

**A NEW SPECIES OF JUPATI, GENUS *Metachirus* Burmeister  
1854 (DIDELPHIMORPHIA, DIDELPHIDAE), FOR THE  
BRAZILIAN AMAZONIA**

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Manuscripts

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53 22 Running title: NEW SPECIES OF *Metachirus*  
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## 26 Abstract

27 The brown-eyed opossums or jupatis represent the genus *Metachirus* with a wide  
28 geographical range in the Neotropics. Although recent studies show distinct  
29 monophyletic clades with high genetic divergence and recognised two species,  
30 *Metachirus nudicaudatus* is to date the only formally recognised species of the  
31 genus. This discrepancy shows a need for systematic revision with multiple sources  
32 of evidence, of the taxonomy of *Metachirus*, which has never been fully revised. We  
33 describe a new species of *Metachirus* for the Brazilian Amazon in the  
34 Xingu/Tocantins interfluve using the unification of concepts and evolutionary  
35 significant units, morphological, genetic, and geographic data. Our analysis reveals a  
36 new species within *Metachirus* as a differentiated Amazonian clade from the Serra  
37 dos Carajás region and the Caxiuanã National Forest, both in the Xingu endemism  
38 centre. This new species can be distinguished from the type species, *M. nudicaudatus*  
39 and *Metachirus mysosuros* through discrete external morphological characters,  
40 including cranium and dentition, and molecular data with an average degree of  
41 divergence, but ancient divergence time for the mitochondrial cytochrome b gene.  
42 The Amazon River delimits the distribution of the new species, and this species  
43 occurs in areas under strong anthropogenic pressure, reinforcing the importance to  
44 guide conservation strategies for the region.

45 Keywords: Taxonomy, Didelphid, Xingu/Tocantins interfluve, Serra dos Carajás.

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3 47 Introduction  
4

5 48 The genus *Metachirus* Burmeister, 1854 includes medium-sized terrestrial  
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7  
8 49 marsupials, with head and body length between 150 and 310 mm and tail longer than  
9  
10 50 head and body, ranging from 178 to 390 mm (Gardner and Dagosto 2007; Voss and  
11  
12 51 Jansa 2009). They can be distinguished from other larger marsupials of the subfamily  
13  
14 52 Didelphinae (*sensu* Voss and Jansa 2009) by a combination of external  
15  
16 53 morphological characters, such as a pair of small cream spots above the eyes, general  
17  
18 54 brown colour, short coat, short extension of hair at the base of the tail, light brown to  
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20  
21 55 dark brown tail colour and absence of marsupium in females of this genus (Emmons  
22  
23 56 1997; Eisenberg and Redford 1999). However, a unique feature of *Metachirus* is the  
24  
25 57 contact between the frontal and squamous bones in the lateral region of the braincase  
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27  
28 58 (Voss and Jansa 2003).  
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31 59 The brown-eyed opossums or jupatis, as representatives of the genus  
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33 60 *Metachirus* are commonly known, have a wide geographical range, and occur from  
34  
35 61 Chiapas in Mexico to the north of Argentina (Gardner and Dagosto 2007; Voss and  
36  
37 62 Jansa 2009). According to Paglia et al. (2012), the brown four-eyed opossum can be  
38  
39 63 found in four different biomes in Brazil: Amazonia, the Atlantic Forest, the Cerrado,  
40  
41 64 and Pantanal. In the Cerrado, its presence appears to be restricted to zones where the  
42  
43 65 Cerrado contacts the Amazonian and the Atlantic Forest domains. Furthermore, the  
44  
45 66 brown four-eyed opossum populations have been recorded in semi-deciduous,  
46  
47 67 transitional forests between the Cerrado and Caatinga domains in northeastern Brazil  
48  
49 68 (Miranda et al. 2009). Thus, increasing the previously known distribution area for  
50  
51 69 this taxon (Miranda et al. 2009). This reinforces the idea that sampling artefact is a  
52  
53 70 probable important determinant of its absence in the eastern most areas of the  
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55 71 Amazon, as well as in forest areas of Central Brazil.  
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3 72 The taxonomy of the genus *Metachirus* has been partially assessed by Voss et  
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5 73 al. (2019) but needs a systematic review including multiple data sources. *Metachirus*  
6  
7 74 was described by Burmestier (1854), from the examination of two specimens, a male  
8  
9 75 and a female deposited in the Naturkunde Museum, Berlin, identified as *Didelphis*  
10  
11 76 *myosuroides* Temminck, 1824 (Hershkovitz 1976). Posteriorly, Thomas (1888)  
12  
13 77 nominated *Didelphis nudicaudata* (= *Metachirus nudicaudatus*; E. Geoffroy 1803), as  
14  
15 78 a type species with Cayenne in French Guiana as type-locality (see Hershkovitz  
16  
17 79 1976; Voss and Jansa 2009).

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21  
22 80 Just one widely distributed species was previously recognized for South  
23  
24 81 America, the brown four-eyed opossum, *Metachirus nudicaudatus* (É. Geoffroy  
25  
26 82 1803). Five subspecies have since been recognised as associated with *M.*  
27  
28 83 *nudicaudatus* (Gardner 2005; Gardner and Dagosto 2007): (1) *M. n. nudicaudatus* E.  
29  
30 84 Geoffroy 1803; (2) *M. n. myosuroides* Temminck 1824, including *personatus* Miranda-  
31  
32 85 Ribeiro 1936; (3) *M. n. colombianus* J. A. Allen 1900 including *antioquiae* J. A.  
33  
34 86 Allen 1916, *dentaneus* Goldman 1912, *imbutus* Thomas 1923 and *phaeurus* Thomas  
35  
36 87 1923; (4) *M. n. tchudii* J. A. Allen 1900 including *bolivianus* J. A. Allen 1921 and  
37  
38 88 *infuscus* Thomas 1923; and (5) *M. n. modestus* Thomas 1923. Voss and Jansa (2009)  
39  
40 89 followed this arrangement proposed by Gardner (2005) and Gardner and Dagosto  
41  
42 90 (2007).

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47  
48 91 Recent phylogeographic studies have shown that *M. nudicaudatus*, comprises  
49  
50 92 distinct monophyletic clades with expressive genetic divergence among them (Costa  
51  
52 93 2003; Patton and Costa 2003; Patton et al. 2000). These studies identified six  
53  
54 94 monophyletic clades for *M. nudicaudatus*. The most divergent clade was formed by  
55  
56 95 the French Guiana haplotypes, with more than 13% of genetic divergence in relation  
57  
58 96 to the other clades (Patton and Costa 2003).

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3 97 In the ethno-zoological article by Voss et al. (2019) several taxonomic  
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5 98 decisions for the genus *Metachirus* were made. The delimitation of the type species,  
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7 99 *M. nudicaudatus* was pointed out, with the revalidation and delimitation of *M.*  
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9  
10 100 *myosuroides* pending review, since the synonymities of several names under *M.*  
11  
12 101 *myosuroides* requires verification (see Gardner and Dagosto 2007). In an annotated  
13  
14 102 checklist of didelphid marsupials recently published by Voss (2022), the author  
15  
16 103 considers the same results obtained by Voss et al. (2019) for *Metachirus* and points  
17  
18 104 to the possibility of a third species from Xingu/Tocantins interfluve.

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22 105 Additionally, a single sequence from an individual collected in the Serra dos  
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24 106 Carajás region in 1999, presented in Patton et al. (2000) and later in Patton and Costa  
25  
26 107 (2003), has shown a high degree of genetic divergence in the presented topologies.  
27  
28 108 These sets of indications led us to investigate more carefully the taxonomic *status* of  
29  
30 109 the populations present in the Serra dos Carajás region and surroundings, through the  
31  
32 110 combination of morphological, morphometric, and molecular data.

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37 111 In the present study, new morphological and molecular data were obtained for  
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39 112 *Metachirus* from Serra dos Carajás, in southeastern Pará, Brazil. The addition of new  
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41 113 specimens allowed us to verify, through an integrative taxonomic approach, whether  
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43 114 the populations of that region represent a new taxon.

