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PENSOFT

New records of the rare little rufous mouse opossum, *Marmosa lepida* (Thomas,1888) (Mammalia, Didelphidae) in southeastern Amazonia, Brazil

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Abstract
Here we present new records of the mouse opossum *Marmosa* (*Stegomamosa*) *lepida* (Thomas, 1888) collected in the recently flooded region of the Xingu River during the construction of the Belo Monte hydropower dam in the state of Pará, Brazil. This taxon is rarely captured, and it has often been misidentified as other similar genera of arboreal marsupials, such as *Gracilinanus*. Our specimens were identified morphologically and genetically using partial DNA of the mitochondrial cytochrome b gene. The new records increase the known distribution by about 250 km to the southeast.

Key words
Didelphimorphia; range extension; *Marmosa* (*Stegomarmosa*); marsupial; Xingu River; Belo Monte dam.

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Introduction

The genus *Marmosa* comprises 19 recognized species allocated to 5 subgenera (Voss et al. 2014), widely distributed in the Neotropical region. *Marmosa* (*Stegomarmosa*) *lepida*, also known as the little rufous mouse opossum, is rarely captured by conventional sampling methods and its habits are therefore poorly known (Brito and Pozo-Zamora 2015).

Species of this genus are usually nocturnal, arboreal, solitary, and feed mainly on fruits and insects (Creighton and Gardner 2008, Voss 2013). *Marmosa lepida* has a broad distribution in the Amazon region, although only a few specimens deposited in museums are known (Fig. 1). It occurs mainly in western Amazonia, in cis-Andean areas from sea level to 1,580 m above sea level (a.s.l.), in Colombia, Venezuela, Ecuador, Peru, Brazil, Suriname, Guyana and French Guiana (Creighton and Gardner 2008, Voss 2013, Voss et al. 2014, Astúa 2015, Brito and Pozo-Zamora 2015).

We present here new records of *M. lepida*, increasing its known distribution area to the state of Pará, Brazil, in eastern Amazonia, to the south of the Amazon river.

Methods

Three dead specimens were collected in 2015 at three locations (Fig. 1) on the left bank of the Xingu River in the municipality of Vitória do Xingu, state of Pará, Brazil, by a wildlife rescue company during the flooding of

