

## An assessment of morphometric and molecular variation in *Monodelphis dimidiata* (Wagner, 1847) (Didelphimorphia: Didelphidae)

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### Abstract

This study aims to clarify the taxonomic status of *Monodelphis dimidiata* and *M. sorex*, using molecular and morphometric data. Cytochrome-*b* sequences were obtained from specimens morphologically assignable to those taxa from the vicinity of type localities, but also representing their presumptive area of sympatry and extremes in their known distributions. These sequences were compared to GenBank downloaded sequences of select *Monodelphis* spp. and also of *Marmosa lepida*, *Micoureus demerarae*, and *Didelphis albiventris*, used as outgroups. Genetic distances and maximum likelihood analyses were performed to recover phylogenetic affinities among taxa. To access morphometric variation, univariate and multivariate statistical methods (Principal Component Analysis and Canonical Variate Analysis) were applied to the covariance matrix of 25 craniometric measurements obtained from a total of 108 specimens sampled across the geographic ranges of the species. The molecular analyses revealed a clade composed by haplotypes assignable to *Monodelphis dimidiata* and *M. sorex* (*sensu* Pine and Handley 2007) as distinct from the remaining *Monodelphis* species sampled to date, with 100% bootstrap support. Within this clade, one haplotype from Itatiaia was the sister clade to the remaining 11 specimens from Montevideo (Uruguay), Rio Grande do Sul and Santa Catarina states (Brazil), which formed an assemblage without clear geographic arrangement. In the morphometric analyses, the first two principal components of morphometric data separated variables associated with general latent size comparably expressed in all samples, and a group of variables including molars widths, molar tooththrow length, and postorbital constriction breadth, which separates the geographic samples in a sequence coincident, to a large extent, with their latitudinal distributions. The sample from Itatiaia, Rio de Janeiro State, was again revealed as clearly distinct from the remaining samples, suggesting that it may represent a peripheral isolated population. With the study of original descriptions and published information on the type material of *M. sorex*, including photographs of syntypes, our analyses point to the recognition of *M. sorex* as a subjective junior synonym of *M. dimidiata*, which is then regarded as a widespread taxon ranging from the latitudes of southern Buenos Aires Province and Córdoba in Argentina to at least Minas Gerais State in southeastern Brazil, through the Pampas and the Atlantic Forest domains.

**Key words:** geographic variation, molecular systematics, multivariate statistics, cranial morphometrics, taxonomy, *Monodelphis sorex*, *Monodelphis henseli*, *Monodelphis dimidiata itatiaiayae*, *Monodelphis brevicaudis*, *Monodelphis tricolor paulensis*, semelparity

### Introduction

The genus *Monodelphis* (Burnett 1830) is the most diverse among South American marsupials, comprising at least 22 recognized species (Gardner 2005, Pine & Handley 2008, Voss & Jansa 2009). A major endemic zone for this New World genus is the Atlantic Forest, which includes more than half of the species in *Monodelphis*. Historically, this diversity has been tentatively sorted among different genera, subgenera, or species groups based on pelage color and patterns (Thomas 1888a, Cabrera 1919, Gilmore 1941, Gomes 1991). Original descriptions have been primarily based on size and pelage colors and patterns, and a number of different

species have been recognized on the basis of one or a few specimens. Despite the progress in the identification of species and species complexes based on morphological (Pine 1975, 1976, 1977, 1980, Pine & Abrawaya 1978, Pine & Handley 1984, Pine *et al.* 1985) and molecular data (Solari 2007), species boundaries and the taxonomic status of several nominal forms remain controversial.

The present paper focuses on the taxonomic status of *Monodelphis dimidiata* (Wagner 1847), a species originally described from Maldonado, Uruguay, with a plain-colored dorsum without dorsal stripes and with flanks of a yellowish color that gradually melt into the ventral coloration (Pine & Handley 2008). Among other nominal forms presenting a plain-colored dorsum, Pine & Handley (2008) currently recognize *M. sorex* (Hensel 1872), originally described from southern Brazil, as distinct from *M. dimidiata*, distinguished by the reddish intensity of pelage on the flanks and feet, as well as by its overall shorter pelage. Currently, these two forms show partially overlapping distributions in southern Brazil and in the region of Misiones, Argentina. An abbreviated history of the usage of these names, as well as of names currently regarded as synonyms of *M. dimidiata* and *M. sorex* was first detailed by Pine *et al.* (1985). Of relevance here, Miranda-Ribeiro (1936) described *P[eromys]. d[imidiata]. itatiayae* primarily on the basis of color in a sample from Itatiaia, Rio de Janeiro State. Previously, Thomas (1888b) had described *Didelphys (Peromys) Henseli* from the state of Rio Grande do Sul in order to distinguish specimens intermediate in size between syntypes of *Didelphis (Microdelphis.) sorex* and larger sympatric specimens which Hensel (1872) had assigned to *M. brachyura* (= *M. dimidiata*). Both *M. dimidiata itatiayae* and *M. henseli* are currently regarded as junior synonyms of *M. sorex* (Pine & Handley 2008). This is also the current taxonomic status of another nominal form described from the state of São Paulo, *Monodelphis tricolor paulensis* Vieira, 1950.

In spite of the apparently straightforward pelage distinction, examination of museum series revealed notable variation in pelage color within populations assigned to either *M. dimidiata* or *M. sorex*. The increase of museum samples from Argentina, southeastern–southern Brazil and Uruguay has revealed variation both in size and pelage coloration in samples from localities in which the two forms have not been previously reported in sympatry (Fig. 1). These findings prompted an analysis of alternative character systems in order to evaluate the taxonomic status of the population samples currently assigned to *M. dimidiata* or to *M. sorex* on the basis of size and pelage color variation.

The aims of this report are therefore (1) to clarify the phylogenetic affinities of samples morphologically assignable to *M. dimidiata* or *M. sorex* (*sensu* Pine & Handley 2008) in the context of the molecular (Cytochrome-*b*) variation in *Monodelphis*, in order to evaluate their taxonomic distinction and to estimate their geographic variability, (2) to analyze the morphometric variability among samples in an attempt to identify trends in craniometric variation among geographical samples, and (3) to contrast molecular and morphological results so as to construct a framework under which the variability documented in type series and topotypes may be interpreted.

## Material and methods

**Molecular methods.** We have sequenced the mitochondrial protein-coding gene Cytochrome-*b* for six specimens morphologically assignable to *Monodelphis dimidiata* / *M. sorex*, from widespread localities throughout the distributions of these putative taxa (Fig. 2, Appendix 1), and also one specimen of *M. americana*. Additionally, 21 sequences deposited in GenBank were included in our dataset, representing *M. adusta* (Thomas, 1897) (2), *M. brevicaudata* (Erleben, 1777) (2), *M. dimidiata* (6), *M. domestica* (Wagner, 1842) (1), *M. emiliae* (Thomas, 1912) (2), *M. handleyi* Solari, 2007 (2), *M. kungsi* Pine, 1975 (2), *M. osgoodi* Doult, 1938 (2), and *M. peruviana* (Osgood, 1913) (2). Our dataset also included *Marmosa lepida* (Thomas, 1888), *Micoureus demerarae* (Thomas, 1905), and *Didelphis albiventris* Lund, 1840 as the outgroups, following Patton *et al.* (1996), Jansa & Voss (2000), Jansa *et al.* (2006), and Voss & Jansa (2009).

