of 22-23 species recognized by recent revisions; Solari, 2007; Pine and Handley, 2008), parsimony and model based analyses failed to recover a monophyletic *Monodelphis*. Although some morphological traits and its unique chromosome number have been used to define the genus (Creighton, 1984; Reig et al., 1987), most of this evidence needs to be reassessed within a phylogenetic context. In a similar way,

the morphological characters proposed by Goin and Rey (1997) do not apply to all *Monodelphis* species (pers. obs.). The most complete morphological dataset for didelphids (Voss and Jansa, 2003, 2009) identified a single discrete character state (maxilloturbinals simple, slender, and unbranched) as a synapomorphy for the four *Monodelphis* species (*brevicaudata*, *emiliae*, *peruviana*, *theresa*) assessed in the analysis. A reassessment of these putative synapomorphies needs to be completed for a larger set of species. Voss and Jansa (2009) also found a well-supported monophyletic genus based on independent and combined analyses of five gene sequences. Therefore, although the monophyly of *Monodelphis* was not clearly supported in the present molecular analysis, keeping this as a working hypothesis seems appropriate (see below).

Indiscriminate use of the cytochrome *b* gene as a genetic marker is criticized because of faster rate of mutation and high saturation (Jansa and Voss, 2000; Springer et al., 2001), which result in an inability to resolve deep divergences. Age estimation based on molecular dating of nuclear genes (Steiner et al., 2005) points to a *Monodelphis* origin as early as the late Oligocene (26.1 ± 3.3 Mya). Therefore, it is possible that the cytochrome *b* could be useful in solving relationships of recently diverged species (see Mustrangi and Patton, 1997; Patton and da Silva, 1997; Costa et al.,

2003; Solari, 2007), but not deeper at or near the base of the genus. Therefore, the inclusion of more slowly nuclear sequences is needed to further test the monophyly of *Monodelphis* and relationships among the eight species groups.

Definition of species and species group

Within the ML tree, eight (*americana*, *adusta*, *domestica*, *emiliae*, *glirina*, *handleyi*, *kunsi*, and species C) out of 17 putative species received high support (BS > 90, PP > 0.95), whereas *osgoodi* had PP > 0.95 but BS < 85 and *peruviana* had low support. Three putative species (*scalops*, *sorex*, and species D) appeared nested within others (*theresa*, *dimidiata*, and *brevicaudata*, respectively) failing the criterion of reciprocal monophyly for specific distinction. Additional studies should be conducted to resolve their status as species. Finally, one undescribed species ('species B', in Pine and Handley, 2008) was represented by a single specimen and its support cannot be established. Genetic divergence among species ranged widely, 0.48-20.6%, depending on the scale of comparison; it was lower within species groups and larger between species from different species groups. This variation makes possible to use the lowest divergence between fully recognized sister species as a threshold useful to tentatively indicate specific level of lineages within some of these species that should be further tested with other analyses; for instance, within the *adusta* and the *brevicaudata* species groups the average divergence between sister species was 10%. Therefore, given that sequence divergence between sister taxa *M. glirina* and 'species B' was 8.65%, and the latter has been morphologically considered similar to *M. domestica* (see Pine and Handley, 2008) these data are indicative of specific status for 'species B'. The implications of these criteria for the recognition of *Monodelphis* species is further discussed below.

It is worthy of mention that four well-supported species groups were corroborated by different tree-building methods. Species groups have been recognized in *Monodelphis* and even given genus-group names (e.g., Matschie,

1916; Cabrera, 1919), but this study constitutes the first test of their monophyly. However, the fact that the phylogenetic analyses lack a number of species must be considered, because their inclusion may affect the composition and limits of some of these groups. Support of the eight species groups in all the phylogenetic analyses is remarkable (Table 1), and merits further assessment of congruence with other nonmolecular datasets (e.g., morphology). This combined approach should enhance the species-level taxonomy in a genus in which it is extremely difficult to correctly identify species (see Solari, 2007).

