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Original investigation

Discovery of the rare Handley's short-tailed opossum, *Monodelphis handleyi*, in the threatened southern Amazonian savanna of Brazil

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ABSTRACT

We report a new locality for *Monodelphis handleyi*, a rare short-tailed opossum species, previously known only from its type locality, in Loreto, northeastern Peru. One adult male was collected using pitfall trap disposed in Humaitá Amazonian savanna of southern Amazonas state, Brazil. Voucher specimen had their identification confirmed by molecular data (mitochondrial gene Cytochrome *b*) and morphological comparison. We provide external and cranial measurements of this specimen and comment on its morphology. The specimen reported here represents the first record of *M. handleyi* in Brazil and is the second known locality for the species, and there is high level of divergence found between the two distant localities (5.1%). This record extends the species range at least 1200 km eastern beyond the type locality and denotes the importance of use of complementary methods for sampling small nonvolant mammals.

Introduction

The genus *Monodelphis* Burnett, 1830 is the more speciose of the order Didelphimorphia, comprising at least 25 species that range from Panama to central Argentina, throughout a variety of environments, from high altitudes in the Andes to Amazonian lowlands, including open savannas and semi-arid scrublands (Pine and Handley, 2008; Pavan et al., 2014; Pavan, 2019). Multispecies clades recovered for the genus *Monodelphis* has laid the basis for its subdivision in five subgenera (Pavan and Voss, 2016), namely *Monodelphis* Burnett, 1830, *Microdelphys* Burmeister, 1856, *Monodelphiops* Matschie, 1916, *Mygalodelphys* Pavan and Voss, 2016, and *Pyrodelphys* Pavan and Voss, 2016. The Handley's short tailed opossum, *Monodelphis handleyi* Solari, 2007, belongs to the subgenus *Mygalodelphys*, together with eight other species (*M. adusta*, *M. kunsi*, *M. osgoodi*, *M. peruviana*, *M. pinocchio*, *M. reigi*, *M. ronaldi*, and *M. saci*). It was described based on eight specimens (Solari, 2007) and it is considered an endemic Peruvian species known only from the type locality, at "Centro de Investigaciones Jenaro Herrera, 2.8 km E of Jenaro Herrera, on the east bank of Río Ucayali, Requena Province, Departament of Loreto" (Solari, 2007, 2016).

Morphologically, this species differs from other *Mygalodelphys* species by a set of characters, like a paler coloration and larger body size and craniodental measurements, except by *M. ronaldi* that is the largest species of the subgenus and very similar to *M. handleyi* (Solari, 2007; Pavan and Voss, 2016). *Monodelphis handleyi* and *M. ronaldi* are morphologically distinct mainly by ventral fur, that in *M. handleyi* has self-whitish ventral markings (Pavan and Voss, 2016), and by skull, that in *M. ronaldi* is flatter, with well-developed sagittal crest, conspicuous temporal lines, and shorter premaxillae with very close 1st and 2nd upper incisors (Solari, 2004, 2007). Few information is available on the natural history of *M. handleyi*, excepting that it was sampled at ground of swamp and well-drained forests with 35-30 m canopy height (Solari, 2007, 2016). It is listed as Data Deficient by the IUCN due to its recent description and be known by few specimens from a single locality (Solari, 2016).

Here, we present the first record of *M. handleyi* beyond its type locality, extending the distribution of the species to eastern, into Amazonian savanna of southern Amazonas state, Brazil. We