DNA was obtained from ethanol-preserved fragments of liver or muscle tissue of all recently collected specimens, by three different extraction protocols: a phenol/chloroform method modified from Sambrook *et al.* (1989), the salt protocol (with sodium chloride 5M) modified from Miller *et al.* (1988), or with QIAGEN® Kit (QIAGEN 2006). PCRs were carried out in 35 denaturation cycles at 94°C for 2 minutes, annealing at

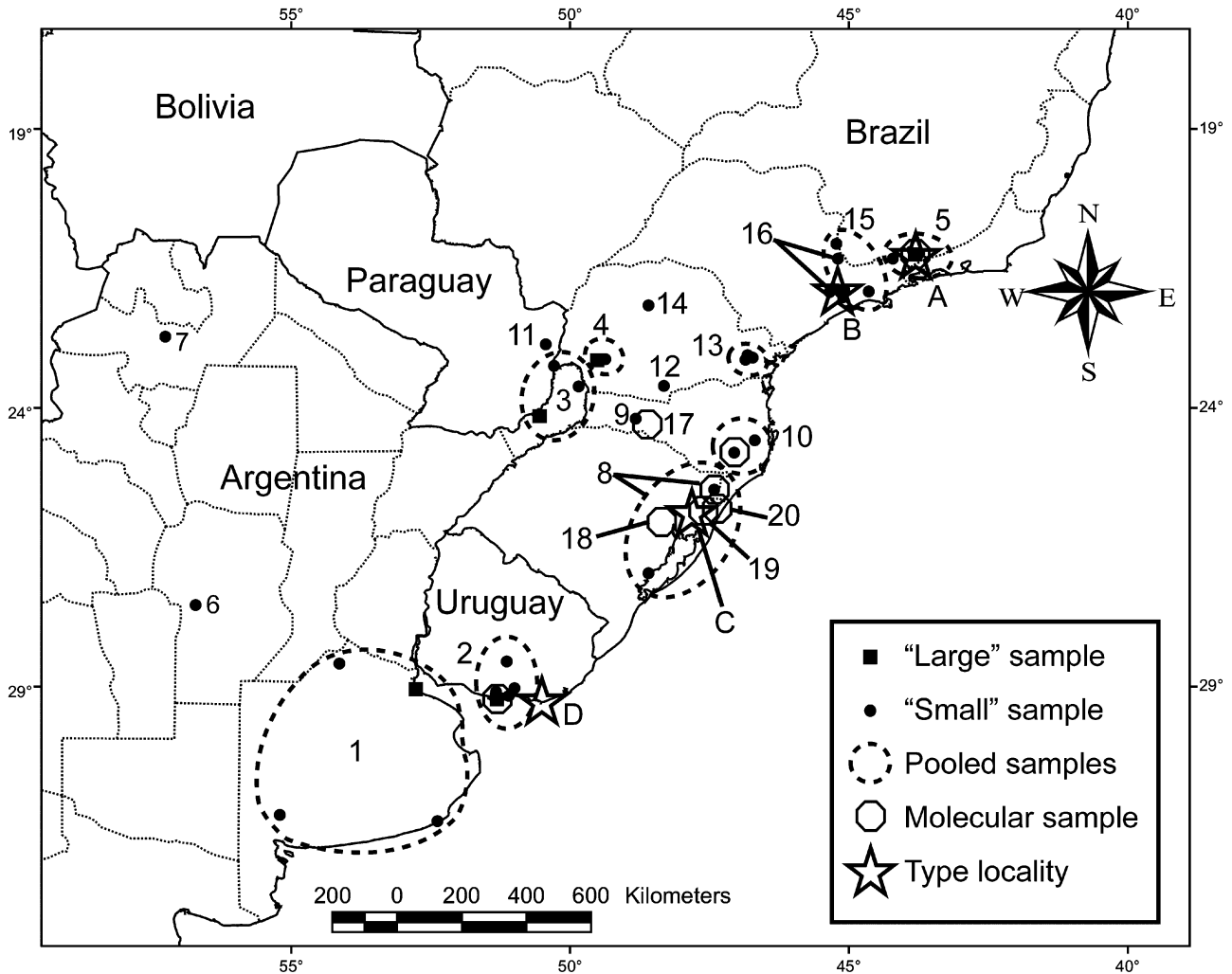
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45°C for 1 ½ minutes and extension at 72°C for 2 minutes, followed by a final extension at 72°C for 5 minutes. Amplified products were either sequenced on an automatic sequencer (Amersham Biosciences ABI 3130) or submitted to MACROGEN (Korea). External primers MVZ05 and MVZ14 (Smith & Patton 1993) were used to amplify the entire *Cytochrome-b* (*Cyt-b*) gene (1149 bp). Another four internal primers were used in sequencing the amplified gene, *CITB1* (Smith & Patton 1993), as well as those designed for this study: *CYTB-MONO-479R* (CCTCARATTCAYTCTACTA), *CYTB-MONO-479F* (TAGTAGARTGAATYTGAGG), and *CYTB-MONO-870F* (RGTAYTAGCYCTCTTAGC). Obtained sequences were then edited and aligned with MEGA 4 program (Tamura *et al.* 2007) and Chromas Pro 1.34 (<http://www.technelysium.com.au/ChromasPro.html>).



**FIGURE 1.** Dorsal, lateral and ventral views of skins from Misiónes, Argentina, illustrating sympatric specimens with the “yellowish” phenotype (MACN-BR22474) and the “reddish” phenotype (MACN-BR22475). Scale bar = 5cm.



**FIGURE 2.** Provenances of *Monodelphis dimidiata* samples used in this study. Locality numbers refer to lists in Appendices 1 and 2. Type localities: A—Itatiaia, Rio de Janeiro State, Brazil (*Monodelphis dimidiata itatiaiae*), B—Pirituba, São Paulo State, Brazil (*Monodelphis tricolor paulensis*), C—Taquara, Rio Grande do Sul State, Brazil (*Monodelphis sorex*) and D—Maldonado, Uruguay (*Monodelphis dimidiata*).

Some individuals were sequenced for this study, one of *M. americana* with a 1036 bp sequence and six specimens of *M. dimidiata* with the complete *Cyt-b* of 1149 bp. Six sequences referred to *M. dimidiata* were downloaded from GenBank, with sequence lengths ranging from 729 to 1122 bp. Remaining ingroup members presented sequences ranging from 630 to 1149 bp. *Cyt-b* exhibited 357 bp parsimony-informative sites. All individuals selected from GenBank had different haplotypes. The single common haplotype in our analyses was shared by LMT290 and LMT317, both specimens from Cambará do Sul, Rio Grande do Sul State.

Genetic distances are often used to tackle the taxonomic status of natural populations. Therefore, we have estimated matrices of pair-wise genetic distances with software MEGA 4 (Tamura *et al.* 2007) using p-distance and K2P with pair-wise deletions to allow comparisons with other studies.

Maximum Likelihood (ML) analyses were done with PhyML v3.0 for server (Guindon & Gascuel 2003). This procedure requires an initial tree that was chosen among five random trees built with the BIONJ (Gascuel 2007) algorithm. ML trees were constructed with heuristic search with the “BEST” option, that improves searches using two branch-swapping algorithms: NNI (Nearest Neighbor Interchange) and SPR (Subtree Pruning and Regrafting). The chosen topology is the one that presents the best solution between both algorithms. Maximum Parsimony (MP) analyses were carried out on PAUP\*4.0b10 (Swofford 1993). MP

trees were also constructed with heuristic search, but using the Tree Bisection-Reconnection algorithm. In this case, the initial trees were constructed with step-wise random addition of taxa, with 1000 replicates.

Node robustness was ascertained with bootstrap proportions based on 1000 pseudo-replicates for both ML and MP (Felsenstein 1985, Russo 1997).

The ML tree requires the input of an evolutionary model. In this case, the GTR+ $\Gamma$ +I model of base substitution (Tavaré 1986, Rodriguez *et al.* 1990) was used, assuming different nucleotide frequencies (A=0.31297901, C=0.31297901, G=0.26549621 and T=0.11192987), a proportion of invariable sites (I parameter = 0.32014527), and correction of distribution gamma (alpha parameter = 0.779309). This model was chosen by MODELTEST 3.07 (Posada & Crandall 1998) on PAUP\*4.0b10 MODELTEST using the Akaike Information Criteria (AIC) in order to select the model that best fits the data matrix (Posada 2003).