#### 44 115 Materials and methods

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50 116 We collected *Metachirus* specimens from the Serra de Carajás region, where  
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52 117 the Belo Monte Hydroelectric Power Plant and the Caxiuanã National Forest are  
53  
54 118 located, in Southeastern Pará, Brazil. Specimens were obtained during several  
55  
56 119 expeditions, by employing pit-fall traps with 60-litre buckets, and conventional live  
57  
58 120 trapping (Sherman and Tomahawk). These specimens were deposited in the mammal

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3 121 collections of the **Emílio Goeldi Museum (MPEG)** in Belém and the National  
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5 122 Museum of Rio de Janeiro (Figure 1; Appendix 1).  
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### 7 123 **Molecular data and analyses**

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9  
10 124 This study used partial sequences of the mitochondrial cytochrome b (cytb)  
11  
12 125 gene for *Metachirus* to evaluate the genetic distance among groups, delimit species  
13  
14 126 and evaluate their time of divergence. Partial sequences of cytb (801 bp) were  
15  
16 127 obtained for 18 specimens of *Metachirus* spp. In addition, available sequences for  
17  
18 128 these species were retrieved from GenBank  
19  
20 129 (<http://www.ncbi.nlm.nih.gov/GenBank/>) and incorporated in the study. Outgroup  
21  
22 130 taxa were based on the phylogenetic results of Voss and Jansa (2009): *Didelphis*  
23  
24 131 *marsupialis* Linnaeus, 1758 and *Philander opossum canus* (Osgood 1913). The  
25  
26 132 sequences obtained in this study will be deposited in GenBank (Table 1).  
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### 30 133 **Laboratory procedures**

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33 134 The DNA was extracted from ethanol-preserved tissues using phenol-  
34  
35 135 chloroform, proteinase K-RNase methods (Sambrook, Fritsch and Maniatis, 1989).  
36  
37 136 The sequences were amplified by Polymerase Chain Reaction (PCR) using primers:  
38  
39 137 GluMarsF1 (5'-AACCATAACCTATGGCATGAA-3') and CytbMarsR2 (5'-  
40  
41 138 GTGGAAKGCRAARAATCGDGT-3'). The PCR were performed with a final  
42  
43 139 volume of 13 µL, comprising: 5.2 µL of H<sub>2</sub>O; 1.5 µL of MgCl<sub>2</sub> (2.5 mM); 1.25 µL  
44  
45 140 of dNTP (2.5 mM); 1.25 µL of 10X buffer (100 mM Tris-HCl, 500 mM KCl); 1 µL  
46  
47 141 of forward primer (0.2 µM); 1 µL of reverse primer (0.2 µM); 0.8 µL of Taq DNA  
48  
49 142 Polimerase (1 U/ul) and 1 ul of genomic DNA. PCR conditions used consisted of a  
50  
51 143 pre-heating step at 94° C for 1 min, followed by 35 cycles of 94°C (1 min), 55°C  
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53 144 (1.30 min); and one minute at 72°C, followed by a final extension at 72°C (10 min).  
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56 145 PCR products were cleaned using Exonuclease I and Shrimp Alkaline Phosphatase  
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3 146 (Fermentas). Sequencing reactions were carried out according to the manufacturer's  
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5 147 recommendation for the ABI BigDye Terminator sequencing kit and resolved on an  
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7  
8 148 ABI 3130xl automated sequencer (Life Technologies).  
9

10  
11 149 Sequence alignments and phylogenetic analyses  
12

13 150 We edited and aligned the sequences in the program *Geneious* 6.1.6 (Kearse et  
14  
15 151 al. 2012). We verified the data saturation through the program *DAMBE5* (Xia 2013)  
16  
17 152 and checked for the presence of stop codons in *Geneious* 6.1.6. (Kearse et al. 2012).  
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20 153 The phylogenetic analyses were performed using Maximum Likelihood (ML)  
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22 154 and Bayesian Inference (BI) phylogenetic analysis. We determined the Hasegawa-  
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24 155 Kishino-Yano + proportion of invariable sites + gamma distribution (HKY+I+G)  
25  
26 156 evolution model by the Bayesian Information Criterion (BIC) and the Akaike  
27  
28 157 information criterion (AIC; Sullivan and Joyce 2005) as implemented in *JModeltest*  
29  
30 158 2 (Darriba et al. 2012).  
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33  
34 159 We performed the ML analysis (Felsenstein 1981) in *PhyML* 3.0 (Guindon et  
35  
36 160 al. 2010). Phylogenetic support was assessed via 1000 bootstrap iterations. Only  
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38 161 groupings with bootstrap values  $\geq 70\%$  were considered significant (Hillis and Bull  
39  
40 162 1993).  
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42

43 163 We ran the BI in the program *MrBayes* 3.1.2 (Ronquist and Huelsenbeck  
44  
45 164 2003). The analysis consisted of an independent run of  $10^7$  generations, sampling the  
46  
47 165 values every 1000 generations. We discarded 10% of the generations and 10% of the  
48  
49 166 trees. We verified the convergence of the effective sample size (ESS) values in the  
50  
51 167 program *Tracer* 1.6 (Rambaut et al. 2014). We created a consensus tree for the BI  
52  
53 168 through the program *TreeAnnotator* 1.7.5 (Drummond et al. 2012). Only groups that  
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55 169 presented values of posterior probabilities (pp)  $\geq 0.95$  were considered significant.  
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3 170 We calculated the genetic distance within and between the different  
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5 171 monophyletic clusters of *Metachirus* that emerged in the consensus tree. For this, we  
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7 172 used the Kimura-2-parameter model (Kimura 1980) in the program *MEGA 6.0*  
8  
9 173 (Tamura et al. 2013). This model has been the most used model in previous work.  
10  
11 174 Employing the Kimura-2-parameter model, therefore, allows better comparisons  
12  
13 175 among our results and those of other studies of *Metachirus*.

14  
15  
16 176 The phylogenetic tree was dated using *BEAST 2.6.0* (Bouckaert et al. 2019)  
17  
18 177 and the same parameters as for the BI tree in *MrBayes* (except the substitution model  
19  
20 178 that was the General Time Reversible + proportion of invariable sites + gamma  
21  
22 179 distribution; GTR+I+G). We incorporated information from two fossils to calibrate  
23  
24 180 the nodes. The calibrations used were 3.3 million years ago (mya) for the separation  
25  
26 181 time between *Didelphis* Linnaeus 1758 and *Philander* Brisson 1762 (Marshall 1976),  
27  
28 182 and between 12.1 to 12.6 mya for the separation between *Monodelphis* Burnett 1829  
29  
30 183 and *Marmosa* Gray 1821, following Jansa et al. (2014). Due to these calibrations, we  
31  
32 184 included two additional taxa as outgroup: *Marmosa murina* (Linnaeus 1758)  
33  
34 185 (Genbank USNM549291) and *Monodelphis domestica* (Wagner 1842) (Genbank  
35  
36 186 OT8047). We also incorporated a substitution rate of 0.04, representing the average,  
37  
38 187 simulated substitution rates of Didelphidae suggested by Leite et al. (2016). We  
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40 188 assumed a Birth-Death speciation model, with estimated base frequencies and an  
41  
42 189 uncorrelated lognormal relaxed-clock model distribution, as tree priors.

#### 190 Morphological and morphometric data

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45 191 We analysed additional skins, skulls, and individuals preserved in fluid and  
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47 192 deposited in the mammalian collections of the following institutions: Emílio Goeldi  
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49 193 Museum (**Museu Paraense Emílio Goeldi; MPEG**), National Institute of Amazonian  
50  
51 194 Research (**Instituto Nacional de Pesquisas da Amazônia; INPA**), Museum of Zoology

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3 195 of the University of São Paulo (**Museu de Zoologia da Universidade de São Paulo;**  
4  
5 196 MZUSP), National Museum of Rio de Janeiro (**Museu Nacional;** MN) and Pontifical  
6  
7 197 Catholic University of Minas Gerais (**Pontifícia Universidade Católica de Minas**  
8  
9 198 **Gerais** MCNM; Figure 1; Appendix 1). In addition to the specimens of the new  
10  
11 199 species described here, we only used characters extracted from specimens that we  
12  
13 200 defined as belonging to the type species, *M. nudicaudatus* and *M. myosuuros* (**Atlantic**  
14  
15 201 **Forest**) A more thorough analysis including characters related to other clades is  
16  
17 202 beyond the scope of the present study, and a broader systematic review of the genus  
18  
19 203 is still pending.