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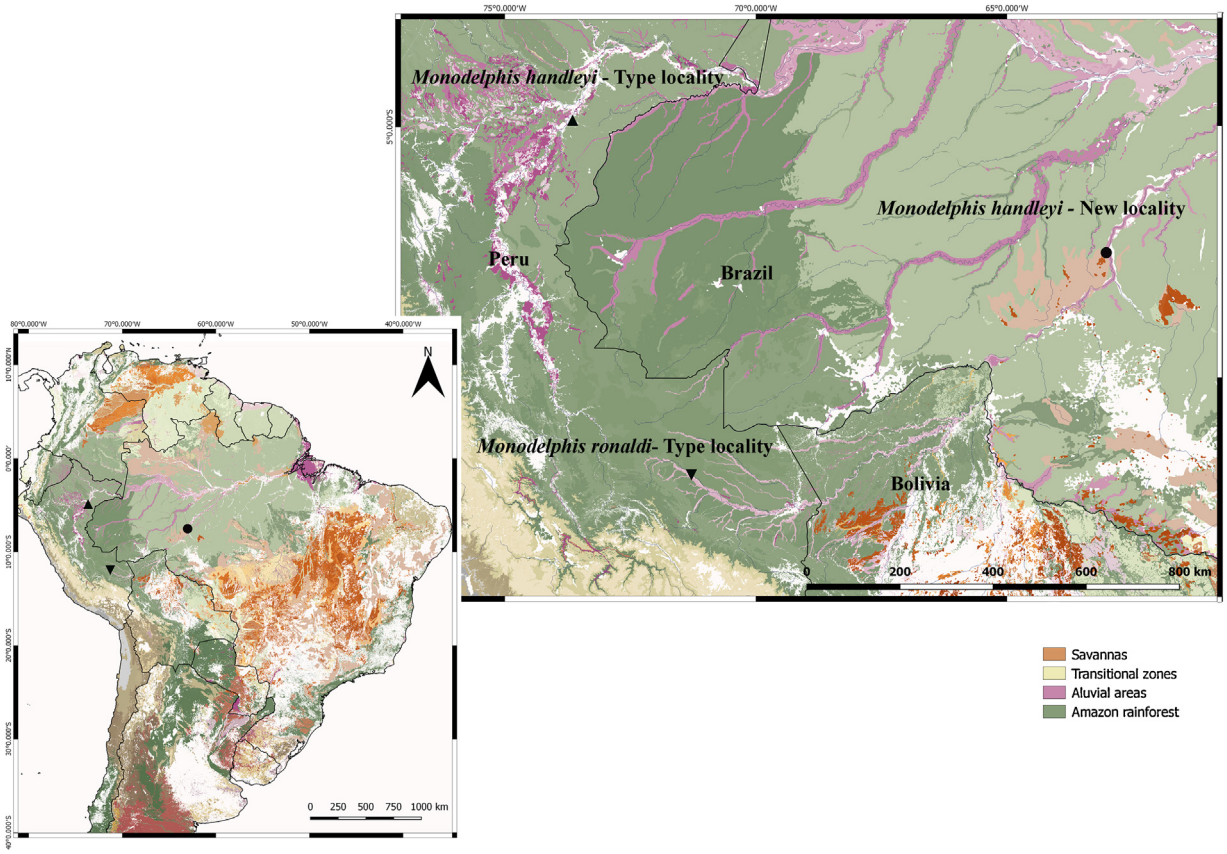

Fig. 1. Map showing the collection localities of *Monodelphis handleyi* and the type locality of the close morphologically related species *M. ronaldi*. Black circle is the present new record of *M. handleyi* from Humaitá, Amazonas state, Brazil; black up-pointing triangle is the previous only known locality, at Requena Province, Loreto, Peru (type locality of *M. handleyi* – Solari, 2007), and black down-pointing triangle the type locality of *M. ronaldi*, at Manu Reserved Zone, Madre de Dios, Peru (Solari, 2004).

also provide measurements and comments about its morphology, molecular data, and conservation status.

Material and methods

Data survey

One specimen of *Monodelphis* (field number ARB 317, of Alexandra M. R. Bezerra) was collected in a pitfall trap, during an inventory carried out in the dry season, between July 17 and August 03, 2003, at the 54° 'Batalhão de Infantaria de Selva' (a military area of Brazil, located at 7° 31′ S, 63° 02′ W, ca. 60 m altitude) (Fig. 1). This locality is at the Amazonian savanna of Humaitá (Gottsberger and Morawetz, 1986), southern Amazonas state, Brazil. In this municipality, the landscape comprises islands of savanna vegetation embedded within a typical Amazon forest matrix (Pires, 1973).

Sampling within the study area was carried out using 20 large size (7.62 × 8.98 x 22.86 cm) Sherman® traps (placed in linear transects) and 100 pitfall traps (= plastic buckets of 20 l arranged in sites, each containing four buckets connected by 6 m × 0.5 m of galvanized plate fences and arranged in a Y-shaped setting with 120° angles, being one central and three peripheral) set in latosol open grassland savanna habitat in transition to open rainforest and latosol tree savanna and adjacent tropical rain forest with some degree of selective lodging (IBGE, 2004), respectively.