**Morphometric methods. Skull measurements:** For each specimen, twenty-five measurements were taken with digital calipers accurate to the nearest 0.01 mm under a stereoscopic microscope: [1] GLS—greatest length of skull, from the anterior tips of nasals to the posteriormost projection of occipital, [2] HBC—height of braincase, measured from the basisphenoid-basioccipital suture to the suture between parietals, including the sagittal crest when present, [3] MSH—anterior skull height, measured across the naso-frontal suture and the maxilla at the posterior limit of the fourth molar, [4] NSL—greatest nasal length, [5] MBM—maximum breadth between lateralmost points of maxillary bones, [6] MBN—maximum breadth across nasals, [7] POC—postorbital constriction, [8] ZGB—zygomatic breadth, measured at a plane defined by the posterior limit of maxilla in its suture with jugal bone, [9] BCB—greatest braincase breadth, measured just anteriorly to the posttympanic processes, [10] LOF—greatest length of ocular foramen, [11] LPM—greatest length of premaxilla, [12] LPP—greatest length of premaxilla + palatine, [13] LPB—greatest length of presphenoid + basisphenoid, [14] BOL—basioccipital length, [15] IPS—anterior length of upper dental series: length from the anterior edge of first incisor to the posterior edge of last premolar, [16] SMT—greatest length of upper molar tooth row, [17] MW1—greatest width of first upper molar, [18] MW2—greatest width of second upper molar, [19] MW3—greatest width of third upper molar, [20] MW4—greatest width of fourth upper molar, [21] MLM—greatest length of mandibular ramus, [22] MHM—greatest height of mandibular ramus, [23] DBC—distance between mandibular condyles, [24] IPI— anterior length of lower dental series: length from the anterior edge of first incisor to the posterior edge of last premolar, [25] IMT—greatest length of lower molar tooth row.

**Missing data estimation:** Specimens missing more than six cranial measurements were excluded from the dataset. It was possible, for 83 of 108 skulls, to obtain all measurements. The remaining specimens (25) had missing values (1.93% of total cells of dataset) estimated by the EM (Expectation-Maximization) method (Dempster *et al.* 1977). In this maximum likelihood procedure, missing cells in the dataset are initially input using the mean for each variable. Means and covariances are then estimated for the complete matrix. Original missing values are then re-estimated based on these new parameters, which are then re-estimated, and the process continues iteratively until estimates converge on final constant values (Strauss *et al.* 2003).

**Sample composition:** Morphometric data were obtained from museum specimens (Appendix 2), including some of the vouchers for sequences used in this study. Only adults, with the third permanent upper and lower premolars totally erupted and functional, were considered for the statistical analyses. This condition refers to age classes 5, 6 and 7 of Pine *et al.* (1985). Specimens with the third premolar erupting but not in occlusion (age class 4, Pine *et al.* 1985), or younger, were excluded from morphometric analyses. In order to increase sample sizes, specimens from geographically close localities were pooled. In the same way, within each sample, sexes were pooled. To reduce possible effects of secondary sexual dimorphism, which was detected in the age interval 5–7 for *M. dimidiata* (Pine *et al.* 1985), very unbalanced samples, such as those in which one sex was represented by only one specimen, were initially excluded from the analyses. A total of 66 adult individuals from five larger geographic samples (each with at least eight specimens, appendix 2) comprise our “larger-samples” morphometric data set. The remaining 52 adult individuals, belonging to 11 small geographic samples, composed the “smaller-samples” dataset. These named “small-samples” consist of samples with seven or fewer individuals and also by those localities represented by males or females only, namely São Paulo (excluding the type specimens of *M. tricolor paulensis*, treated as a separated small sample)

and Urubici. This distinction of “large” and “small” samples was relevant for the statistical analyses, as detailed below.

**Statistical analyses:** Main trends of craniometric variation, both for the larger-samples total dataset regarded as belonging to a single population and among larger geographic samples treated as distinct groups, were summarized, respectively, by Principal Component (PCA) and Canonical Variate (CVA) Analyses. PCA was performed using the covariance matrix generated with all 25 log-transformed measurements. Individual scores on the first two principal components were then labeled by geographic provenance, and correlations of original variables with principal components were computed to access variables most associated with the main tendencies of variation. Discriminant functions among geographic samples and variables most associated with them were assessed by CVA of the same 25 craniometric variables. The variation in craniometrical characters most associated with discriminatory principal components and canonical axes was portrayed as frequency histograms in order to allow objective comparisons among samples.

Small samples (those with seven or fewer individuals) were then probabilistically allocated to the larger samples on the basis of their shortest Mahalanobis distances, estimated in 1000 bootstrap iterations. All multivariate analyses were performed on MATLAB 4 (Mathworks 1992, routines by R.E. Strauss at <http://www.biol.ttu.edu/Strauss/Matlab/matlab.htm>).

## Results

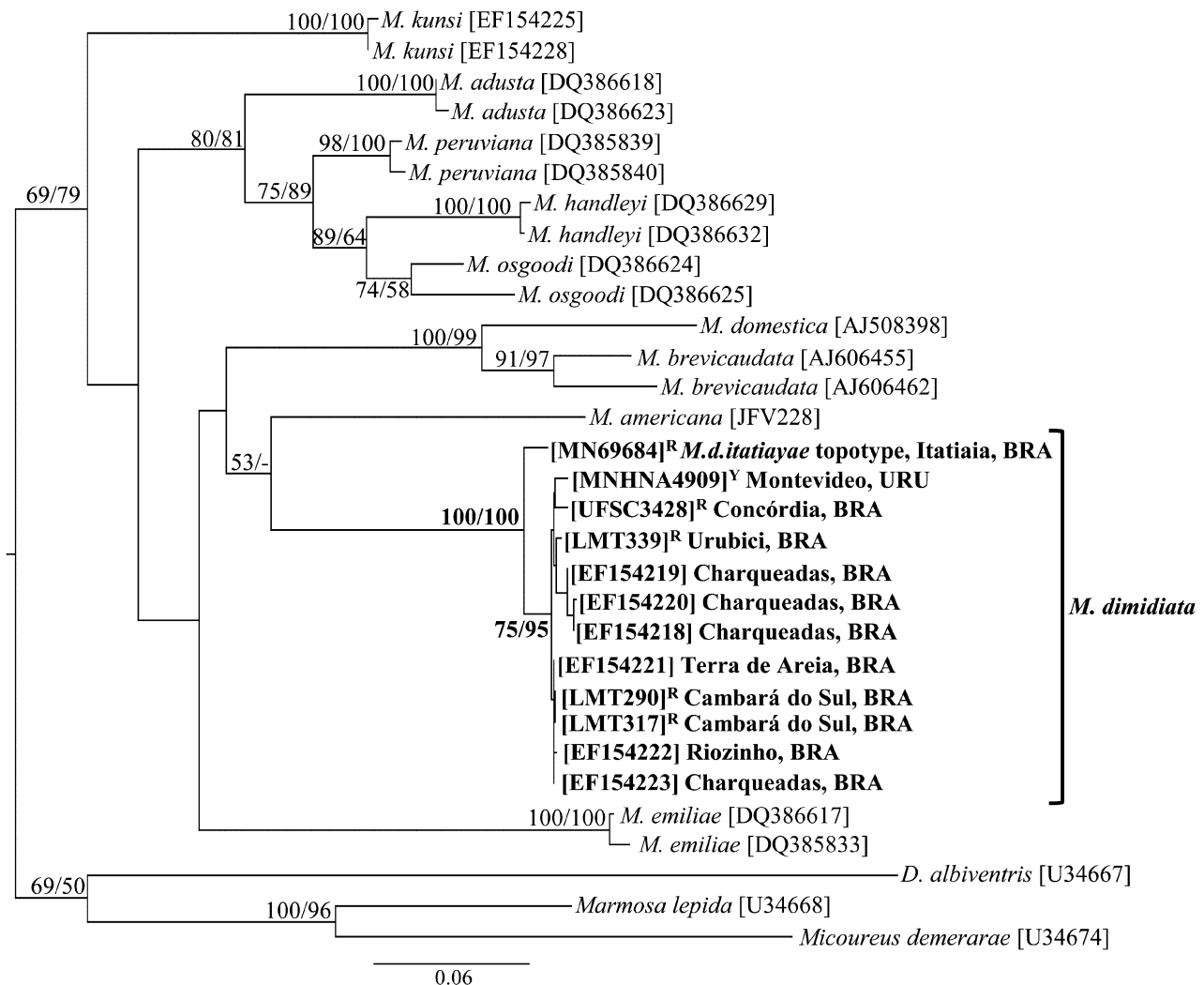
### Molecular analyses

The two most parsimonious trees constructed under 357 parsimony informative sites, presented 1226 steps, CI=0.52 and RI=0.69. Phylogenetic relationships recovered with MP and ML analyses results were very similar, so only the ML tree is shown (Fig. 3). The bootstrap value for Maximum Parsimony is listed following the Maximum Likelihood bootstrap value in the ML tree and throughout the text.