204 We examined detailed high-resolution photographs of the holotype of *M.*  
205 *nudicaudatus nudicaudatus* (É. Geoffroy 1803) deposited at the Natural History  
206 Museum in Paris (**Muséum National d'Histoire Naturelle;** MNHN). We obtained  
207 high-resolution photos of three specimens collected by Voss et al. (2001) in Paracou,  
208 French Guiana (topotypic samples of *M. n. nudicaudatus*) **and deposited at the**  
209 **American Museum of Natural History (AMNH): AMNH 266451, 267009 and**  
210 **267010.** Additionally, we examined photographs of the holotypes of the subspecies  
211 recognised by Gardner (2005) and Gardner and Dagosto (2007): *M. n. nudicaudatus*,  
212 *M. n. myosuuros*, *M. n. colombianus*, *M. n. tchudii* and *M. n. modestus*.

213 We used the original descriptions of the nominal taxa for *Metachirus*,  
214 according to Gardner (2005), Gardner and Dagosto (2007), and Voss and Jansa  
215 (2009), as well as the description by Husson (1978) for topotypic samples of *M. n.*  
216 *nudicaudatus* from Suriname, the transcriptions of the original descriptions of the  
217 nominal *Metachirus* taxa by Miranda Ribeiro (1936), **and topotypical samples of *M.***  
218 ***myosuuros* deposited in Brazilian mammal collections (see appendix 1).**

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3 219 Species delimitation is considered fundamental in Biology since species are  
4  
5 220 essential units for analysis in many areas of the biological sciences. However,  
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7  
8 221 delimitating interspecific boundaries is a complex task. There are several concepts  
9  
10 222 and criteria to define and delimit species, some of which are still quite controversial  
11  
12 223 (de Queiroz 2007). In this study, we adopt the General Species Concept proposed by  
13  
14 224 de Queiroz (2007) and an integrative approach (molecular, morphological, and  
15  
16 225 morphometric data).

17  
18  
19 226 Age classes

20  
21 227 We classified individuals into seven age groups according to Tyndale-Byscoe  
22  
23 228 and Mackenzie (1976). We performed morphometrical analyses with only adult  
24  
25 229 specimens and commented on sexual and ontogenetical variations for external  
26  
27 230 characters. Both were based on the age definition of Tyndale-Byscoe and Mackenzie  
28  
29 231 (1976). We followed Voss and Jansa (2009) for the nomenclature of external and  
30  
31 232 craniodental structures.

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33 233 Morphometric data

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36 234 We measured 16 craniodental dimensions to the nearest 0.01 mm with digital  
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38 235 callipers while adult specimens were viewed at low magnification under a  
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40 236 stereomicroscope. Only specimens belonging to the type species of *Metachirus*  
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42 237 *myosuros* (Atlantic Forest) and to the new species herein described were measured  
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44 238 (for further details see Results and Discussion).

45  
46  
47 239 The measurements were as follows: MCC (maximal cranial circumference),  
48  
49 240 NAS (nasal bone length), RWJ (rostrum width between the jugal bones), RWF  
50  
51 241 (rostrum width between the frontal bones), NW (greater nasal width), CI (interorbital  
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53 242 constriction), PC (postorbital constriction), WZ (width between zygomatic arches),  
54  
55 243 GCW (greatest cranial width), CB (condyle-basal length), PL (palate length), MAX

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3 244 (length of the maxillary row of teeth), LUM (length of the upper molar series), CAN  
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5 245 (width between the lateral margins of the upper canines), WPP (width of the palatal  
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7 246 projection), WM (width between the external faces of the third molars), LLM (length  
8  
9 247 of the lower molar series) and JL (jaw length).

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11  
12 248 We performed a Principal Component Analysis (PCA; Manly 1994) to verify  
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14 249 the behaviour of morphometric data in the multivariate space. The tests and statistical  
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16 250 analysis were performed using *PAST 4.02* (Hammer et al. 2013) considering a  
17  
18 251 significance level of 0.05. Male and female individuals allocated to age groups 6 and  
19  
20 252 7 were considered adults and grouped together to carry out the relevant  
21  
22 253 morphometric analyses.

## 24 254 Results

### 25 255 Molecular data

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27  
28 256 The consensus trees obtained through the ML and the Bayesian analyses were  
29  
30 257 completely congruent in relation to the recovery of clades. The genetic divergence  
31  
32 258 between *Metachirus* and external groups was 21.3% (Table 2). We identified seven  
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34 259 mutually monophyletic and strongly supported clades for *Metachirus* (Figure 2).

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36  
37 260 The first split in the consensus tree is highly sustained ( $pp = 1.0/95$ ) and  
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39 261 separates the two basal clades (the Guiana Shield and Xingu/Tocantins clades) from  
40  
41 262 the four remaining ones.

42  
43  
44 263 The Xingu/Tocantins interfluve clade was formed by haplotypes from  
45  
46 264 Southeastern Amazonia. This clade showed a significant support value ( $pp =$   
47  
48 265  $0.99/91$ ). The degree of genetic divergence between the Xingu/Tocantins clade and  
49  
50 266 the other clades ranged between 8.2% and 16.1%. The level of internal divergence  
51  
52 267 was 2% (see Table 2; Figure 2).

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3 268 The Guiana Shield clade is composed of haplotypes from French Guiana and  
4  
5 269 Jari River (on the border between the states of Pará and Amapá, in the Brazilian  
6  
7 270 Amazon), diverging 8.2% in relation to its sister group, the Xingu/Tocantins clade.  
8  
9  
10 271 The Guiana Shield clade differs between 8.2% and 14.9% in relation to all others.  
11  
12 272 This clade showed a significant support value (pp = 0.96/98). The morphological  
13  
14 273 characters observed for the representative specimens of this clade are congruent with  
15  
16 274 the original description of the type-species, *M. nudicaudatus* and topotypic samples  
17  
18 275 from the Guiana shield (see Table 2; Figure 2).

21 276 The Central/Northwestern Amazonian clade, broader, both in geographic and  
22  
23 277 probably in taxonomic terms, was composed of five internal clades. This clade  
24  
25 278 contained haplotypes from central and western Amazonia, north of the Amazon  
26  
27 279 River, including a location in northern Peru, and haplotypes from the Jaú River, a  
28  
29 280 tributary of the Negro River, Amazonas State, Brazil. This clade was quite cohesive,  
30  
31 281 with significant support (pp = 0.99/100), as well as a low degree of internal  
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33 282 divergence (1%). This suggests that it might represent a single taxonomic entity. The  
34  
35 283 genetic divergence between the Central/Northwestern Amazonian clade and each of  
36  
37 284 the others ranged from 8.1% to 16.1%. The external morphology presented by the  
38  
39 285 sequenced vouchers refers to that of *M. n. colombianus sensu* Gardner (2005) and  
40  
41 286 Gardner and Dagosto (2007) and to taxa that the authors considered synonymous  
42  
43 287 with this subspecies (Table 2; Figure 2).

48 288 The Central American clade is formed by only two sequences from Panama,  
49  
50 289 showing high support (1/100), with genetic divergence among clades ranging from  
51  
52 290 8.3% to 16.1%. The intra clade genetic divergence was 0.0%. We did not examine  
53  
54 291 the morphology of these vouchers. Voss et al. (2019) did not provide a description of

1  
2  
3 292 the Central American clade, which they synonymized with *M. myosuuros* (cf.  
4  
5 293 Discussion; Table 2; Figure 2).

6  
7 294 The Southwestern Amazonian clade showed a high support value ( $pp = 1/100$ ),  
8  
9 295 with an intra clade genetic divergence of 1%. The genetic divergence between this  
10  
11 296 clade and each of the others ranged from 5.3 to 14.7%. The specimens of this clade  
12  
13 297 have an external morphology that refers to the subspecies *M. n. tschudii sensu*  
14  
15 298 Gardner (2005) and Gardner and Dagosto (2007) and to the nominal taxa considered  
16  
17 299 synonymous with this subspecies by these authors (Table 2; Figure 2).

18  
19 300 The Eastern Amazonian/Teles Pires River clade from the Belém vicinities, Pará  
20  
21 301 State, and Cristalino National Park on the left bank of the upper Teles Pires River,  
22  
23 302 Mato Grosso State, Brazil was well supported (0.95/87). This clade differed with  
24  
25 303 5.6% to 14.1% in relation to the other clades recovered in the analysis. For  
26  
27 304 taxonomic possibilities for this clade see the Discussion (Table 2; Figure 2).