The following accounts are provided as a first step toward the definition of the groups recovered in this study. For each species group, a provisional name based on the oldest species name available within each is provided, along with previous references to similar arrangements. Taxonomic composition and geographic distribution are based on specimens studied, but also on information included in earlier works. Departures from current taxonomic usage (Gardner, 2005; Pine and Handley, 2008) are indicated.

The brevicaudata species group

Species in this group occur throughout the tropical dry forests of northern South America, eastern Amazonia from the Guiana Shield to central Brazil, the Cerrado grasslands, and the lowlands of western Amazonia (Fig. 1). Species included in this species group are *brevicaudata* Erxleben, *glirina* Wagner, *domestica* Wagner, plus 'species B' and 'species D' of Pine and Handley (2008); however, because the specimen representing the latter was not recovered as distinct and clustered within *brevicaudata*, it is no longer considered as distinct. The only indication of a likely affinity among the included species is that many of them were listed as synonyms or subspecies of *brevicaudata* (e.g., Cabrera, 1958). Similarly, *domestica* was suggested as close to *brevicaudata* sensu lato when Pine (1980) compared these forms to *maraxina*—see also Patton and Costa (2003), Pine and Handley (2008). The following taxa should

also be included in this species group: *palliolata* Osgood and *maraxina* Thomas (see Pine, 1980; Voss et al., 2001). Individuals allocated to *orinoci* by Reig et al. (1977), Linares (1998), and Ventura et al. (1998) actually represent an unnamed form referred to as 'species A' by Pine and Handley (2008); however, *orinoci* is a synonym of *brevicaudata* (Voss et al., 2001). Node support for this species group was over 90 in both ML and MP, and 1.00 for PP (Table 1).

Gomes (1991) allocated the taxa of this species group to three different groups: *touan* (including *glirina*, *touan*, and 'amazonica' [nomen nudum]), *brevicaudata* (*brevicaudata*, *rubidus* [sic], and 'macae' [nomen nudum]), and *domestica*. His definitions for these taxa are not comparable to those in use by other authors (Voss et al., 2001; Gardner, 2005; Pine and Handley, 2008), and will not be discussed further. In addition, Gomes (1991) listed all of the Andean species (*adusta* plus *kunsi* groups of this analysis) under his *brevicaudata*. The tricolored and bicolored forms of *brevicaudata* sensu Voss et al. (2001) are the basis for a restricted use of *touan* and *brevicaudata*, respectively. A putative relationship between *emiliae* and *b. touan* (proposed by Carvalho, 1960) was discussed and refuted by Pine and Handley (1984).

Relationships among species are consistent in all analyses, with *brevicaudata* as sister to *domestica* and to (*glirina* + species B). Sequence divergence ranges from 8.65% (*glirina* - species B) to 13.99% (*brevicaudata* - *glirina*). Average within divergence ranges from 2.69% (*domestica*) to 4.84% (*brevicaudata*). Sub-clades of *M. brevicaudata* (6.9% of divergence) correspond to the "tricolor" and "bicolor" forms discussed by Voss et al. (2001) and the phylogroups identified by Steiner and Catzeflis (2004). Sub-clades of *M. glirina* are geographically separated by the Beni-Madeira drainage, which is a major barrier in lowland Amazonia (Cracraft, 1985), and diverge by 7.7%. Samples of *M. domestica* come from Brazil, Bolivia, and Paraguay; minor geographic structure is caused by samples from central Brazil being sister to the

clade formed by the other sequences. The single specimen of 'species B' of Pine and Handley (2008) comes from eastern Bolivia, and represents an undescribed species.

The adusta species group

These taxa are associated with the Andean Cordillera, along an elevational range that includes the lowland rainforests to montane forests, with *M. reigi* being a geographic outlier (Fig. 1; see also Lim et al., 2010) to this pattern. This species group includes *adusta* Thomas, *peruviana* Osgood, *osgoodi* Doutt, and *handleyi* Solari. This species group was suggested by Cabrera (1958) and Anderson (1997), but defined and restricted by Solari (2007); as delimited here, this group does not include *kunsi* (see Anderson, 1997). Other taxa, not sampled but considered as part of this group, based on morphological traits, are *melanops* Goldman, *ronaldi* Solari, and *reigi* Lew and Perez-Hernandez. Lim et al. (2010) supported both, the recognition of the *adusta* group of species (node support was 95 for BS and 1.00 for PP) and the position of *reigi* within this group. In the present topology, node support was rather low (BS < 70; PP < 0.80; Table 1) in all analyses.