The maximum likelihood analysis of Cyt-*b* sequence data revealed three distinct groups within the *Monodelphis* diversity represented in our molecular data set, although with a generally low bootstrap support. The first group separates two individuals of *M. kunsi* from the remaining forms of the genus. *Monodelphis kunsi* is found in Bolivia, central–southern Brazil, Paraguay and northwest Argentina (Vargas *et al.* 2003, Jayat & Miotti 2006, de la Sancha *et al.* 2007, Solari 2007). It is here represented by two sequences of individuals from Goiás, Brazil, composing a well-supported clade with 100% bootstrap support both in ML and MP analyses.

The second group is composed of Andean and western Amazonian species, namely the *adusta* complex that clusters with 80/81% bootstrap support (Solari 2007). Within this complex, *M. adusta*, *M. peruviana*, and *M. handleyi* form a paraphyletic assemblage with *M. osgoodi* as the sister group to *M. handleyi*. Our results are fairly congruent to previous findings by Solari (2007), but differ by not revealing *M. emiliae*, an exclusively Amazonian taxon, as the sister group of the *adusta* complex. Instead, *M. emiliae* is basal to another clade that joins *M. domestica* + *M. brevicaudata* and *M. americana* + *M. dimidiata*, composing the third major group on our tree. This latter arrangement is also geographically consistent, because *M. domestica* and *M. brevicaudata* are respectively from Cerrado-Caatinga and Amazonian habitats, whereas *M. americana* and *M. dimidiata* are mainly Atlantic Forest species.

In contrast with the generally low confidence values for basal relationships in our tree, all 12 individuals of *M. dimidiata* are clustered with 100% bootstrap support in both ML and MP analyses. Within this clade, one individual (MN69684) from Itatiaia (Rio de Janeiro State), is sister to the remaining 11 samples, with 75/95% bootstrap support. The haplotypes formed a non-distinct assemblage with no geographic arrangement. Localities from which samples were taken ranged from the states of Santa Catarina (UFSC3428, LMT339) and Rio Grande do Sul (DS019, LMT290, 317, MN37852–54, 37856, ZE97) in Brazil, to Montevideo, Uruguay (MNHNA4909), and this area includes the area of sympatry of *M. dimidiata* and *M. sorex*, according to the distribution maps of Pine & Handley (2008).



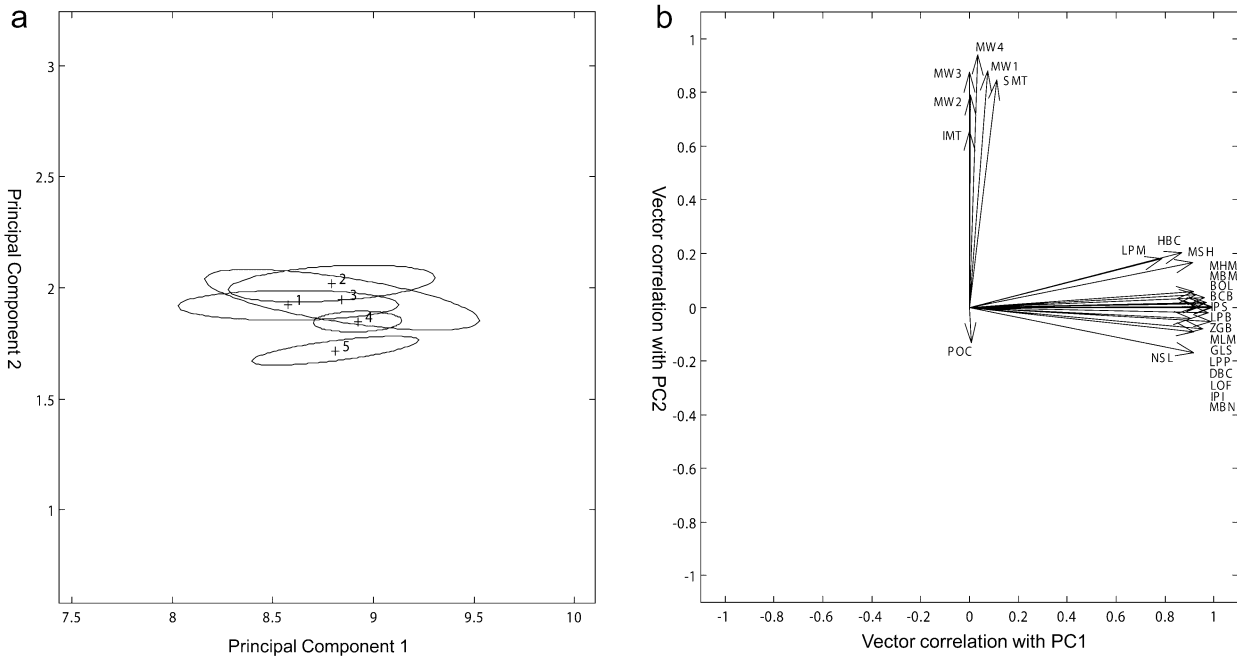
**FIGURE 3.** Optimal tree obtained by maximum likelihood analysis based on 1149pb (see appendix 1 for correct length of each individual) of Cytochrome-*b* under GTR + $\Gamma$ +I model. Numbers above branches are bootstrap values based on 1000 replicates (ML/MP respectively). Inside square brackets are GenBank, museum or collector's identification voucher numbers. Y = Yellowish flanks, R = Reddish flanks.

### Morphometric analyses

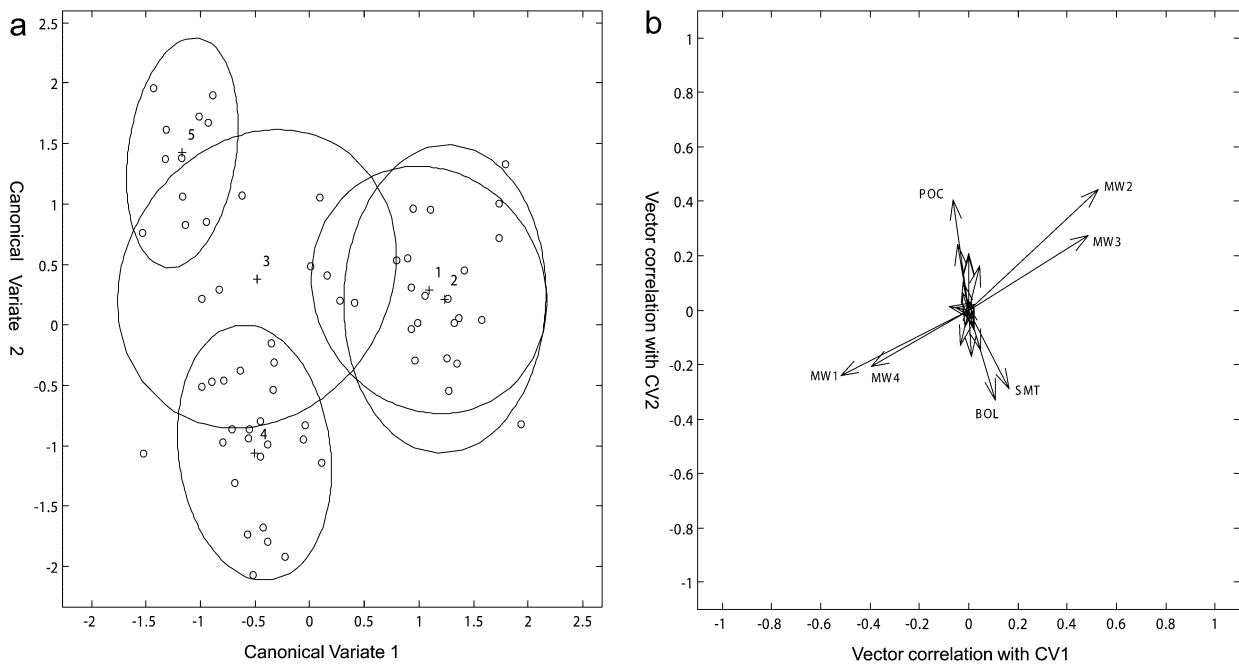
The five larger morphometric samples represent large areas within the geographic range of *M. dimidiata* / *M. sorex* and included specimens from type localities or their vicinities (Appendix 2). Principal Component Analysis revealed that more than 87% of the total variation in the 25 original variables was summarized by the first two principal components, 83% being explained by the first axis alone (Fig. 4). High and positive correlations of most variables with this particular axis indicate that it may be interpreted as the size vector. No distinction among scores of different populations is revealed in relation to this axis, indicating that a wide range of cranial sizes is present in all samples, regardless of their geographical origins.