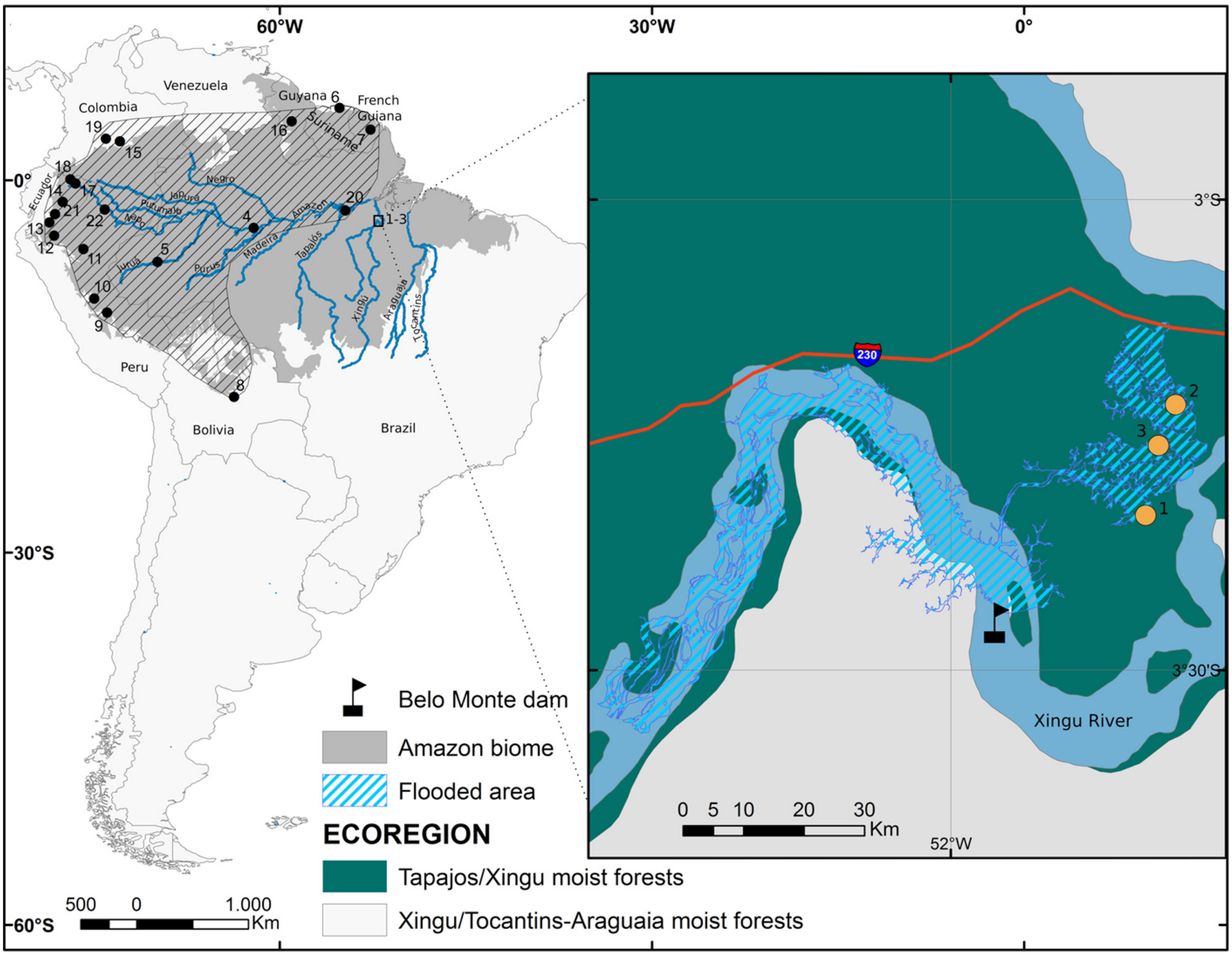

Figure 1. Map showing known records occurrence for *Marmosa lepida*. Hatched area represents occurrence range from IUCN red list. Gray area corresponds to Amazonia biome. Black circles correspond to locations of specimens found at on line databases (GBIF and GenBank) and/or literature (numbers correspond to localities listed in Table 1). Orange circles in the amplified map correspond to collecting localities of the three new specimens of *Marmosa lepida*. Red solid line is the interstate highway (BR-230).

the Belo Monte hydropower dam reservoir. This region is known as the great curve of the Xingu River and encompasses the Xingu-Tapajós moist forest ecoregion (Fig. 1), formed by open ombrophilous forests of low-altitude close to sea level (Salomão et al. 2007).

The 3 specimens are deposited and catalogued at the Mammal Collection of the Universidade Federal do Espírito Santo (UFES-MAM). Age criteria were determined by tooth eruption and wear following Voss and Jansa (2009).

The mitochondrial cytochrome b (Cyt b) gene was sequenced for confirmation of taxonomic identification. This marker was chosen because of its efficiency in diagnosing mammals at the species level (Bradley and Baker 2001, Agrizzi et al. 2012). Polymerase chain reactions (PCR) were carried out using 2.5 µl of 10× buffer, 1.0 µl of $MgCl_2$ at 50 mM, 0.5 µl of deoxynucleotide triphosphate mix (10 mM for each nucleotide), 0.3 µl of each primer at 10 mM, 3 units of Taq Platinum (Invitrogen Corporation, Carlsbad, California), and 1.0 µL template DNA with the final volume of 25 µL. We used primers MVZ05 and MVZ16 (Smith and Patton 1993) to amplify the first 801 base pairs (bp) of the Cytochrome b gene (Cyt b). PCRs were performed with an initial denaturation temperature of 94 °C for 5 min followed by 39 cycles (30 s denaturation at 94 °C, 45 s annealing at 48 °C and extension for 45 s at 72 °C) and a final extension at 72 °C for 10 min. Samples were sequenced in both directions using the same PCR primers listed before. Sequences were aligned in Geneious R9.1 (Biomatters, Auckland, New Zealand) using ClustalW algoritm and deposited in GenBank (Benson et al. 2004) (under accession numbers MG586948, MG586949 and MG586949). We used pairwise p-distance calculated in Geneious to compare sequence divergence to other sequences of *M. lepida* deposited in GenBank (accession numbers HM106376, U34668, AJ606452 and HM106377) and previously identified by experts in the field such as James L. Patton and Robert Voss, as mentioned on the GBIF website (see Table 1 for GBIF specimens).