Although this group has generally been recognized by other authors, its integrity was challenged when Gomes (1991) listed some of the above-listed names under his concept of *M. brevicaudata*. Morphometric affinities were recognized between *adusta* and *reigi* (Ventura et al., 2005), as opposed to members of the *brevicaudata* group (as defined here), based on Venezuelan specimens only. Morphological resemblance and nearby distribution suggested a relationship between members of the *adusta* group and *kunsi* (see Anderson, 1997; Vargas et al., 2003) but analyses of genetic data show these as distantly related lineages.

The internal topology is the same as that presented by Solari (2007) and Lim et al. (2010), with *adusta* being sister to the remaining species, followed by *reigi*, and with a sister relationship between *peruviana* and *osgoodi* plus *handleyi*. Genetic divergences among

species range from 8.97% for *peruviana* - *osgoodi*, to 14.55% for *adusta* - *handleyi*. The geographic sub-clades in *osgoodi* diverge by over 6%, which is similar to the average internal divergence in *peruviana* (Solari, 2007). Samples of *M. adusta* come from Ecuador and northern Peru, whereas *handleyi* come from a single locality in northeastern Peru. Samples of *peruviana* come from northern through southern Peru and central Bolivia; this species is parapatric with *osgoodi* at the southern part of its range.

The dimidiata species group

The range of this species group includes very different habitats: the Pampa, the Chaco, and Atlantic forests (Flores, 2006; Pine and Handley, 2008), all located around southeastern South America (Fig. 1). Only two species are included in this group, *dimidiata* Wagner, and *sorex* Hensel. These taxa were grouped together by Pine et al. (1985), also including *fosteri* Thomas and *henseli* Thomas, the former tentatively regarded as a full species and the latter as a synonym of *sorex*. It is worth noting that *brevicaudis* Olfers 1818 (a nomen oblitum according to Pine and Handley, 2008) is sometimes used as the oldest name for *sorex* or for a distinct species (Gomes, 1991; Brown, 2004 [following Hershkovitz, 1959]). This group has bootstrap support of 100 for MP and ML, and 1.00 for PP (Table 1).

Although *sorex* is recognized as a distinct species (Pine et al., 1985; Gardner, 2005; Pine and Handley, 2008), the taxa do not form reciprocal monophyletic groups as well as show very low internal divergence (< 0.5%), similar to the range found in among populations of a single species of recent divergence. Miranda et al. (2007) found low levels of divergence among oryzomyine rodents in the transition between Atlantic forests and Pampa of southeastern Brazil; these authors suggested that recent changes molded by ocean level fluctuations may resulted in low geographic structure over this large distribution (despite habitat distinctions). It is possible that *dimidiata* and *sorex* (or even *fosteri*, as suggested by Pine et al., 1985) represent a single

species with disjoint geographic ranges and distinct coloration patterns. If that is the case, the oldest available name would be *dimidiata* Wagner 1847.

The theresa species group

This is the only species group restricted to the Atlantic forests. There are two species included in this group, *theresa* Thomas and *scalops* Thomas. A relationship of these names had not been suggested, although both occur in southeastern Brazil. However, Gomes (1991) suggested that dorsal stripes and brownish to reddish dorsal coloration (as seen in *theresa*) represent a juvenile pelage that change into an adult pattern, with gray on the dorsum, reddish to orangish head, rump and limbs, and no dorsal stripes (as seen in *scalops*). Recent findings by L. P. Costa and collaborators (pers. comm.) indicate this could be an age dependent trait, thus supporting Gomes (1991) hypothesis. Because the presence of dorsal stripes is recorded in at least other two groups, this state character may have evolved more than once within *Monodelphis*. Node support is over 90 for the ML and MP analyses, and 1.00 for PP (Table 1).