Specimen identification and distribution

The identification of the *Monodelphis* specimen was based on molecular data (mitochondrial Cytochrome *b* gene) and morphological characters following Pavan and Voss (2016), Pine and Handley (2008), and, later, Solari (2004, 2007). Color nomenclature follows Smithe (1974) and skull morphology nomenclature follows Voss and Jansa (2009). External measurements of the new specimen were taken in flesh and are as follow: total length (TL), head-body length (HBL), tail length (T), ear length (E), hindfoot without claws (HF) and with claws (HFC) are given in millimeters (mm), and weight in grams (g). Twenty one craniodental measurements based on Carmignotto and Monfort (2006), greatest distance between upper third molars (M3M3, based on Pine, 1981), and mandibular ramus length (MRL), were taken with a digital calliper (Mitutoyo ®) to 0.01 mm precision and are described as follows: BAB, breadth across bullae; BB, breadth of braincase; BBB, breadth between bullae; BBr, least breadth of pterygoid bridge; CB, upper canine breadth; CBL, condylobasal length; CD, cranial depth; CL, upper canine length; GSL, greatest skull length, measured from anterior most nasal end to occipital condyles; LIB, least interorbital breadth; LM, length of the upper molar series (M1–M4); LM1, length of the lower molar series (m1–m4); LPB, least postorbital breadth; MAD, length of the mandible; MRL, mandible ramus length, measured from top coronoid process to below angular process; MTR, maxillary toothrow (C-M4); M3M3, greatest distance between the most lateral point cross the upper third molars; NAS, nasal length; NB, nasal breadth, at frontal-maxillary sutures; PB, palatal breadth; P3L, upper third premolar length; ROS, breadth of rostrum; and ZB, zygomatic breadth.

Sequence of the mitochondrial Cytochrome *b* (1149 bp, CYTB) gene was obtained from the specimen from the new locality (Fig. 1). Genomic DNA was extracted from ethanol-preserved liver tissue using the proteinase-K/phenol-chloroform protocol (Sambrook et al., 1989). The CYTB was amplified using primers L14724 [5′-CGAAGCTTGATATGAAAAACCATCGTTG-3′ (Irwin et al.,

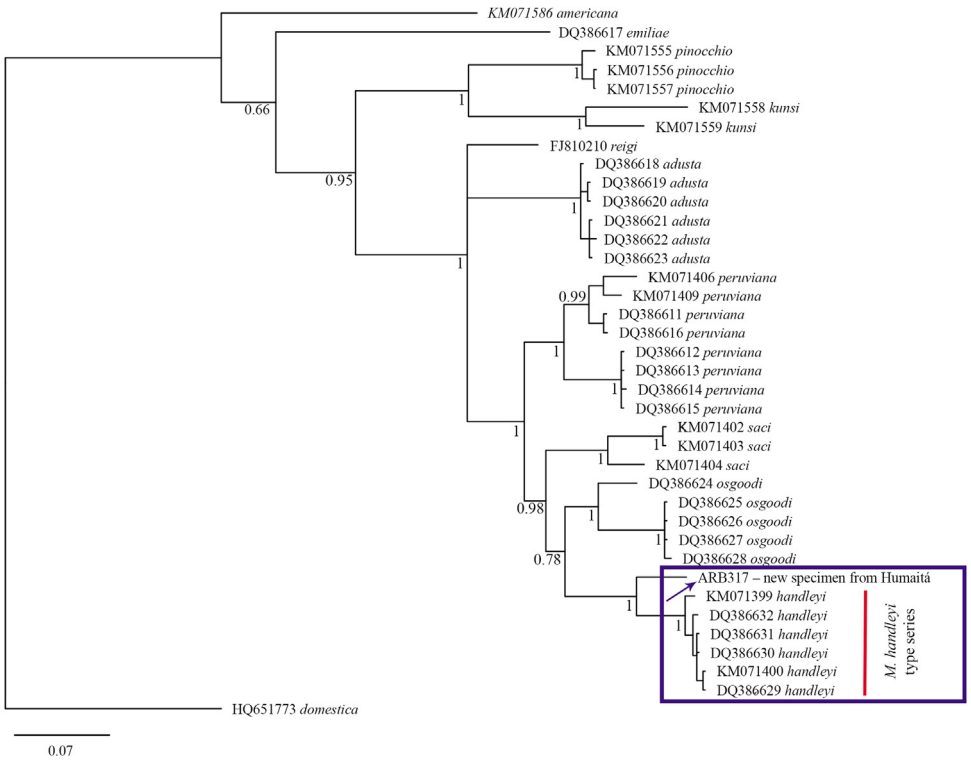

Fig. 2. Genetic relationships of *Monodelphis handleyi* samples based on Bayesian Inference (BI) of Cytochrome *b* (CYTB) sequence data (420–1149 bp) considering GTR + G +I model. Node support indices are shown next to the branches. Purple squared show the clustered sequence of the new specimen (arrow) with sequences from the type specimens of *M. handleyi* (red line), including the holotype (GenBank sequence number DQ386629) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

1991)] and CIT-REV [5′-GAATATCAGCTTTGG-3′ (Casado et al., 2010). Amplicons were purified using GFX™ PCR DNA and Gel Band Purification Kit (GE Healthcare, Brazil), and sequenced using the same amplification primers besides internal primers MVZ16 [5′-AAATAGGAARTATCATTCTGGTTTRAT-3′ (Smith and Patton, 1993)] and CB-in2 [5′-TGAGGACAAATATCATTYTGAG-3′ (Cassens et al., 2000)]. Electropherograms were manually checked using Chromas 1.45 and Chromas Pro 1.41 (Technelysium Pty Ltd, Tewantia, Queensland, Australia).