The nomenclature of cranial structures measured followed Voss and Jansa (2009). We took 29 cranial measurements with a digital caliper following Rossi et al. (2010) (Table 2).

Table 1. Records of *Marmosa lepida* sorted according to° Numbered localities mapped in Figure 1, and corresponding reference and/or voucher° Number and Genbank accession° Number when available. Global Biodiversity Information Facility = GBIF; Field Museum of° Natural History = FMNH; Museum of Vertebrate Zoology = MVZ; Royal Ontario Museum = ROM; Muséum d'histoire° Naturelle de la Ville de Genève = MHNG; Kansas University Museum = KU; Colección de Mamíferos del Instituto de Ciencias° Naturales = ICN-MHN-Ma; University of Michigan Museum of Zoology = UMMZ; Museo de la Escuela Politécnica° Nacional = MEPN.

Locality no.	Specific locality	Country	Latitude	Longitude	Reference/Voucher/ Accession° Number
1	Left bank of Xingu River	Brazil	03.335° S	051.795° W	This study/ UFES-MAM 2921 (MG586948)
2	Left bank of Xingu River	Brazil	03.217° S	051.763° W	This study/ UFES-MAM 2927 (MG586950)
3	Left bank of Xingu River	Brazil	03.261° S	051.781° W	This study/ UFES-MAM 2948 (MG586949)
4	Codajás	Brazil	03.833° S	062.083° W	Creighton and Gardner (2008)
5	Igarapé Grande	Brazil	06.583° S	069.833° W	Creighton and Gardner (2008)/ GBIF: FMNH 140824
6	Paramaribo	Suriname	05.833° N	055.166° W	Creighton and Gardner (2008)
7	Les Nouragues	French Guiana	04.083° N	052.666° W	Creighton and Gardner (2008)
8	Buenavista	Bolivia	17.450° S	063.666° W	Creighton and Gardner (2008)
9	Lagarto	Peru	10.660° S	073.900° W	Creighton and Gardner (2008)
10	Puerto Marquez	Peru	09.533° S	074.933° W	Creighton and Gardner (2008)
11	Santa Cruz	Peru	05.550° S	075.800° W	Creighton and Gardner (2008)
12	Amazonas	Peru	04.466° S	078.166° W	Creighton and Gardner (2008); Voss et al. (2014): JLP 7844/ GBIF: MVZ 154750, MVZ 154752, MVZ 155245 (HM106378/U34668) and MVZ 181583
13	Galacquiza	Ecuador	03.400° S	078.550° W	Creighton and Gardner (2008)
14	Pastaza	Ecuador	01.733° S	077.483° W	Creighton and Gardner (2008)
15	Meta	Colombia	03.133° N	072.866° W	Creighton and Gardner (2008)
16	Potaro-Siparuni	Guyana	04.750° N	059.020° W	Jansa and Voss (2000); Steiner and Catzeflis (2004); Borisenko et al. (2008); Voss and Jansa (2009); Voss et al. (2014); Díaz-Nieto et al. (2015)/ GBIF: ROM 107034, MHNG-MAM-1705.096 (EU095427/ HM106376/KU171146/ FJ159293/FJ159343/AF257681/AJ606453/ AJ606452)
17	San Pablo de Kantesiya	Ecuador	00.26° S	076.44° W	GBIF: MHNG-MAM-1705.096, MHNG-MAM-170.004
18	Santa Cecilia	Ecuador	00.08° N	076.86° W	GBIF: KU 135118, KU 135102
19	Trocha de Guapayita	Colombia	03.35° N	073.99° W	GBIF: ICN-MHN-Ma 1092
20	Near Santarém	Brazil	02.43° S	054.07° W	GBIF: UMMZ 62449
21	Morona Santiago	Ecuador	02.720° S	078.088° W	Brito and Pozo-Zamora (2015)/ MEPN 12313
22	Loreto	Peru	02.366° S	074.083° W	Steiner et al. (2005); Voss et al. (2014)/ AMNH 273186 (HM106377/ KM819020/KJ129918/KJ129973/ KJ129870/AJ606452/AJ628376/AJ628395/ AF257681)

The current distribution area of the species used in the present study follows Cáceres et al. (2016).

Results

New records. Brazil, North Region, state of Pará: left bank of Xingú River (03°20′06″ S, 051°47′42″ W), an adult female collected on 23 January 2015, UFES-MAM 2921 (field number BM 54809); left bank of Xingú River (03°13′01″ S, 051°45′47″ W), an young female collected on 24 February 2015, UFES-MAM 2927 (field number BM 58834); left bank of Xingú River (03°15′40″ S, 051°46′52″ W), a subadult male collected on 3 June 2015, UFES-MAM 2948 (field number BM 80318).