Average genetic divergence between *theresa* and *scalops* is only 3.4%, whereas internal divergence in *theresa* reaches 2.5%; these values are within the range seen in species with large geographic ranges (e.g., *brevicaudata*, *peruviana*). All the *theresa* specimens come from Sao Paulo, whereas the single *scalops* comes from Minas Gerais (Fig. 1). If further examination of the animal from Minas Gerais reveals it represents an adult with "typical" *scalops* coloration, the affinities suggested by Gomes (1991) would be verified.

The emiliae complex

The species is restricted to the lowland forests in a west-east band of Amazonia in Brazil, Bolivia, and Peru (Fig 1). Only one species is included, *emiliae* Thomas. No other taxon has been associated with *emiliae*, except by erroneous interpretations (*tricolor emiliae* in Miranda-Ribeiro, 1936, or *touan emiliae* in

Cabrera, 1958) that made *emiliae* a subspecies within the *brevicaudata* group, an issue addressed by Pine and Handley (1984). Linares (1998) also treated this species as *M. touan*, which is a synonym of *M. brevicaudata* (see Voss et al., 2001). Resemblance between *emiliae* and *scalops* only applies to overall color pattern (see Pine and Abravaya, 1978). Node support is > 95 in both MP and ML bootstrap, and 1.00 for PP (**Table 1**). Average within divergence is 0.5%, with little geographic structure; although it might be explained by inadequate sampling.

The kungsi complex

The geographic range shown by the samples includes southern Bolivia, northern Argentina, and eastern Paraguay (**Fig. 1**), which, when combined with verified records from northern Bolivia and central Brazil (see De la Sancha et al., 2007), makes it rather biogeographically complex. This is another species group with only one species, *kungsi* Pine, which has sometimes been associated with *adusta* and related taxa (Anderson, 1997), but the latter form a distant cluster herein recognized as a species group. Although *kungsi* and some members of the *adusta* group share a uniform buffy to light-brownish overall coloration, fur color seems to be homoplastic as it is seen in other taxa (e.g., red sides in *brevicaudata* and *sorex*, or black dorsal stripes in *theresa* and *americana*). Node support is 100 for MP and ML trees, and 1.00 for PP (**Table 1**).

The average internal divergence is 0.8%, compatible with the fact that only one species is represented; and, if some geographic structure can be recovered, it would account for only 1.5% between Bolivia-Argentina and Paraguay. A recent comparison with GenBank sequences from central Brazil assigned to *kungsi* (submitted by B. A. Carvalho et al.) clusters these as sister to the group recovered in this study with almost 6.5% divergence, which is a high value but below the average among recognized species in this study (above 8.5% in the *adusta* or *brevicaudata* groups). This may indicate a biogeographic division between those populations.

The americana complex

For this species, Pine and Handley (2008) documented an extensive range in eastern Brazil. Although only one species, *americana* Muller, forms this group, more taxa may be included. Currently, this species name is used for the three-dorsal-striped taxon from eastern Brazil, extending from north to south (as opposed to others restricted to the Atlantic forest, such as *iheringi* or *theresa*). Node support is > 90 in the MP and ML trees, and 1.00 for PP (**Table 1**). Voss and Jansa (2003) suggested that an *americana* species group could be associated with other dorsally striped animals (such as *theresa*), but these taxa did not fall together in the present phylogenetic analyses.

Samples from two localities, Bahia and São Paulo (**Fig. 1**), clustered together with 8.1% of divergence, which is larger than average for other species in this study and could then represent a subspecific division. In Gomes's (1991) view, *americana* includes a northern and a southern group which represent two different species, but provided no names for these.

The 'species C' complex

The only species in this group has not been named. So far, the species is known from four montane forests at elevations above 1800 m in central Peru (Solari et al., in prep.). Although this form was called *theresa* by Gardner (1993), Pacheco et al. (1995), and Patton and Costa (2003), no relationship with that species has been found. Node support is 100 in MP and ML trees, and 1.00 for PP (**Table 1**). The present analysis verified that distinction at a genetic level; in fact, although the 'species C' of Pine and Handley (2008) is remarkably similar to *americana*, these two exhibit a high genetic divergence (> 15%) from each other. Samples from two localities in central Peru (**Fig. 1**) diverge by 3.5%; similar geographic divergence was found when compared with shorter sequences (~400 bp) from other localities. Although the unnamed species appeared as sister to *americana* in the Bayesian topology, this relationship had low support

(< 0.65) so they are recognized as independent lineages in this arrangement.