Conversely, PC2 explains a mere 4% of the total variation and summarizes morphometric variation that is not correlated with cranial size, namely molar series lengths (SMT, IMT), superior molar widths (MW1–4) and postorbital constriction (POC), this last character presenting a weak negative loading with respect to this component. Except for the Buenos Aires sample, centroids are distributed in a latitudinal sequence. The 95%

confidence intervals of sample scores are quite superimposed, but the ellipse from Itatiaia is bluntly distinct from the remaining ones (Fig. 4), suggesting that this sample may represent a peripherally isolated population. The positive loadings of molar length and width measurements with PC2 indicate that individuals from higher latitudes (those from Montevideo + Buenos Aires) have greater molar widths and greater molar series lengths, while the specimens from Itatiaia have slightly narrower and shorter molar rows.



**FIGURE 4.** a) Plot of 95% confidence intervals of scores in the first two principal components, and respective centroids, of each geographic sample: (1) Buenos Aires, (2) Montevideo, (3) Misiones, (4) Boa Vista da Aparecida, (5) Itatiaia; b) respective vector correlations of original variables (acronyms defined in Material and Methods).



**FIGURE 5.** a) Plot of individual scores and 95% confidence ellipses for each geographic sample in the first two canonical variates: (1) Buenos Aires, (2) Montevideo, (3) Misiones, (4) Boa Vista da Aparecida, (5) Itatiaia. Crosses represent the centroid of each sample; b) respective vector correlations of original variables (acronyms defined in Material and Methods).

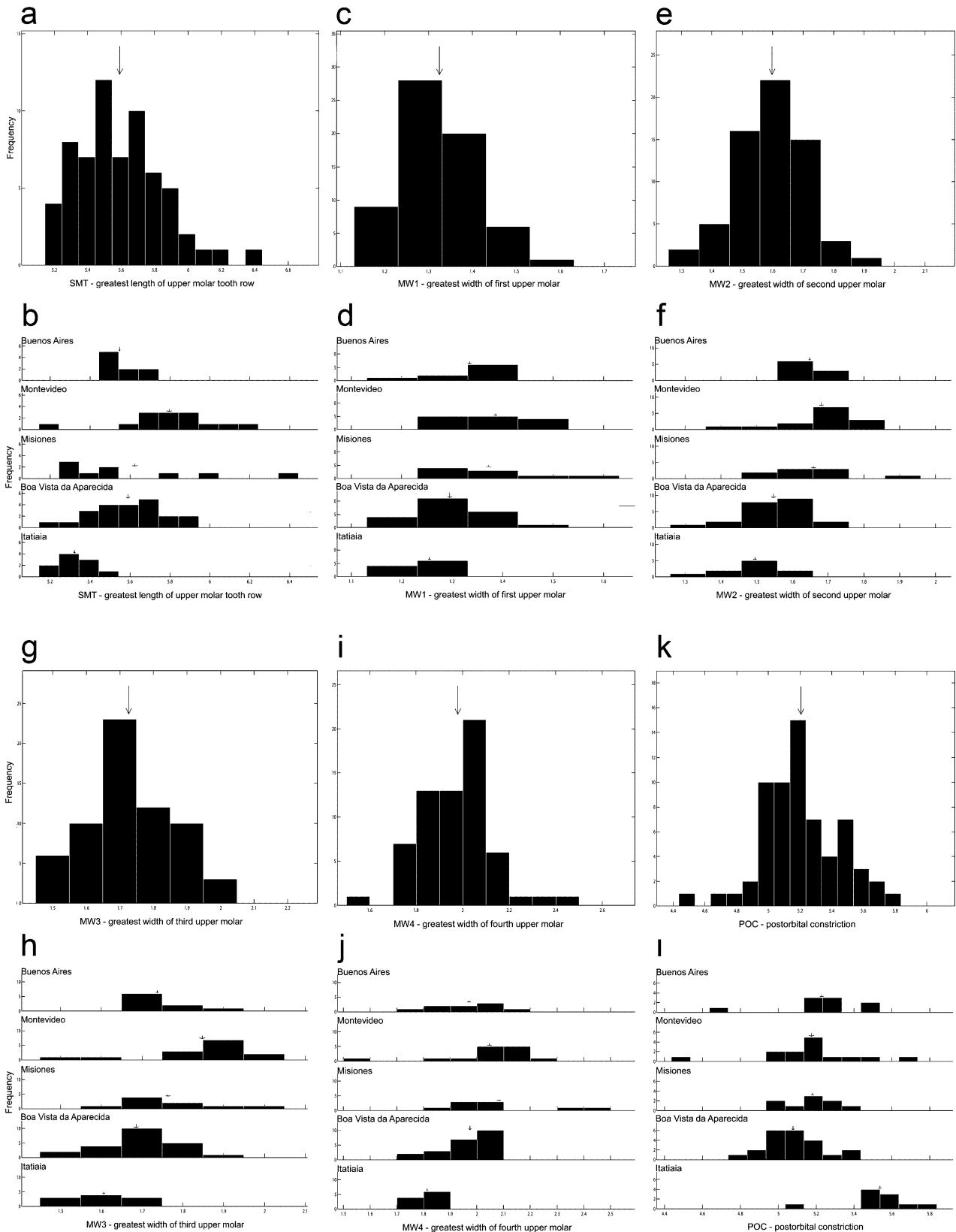
The Canonical Variate Analysis of the same dataset also identified the molar width and molar series length characters as well as the postorbital constriction (POC) as the most distinctive morphometric traits among population samples. The first canonical variate, which accounts for almost 50% of the variation among geographical groups, separated samples from Buenos Aires and Montevideo from the remaining ones, except for a few overlapping scores with samples from Misiones and Boa Vista da Aparecida (Fig. 5). Molar width characters were the only ones correlated with this axis. Separation of the Itatiaia sample from the Boa Vista da Aparecida and Montevideo + Buenos Aires samples was supported by postorbital constriction (POC), upper molar tooththrow length (SMT), and, in addition, by basioccipital length (BOL). The sample from Misiones presents overlapping scores with all other samples, indicating that it has intermediate values for the diagnostic characters separating samples from the extremes of the geographical range (Itatiaia and Montevideo+Buenos Aires) and also from the sample from Boa Vista da Aparecida.

Frequency histograms illustrate the differences revealed by characters most associated with discrimination among large samples (Fig. 6). The sample from Itatiaia shows the smallest absolute values and means for the molar widths and upper molar tooththrow length, and the largest values and mean for postorbital constriction (POC) (Fig. 6, k-l). Congruent to a clinal pattern of variation, the sample from Boa Vista da Aparecida usually presented the second smaller means for upper molar widths (Fig. 6 d,f,h), but revealed the lowest mean for postorbital constriction (Fig. 6, l). Distributions of this variable in the remaining samples, except Itatiaia, are nevertheless very similar, suggesting that mean differences may be due to their small sample sizes.