Identification. We examined the 3 specimens and compared external and cranial morphology with descriptions available in the literature (Creighton and Gardner 2008, Voss et al. 2014, Astúa 2015) to confirm the genetic identification of all 3 individuals (pairwise identity of 98.4% and query cover of 98.33% with reference sequence under Genbank accession number HM106376 for all 3 sequences of *M. lepida* from this study). Significant features of the genus, which distinguish it from other similar genera (e.g. *Gracilinanu*s and *Thylamy*s), are the presence of a strong supra-orbital process and the absence of an anteromedial process of the alisphenoid portion of the auditory bulla and generally spiral tail scales pattern (Figs 2, 4) (Creighton and Gardner 2008). *Marmosa lepida* is distinguished from other congeners by its smaller size (head and body length 97–120 mm and tail length 140–150 mm) (Table 2), brighter reddish dorsal pelage and brighter chestnut fur (Fig. 3) (Voss et al. 2014). Voss et al. (2001) warned about the problems of morphological identification of young individuals, as they could be easily misidentified as *Gracilinanus emilae*. Therefore, our 2 younger specimens (UFES-MAM 2921 and 2948) were analyzed morphologically, with only a few measurements (Table 1). The karyotype is unknown and no sexual dimorphism has been recorded for skull size and shape (Astúa 2015).

Table 2. Selected measurements (mm) and weight (g) of 3 specimens of *Marmosa lepida* from the Belo Monte flooded area, Vitória do Xingu, Pará, Brazil.

	UFES-MAM 2921	UFES-MAM 2927	UFES-MAM 2948
Sex	Female	Male	Male
Age category	6	3	4
Greatest length of skull (GLS)	27.97	23.69	27.78
Condylobasal length (CBL)	27.28	23.43	26.86
Rostral length (RL)	10.39	9.32	10.37
Nasal length (NL)	10.32	8.38	10.75
Palatal length (PL)	15.97	13.37	15.60
Length of maxillary tooth row (MTR)	11.10	*	11.02
Length of upper molar series (UMS)	5.81	*	5.80
Length of M4 (LM4)	1.37	*	0.87
Width of M2 (WM2)	1.47	*	1.40
Width of M4 (WM4)	2.35	*	1.87
Heigth of upper canine (HC)	2.15	1.98	1.77
Palatal breadth (PB)	8.91	*	8.63
Postpalatal breadth (PPB)	10.63	10.19	10.68
Breadth of basicranium (BB)	4.12	1.99	3.33
Breadth across tympanic bullae (BTB)	8.50	8.84	8.09
Length of tympanic bulla (LTB)	4.90	4.57	4.59
Tympanic bulla opening (TBO)	1.42	1.62	1.54
Width of ectotympanic (WET)	2.40	2.18	2.26
Nasal breadth (NB)	2.82	2.66	3.03
Breadth of rostrum across canines (BRC)	4.22	2.44	4.06
Breadth of rostrum between jugals (BRJ)	9.76	7.96	7.07
Least interorbital breadth (LIB)	4.47	3.92	4.55
Postorbital constriction (POC)	6.66	4.46	6.30
Breadth of braincase (BBC)	10.80	10.23	11.24
Zygomatic breadth (ZB)	15.42	12.45	14.41
Length of mandible (LM)	19.54	15.93	18.15
Length of lower molar series (LMS)	5.67	*	6.35
Length of m4 (Lm4)	1.28	*	1.78
Width of m2 (Wm2)	0.86	*	0.91
Head-body (H-B)	99	93	99
Tail (T)	142	82	136
Hind foot (HF)	16	12	16
Ear (E)	17	14	13
Mass (M)	16	6	12

* Measurements not taken.

Figure 2. Dorsal, ventral and lateral views skulls and mandibles of the 3 specimens of *Marmosa* (*Stegomarmosa*) *lepida* collected at Vitória do Xingu, Pará, Brazil: UFES-MAM 2927 (left), 2921 (middle), 2948 (right).

Figure 3. Ventral (above) and dorsal (below) views of 2 specimens of *Marmosa (Stegomarmosa) lepida* collected at Vitória do Xingu, Pará, Brazil. A) UFES-MAM 2921 and B) UFES-MAM 2948.