TAXONOMIC CONCLUSIONS

Based on these results, it is clear that most of the species groups recognized by some authors (e.g., Matschie, 1916; Cabrera, 1919; Gilmore, 1941; Gomes, 1991) do not correspond to monophyletic groups. Therefore, a taxonomic arrangement consistent with the hypotheses of relationship for the included taxa is presented and discussed in the following section.

The oldest available name for the genus of short-tailed opossums is *Monodelphis* Burnett 1830, with *Monodelphis brachyura*: Burnett (= *Didelphis breviceaudata* Erxleben 1777) as type species. Another genus name, *Hemiuirus* Gervais 1855, was coined to include *brachyurus*, *tristriatus*, and *tricolor*, but the name is preoccupied by *Hemiuirus* Rudolphi 1809 (Platyhelminthes) and therefore is unavailable (see Pine and Handley, 2008).

Peramys Lesson 1842 was proposed with no type species; later Thomas (1888) designated *P. brachyura*: Lesson (= *M. dimidiata*, see Pine and Handley, 2008) as type species. Cabrera (1919) proposed *Minuania* as a subgenus with *Didelphys dimidiata* Wagner 1847 as type species, and it is therefore a subjective junior synonym of *Peramys*. Then, Miranda-Ribeiro (1936) described and included *dimidiata itatiayae* and *umbristriata* (*goyana* of the same author is a synonym), and Cabrera and Yepes (1940) referred *fosteri* to *Minuania*. Haltenorth (1958) listed *Minuania* as full genus including *dimidiata*, *fosteri*, and *umbristriata*, whereas Pine (1976) treated it as a valid subgenus with *dimidiata* and *umbristriata* only. Kirsch and Calaby (1977) did not recognize *Minuania* as separate from *Monodelphis*, and listed *dimidiata* (with *fosteri* as a synonym) but not *umbristriata*. Pine et al. (1985) tentatively treated *henseli* Thomas as a synonym of *sorex*, and *fosteri* as a full species. Another genus-group name is *Monodelphiops* Matschie 1916, with *Microdelphys sorex* Hensel 1872 as type spe-

cies; however, because of the close relationships between *M. dimidiata* and *M. sorex*, *Monodelphiops* Matschie, with *M. sorex* as type species, should be a synonym of *Peramys*.

Microdelphys Burmeister 1856 was proposed as a subgenus of *Didelphis*, with *tristriata*: Burmeister (= *Sorex americanus* Müller 1776) as type species by subsequent designation (Thomas 1888). Miranda-Ribeiro (1936) listed *americana*, *iheringi*, *theresa*, and *unistriata* in a key for subgenus *Microdelphys*; however, this arrangement conflicts with results presented here by inclusion of *theresa*. In a restricted sense, *Microdelphys* would include short-tailed opossums with blackish dorsal stripes that are conspecific with or that share a close relationship with the type species (*M. americana*).

Gilmore's (1941) use of names *Monodelphis* ("tricolor" group), *Lestodelphys* ("bicolor" group), *Microdelphys* ("striped" group), and *Minuania* ("variegated" group), as subgenera of *Monodelphis* is difficult to evaluate because no species were listed under them. In any case, his definition of *Monodelphis* is polyphyletic by inclusion of *Lestodelphys*. Dismissing Gilmore's system, the arrangement for the five available genus-group names would stand as follows:

1. *Monodelphis* Burnett 1830 (Type species: *brachyura*: Burnett = *brevicaudata* Erxleben)
2. *Peramys* Lesson 1842 (Type species: *brachyurus*: Lesson = *dimidiata* Wagner)
Monodelphiops Matschie 1916 (Type species: *sorex* Hensel)
Minuania Cabrera 1919 (Type species: *dimidiata* Wagner)
3. *Microdelphys* Burmeister 1856 (Type species: *tristriata*: Burmeister = *americana* Muller).