28  
29 305 Finally, the Atlantic Forest clade was composed of samples from locations  
30  
31 306 within the Atlantic Forest biome in Brazil, whose individuals can be morphologically  
32  
33 307 associated to *M. n. myosuuros* sensu Gardner (2005) and Gardner and Dagosto (2007),  
34  
35 308 including *personatus* Miranda-Ribeiro 1936 as a synonym. The degree of intra-clade  
36  
37 309 divergence was 1%, and between this clade and each of the others, the divergence  
38  
39 310 ranged from 5.3% to 14.4% (Table 2; Figure 2).

40  
41 311 The dated phylogenetic tree showed that the lineages which originated in the  
42  
43 312 Xingu/Tocantins and the Guiana Shield (the *M. nudicaudatus* and Xingu/Tocantins  
44  
45 313 interfluvial clades) diverged from the others 6.34 mya (Figure 3). The  
46  
47 314 Xingu/Tocantins and Guiana Shield clades then diverged from one another 3.3 mya,  
48  
49 315 during the Pliocene. The other clades, comprising Central America,  
50  
51 316 Central/Northwestern Amazonia, Southwestern Amazonia, Eastern Amazonia/Teles  
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3 317 Pires River, and the Atlantic Forest clades (*M. myosuuros*) diverged about 3.5 mya  
4  
5 318 (Figure 3).

6  
7  
8 319 Morphometric data

9  
10 320 Only one adult male skull for each of the two species and two of *M. myosuuros*  
11  
12 321 was available for us to analyse. Therefore, we consider the results cautiously,  
13  
14 322 stressing the need to increase the number of adult male specimens in future  
15  
16 323 morphometric analyses (Table 3). Nevertheless, females already show a clear  
17  
18 324 tendency to differ between the three species.

19  
20  
21 325 The morphometric data had a normal distribution, so they were not log  
22  
23 326 transformed for the PCA. The first component explained 67% of the craniometric  
24  
25 327 variation observed among samples and the second component explained 17%; the  
26  
27 328 remaining 16% were divided among the other principal components (Table 4).

28  
29  
30 329 We observe a strong separation in the morphometric space for females of the  
31  
32 330 three species in relation to principal components 1 and 2, demonstrating that there  
33  
34 331 are differences related to size and shape (Figure 4). The single male of the new  
35  
36 332 species suggests differences mainly in the shape of the skull and other more robust  
37  
38 333 and prominent structures. Regarding the male of *M. nudicaudatus*, we found? an  
39  
40 334 unexpected result based on the literature for didelphid marsupials. A sampling  
41  
42 335 artefact, combined with an age effect, may explain the position of the circle referring  
43  
44 336 to the male of *M. nudicaudatus* (possibly a younger adult). Despite the differentiation  
45  
46 337 of the two male specimens of *M. myosuuros* from the females, which suggest sexual  
47  
48 338 morphometric dimorphism, and from the other species, which suggest taxonomic  
49  
50 339 differentiation, their number (N = 2) is low then conclusions must be cautious. Thus,  
51  
52 340 there is a strong need for a larger sample of male specimens. In the case of the  
53  
54 341 females, the pattern more clearly separates the three species in the multivariate space.  
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3 342 Based on the above integrative analysis, we proceed to formally describe a new  
4  
5 343 species for the genus *Metachirus*. We reinforce the adopted Species Concept (de  
6  
7 344 Queiroz 2007) and provide a simplified synonymy, followed by a discussion.  
8  
9

10 345 Taxonomy

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12 346 *Metachirus aritanai* new species

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15 347 *Metachirus nudicaudatus* Cabrera 1961

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17  
18 348 *Metachirus nudicaudatus* Emmons and Feer 1990

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21 349 *Metachirus nudicaudatus* Eisenberg and Redford 1999

22  
23  
24 350 *Metachirus nudicaudatus* Emmons and Feer 1997

25  
26  
27 351 *Metachirus nudicaudatus* Patton et al. 2000

28  
29  
30 352 *Metachirus nudicaudatus* Costa 2003

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32  
33 353 *Metachirus nudicaudatus* Patton and Costa 2003

34  
35  
36 354 *Metachirus nudicaudatus* Gardner and Dagosto 2007

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39 355 *Metachirus nudicaudatus* Rocha et al. 2011

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46  
47 357 Holotype: an adult male, consists of skin and skull (INPA 2831, original number CS  
48  
49 358 83), in addition to tissue samples preserved in ethanol, collected in 1999 by Maria  
50  
51 359 Nazareth F. da Silva in the Tapirapé-Aquiri National Forest, Marabá, Pará, Brazil  
52  
53 360 (05° 46' 53.10" S; 50° 31' 48.60" W; Figure 1).  
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3 362 Paratypes (N = 19): Eight adult females (N = 8) consisting of skins, skulls and tissues  
4  
5 363 preserved in ethanol; two adult males (N= 2) consisting of skins, skulls and tissues  
6  
7 364 preserved in ethanol; and five sub-adult and young individuals (N= 9) consisting of  
8  
9 365 specimns preserved in fluid, skull and tissues. Adult female specimens: Two of the  
10  
11 366 specimens were collected by Cleuton L. Miranda in the Tapirapé-Aquiri National  
12  
13 367 Forest, municipality of Marabá, Pará, Brazil (PSA 06; PSA 200) and will be  
14  
15 368 catalogued in the mammal collection of MPEG. Three specimens were collected by  
16  
17 369 Natalia C. Ardente, two in the Carajás National Forest (MN 78307; MN 79953),  
18  
19 370 municipality of Marabá, Pará, Brazil, and one in the Tapirapé-Aquiri National Forest,  
20  
21 371 municipality of Marabá, Pará, Brazil (MN 75549). The final three specimens were  
22  
23 372 collected by Fabiana Caramaschi, Cibele R. Bonvicino and Paulo Sérgio D'Andrea  
24  
25 373 in the Vila Santa Cruz, Araguatins municipality, Tocantins State, Brazil (LBCE  
26  
27 374 14843, 14845, 14859) and will be catalogued in the mammal collection of MN.  
28  
29 375 Adult male specimens: The two specimens were collected by Natalia C. Ardente in  
30  
31 376 the Carajás National Forest, Pará State, Brazil (MN 73864; MN 78309). Sub-adult  
32  
33 377 and young specimens: The nine specimens were collected by Marco Antonio R.  
34  
35 378 Junior and Stepheson Abrantes. Five specimens were collected in the Caxiuanã  
36  
37 379 National Forest, municipality of Portel, Pará, Brazil, and are cataloged in the  
38  
39 380 mammal collection of MPEG: MAR 890, 1165, 1292, 1407, 1409. Four specimens  
40  
41 381 were collected in Bom Futuro on the left margin of the middle Xingu River, Pará,  
42  
43 382 Brazil, and are catalogued in MPEG: CN 78, 83, 91, 240.  
44  
45 383  
46  
47 384 Type-locality: Tapirapé-Aquiri National Forest, municipality of Marabá, State of  
48  
49 385 Pará, Brazil (05° 46' 53.10" S; 50° 31' 48.60" W; Figure 1).  
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3 387 Etymology: the name *Metachirus aritanai* is given in honor of the great leader  
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5 388 Aritana Yawalapiti (1949 – 2020), of the Yawalapiti ethnicity, from the upper Xingu  
6  
7 389 River region and in honour of all indigenous peoples in Brazil. The leader Aritana  
8  
9  
10 390 died in August 2020, a victim of covid-19, representing a great loss for the Xingu  
11  
12 391 people, for all indigenous peoples, and for all Brazilians.

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15 392  
16  
17 393 Distribution: *M. aritanai* new species is restricted to the Xingu-Tocantins interfluve,  
18  
19 394 the endemism centre Xingu *sensu* Silva (2005). As far as we know, *M. aritanai* new  
20  
21 395 species, is endemic to this biogeographic region (Figure 1). The species is bounded  
22  
23 396 to the north by the Amazon River, to the west by the Xingu River and to the east by  
24  
25 397 the Tocantins River. The southern boundary cannot yet be clearly defined. According  
26  
27 398 to the consensus topology and times of divergence presented in this study, a sister  
28  
29 399 species of *M. aritanai* new species, *M. nudicaudatus*, occurs north of the Amazon  
30  
31 400 River (Figure 2 and Figure 3).