Molecular identification of the specimen was performed with the BLAST algorithm (https://blast.ncbi.nlm.nih.gov/Blast.cgi) using, as query, the obtained sequence and searching for highly similar sequences (Mega BLAST) on the entire nucleotide collection database. Moreover, the new CYTB DNA sequences were aligned with CYTB sequences of *Mygalodelphys* species (identification of the subgenus based on morphological characters of the new specimen - Pavan and Voss, 2016) available in GenBank (Appendix A), namely *M. adusta*, *M. handleyi*, *M. kunsi*, *M. osgoodi*, *M. peruviana*, *M. pinocchio*, *M. reigi*, and *M. saci*. As outgroup were used *M. americana* (subgenus *Microdelphys*), *M. domestica* (subgenus *Monodelphis*), and *M. emiliae* (subgenus *Pyrodelphys*) (Appendix A). Alignment of the sequences (ranging from 420 to 1149 bp) was carried out with MEGA 6.0 (Tamura et al., 2013) using the Clustal algorithm (Thompson et al., 1994). Alignment was also verified at amino acid level to check the occurrence of spurious stop codons.

Cytochrome *b* haplotypes genealogies were estimated using Bayesian inference (BI) phylogenetic tree. The BI tree was built with the software MrBayes v3.2.1 (Ronquist and Huelsenbeck, 2003), under the assumption of a GTR model of sequence evolution considering gamma rate of substitutions plus invariant sites (GTR + G +I). This model was chosen using the software jModeltest 2.1 (Darriba et al., 2012) and using the Bayesian information criterion (BIC). Two independent Markov Chain Monte Carlo (MCMC) analyses were run with four chains and 1 million generations sampling the chains every 1000 generations. A burn-in of 10% of generated trees was applied. A Neighbor-joining (NJ) tree as based on genetic distance (*p*-distance in this case) was performed with MEGA 6.0. Node support was obtained by means of bootstrap re-sampling (1000 replicates).

Pairwise genetic distance (*p*-distance) among clades and lineages were calculated with Mega 6.0 (Tamura et al., 2013). Gene abbreviation follows ẗHUGO Gene Nomenclature Committee at the European Bioinformatics Institute(HGNC, 2018).

The voucher specimen was deposited in the Mammal Collection of the Museu Paraense Emílio Goeldi (MPEG), in Belém, Pará state, Brazil (voucher number MPEG 45648). Maps were generated in QGIS version 2.18.9 'Las Palmas' (QGIS, 2017), and map shapes from IBGE (1992) for Brazilian ecological tension areas and from USGS (2017) for South American ecosystems.

Other institutional abbreviations used along the text: AMNH (American Museum of Natural History, New York), CBF (Colección Boliviana de Fauna, La Paz), FMNH (Field Museum of Natural History, Chicago), KU (University of Kansas Museum of Natural History, Lawrence), MN (Museu Nacional/Universidade Federal do Rio de Janeiro, Rio de Janeiro), MSB (Museum of Southwestern Biology, Albuquerque), MUSM (Museo de Historia Natural, Universidad Nacional Mayor de San Marcos, Lima), MVZ (Museum of Vertebrate Zoology, Berkeley), MZUSP (Museu de Zoologia da Universidade de São Paulo, São Paulo), ROM (Royal Ontario Museum, Ontario), UNB (Universidade de Brasília, Brasília, Distrito Federal), USNM (National Museum of Natural History, Washington D.C.), TK (Tissue catalogue number of TTU – Museum of Texas Tech University, Lubbock). Other field numbers used along the text: APC (Ana Paula Carmignotto), CRB (Cibele R. Bonvicino), LHE (Louise H. Emmons), MTR (Miguel T. Rodrigues), and PHA (Paulo H. Asfora).

Fig. 3. Skin of *Monodelphis handleyi* (ARB 317/MPEG 45648) from Humaitá, Amazonas state Brazil in dorsal, ventral and left lateral views.

Fig. 4. Skull of *Monodelphis handleyi* (ARB 317/MPEG 45648) from Humaitá, Amazonas state Brazil in dorsal, ventral and right lateral views, respectively.