Discussion

These new records of *M. lepida* increase the known distribution of the species by about 250 km to the southeast, to the south of Amazon (Fig. 1). This species is currently known from ca 20 localities (Table 1), and they are difficult to capture by conventional methods (Ochoa et al. 2009). Despite a small number of museum specimens of *M. lepida* (Creighton and Gardner 2008), its conservation status in the IUCN red list is Least Concern (Cáceres et al. 2016) because of its wide geographic range (Fig. 1).

This arboreal genus occurs in moist forest areas throughout the neotropical region, being replaced by *Gracilinanus,* another genus of arboreal marsupial, in more open and dry areas (e.g. Cerrado of central Brazil). Although *Gracilinanus* also occurs in moist forests, the 2 genera do not appear to co-occur (Creighton and Gardner 2008, Voss et al. 2014). However, we have found one individual of *Gracilinanus emilae* in the same area (BM 63456 = UFES-MAM 2937, Genbank accession number of Cyt-b sequence MG586951), showing that these 2 genera do occur in sympatry and reinforcing the use of reliable taxonomic methods to diagnose them.

The sampling area of the specimens reported here corresponds to the flooded area to impound water for the operation of the Belo Monte hydropower dam. The total flooded area reaches 516 km^2, spreading across 3 municipalities in the state of Pará, Brazil: Altamira, Vitória do Xingu, and Brasil Novo (Ministério das Minas e energia 2011, Ministério do Meio Ambiente 2017) (Fig. 1). This project was very controversial, with low social acceptance, including the scientific community, which

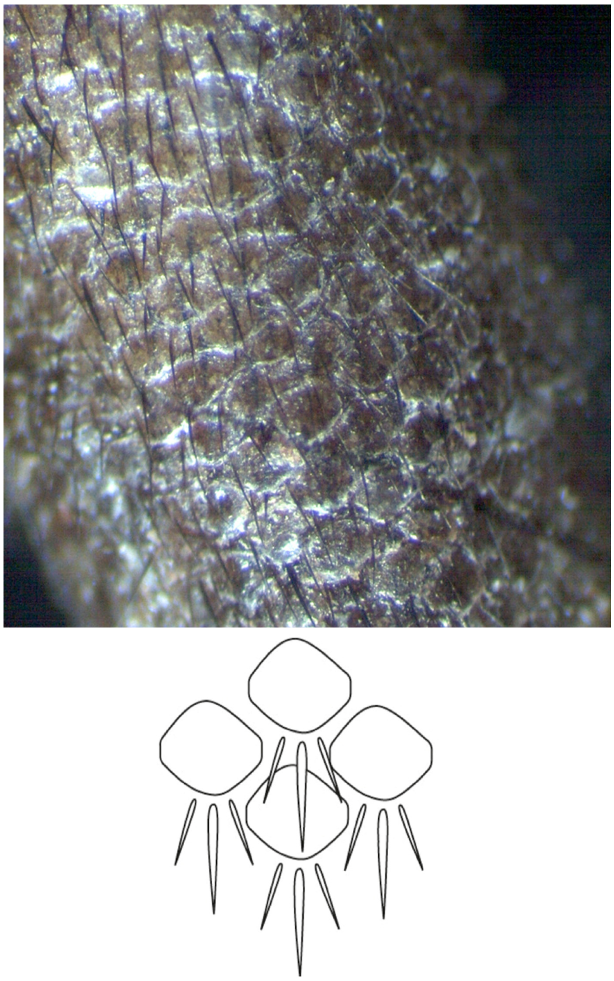

Figure 4. Scheme of the scales on the tail and dorsal view of these scales of *Marmosa* (*Stegomarmosa*) *lepida* (UFES-MAM 2921) collected at Vitória do Xingu, Pará, Brazil.

argued strongly against the construction of the dam in this region (Nazareno and Lovejoy 2011). The environmental impacts generated by flooding the forest cause not only the loss of habitat, but also changes in flood and droughts dynamics (characteristic of the Amazon region), which directly affect the cycle of the fluvial fauna and the exploitation of the river by local communities and other predators (Winemiller et al. 2016).

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Authors' Contributions

RRG collected morphological data, wrote the text, and prepared the figures and maps; ACL collected the genetic data and conducted the laboratory work; and LPC organized the manuscript and identified the specimens. All authors contributed to the text.

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