32  
33 401  
34  
35 402 Diagnosis: *Metachirus aritanai* new species is characterized by the combination of  
36  
37 403 the following characters: general colouration light brown with speckles of gold on  
38  
39 404 the back; small amount of rust hair behind the ears; supra-ocular spots of cream  
40  
41 405 colour; cheeks with the same colouration as supra-ocular spots; brown and gold-  
42  
43 406 speckled face; absence of median stripe on the rostrum; presence of a narrow,  
44  
45 407 blackened dorsal line at the top of the head, usually extending to the nape of the  
46  
47 408 neck; clear belly, usually pure cream, wide interorbital region (average = 12.12 mm)  
48  
49 409 and with incipient ridges; rounded and inflated cranial cavity, with large  
50  
51 410 maxillopalatine (average = 24.68 mm) and fenestrae; small entoconids; strongly  
52  
53 411 convergent temporalis scars. Figure 5 to Figure 7 show the mentioned characters,  
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3 412 except for the morphology of the entoconid, which is similar to that of *M.*

4  
5 413 *nudicaudatus* and may be seen in Figure 15, page 46 in Voss et al. (2019).

6  
7 414 Description

8  
9 415 External morphology

10  
11 416 Combined length of adult head and body ca. 230 mm on average, (ranging 243-

12  
13 417 275 mm); adult weight ca. 380g. Mid-rostral fur cream coloured with apical portion

14  
15 418 dark brown (most of the cream-coloured hairs have black tips, but there are also

16  
17 419 completely blackened or cream hairs in lesser quantities, somewhat like the dorsal

18  
19 420 coat). Dark median rostral stripe absent or inconspicuous. Dark circumocular mask

20  
21 421 present (blackened), extending posteriorly to the base of the ear, continuous with the

22  
23 422 coronal fur. Narrow dark brown dorsal line starting at the coronal spot and extending

24  
25 423 to the end of the nape. Throat gland present in adult males. Small pure cream

26  
27 424 supraocular spots present, usually in the shape of an inverted triangle, varying in

28  
29 425 size. Pure cream-coloured cheeks, of the same colour as the supraocular spots.

30  
31 426 Completely brown ears. Presence of small amounts of rust hair behind the base of the

32  
33 427 ears. General colouration light brown speckled with gold. Dorsal colour pale brown,

34  
35 428 with grey based hairs, cream stripe over most of its length and short blackish apex,

36  
37 429 giving the back a golden speckled appearance. Dorsal hairs usually do not exceed 10

38  
39 430 mm in length. Dorsal guard hairs short, dark, and inconspicuous. Lateral portions of

40  
41 431 the body brown and lighter than the back. Ventral pelage usually pure cream, similar

42  
43 432 in colour to the supra-ocular spots and cheeks. Mammae 4–1–4 = 9, all abdominal

44  
45 433 inguinal. Pouch absent. Tail is light brown to dark brown, dorsal discolouring

46  
47 434 gradually towards the distal portion; ventral surface of the tail not pigmented. Scales

48  
49 435 without a consistent arrangement (both spiral and annular patterns coexisting). Tail

50  
51 436 longer than the length of the head and body, covered with 5 to 10 mm long hairs on

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2  
3 437 all sides at the base. Hands and feet covered with cream coloured hair, like the  
4  
5 438 ventral coat; Manus mesaxonic ( $d_{III} > d_{IV}$ ); Carpal tubercles absent in mature adult  
6  
7  
8 439 males.

9  
10 440 Cranium-dental morphology

11  
12  
13 441 Cranium wide and robust. Relatively elongated face. Nasal branch of the  
14  
15 442 premaxilla developed. Rostral process of the premaxilla absent. Long nasal bone,  
16  
17 443 widely enlarged in the region of the maxillary/frontal suture. Mandible developed,  
18  
19 444 with elongated and deep infraorbital foramen at the height of the Pm2. Interorbital  
20  
21 445 region wide (average = 12.12 mm) with incipient ridges. Lacrimal bone extends in  
22  
23 446 the rostrum to the anterior margin of the orbital cavity, surpassing the two exposed  
24  
25 447 lacrimal foramina. Zygomatic arch well developed, composed by the jugal and  
26  
27 448 squamosal. Postorbital process of the jugal absent. Postorbital process of the frontal  
28  
29 449 absent. Palatine articulates ventrally with mandible and dorsally with frontal. Frontal  
30  
31 450 quite developed extending from the posterior region of the nasal branch of the  
32  
33 451 mandible to the posterior region of the cranium, where it articulates with the parietal.  
34  
35 452 Supraorbital crest imperceptible. Absent or incipient temporal crest. When incipient,  
36  
37 453 the crest most often extends from the frontal to the parietal, where it can be joined to  
38  
39 454 the midline of the cranium and extend to the lambdoidal crest. Sagittal crests absent  
40  
41 455 in the anterior part of the occiput. Parietal articulates anteriorly with the frontal, but  
42  
43 456 they are separated by median sutures on the right and on the left. Parietal articulates  
44  
45 457 posteriorly with the supra-occipital and mastoid. Lamboidal crest not developed.  
46  
47 458 Squamosal in contact with the frontal on the side of the cranium. Parietal and  
48  
49 459 alisphenoid not in contact. Petrosal not exposed laterally. Squamosal parietal fenestra  
50  
51 460 absent. Parietal and mastoid normally in contact. Interparietal not in contact with  
52  
53 461 squamosal. Palate long, wide and composed mostly by the mandible, which  
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3 462 articulates with the palatal branch of the pre-mandible at the height of the well-  
4  
5 463 developed incisor foramen. Mandible articulates anteriorly with the palate.  
6  
7 464 Maxillopalatine large (average = 24.68) and fenestrated. Mandible and alisphenoid  
8  
9 465 not connected at the bottom of the orbital region, separated by the palatine. Foramen  
10  
11 466 of transverse canal absent. Tympanic process of alisphenoid laterally compressed and  
12  
13 467 non-globular, slightly pointed or rounded. Anterior member of ectotympanic  
14  
15 468 suspended directly from the basicranium. Paraoccipital process broad, erect, and  
16  
17 469 ventrally projected. Dorsal margin of foramen magnum in contact with exoccipital.  
18  
19 470 Well-developed mandible. Dental formula  $i5/4, c1/1, pm3/3, m4/4$ . First upper  
20  
21 471 premolar is smaller than the second, well developed and non-vestigial. Second and  
22  
23 472 third upper premolars equivalent in height. Post-metacrist bigger than post-protocrist  
24  
25 473 in molars. The lower third premolar, when deciduous, has three cusps. Entoconids  
26  
27 474 small and temporalis scars (*sensu* Voss *et al.* 2019) strongly convergent.  
28  
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33

#### 34 475 Morphological variation

35  
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37 476 We did not observe differences in the external morphology of males and  
38  
39 477 females within any of the considered age groups (young, sub-adult or adult).  
40  
41 478 However, there were significant age-related morphological differences. Young and  
42  
43 479 sub-adult individuals had a general greyish and darker colour than adult individuals.  
44  
45 480 Yet, sub-adults also exhibit some diagnostic characters of *M. aritanai* new species,  
46  
47 481 such as cream supra-ocular spots and cheeks of the same colour, absence of median  
48  
49 482 stripe on the rostrum and cream-pure belly (Table 5).  
50  
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53