Results

Molecular identification

The BLAST algorithm retrieved, unequivocally, the CYTB sequences of *M. handleyi* as the most similar sequences (95% identity). The second most similar sequences belonged to *M. osgoodi* and *M. peruviana* (92% identity). The BI and NJ trees confirmed these results (Fig. 2; Appendix B): the CYTB sequence of new specimen cluster (bootstrap value = 99%; and posterior probability = 1) with the six *M. handleyi* sequences belonging to Peru. However, while these Peruvian sequences are very close one to each other (1.2% maximum divergence) the haplotype from Brazil is distinctly different and it diverges from the others by 5.1% (*p*-distance).

Updated distribution

Humaitá municipality [Fig. 1], southern Amazonas state (AM), Brazil, at the military area 54° 'Batalhão de Infantaria de Selva': one adult specimen of *M. handleyi* was captured in a pitfall trap in the forest at July 24, 2003. The sampling effort comprised 1920 trap-nights.

The following non-volant mammal species (here Didelphimorphia and Rodentia) were also captured during this inventory: *Didelphis marsupialis* (UNB 2038), *Marmosa murina* (UNB 2045, UNB 2059), *Marmosops* cf. *noctivagus* (UNB 2043), *Marmosops* cf. *pinheiroi* (UNB 2039 – also a possible range extension, needs species confirmation), *Monodelphis glirina* (Bezerra et al., 2018), *Cerradomys maracajuensis* (ARB 322), *Necromys lenguarum* (UNB 2037 – also a range extension, in preparation), *Cavia* sp. (UNB 2042, UNB 2044 - in study by collaborators for phylogenetic inferences based on DNA sequence data), and a roadkilled *Cuniculus paca* (UNB 2060).

Morphological description and comparison

The specimen (Fig. 3) has short fur (3.5 mm dorsal side, 2 mm ventral side), with unpattern brownish dorsal pelage (Brussels Brown), with rump a bit darker (Warm Sepia), hair pale-gray base (ca. 2 mm) and buffy-brown in tips; ventral pelage self-colored cream (Light Drab) with a longitudinal self-whitish median markings (Pale Horn Color) and orange (Warm Buffy) gular gland mark. There is a small spot of white hairs in dorsal side, at the left rump (4 × 2 mm). The skull (Fig. 4) is slightly convex, with low sagittal crest and subtle scars of *temporalis* muscle on each side, posterior edge of infraorbital foramen in oblique angle to the horizontal plane, diastema (0.6 mm) between 1st and 2nd upper incisors. *Monodelphis ronaldi* self-colored ventral fur without median markings, flat skull in lateral view, well-developed sagittal crest, infraorbital foramen in right angle, and very close 1st and 2nd upper incisors (Solari, 2004, 2007). The skull of the specimen from Humaitá has pointing posterior border of the incisive foramen, that is lyriform and posteriorly extends to middle of upper canines, instead the rounded posterior border of the incisive foramen and that extents back only as far as the anterior based of canines in the holotype of *M. handleyi* (Solari, 2007).

External and craniodental measurements are in Table 1. Few morphometric data are available for *M. handleyi* in the literature (holotype and mean for five paratypes – Solari, 2007) and for *M. ronaldi* (holotype – Solari, 2004). Comparisons with these data and our specimen from Humaitá, showed that our specimen has almost the same body size as the holotype of *M. handleyi* and has larger tail and remarkably larger hindfeet than *M. ronaldi* and all known *M. handleyi* specimens (see Solari, 2007); however, *Monodelphis ronaldi* has larger body size and largest skull length, mandible ramus height, and zygomatic breadth. There is no remarkable data regards to craniodental measurements of our specimen compared to *M. handleyi* (see Solari, 2007, Table 1, page 324).

Table 1
External and craniodental measurements of the new record of *Monodelphis handleyi*, an adult male from Humaitá, Amazonas state (AM), Brazil, and the holotypes of *M. handleyi* (from Solari, 2007) and *M. ronaldi* (from Solari, 2004, except LIB from Solari, 2007). For acronyms see Material and Methods.