Most of the small geographic samples were allocated to proximal larger samples, indicating a strong geographic basis for the morphometric variation summarized by Mahalanobis distances (Table 1). Thus, the sample from Córdoba (Argentina) was allocated to the Buenos Aires + Montevideo cluster in 100% of the bootstrap iterations: a similar allocation pattern was revealed for the small pooled sample from Rio Grande do Sul, the southernmost Brazilian sample. The specimen from Salta presented a larger frequency of bootstrap allocation to the large sample from Misiones (63%) but also clustered to Buenos Aires (22%) and Boa Vista da Aparecida (13%). Small samples from Santa Catarina State (Xavantina and Urubici) were allocated with similar bootstrap frequencies to the large samples of Boa Vista da Aparecida and Itatiaia. Samples from Paraná State (Bituruna, Curitiba and Fênix), and the specimen from Paraguay (Alto Paraná) were also allocated to the sample from Boa Vista da Aparecida, a result also found for the type specimens of *M. tricolor paulensis*. The small pooled sample from São Paulo State, composed by specimens from Itapevi, Salesópolis, Guarulhos, Pinhal, São Bernardo do Campo, São Paulo and Serra Negra, clustered with the large sample from Itatiaia in 92% of the bootstrap iterations.

**TABLE1.** Probabilistic values for allocations of “small” samples (rows) to “large” samples (columns) based on shortest Mahalanobis distances obtained in 1000 bootstrap iterations. Values in bold caption suggest most probable allocation(s). Numbers in square brackets correspond to the localities listed in Appendix 2 and mapped in Figure 2.

“small” / “large”	[1] Buenos Aires	[2] Montevideo	[3] Misiones	[4] Boa Vista da Aparecida	[5] Itatiaia
[6] Córdoba	.242	<b>.758</b>	-	-	-
[7] Salta	.221	-	<b>.625</b>	.128	.026
[8] Rio Grande do Sul	.351	<b>.622</b>	-	.026	.001
[9] Xavantina	-	-	.003	<b>.559</b>	.438
[10] Urubici	.002	-	-	.436	<b>.562</b>
[11] Alto Paraná (Paraguay)	-	-	-	<b>1</b>	-
[12] Bituruna	-	-	.322	<b>.656</b>	.022
[13] Curitiba	-	-	-	<b>1</b>	-
[14] Fênix	-	.003	.020	<b>.977</b>	-
[15] São Paulo	-	-	.015	.089	<b>.896</b>
[16] São Paulo ( <i>M.t.paulensis</i> )	-	-	.071	<b>.924</b>	.005



**FIGURE 6.** Absolute frequency distributions for select characters most associated with the second principal component and the first canonical variate. Upper—pooled distributions for all “large” samples; Lower—distributions for each “large” sample. (a-b) SMT, (c-d) MW1, (e-f) MW2, (g-h) MW3, (i-j) MW4 and (k-l) POC . Arrows indicate the mean for each distribution.

## Discussion

Our molecular analysis distinguishes a clade, composed of the forms currently recognized as *Monodelphis dimidiata* and *M. sorex*, from the remaining *Monodelphis* species sampled, including another plain-colored form with reddish flanks, *M. brevicaudata*, from the Amazon region, as well as the largely Atlantic forest species *M. americana*.

A maximum p-distance of 2.9% (K2P = 3.0%) between specimens referable to *M. dimidiata* and *M. sorex* was found in the comparison of haplotypes MNHNA4909 and MN69684. These specimens constitute the geographic extremes of our molecular samples, the former from the southernmost locality, Montevideo, which is close to the type locality of *Monodelphis dimidiata* (Maldonado, Uruguay), and the latter from Itatiaia, comprising the northernmost sample. The Itatiaia mountain range includes the type locality of *M. dimidiata itatiayae*, a nominal form that has been referred to *M. sorex* since Pine (1980). Excluding this more divergent haplotype (MN69684), p-distances between haplotypes from localities in southern Brazil and in Uruguay did not reach values over 1.6% (K2P also 1.6%). These localities (Cambará do Sul, Charqueadas, Riozinho, and Terra de Areia—all in Rio Grande do Sul State, Brazil) are circumscribed in areas of presumptive sympatry of *M. dimidiata* with *M. sorex*, and are also in the vicinity of the type locality of *M. sorex*, Taquara, Rio Grande do Sul State, Brazil. Moreover, specimens used in the molecular analyses had skins with different intensities of reddish in the flanks, varying from pale yellowish in individuals from Uruguay to reddish and deep reddish skins from Cambará do Sul, Urubici, and Itatiaia.

Such specimens, which would otherwise be identified as *M. dimidiata* and *M. sorex* on the basis of pelage color, following the recent key provided by Pine and Handley (2008), did not present sufficient differences in their *Cyt-b* sequences to justify their separation into two distinct lineages.

**Taxonomic status of *Monodelphis sorex* and *M. henseli*:** Hensel (1872) described *Monodelphis sorex* based on a notable difference in body size and pelage color of small and dark specimens from Taquara, Rio Grande do Sul, which he recognized as adults, in relation to a larger form from the same locality, referred by him to *M. brachyura* (= *M. dimidiata*). Syntypes of *M. sorex* are deposited in the Zoologisches Museum, Berlin, under entries ZMB 35498 and 35499, the skulls of which were depicted by Avila-Pires (1994). Examination of the original plates that were published in Figs. 4 and 5 of Avila-Pires (1994) shows that the permanent lower and upper third premolars (P3 and p3) are not completely erupted in both syntypes of *M. sorex*. In both specimens, the deciduous premolars were already lost, but ZMB 35498 has the third premolar already erupted but not completely in place, whereas ZMB 35499 has P3 and p3 still in the alveoli.

The presence of P3 and p3 completely in occlusion was chosen as a criterion to include specimens in our samples following the findings of Pine *et al.* (1985) that *M. dimidiata* presents a wide variation in ontogenetic size, which could result in unreliable patterns of discrimination among geographic samples of mixed age.

Thus, following the age criterion used in our study, and in contrast to the original conclusions of Hensel (1872), the syntypes of *M. sorex* cannot be regarded as fully grown adult individuals, and are interpreted here as young specimens of the larger form from Taquara studied by Hensel, (*i.e.*, *M. dimidiata*). Pine and Handley (2008) came to the same conclusion with respect to the age of type specimens pictured in Avila-Pires's published figures. Our molecular results, coupled with the analyses of type specimens and of the original description by Hensel (1872), point to the conclusion that *M. sorex* is a junior synonym of *M. dimidiata*. Another form from southern Brazil, originally recognized by Hensel (1872) among his specimens from Taquara, and formally described by Thomas (1888b) as *M. henseli*, was considered to be indistinguishable from *M. sorex* by Pine *et al.* (1985). We did not examine the type specimen, which is maintained in the Natural History Museum, London, but to judge from the original description as a form intermediate in size between *M. sorex* and the larger form from Taquara (Hensel 1872, Thomas 1888a;b) we conclude that it must also be regarded as a junior synonym of *M. dimidiata*.

**Taxonomic status of *M. dimidiata itatiayae*:** The p-distance (and K2P) for the haplotype from Itatiaia, and the meager bootstrap support for its branch (75%) in the maximum likelihood analysis, are indicative of an incipient differentiation for this population.

Our morphometric analyses revealed a wide overlapping of scores from different regions of the geographic range of *M. dimidiata*, with a subtle separation of the Itatiaia population with respect to the remaining large samples. The molar widths and the postorbital constriction measurements distinguish Itatiaia specimens from the remaining samples, despite the fact that this variation seems to be structured in a latitudinal continuum.

Molar widths and molar row lengths, as well as postorbital constriction (POC), usually show low variation related to age and sex, and thus are probably reliable markers for the differentiation of samples. Of 13 cranial characters included in an analysis of variance between adult males and adult females of *M. dimidiata* from Balcarce, Buenos Aires, Argentina, by Pine *et al.* (1985), only two, namely M1–M4 length and postorbital constriction, did not show significant sex variation, and males and females showed a notable overlap in these measurements. In the same way, in contrast with 11 other cranial characters, only M1–M4 length and postorbital constriction did not show notable differences in means for age classes 5, 6, and 7 in separate comparisons for each sex (Pine *et al.* 1985).

In spite of these findings, and to further evaluate the possible impact of secondary sexual dimorphism in our results, we performed the same statistical analyses for a reduced dataset including only adult males. Even with the minimum sample sizes reduced to four individuals, it was possible to compose five geographic “large” samples, namely Montevideo, Misiones, Boa Vista da Aparecida, São Paulo and Itatiaia. Because the overall distribution of scores in both PCA and CVA was similar to those of pooled sexes, and vector correlations indicated the same molar and postorbital measurements to be the most distinctive characters, only the results of pooled sexes analyses are shown here.