Strict application of the resulting phylogenies to update the subgeneric arrangement would result in a restricted meaning for the three genus-group names. Thus, *Monodelphis* would include the *brevicaudata* group as defined above, and finally, *Microdelphys* should

be restricted to the *americana* group, as defined above, and excluding the other species groups (*M. theresa* and 'species C') displaying dorsal stripes. Four recognized species that were not included in the phylogenetic analyses (*iheringi*, *rubida*, *umbristriata*, *unistriata*) would remain as *incertae sedis* within *Monodelphis* (sensu lato).

Given the low resolution of the topologies in regard to relationships among the eight species groups, *Monodelphis* (sensu stricto), *Peramys*, *Microdelphys*, and the remaining five (still unnamed), they should be considered of similar rank and not to be arranged into more inclusive groups. Under a conservative approach, *Monodelphis* (sensu lato) would stand as the valid name for the genus and these eight species groups could be regarded as equivalent to subgenera but without use of formal taxon names. Use of a single genus name seems to be the best choice to promote stability, and this still allows for recognition of the group's extensive supraspecific diversity through explicit treatment of its contained species groups. Use of a different genetic marker, one with a slower rate of substitution (such as a nuclear intron; e.g., DMP1, see Jansa et al., 2006), for the same set of taxa studied here should provide a powerful starting point to assess the constraints and informative value of the cytochrome *b* gene for *Monodelphis*. This, along with an improved taxon sampling, would be necessary to resolve definitions and limits for *Monodelphis* and its included species.

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APPENDIX 1

List of specimens examined including species name, geographic locality, GenBank accession and voucher numbers for 60 *Monodelphis* and six outgroup cytochrome-*b* sequences. Asterisks (*) by tissue numbers denote 25 sequences (20 for ingroup and five for outgroup) obtained from GenBank. Sequences by J. L. Patton and collaborators are indicated by a karat (^). Museum catalog numbers (see 'Material and Methods' for acronyms) are missing for vouchers housed but not yet cataloged or for which the number is unknown. Numbers in parentheses following the localities make reference to symbols used in **Fig. 1**. *Ma.* = *Marmosa*, *M.* = *Monodelphis*; JPJ = J. P. Jayat (Laboratorio de Investigaciones Ecológicas de las Yungas, Universidad Nacional de Tucumán, Argentina); JAA = J. Amanzo A. (MUSM, Lima, Peru).

Species	Locality	Tissue no.	Voucher no.
<i>Glironia venusta</i>	BRAZIL: Amazonas	U34666*	INPA (MNFS 75)
<i>Didelphis marsupialis</i>	BRAZIL: Acre	U34665*	---
<i>Philander opossum</i>	BRAZIL: Acre	U34678*	---
<i>Tlacuatzin canescens</i>	MEXICO: Jalisco	HM998562	TTU 37700
<i>Ma. (Marmosa) murina</i>	GUYANA:	AJ486978*	ISEM T-1698
<i>Ma. (Micoureus) regina</i>	PERU: Amazonas	U34675*	MVZ 154766
<i>Monodelphis adusta</i>	PERU: Loreto (1)	HM998563	TTU 98864
	PERU: Loreto (2)	HM998564	KU 157978
<i>M. americana</i>	BRAZIL: Bahía (3)	HM998558^	UFMG (RM 86)
	BRAZIL: São Paulo (4)	HM998565	MZUSP 29202
<i>M. brevicaudata</i>	FRENCH GUIANA: Macouria (5)	AJ606455*	ISEM V-937
	FRENCH GUIANA: Sãoul (6)	AJ606456*	ISEM N-540
	GUYANA: Barima Waini (7)	AJ606457*	USNM 568009
	GUYANA: Barima Waini (8)	AJ606461*	ROM (FN 31731)
	GUYANA: Potaro Siparuni (9)	AJ606462*	ROM (FN 43457)
	SURINAME: Marowijne (10)	HM998566	TK 21015
	VENEZUELA: Bolívar (11)	AJ606458*	EBRG (L 1920)
	VENEZUELA: Bolívar (11)	AJ606459*	EBRG (L 1918)
	VENEZUELA: Bolívar (11)	AJ606460*	EBRG (L 1917)
<i>M. dimidiata</i>	ARGENTINA: Santa Fe (12)	HM998568	MSB (NK 109725)
	ARGENTINA: Santa Fe (12)	HM998569	MSB (NK 113559)
	URUGUAY: Montevideo (13)	HM998567	EV 1025
<i>M. domestica</i>	BOLIVIA: Chuquisaca (14)	HM998571	MSB 55853
	BOLIVIA: Chuquisaca (14)	HM998572	MSB 56112
	BOLIVIA: Santa Cruz (15)	HM998573	MSB 67023
	BOLIVIA: Santa Cruz (16)	HM998574	MSB (NK 23288)
	BOLIVIA: Tarija (17)	HM998575	MSB (NK 25144)
	BOLIVIA: Tarija (17)	HM998576	MSB (NK 23349)
	BRAZIL: Mato Grosso do Sul (18)	HM998570	MVZ 197457