Characters	*Monodelphis handleyi* Humaitá, AM ARB 317/MPEG 45648	*Monodelphis handleyi* Holotype	*Monodelphis ronaldi* Holotype
Sex	♂	♂	♂
W	32.5	–	–
TL	198.00	–	–
HBL	124.50[1]	124.00	141.5
T	73.50	68.00	72.50
HF	27.00	16.00	20.00
HFC	28.50	–	–
E	14.00	–	–
BAB	10.09	–	–
BB	11.42	–	–
BBB	5.18	–	–
BBr	2.14	–	–
CB	1.23	–	–
CBL	31.60	31.90	35.32
CD	8.75	–	–
CL	3.34	–	–
GSL	32.10	–	–
LIB	5.66	4.80	5.30
LM	7.68	7.40	8.08
LM1	7.92	–	–
LPB	5.54	–	5.31
MAD	24.19	–	–
MRL	8.88	–	–
MTR	12.71	13.10	14.50
M3M3	10.64	11.20	12.79
NAS	14.57	–	–
NB	4.42	–	–
PB	10.96	–	–
P3L	2.10	–	–
ROS	5.02	–	–
ZB	17.04	17.80	20.70

HBL for this specimen = Total length (TL) less Tail length (T).

Discussion

Distribution and the use of pitfall traps

The specimen reported here represents the first record of *M. handleyi* in Brazil and is the second known locality for the species, extending its eastern distribution limit by ca. 1.200 km. Both records of *M. handleyi* are from primary (this study and Solari, 2007) and secondary forests (Solari, 2007) of Amazon domain, although the Humaitá municipality (locality for the new record) is within an Amazonian savanna, characterized by poor soils and unique structural and phytogeographical complexity (Gottsberger and Morawetz, 1986). The only previously known record of this species, the original description (Solari, 2007), was mainly based on specimens captured with pitfall traps (seven of eight specimens, being one individual caught with snap trap).

The record here shown is one among several new records of rare or shy small nonvolant mammalian species recently obtained with the use of pitfall traps (e.g., Bezerra et al., 2014; Maestri et al., 2015; Delciellos et al., 2016; Percequillo et al., 2017). In fact, more than two decades ago, Voss and Emmons (1996) stated on the importance of the use complimentary methods (live traps, such as Sherman and Tomahawk + pitfall traps) to survey small nonvolant mammals in Neotropical Rainforest, methods that have shown be efficient also in open environments, such as the savannas of Cerrado domain (e.g., Carmignotto and Aires, 2011; Bonvicino et al., 2014) and the semi-arid of Caatinga (e.g., Bezerra et al., 2014). Therefore, is mandatory that any serious study uses such complimentary sampling methods for inventorying and monitoring small nonvolant mammal communities in the Neotropical region.

Diversity and conservation

Monodelphis is the most speciose genus of the order Didelphimorphia (Pine and Handley, 2008; Solari, 2010; Pavan et al., 2014). Recent studies based on molecular data have found highly divergent lineages (Caramaschi et al., 2011; Pavan et al., 2014; Vilela et al., 2015), which have been base for new species description while other were revalidated (Solari et al., 2012; Voss et al., 2012; Pavan et al., 2014). *Monodelphis handleyi* is morphologically very similar to the only specimen known for *M. ronaldi* from southeastern Peru (Solari, 2004), and so far there is no molecular sequence data for the latter species. Both species are included in a species group (subgenus *Mygalodelphys*) that yet needs a comprehensive taxonomic revision (Pavan and Voss, 2016). The small number of specimens known for both taxa (*M. handleyi* – nine specimens including the presently record, and *M. ronaldi* – only the holotype), and the morphological similarity between them (Solari, 2007; Pavan and Voss, 2016; this study), denotes that these species deserve attention in further investigations for new specimens and a possible sample extraction from holotype of *M. ronaldi* for DNA molecular analysis.

The moderately high level of divergence found between the two distant localities (5.1%) of *M. handleyi* is worth of note. Similar situation of high intraspecific divergence is reported for other species of the genus *Monodelphis* (Pavan et al., 2014; Bezerra et al., 2018), suggesting the needing of detailed analyses to determine whether any represent cryptic taxa, as also observed by Pavan (2019) for the complex *M. glirina* group. *Monodelphis handleyi* is another species which may bring up additional surprises in term of taxonomy and species limits if new populations will be found in the future.

Neotropical diversity, including the marsupials, in South America is still far from being completely described. Recently published new records, phylogenetic analyses and taxonomic revisions have changed the distribution patterns of several Neotropical small nonvolant mammals (*e.g.*, Díaz-Nieto et al., 2016; Miranda et al., 2018; Rocha et al., 2018; Voss et al., 2018). In this context, the conservation status of these taxa needs revalidation, since numerous taxa changed to smaller or fragmented distribution ranges. Besides, should be considering that many those studies are mainly based on specimens housed in scientific collections by almost a century (*e.g.*, Miranda et al., 2018). Despite of the extension of range of *M. handleyi* after this study, its conservation status remain critical. Solari (2016) claimed attention for the selective logging and exploitation as possible threats to the species, and the southern of the Brazilian Amazon is one of more threatened region due to the continuous deforestation (Colli et al., 2003; Mantovanelli et al., 2015). Further threatened species lists revaluation should consider the conservation status of *M. handleyi*.