#### 54 483 Comparisons

55  
56 484 *Metachirus aritanai* new species differs from the nominal form, *M.*  
57  
58 485 *nudicaudatus* (*sensu* Voss *et al.* 2019 and present study) and *Metachirus myosuros*  
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3 486 from the Atlantic Forest (*sensu present study*) by their dorsal colouring (light brown  
4  
5 487 speckled with gold in *M. aritanai* vs. brown speckled with black and light orange in  
6  
7 488 *M. nudicaudatus*, vs. brown-orange to light brown in *M. myosuuros*); belly colouring  
8  
9 489 (pure-cream in *M. aritanai* vs. yellow-cream in *M. nudicaudatus*, vs. yellowish hues  
10  
11 490 in *M. myosuuros*); colouring of the sides of the body (similar to the dorsum but  
12  
13 491 slightly lighter in *M. aritanai* vs. orange-brown, visibly lighter than the dorsum with  
14  
15 492 conspicuous blackish and orange hairs in *M. nudicaudatus* vs. yellow with a variety  
16  
17 493 of light greyish and even golden hues of grey and golden in *M. myosuuros*); colouring  
18  
19 494 of the supra-ocular spots (cream in *M. aritanai* vs. cream and slightly orange,  
20  
21 495 especially at the edges in *M. nudicaudatus*, vs. yellow to cream in *M. myosuuros*);  
22  
23 496 lighter hair behind the ears (yellowish and in small amounts in *M. aritanai* vs. rust  
24  
25 497 and in large quantities in *M. nudicaudatus*, vs. yellowish hues in *M. myosuuros*); hair  
26  
27 498 colour on the tarsus (cream in *M. aritanai* vs. brownish-orange in *M. nudicaudatus*,  
28  
29 499 vs. varying hues from blackish to brownish-grey with golden fur in *M. myosuuros*);  
30  
31 500 width and robustness of the skull (wider and more robust in *M. aritanai* vs. narrower  
32  
33 501 and more delicate in both *M. nudicaudatus* and *M. myosuuros*); nasal width in relation  
34  
35 502 to the maxillary (much narrower than the pre-maxillary in the initial portion and  
36  
37 503 similar width in the end portion with a rounder end near frontal bone in *M. aritanai*  
38  
39 504 vs. much narrower than the pre-maxillary in the initial portion and similar width in  
40  
41 505 the end portion with a tapered end near frontal in both *M. nudicaudatus* and *M.*  
42  
43 506 *myosuuros*); contour of the pre-maxillary (wider, short and pointed in *M. aritanai* vs.  
44  
45 507 narrower, long and rounded in *M. nudicaudatus*, vs. narrower, long and pointed in *M.*  
46  
47 508 *myosuuros*); frontal edges (irregular in the posterior portion with the presence of a  
48  
49 509 protuberance in the median region in *M. aritanai* vs. more regular and rounded limit,  
50  
51 510 without the presence of a median protuberance in *M. nudicaudatus*, vs. usually  
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3 511 developed and pointed in *M. myosuroides*); average differences in mitochondrial  
4  
5 512 sequences of the *cytb* gene (between *M. aritanai* and *M. nudicaudatus* = 8.2%;  
6  
7  
8 513 between *M. aritanai* and *M. myosuroides* = 14.4%; Table 5; Figure 5 to Figure 8).  
9

10 514 Discussion

11  
12 515 The possibility of proposing a new species for the genus *Metachirus* with  
13  
14 516 occurrence in the Xingu/Tocantins interfluvium was first raised from the high degree of  
15  
16  
17 517 genetic divergence presented by a single sequence from Serra dos Carajás region in  
18  
19 518 the topologies for *Metachirus* originally presented by Patton et al. (2000), and later  
20  
21 519 by Costa (2003) and Patton and Costa (2003).  
22

23  
24 520 More recently, new samples were collected independently by some authors of  
25  
26 521 this paper in several locations, such as in the region of Serra dos Carajás, on the right  
27  
28 522 bank of the middle Xingu River (the region where the Belo Monte Hydroelectric  
29  
30 523 Plant is currently installed) and in the Caxiuanã National Forest, all in Pará State,  
31  
32  
33 524 Brazil. These specimens showed morphological similarities, which led us to suspect  
34  
35 525 that they could belong to the same taxonomic entity that had not yet been described.  
36

37 526 A detailed examination of the original descriptions of *Metachirus* revealed that  
38  
39 527 the populations of jupatis present in the Xingu/Tocantins interfluvium could not be  
40  
41 528 morphologically associated to any nominal taxa previously associated with this  
42  
43 529 genus. Given the absence of a taxonomic revision for *Metachirus* and the availability  
44  
45 530 of only provisional arrangements proposed in compilation studies (Gardner 2005;  
46  
47 531 Gardner and Dagosto 2007), we did not make in-depth comparisons among *M.*  
48  
49 532 *aritanai* new species, and other subspecies and taxa so far associated with the genus.  
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52  
53 533 Our results reveal that the taxonomic status of taxa in *Metachirus* urgently  
54  
55 534 needs to be accurately assessed including by more morphological data. Although  
56  
57 535 superficial, the examination of external morphological characters of sequenced  
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3 536 specimens belonging to other clades showed significant morphological variation. In  
4  
5 537 some cases, the external morphology resembled those present in original descriptions  
6  
7 538 of Amazonian taxa (Figure 3). Therefore, in the presentation of the molecular results,  
8  
9  
10 539 we only mentioned the similarity with descriptions of available nominal taxa (Table  
11  
12 540 6). We stress that defining the identities of these taxa is outside the scope of the  
13  
14 541 present study and should be the subject of a wide-ranging systematic review. The  
15  
16 542 table is therefore presented to show the expressive morphological variation of  
17  
18 543 Amazonian populations all synonymized by Voss et al. (2019) as *M. myosuuros*.  
19  
20 544 Combined with genetic data, this morphological diversity reinforces the importance  
21  
22 545 of a systematic assessment of the genus as a whole, for better taxonomic definitions.  
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25  
26 546 In this study we evaluate morphological and molecular data for *Metachirus* by  
27  
28 547 following the General Species Concept, which proposes the unification of concepts  
29  
30 548 and evolutionary significant units (De Queiroz 2007). Thus, the comparison of  
31  
32 549 specimens of the new species, *Metachirus aritanai* new species with type species of  
33  
34 550 *M. nudicaudatus* and *M. myosuuros* (from the Atlantic Forest biome), was done using  
35  
36 551 an integrative approach. The analysis of this new data set corroborates the status of  
37  
38 552 *Metachirus aritanai* new species as a full species and a formal description is  
39  
40 553 presented. We corroborated the delimitation for *M. nudicaudatus* proposed by Voss  
41  
42 554 et al. (2019), for which there are no synonyms included and we could safely identify  
43  
44 555 specimens belonging to our samples based on those criteria. Regarding the  
45  
46 556 application of the name *M. myosuuros* by Voss et al. (2019) we partially disagree. Our  
47  
48 557 analysis shows that this name can be applicable to the populations of the Atlantic  
49  
50 558 Forest which stretch from Rio Grande do Norte in northeastern Brazil to Misiones in  
51  
52 559 northern Argentina. In fact, the type locality of this nominal taxon is found in the  
53  
54 560 Ipanema, São Paulo, Brazil (Gardner 2005; Gardner and Dagosto 2007).  
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3 561 Based on a few samples (mainly from the Brazilian Amazon, Chaco, and  
4  
5 562 Pantanal) and with expressive degrees of genetic divergence between clades Voss et  
6  
7 563 al. (2019) synonymized several subspecies and synonyms under the name *M.*  
8  
9 564 *myosuuros* (Voss 2022). Due to the lack of sufficient samples, geographic coverage,  
10  
11 565 and a presentation of morphological characters that should have provided the basis  
12  
13 566 for these taxonomic decisions we disagree with their decision to do so. Until a  
14  
15 567 careful review is conducted and published, we disagree with the provisional  
16  
17 568 application of the name *M. myosuuros* to populations from outside the Atlantic Forest  
18  
19 569 biome.

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23  
24 570 Another relevant point is that we excluded the sequence MNU 051 from the  
25  
26 571 Sinnamary River, French Guiana (locality close to the type-locality of *M.*  
27  
28 572 *nudicaudatus*, Cayenne, French Guiana), from our molecular analysis. This sequence  
29  
30 573 was the most divergent in the analyses presented above and in other studies (Patton  
31  
32 574 and Costa 2003; Patton et al. 2000). The sequence was obtained from a biopsy of an  
33  
34 575 earpiece from an individual captured during a wildlife rescue in the Sinnamary River  
35  
36 576 (François Catzeflis, personal communication). The MNU 051 sequence presented a  
37  
38 577 series of problems, among which, short size (less than 400 bp) and several gaps  
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40 578 influencing the uncertainty of the results regarding its relationship. For these reasons,  
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42 579 we excluded the sequence from our analyses.

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47 580 *Metachirus aritanai* new species can be differentiated from the type species  
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49 581 and *M. myosuuros* (*sensu present study*) using an integrative approach based on  
50  
51 582 morphological data (external and craniodental morphology), photography of type  
52  
53 583 specimens and original descriptions, in addition to molecular data (degrees of  
54  
55 584 divergence and expressive time of divergence). Therefore, based on the general  
56  
57 585 Species Concept proposed by de Queiroz (2007), we recognize *M. aritanai* as a new  
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3 586 species. Furthermore, the comparison of the original descriptions of the nominal  
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5 587 taxa, with the external morphology of some specimens within the proposed  
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7  
8 588 distribution area for subspecies according to Gardner and Dagosto (2007), allowed us  
9  
10 589 to safely verify that the new species has a very distinct external morphology. This set  
11  
12 590 of evidence reinforces a full-species hypothesis.