The type specimen of *M. dimidiata itatiayae* (described as *P[eramys]. d[imidiata]. itatiayae*), a male caught at Campo Belo, Itatiaia, and described by Miranda-Ribeiro (1936) as a color variant of *M. dimidiata*, has been regarded as a junior-synonym of *M. sorex* (Pine 1980, Pine & Handley 2008). Ximénez *et al.* (1972) found this specimen indistinguishable from topotypic specimens of *M. dimidiata* from Uruguay, among which the color variation encompassed that of the darker and more reddish holotype of *M. d. itatiayae*. We were not able to examine this specimen, currently maintained at the Smithsonian Institution (Pine 1980), but available measurements provided by Pine (1980) are indicative of an adult individual of *M. dimidiata*. The published measurements for the holotype are not conclusive, however, as to their falling within the range of those of available specimens from Itatiaia included in our morphometric analyses: the reported M1–M4 length (5.8 mm) is outside the range of our Itatiaia sample (5.2–5.5 mm, Fig.6), whereas that of postorbital constriction (5.2 mm) is near the lower extreme.

The lack of intermediate geographic samples, either molecular and morphological, between those from southern Brazil and Uruguay and that of the Itatiaia population prevents a conclusive determination of the taxonomic status of *M. dimidiata itatiayae*. The allocation of the small pooled sample from São Paulo State, to the Itatiaia “large” sample may constitute additional evidence that the morphological and molecular differences revealed between the Itatiaia sample and those from southern Brazil and Uruguay are due to the large hiatus of representation in our samples relative to São Paulo and northern Paraná states.

**The identity of Azara’s “Micouré a queue courte”:** In a recent article, Voss *et al.* (2009) called the attention for the nominal form *Didelphis breviceaudis* Olfers, 1818, based on Azara’s (1801) “Micouré a queue courte”. The name has been entirely forgotten in the literature until rediscovered by Hershkovitz (1959) and more recently used as a valid name by Wetzel and Lovett (1974) and Brown (2004). The original material used by Azara (1801) to describe the “Micouré a queue courte” and subsequently “el colicorto” (Azara 1802), is not known to be in existence, but both descriptions refer to a *Monodelphis* species with a grizzled grayish brown dorsum and reddish flanks (Voss *et al.* 2009). The specimen for which Azara’s (1801) description was based had been obtained by Don Pedro Blas Nosedá, a priest serving at the Jesuit mission settlement of San Ignacio Güazú, in the Department of Misiones, Paraguay.

In the present study, only one specimen from Paraguay (Alto Paraná, Puerto Yaguarazapá, MZUSP2823), a red-flanked male individual, was available for examination. DNA samples from Paraguayan specimens were not available, but the referred specimen was morphometrically allocated to the large sample from Boa Vista da Aparecida, Paraná, Brazil, in 100% of 1000 bootstrap iterations (Table 1). Similarly to the large sample

from Misiones, Argentina, it presented intermediate values for the craniodental measurements of samples from the extremes of the geographical range, an indication that it belongs to the same widespread biological species identified here as *M. dimidiata*.

Due to its infrequent use in the literature, *Didelphis brevicaudis* Olfers, 1818 has been regarded as a *nomem oblitum* (e.g., Gardner 2005). Despite the fact that one of the conditions for a prevailing usage of a younger name (ICZN 1999; articles 23.9.1.1) is clearly not fulfilled in this case, we consider that the use of the older synonym would threaten stability and cause taxonomic confusion. Thus we consider that the matter should be referred to the Commission for a ruling under plenary powers to maintain use of the younger synonym, in this case *Monodelphis dimidiata* Wagner, 1847. Meanwhile, following article 23.9.3 (ICZN 1999), we maintain usage of the junior name.

**Concluding remarks:** Our analysis points to the recognition of *M. dimidiata* as a widespread taxon ranging from the latitudes of southern Buenos Aires Province and Córdoba in Argentina to Minas Gerais State in southeastern Brazil. It ranges through both the Pampas and the Atlantic Forest domains, where it generally appears to be most associated with open formations such as in the interfaces with grasslands and *campos de altitude* (montane grasslands).

An interesting feature of the reproductive biology of *Monodelphis dimidiata* in the region of Balcarce, Buenos Aires Province, Argentina was revealed in a long-term study by Dalby, and reported by Pine *et al.* (1985). Dalby noticed that adults did not survive the winter in that region, and concluded that *M. dimidiata* was “one of the few warm-blooded vertebrates in which both sexes are thought to be annuals”. Since then, other studies have documented a similar pattern of complete annual turnover of generations in at least another species of South American marsupial, namely *Marmosops incanus* (Lund 1840), a form restricted to eastern Brazil (Lorini *et al.* 1994). Similarly to *Monodelphis dimidiata* population in Balcarce, *Marmosops incanus* has considerable secondary sexual dimorphism in size, with males growing much larger than females in the later age classes, and no adult males being recorded in any population sample taken between January and May, when young adult males are first recorded (Lorini *et al.* 1994). Like the population of *Monodelphis dimidiata* from Balcarce, Argentina, a strong age structure was detected for *Marmosops incanus*, suggesting that records from short periods of the year will document specimens belonging to a restricted age interval, and consequently of a similar size. This fact presents difficulties for studies of geographic variation based on museum series, inasmuch as some age classes may not be represented in samples obtained during certain restricted intervals.

On the other hand, owing to the differences in size found in specimens from similar age classes (e.g., adult males compared to adult females); or different age classes (e.g., young males compared to adult males), some specimens from the same series have been identified as belonging to different species. As noted above, this was the case with the sample of *M. dimidiata* from Taquara, Rio Grande do Sul State, studied by Hensel. Similarly, Bertoni (1914) reported animals identified as both *M. sorex* and *M. henseli* from Puerto Bertoni, Paraguay.

The fact that *M. dimidiata* occupies a wider range than previously realized, with populations living in areas of subtropical and tropical climate, where winters are less severe than in the latitude of Balcarce, suggests that further examination of its reproductive biology is needed, in the attempt to document the generality of semelparity as the species reproductive strategy, and to further gain insight on its ecological and historical determinants.

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## Appendix 1.

Sequences used in molecular analyses, referred by the GenBank accession number followed by museum or collector acronym and number (when available) of voucher, provenance, number of base pairs (bp) and bibliographical citation. Numbers in bold refer to localities of *M. dimidiata* mapped in Fig. 2. Equivalent administrative units in the different countries (states, provinces, and departments) are underlined>. Voucher specimens are referred to by numbers and museum or collector acronyms, as follows: MACN-BR—Museo Argentino de Ciencias Naturales “Bernardino Rivadavia”, Buenos Aires, Argentina; MN—Museu Nacional—UFRJ, Rio de Janeiro, Brazil; MNHNA—Museo Nacional de Historia Natural y Antropológica, Montevideo, Uruguay; UFSC—Universidade Federal de Santa Catarina (mammal collection), Santa Catarina, Brazil; Museu de História Natural do Capão da Imbuia, Curitiba, Paraná, Brasil; LMT—Liliani Marília Tiepolo, and JFV—Júlio Fernando Vilela, to be deposited in MN; DS—Denis Sana, and ZE—José Luiz Passos Cordeiro, to be deposited in UFRGS; EMG—Enrique González. PNI-CIES—Parque Nacional Iguazu—Centro de Investigaciones Ecológicas Subtropicales, to be deposited in MACN-BR. Geographic coordinates (from USGBN 1992, Paynter 1989, Paynter 1995, Paynter & Traylor 1991) follow the toponym to which they refer. Carvalho et al. (unpublished) refer to sequences deposited in the GenBank by B.A.Carvalho, L.F.B. Oliveira, & M.S. Mattevi under the title “*Monodelphis* Phylogeny”.