Contribution of authors

ARB collected field data, delineated the study, wrote the paper, and prepared the figures; FPC analyzed part of molecular data; CRB provided laboratory facilities and wrote the paper; RC analyzed the molecular data and wrote the paper.

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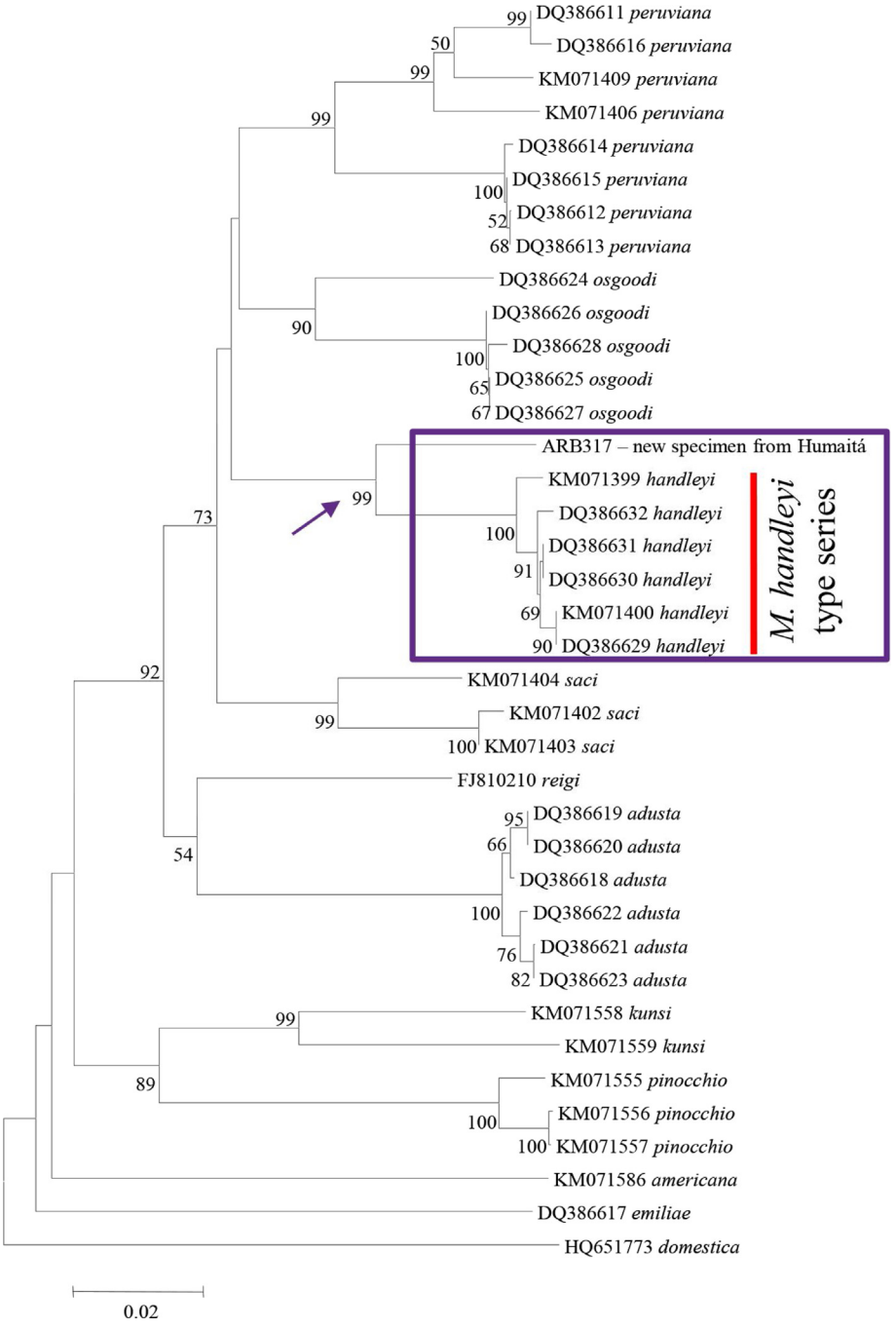
Appendix A

Information of the Cytochrome *b* gene sequences of *Monodelphis* specimens used for analyses with species identification, voucher number (field and/or collection numbers), locality, GenBank, nucleotide accession number; number of base pair sequenced (bp), and source. See Materials and Methods for voucher acronyms.