13  
14 591 Regarding the morphometric data, we recognize the low number of adult male  
15  
16 592 specimens (N = 2) for *M. aritanai* new species, *M. nudicaudatus* (N = 1), for *M.*  
17  
18 593 *mysouros* (N = 2). Even so, we performed a PCA using mostly females to get a sense  
19  
20 594 of how the data behaves in the morphometric space. The results for females  
21  
22 595 demonstrated a clear separation between *M. nudicaudatus*, *M. mysouros* and *M.*  
23  
24 596 *aritanai* new species (Figure 4). We assume that differentiation between males is  
25  
26 597 also plausible. The absence of a greater number of measured individuals is because  
27  
28 598 most of the samples were young or sub-adult individuals, and thus not fully  
29  
30 599 developed, making them unfit for type descriptions of the species. In addition, an  
31  
32 600 important series with some adult individuals of the new species deposited at MZUSP  
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34 601 (see list of examined material), coming from the works of Belo Monte Hydroelectric  
35  
36 602 Power Plant, did not have their skulls cleaned and available for use in morphometry.  
37  
38 603 Therefore, we suggest future morphometric analyses with a greater number of adult  
39  
40 604 skull specimens. Morphometric data was not used to diagnose the new species, but as  
41  
42 605 complementary data to reinforce trends already visualized through morphology and  
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44 606 other used tools.

45  
46 607 In relation to the geographic distributional limits of the new species compared  
47  
48 608 to the distributions of the type species and other potential species, the northern limit  
49  
50 609 coincides with the Amazon River (right bank). North of this river, *M. nudicaudatus*  
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52 610 occurs (left bank). On the west bank of the Xingu River (left bank), occurs another  
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3 611 molecularly distinct clade with external morphology distinct from *Metachirus*  
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5 612 *aritanai* new species. However, the taxonomic definition of this western taxon is  
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7  
8 613 beyond the scope of this paper and requires a generic systematic review.  
9

10 614 As for the southern limit of the distribution of *M. aritanai* new species, the  
11  
12 615 samplings carried out to date point to the north of the Tocantins State, in the  
13  
14 616 Araguaia and Bico do Papagaio regions, in transition areas between the Amazon and  
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16  
17 617 Cerrado domains (Rocha et al. 2011; present study). There is a lack of sampling in  
18  
19 618 the northeastern Mato Grosso State, where *M. aritanai* new species may occur given  
20  
21 619 the presence of areas dominated by semi-deciduous forests. It is worth mentioning  
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23  
24 620 that in the northern central portion of Mato Grosso State the presence of two distinct  
25  
26 621 clades have already been verified, represented in this study by the Southwestern  
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28 622 Amazonian clade and Eastern Amazonian/Alta Floresta clade. The results also show  
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30 623 sympatry between two distinct clades in the region of the Teles Pires River, here  
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32  
33 624 called Eastern Amazonia/Alta Floresta and Southwestern Amazonia with on average  
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35 625 6.5% genetic divergence between them.  
36

37 626 There is no evident geographical barrier between the northeastern and  
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40 627 northwestern areas of Mato Grosso State. One possibility for diversification of this  
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42 628 species would be that the predominant vegetation type is an important factor  
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44 629 controlling the distributions of both taxa in this close geographic region: the eastern  
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46  
47 630 region is dominated by more open and transitional vegetation, while the northwestern  
48  
49 631 region is covered by more robust vegetation and closer to upland, “*terra firme*”  
50  
51 632 forests of the Amazon domain (Dinerstein et al. 2017). However, this hypothesis is  
52  
53 633 speculative and needs further investigation through targeted sampling in that region.  
54

55  
56 634 So far, the seven different clades of *Metachirus* that emerged in our topology  
57  
58 635 are delimited by the great Amazon rivers, a reality different from, for example, that  
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1  
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3 636 of the genus *Philander* (Patton and Costa 2003; Patton et al. 2000) whose taxa go  
4  
5 637 beyond large rivers. The first split of *Metachirus* was dated to 6.34 mya during the  
6  
7 638 late Miocene and separated *M. nudicaudatus* (represented by the Guiana Shield  
8  
9 639 clade) and *M. aritanai* new species (Xingu/Tocantins clade) from the other clades  
10  
11 640 (i.e., the Central American, Central/Western Amazonian, Southwestern Amazonian,  
12  
13 641 Eastern Amazonian/Alta Floresta, and the Atlantic Forest [*M. myosuroides*] clades).

16 642 During the late Miocene, the Amazon region was under the influence of the  
17  
18 643 Purus Arch which is believed to have acted as a barrier between the eastern and  
19  
20 644 western Amazon (Figueiredo et al. 2009; Hoorn et al. 2017). This first divergence of  
21  
22 645 the group may be related to this scenario, separating the Eastern clades (*M.*  
23  
24 646 *nudicaudatus* and *M. aritanai* new species) from the other recovered clades. The  
25  
26 647 Central and Western Amazon clades which later would have expanded to Central  
27  
28 648 America and the Atlantic Forest. The separation between *M. nudicaudatus* (from the  
29  
30 649 Guiana Shield) and *M. aritanai* new species (Xingu/Tocantins interfluve) was dated  
31  
32 650 to 3.3 mya during the Pliocene. The Guiana Shield and the Xingu/Tocantins  
33  
34 651 interfluve are separated by the Amazon River, and thus the evolutionary history of  
35  
36 652 these species supports the Amazon paleogeographic model of this river acting as  
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38 653 barrier until the Plio-Pleistocene period (Ribas et al. 2012). Even so, we recognize  
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40 654 that the geological history of the drainage system of the Amazon River is complex,  
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42 655 starting gradually with the uplift of the Andes Mountain range from the Oligocene to  
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44 656 the Plio-Pleistocene.

49 657 The discovery of a new species in areas under strong anthropic pressure (e.g.,  
50  
51 658 hydroelectric plants, areas destined for the exploitation of minerals, among other  
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53 659 anthropic impacts) reinforces the importance of continued sampling in the region. It  
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55 660 will be important to obtain representative series of this faunal component for  
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3 661 scientific collections (few available specimens compared to other marsupial genera),  
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5 662 to promote studies of different natures and for obtaining reliable information about  
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7 663 the extant fauna of small non-flying mammals, a group that is poorly known from the  
8  
9 664 Xingu/Tocantins region. This would help direct conservation strategies for the fauna  
10  
11 665 of this region.

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32 676 could not be included in this study as they are not yet published. To Dr. Silvia Pavan  
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35  
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41  
42 681 CNPq/FAPEAM).