Species	Locality	Tissue no.	Voucher no.
	PARAGUAY: Concepción (19)	HM998577	MSB 82534
	UNKNOWN: lab colonies	AJ508398*	—
	UNKNOWN: lab colonies	X70673*	—
<i>M. emiliae</i>	BOLIVIA: Pando (20)	DQ385835*	USNM 579574
	BRAZIL: Acre (21)	DQ385833*	MPEG 28113
	BRAZIL: Acre (21)	DQ385834*	MVZ 190335
	BRAZIL: Amazonas (22)	DQ385832*	INPA 3040
<i>M. glirina</i>	BOLIVIA: Pando (24)	HM998580	AMNH 262398
	BOLIVIA: Pando (23)	HM998579	MSB 57005
	BOLIVIA: Pando (24)	HM998581	AMNH 262397
	BRAZIL: Mato Grosso (25)	HM998578	MVZ 197456
	BRAZIL: Mato Grosso (25)	HM998559^	MVZ 197990
<i>M. handleyi</i>	PERU: Loreto (26)	DQ386631*	AMNH (JAA 849)
	PERU: Loreto (26)	DQ386632*	MUSM 23809
	PERU: Loreto (26)	DQ386630*	MUSM 23810
<i>M. kunsi</i>	ARGENTINA: Salta (27)	HM998582	JPJ 1394
	ARGENTINA: Salta (27)	HM998583	JPJ 1508
	ARGENTINA: Salta (27)	HM998584	JPJ 1544
	BOLIVIA: Tarija (28)	HM998585	AMNH 263968
	PARAGUAY: Canindeyú (29)	HM998586	TTU (TK 6127)
<i>M. osgoodi</i>	BOLIVIA: Cochabamba (30)	HM998588	CBF 7552
	BOLIVIA: La Paz (31)	HM998587	CBF 7640
<i>M. peruviana</i>	BOLIVIA: Cochabamba (30)	HM998589	CBF 7611
	PERU: Ayacucho (32)	HM998590	MUSM 21427
	PERU: Cusco (33)	U34677*	MVZ 171412
	PERU: Cusco (34)	U34676*	MVZ 173928
	PERU: Loreto (35)	DQ385836*	AMNH 272695
<i>M. scalops</i>	BRAZIL: Minas Gerais (36)	HM998560^	UFMG 2251
<i>M. sorex</i>	PARAGUAY: Itapúa (37)	HM998591	TTU (TK 65944)
	PARAGUAY: Itapúa (38)	HM998592	TTU (TK 66283)
<i>M. theresa</i>	BRAZIL: São Paulo (39)	HM998594	MZUSP 29200
	BRAZIL: São Paulo (39)	HM998595	MVZ 182776
	BRAZIL: São Paulo (40)	HM998593	FMNH 141587
<i>M. Species B</i>	BOLIVIA: Santa Cruz (41)	HM998596	AMNH 263548
<i>M. Species C</i>	PERU: Huánuco (42)	HM998598	MUSM 18943
	PERU: Pasco (43)	HM998597	MUSM 11334
<i>M. Species D</i>	BRAZIL: Pará (44)	HM998561^	INPA (CS 49)