Species	Voucher	Locality	GenBank	bp	Source
M. adusta	TK125211/KU157978	Loreto, Peru	DQ386618	630	Solari, 2007
M. adusta	TK104093/TTU84865	Pastaza, Ecuador	DQ386619	618	Solari, 2007
M. adusta	TK104127/TTU84899	Pastaza, Ecuador	DQ386620	618	Solari, 2007
M. adusta	TK73228/TTU98686	Loreto, Peru	DQ386621	702	Solari, 2007
M. adusta	TK73496/TTU101019	Loreto, Peru	DQ386622	616	Solari, 2007
M. adusta	TK73633/TTU98864	Loreto, Peru	DQ386623	630	Solari, 2007
M. handleyi	ARB317/MPEG45648	Humaitá, Amazonas, Brazil	MK867353	1149	this study
M. handleyi	MUSM23810/TK82891	Loreto, Peru	DQ386630	801	Solari, 2007
M. handleyi	AMNH276704/TK82889	Loreto, Peru	DQ386631	1149	Solari, 2007
M. handleyi	MUSM15991 (holotype)	Loreto, Peru	DQ386629	690	Solari, 2007
M. handleyi	MUSM23809/TK82890	Loreto, Peru	DQ386632	1149	Solari, 2007
M. handleyi	AMNH2766998	Loreto, Peru	KM071400	763	Pavan et al., 2014
M. handleyi	AMNH276709	Loreto, Peru	KM071399	773	Pavan et al., 2014
M. kunsi	AMNH263968	Tarija, Bolivia	KM071558	1149	Pavan et al., 2014
M. kunsi	APC860/MZUSP35059	Tocantins, Brazil	KM071559	1149	Pavan et al., 2014
M. osgoodi	CBF7640/TK125204	La Paz, Bolivia	DQ386624	651	Solari, 2007
M. osgoodi	TK125207	Cochabamba, Bolivia	DQ386626	651	Solari, 2007
M. osgoodi	AMNH264922	Cochabamba, Bolivia	DQ386628	660/	Solari, 2007
M. osgoodi	TK125205	Cochabamba, Bolivia	DQ386625	660	Solari, 2007
M. osgoodi	TK125209	Cochabamba, Bolivia	DQ386627	660	Solari, 2007
M. peruviana	FMNH172032	Cusco, Peru	DQ386611	660	Solari, 2007
M. peruviana	MSB68336	La Paz, Bolivia	DQ386612	600	Solari, 2007
M. peruviana	AMNH264562	La Paz, Bolivia	DQ386613	622	Solari, 2007
M. peruviana	TK125206	Cochabamba, Bolivia	DQ386614	624	Solari, 2007
M. peruviana	TK125210	Cochabamba, Bolivia	DQ386615	630	Solari, 2007
M. peruviana	FMNH169812	Cusco, Peru	DQ386616	420	Solari, 2007
M. peruviana	AMNH272695	Loreto, Peru	KM071406	795	Pavan et al., 2014
M. peruviana	LHE1395/USNM582110	Junín, Peru	KM071409	795	Pavan et al., 2014
M. pinocchio	MZUSP30740	São Paulo, Brazil	KM071555	1149	Pavan et al., 2014
M. pinocchio	MTR10770/MZUSP s/n°	Minas Gerais, Brazil	KM071556	1149	Pavan et al., 2014
M. pinocchio	MTR11578/MZUSP s/n°	Espírito Santo, Brazil	KM071557	1149	Pavan et al., 2014
M. reigi	ROM114699	Mount Ayanganna, Guyana	FJ810210	1149	Lim et al., 2010
M. saci	MPEG40575	Pará, Brazil	KM071402	795	Pavan et al., 2014
M. saci	MPEG42956	Pará, Brazil	KM071403	517	Pavan et al., 2014
M. saci	MPEG38947	Pará, Brazil	KM071404	517	Pavan et al., 2014
M. americana	PHA467/UFPE	Pernambuco, Brazil	KM071586	1149	Pavan et al., 2014
M. emiliae	TK125201/MUSM13298	Loreto, Peru	DQ386617	750	Solari, 2007
M. domestica	CRB2372/MN67084	Goiás, Brazil	HQ651773	1149	Caramaschi et al., 2011

Appendix B

Genetic relationships of *Monodelphis handleyi* samples based on Neighbor Joining analysis of Cytochrome *b* (CYTB) sequence data (420–1149 bp) using *p*-distance model. Nodal support indices are shown next to the branches. Purple squared show the clustered sequence of the new specimen (arrow) with sequences from the type specimens of *M. handleyi* (red line), including the holotype (GenBank sequence number DQ386629).



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