- Outgroups: *Didelphis albiventris* (U34667, USNM (unnumbered), Venezuela: Aragua, 896 bp, Patton *et al.* 1996), *Marmosa lepida* (U34668, MVZ155245, Peru: Amazonas, 1149 bp, Patton *et al.* 1996), *Micoureus demerarae* (U34674, MNFS187, Brasil: Amazonas, 1149 bp, Patton *et al.* 1996);
- Monodelphis adusta*: (DQ386618, KU157978, Peru: Loreto, 630 bp, Solari 2007), (DQ386623, TTU84865, Ecuador: Pastaza, 630 bp, Solari, 2007); *Monodelphis breviceaudata*: (AJ606455, ISEM-V937, French Guiana: Macouria, 800 bp, Steiner & Catzeflis 2004), (AJ606462, ROM-F43457, Guiana: Potaro-Siparuni, 799 bp, Steiner & Catzeflis 2004);
- Monodelphis americana*: JFV228 (Brazil: Rio de Janeiro, Itatiaia, 1036 bp, this study);
- Monodelphis domestica*: (AJ508398, 1149 bp, Nilson *et al.* 2004), *Monodelphis emiliae*: (DQ386617, MUSM13298, Peru: Loreto, 750 bp, Solari 2007), (DQ385833, MPEG28113, Brazil: Acre, 805 bp, Solari 2007); *Monodelphis*

*handleyi*: (DQ386629, MUSM15991, Peru: Loreto, 690 bp, Solari 2007), (DQ386632, MVC362, Peru: Loreto, 1149 bp, Solari 2007); *Monodelphis kunsii*: (EF154228, MN36265, Brazil: Goiás, Niquelândia, 732 bp, Carvalho *et al.* unpublished), (EF154225, MN37313, Brazil: Goiás, Colinas do Sul, 737 bp, Carvalho *et al.* unpublished); *Monodelphis osgoodi*: (DQ386624, CBF7640, Bolivia: La Paz, 651 bp, Solari 2007), (DQ386625, EY1915, Bolivia: Cochabamba, 660 bp, Solari 2007); *Monodelphis peruviana*: (DQ385839, MUSM13416, Peru: Cuzco, 743 bp, Solari 2007), (DQ385840, USNM582782, Peru: Cuzco, 639 bp, Solari 2007).

*Monodelphis dimidiata*: **Brazil**: Rio de Janeiro: Itatiaia [22°23'S 44°40'W] (**5**)—MN69684 (1149 bp, this study); Santa Catarina: Concórdia [27°14'S;52°01'W] (**17**)—UFSC3428 (1149 bp, this study); Urubici [28°01'S;49°35'W] (**10**)—LMT339 (1149 bp, this study); Rio Grande do Sul: Cambará do Sul, [29°03'S;50°09'W] (**8**)—LMT 290 (1149 bp, this study), LMT 317 (1149 bp, this study); Charqueadas [29°57'S;51°37'W] (**18**)—MN37852 (EF154223, 730 bp, Carvalho *et al.* unpublished), MN37853 (EF154220, 744 bp, Carvalho *et al.* unpublished), MN37854 (EF154219, 789 bp, Carvalho *et al.* unpublished), MN37856 (EF154218, 733 bp, Carvalho *et al.* unpublished); Riozinho [29°38'S;50°27'W] (**19**)—DS019 (EF154222, 729 bp, Carvalho *et al.* unpublished); Terra de Areia [29°35'S;50°04'W] (**20**)—ZE97 (EF154221, 1122 bp, Carvalho *et al.* unpublished); **Uruguay**: Montevideo: Montevideo Parque Lecocq [34°53'S 56°11'W] (**2**)—MNHNA4909 (EMG1330) (1149 bp, this study).

## Appendix 2.

Specimens used in morphometric analyses. Equivalent administrative units in the different countries (states, provinces, and departments) are underlined. Samples (either from isolated or pooled localities) are identified by numbers in bold, also used in reference to Figs. 2, 4 and 5. Museum and collectors acronyms are explained in Appendix 1.

**Argentina**: Buenos Aires (**1**): Miramar [34°36'S 58°27'W] (♀♀) MACN-BR17281,17282, Pergamino [33°53'S 60°35'W] (♂) MACN-BR18998, Tornquist Sierra de la Ventana [38°09'S 61°48'W] (♀♀) MNHNA3731–3733, (♂) MNHNA14961, No specific locality (♀) MACN-BR15719, (♂) MACN-BR14961; Córdoba (**6**): Calamuchita [32°15'S 64°35'W] (♀♀) MACN-BR14752-53,14755, (♂) MACN-BR14751; Misiones (**3**): General Manuel Belgrano Dept. [26°10'S 53°55'W] (♀) MACN-BR18882, Iguazú [25°36'S 54°36'W] (♂) PNI-CIES79, No specific locality (♀) MACN-BR22476, (♂♂) MACN-BR52.52, 52.58, 51.177, 22474, 52.70, 22475; Salta (**7**): Salta [24°47'S 65°25'W] (♀) MACN-BR49.212. **Brazil**: Paraná: Bituruna (**12**) [26°09'S 51°33'W], (♂) MHNCI4410; Boa Vista da Aparecida (**4**) [25°26'S 53°24'W] (♀♀) MHNCI4553, 4556, 4652, 4732, 4736, (♂♂) MHNCI4352, 4552, 4555, 4557, 4837, 4840, 5617, 5844–5847, 4734–4735, Três Barras do Paraná [25°25'S 53°11'W] (♀) MHNCI5511, (♂♂) MHNCI4558, 4563, 5510; Curitiba (**13**) [25°25'S 49°16'W] (♀) MHNCI4417, (♂) MHNCI4846, Colombo [25°17'S 49°13'W] (♀) MHNCI4831, Quatro Barras [25°21'S 49°04'W] (♀) MHNCI4737, (♂) MHNCI4825; Fênix (**14**) [23°55'S 51°59'W] (♂♂) MHNCI4742, 5483, 5492; Rio de Janeiro: Itatiaia (**5**) [22°23'S 44°40'W] (♀♀) MN42024, 69694, (♂♂) MN42021, 69678, 69684, 69747, MN71746–71747, Resende [22°28'S 44°27'W] (♂) MN29814, 71865; Rio Grande do Sul (**8**): Cambará do Sul [29°03'S 50°09'W] (♀♀) LMT263, 290, 306, (♂) LMT328, São Lourenço do Sul [31°22'S 51°59'W] (♂♂) MZUSP1435, 1527; No specific locality (♀) MN1312; Santa Catarina: Urubici (**10**) [28°01'S 49°35'W] (♀♀) LMT345, 348, 357–358, 365, 373; Rancho Queimado [27°40'S 49°01'W] (♀) UFSC3696; Xavantina (**9**) [27°04'S 52°21'W] (♀) UFSC3778, (♂) UFSC3777; São Paulo (**15**): Itapevi [23°33'S 46°56'W] (♂) MHNCI4900; Salesópolis [23°32'S 45°51'W] (♂) MN24549; Guarulhos [23°28'S 46°32'W] (♂) MZUSP31865; Pinhal [22°12'S 46°45'W] (not sexed) MZUSP7434; São Bernardo do Campo [23°42'S 46°34'W] (♂♂) MZUSP30698, 30726; São Paulo [23°33'S 46°38'W] (♂) MZUSP7671; Serra Negra [22°37'S 46°42'W] (not sexed) MZUSP6611 (**16**) type specimens of *M. tricolor paulensis*: Pirituba [23°29'S 46°43'W] (♂) MZUSP2557 (holotype), Piquete [22°37'S 45°11'W] (♀) MZUSP25 (paratype). **Paraguay** (**11**): Alto Paraná Puerto Yaguarazapá [not located, plotted as Alto Paraná] (♂) MZUSP2823. **Uruguay** (**2**): Canelones: Bañado Tropas Viejas [34°47'S 55°52'W] (♂) MNHNA1042; Progreso [34°40'S 56°13'W] (♂) MNHNA3419; Tapia [34°34'S 55°42'W] (♂) MNHNA2916; Florida: Paso del Sordo [33°50'S 55°55'W] (♂♂) MNHNA3730, 3744. Montevideo: Montevideo - Parque Lecocq [34°53'S 56°11'W] (♀♀) MNHNA4108–4110, (♂♂) MNHNA4107, 4111, 4113, 2024, 2289, 3705; No specific locality (♂) MACN-BR33.174, (♀) MNHNA4116.