#### 43 682 Statement on ethics

44  
45 683 We warrant that we conducted this work with integrity, fidelity and honesty and we  
46  
47 684 openly assume responsibility for this work by not knowingly involving or  
48  
49 685 participating in any form of malicious harm to another person or animal.  
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3 816 Tables  
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6 817 Table 1: List of samples included in molecular analyses of *cytochrome b*. For each  
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8 818 sample, the voucher number, GenBank accession number and references. See  
9  
10 819 Appendix I for locality names plotted on the map (Figure 1).  
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<b>ID present study</b>	<b>Voucher</b>	<b>Accession</b>	<b>Reference</b>
<i>Metachirus myosuroides</i>	MAM187	KJ129967	Giarla & Jansa, 2014
<i>Metachirus myosuroides</i>	MAM193	In submission	This study
<i>Metachirus myosuroides</i>	MAM88	In submission	This study
<i>Metachirus myosuroides</i>	ML88	In submission	This study
<i>Metachirus myosuroides</i>	MAM246	In submission	This study
<i>Metachirus myosuroides</i>	ML114	In submission	This study
<i>Metachirus myosuroides</i>	MF27	In submission	This study
<i>Metachirus myosuroides</i>	YL224	In submission	This study
<i>Metachirus myosuroides</i>	YL268	GU112913	Agrizzi et al. 2013
<i>Metachirus myosuroides</i>	YL35	GU112911	Agrizzi et al. 2013
<i>Metachirus myosuroides</i>	YL577	GU112912	Agrizzi et al. 2013
<i>Metachirus myosuroides</i>	YL82	GU112909	Agrizzi et al. 2013
<i>Metachirus myosuroides</i>	LPC935	-	Patton et al. 2001
<i>Metachirus myosuroides</i>	MBML2447	GU112907	Agrizzi et al. 2013
<i>Metachirus myosuroides</i>	ML89	In submission	This study
<i>Metachirus myosuroides</i>	EDH23	MK817282	Voss et al. 2019
<i>Metachirus myosuroides</i>	YL77	GU112910	Agrizzi et al. 2013
<i>Metachirus myosuroides</i>	RM113	In submission	This study
<i>Metachirus myosuroides</i>	RM118	In submission	This study
<i>Metachirus myosuroides</i>	RM91	In submission	This study
<i>Metachirus sp.</i>	MCNM1200	In submission	This study
<i>Metachirus sp.</i>	TGP14	In submission	This study
<i>Metachirus sp.</i>	TGP33	In submission	This study
<i>Metachirus sp.</i>	LPC548	GU112908	Agrizzi et al. 2013
<i>Metachirus sp.</i>	MF1618	In submission	This study
<i>Metachirus sp.</i>	UFMT1353	In submission	This study
<i>Metachirus sp.</i>	MSF1552	In submission	This study
<i>Metachirus sp.</i>	MF2165	In submission	This study
<i>Metachirus sp.</i>	MF2374	In submission	This study
<i>Metachirus sp.</i>	MF2211	In submission	This study
<i>Metachirus sp.</i>	UFMT1357	In submission	This study
<i>Metachirus sp.</i>	MVZ190290	-	Patton et al. 2001
<i>Metachirus sp.</i>	INPA2984 or MVZ193511	-	Patton et al. 2001
<i>Metachirus sp.</i>	MNSF1394	In submission	This study
<i>Metachirus sp.</i>	MNFS40	In submission	This study
<i>Metachirus sp.</i>	MNFS91	In submission	This study
<i>Metachirus sp.</i>	INPA2994 or MVZ193533	-	Patton et al. 2001

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3	<i>Metachirus sp.</i>	MVZ190298	-		Patton et al. 2001
4	<i>Metachirus sp.</i>	INPA2983	-		Patton et al. 2001
5	<i>Metachirus sp.</i>	FC4896	In submission		This study
6	<i>Metachirus sp.</i>	INPA2516	-		Patton et al. 2001
7	<i>Metachirus sp.</i>	INPA2517	-		Patton et al. 2001
8	<i>Metachirus sp.</i>	LPC121	In submission		This study
9	<i>Metachirus sp.</i>	LPC124	In submission		This study
10	<i>Metachirus sp.</i>	INPA4377	-		Patton et al. 2001
11	<i>Metachirus sp.</i>	INPA4378	-		Patton et al. 2001
12	<i>Metachirus sp.</i>	INPA4160	-		Patton et al. 2001
13	<i>Metachirus sp.</i>	INPA4159	-		Patton et al. 2001
14	<i>Metachirus sp.</i>	INPA4493	-		Patton et al. 2001
15	<i>Metachirus sp.</i>	INPA4494	-		Patton et al. 2001
16	<i>Metachirus sp.</i>	INPA4528	-		Patton et al. 2001
17	<i>Metachirus nudicaudatus</i>	RNL47	In submission		This study
18	<i>Metachirus nudicaudatus</i>	MAR1409	In submission		This study
19	<i>Metachirus nudicaudatus</i>	MCNM1494	In submission		This study
20	<i>Metachirus nudicaudatus</i>	PSA06	In submission		This study
21	<i>Metachirus new species</i>	PSA94	In submission		This study
22	<i>Metachirus new species</i>	MAR1165	In submission		This study
23	<i>Metachirus new species</i>	PSA200	In submission		This study
24	<i>Metachirus new species</i>	CS83	In submission		This study
25	<i>Metachirus new species</i>	MPEG1130	In submission		This study
26	<i>Metachirus new species</i>	MCNM1496	In submission		This study
27	<i>Didelphis marsupialis</i>	DMU34665	U34665		Patton et al. 2001
28	<i>Philander opossum canus</i>	JLP169868	JQ778972		Patton et al. 2001

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821 Table 2: Genetic distances calculated from the Kimura-2-parameter model for the  
822 different *Metachirus* clades that emerged in the Maximum Likelihood and Bayesian  
823 Inference trees. In bold, average distances in percentages for the seven recovered  
824 clades.

Clades	1	2	3	4	5	6	7	Intra clade
1. Xingu/ Tocanti ns	0.02							2
2. Guiana Shield	<b>8.2</b>	0.01						1
3. Northw estern	<b>16.1</b>	<b>14.9</b>	0.01					1

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3		Amazo						
4		nia						
5		4.						
6		Central	<b>16.1</b>	<b>14.7</b>	<b>9.6</b>	<b>0</b>		<b>1</b>
7		Americ						
8		a						
9		5.						
10		Southw	<b>14.7</b>	<b>13.1</b>	<b>9.5</b>	<b>9.6</b>	<b>0.01</b>	<b>1</b>
11		estern						
12		Amazo						
13		nia						
14		6.						
15		Eastern	<b>14.1</b>	<b>12.9</b>	<b>9.8</b>	<b>9.9</b>	<b>6.5</b>	<b>0.01</b>
16		Amazo						<b>1</b>
17		nia						
18		7.						
19		Atlantic	<b>14.4</b>	<b>11.6</b>	<b>8.1</b>	<b>8.3</b>	<b>5.3</b>	<b>5.6</b>
20		Forest					<b>0.01</b>	<b>1</b>
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829 Table 3: Descriptive statistics for adult females and males of *Metachirus*  
 830 *nudicaudatus*, *M. myosuuros* (sensu present study) and *Metachirus* (potential new  
 831 species, Serra de Carajás region). MC = male from the Carajás region; FC = females  
 832 from the Carajás region; MN = male of *M. nudicaudatus*; FN = females of *M.*  
 833 *nudicaudatus*; **MM= males of *M. myosuuros*; FM= females of *M. myosuuros***. MCC =  
 834 maximal cranial circumference. NAS = nasal bone length; RWJ = rostrum width  
 835 between the jugal bones; RWF = rostrum width between the frontal bones; NW =  
 836 greater nasal width; CI = interorbital constriction; PC = postorbital constriction; WZ  
 837 = width between zygomatic arches; GCW = greatest cranial width; MAX = length of  
 838 the maxillary row of teeth; LUM = length of the upper molar series; CAN = width  
 839 between the lateral margins of the upper canines; WPP = width of the palatal  
 840 projection; WM = width between the external faces of the third molars; LLM =  
 841 length of the lower molar series; JL = jaw length.

	<i>Metachirus new species</i>		<i>M. nudicaudatus</i>		<i>M. myosuuros</i>	
	♂ (N= 1)	♀ (N= 3)	♂ (N= 1)	♀ (N= 5)	♂ (N= 4)	♀ (N= 2)
<b>MCC</b>	59.35	53.40 ±3.22	59.91	59.75±2.56	56.73±2.28	55.92±1.47
<b>WZ</b>	29.13	25.87±1.86	29.53	29.66±2.48	27.77±0.70	27.31±0.84
<b>NAS</b>	16.57	14.93±0.81	15.8	16.37±0.64	17.53±0.74	16.97±0.11
<b>RWJ</b>	11.02	9.88±0.70	11	10.85±0.31	11.47±1.08	12.06±0.25
<b>RWF</b>	8.12	7.62±0.52	8.4	8.33±0.47	7.65±1.21	7.48±0.37
<b>NW</b>	11.71	11.42 ±0.84	11.68	12.09±0.39	14.49±0.98	12.69±0.52
<b>CI</b>	8.42	8.5 ±0.49	8.32	8.99±0.30	9.61±0.30	9.56±0.14
<b>PC</b>	28.49	25.51 ±1.06	28.16	27.32±0.97	29.14±1.42	28.52±0.32
<b>GCW</b>	16.91	15.79 ±0.71	12.95	13.16±0.51	12.48±0.53	12.07±0.26
<b>MAX</b>	25.47	23.02 ±1.29	24.72	25.28±0.73	24.27±0.50	23.85±0.19
<b>LUM</b>	11.93	10.98 ±1.15	11.93	11.79±0.42	11.54±0.55	10.77±0.26
<b>CAN</b>	8.98	7.70 ±0.65	8.41	8.12±0.40	9.47±0.71	9.14±0.08
<b>WPP</b>	10.7	9.51 ±0.09	10.75	10.40±0.86	11.56±0.49	11.29±0.26
<b>WM</b>	17.14	15.76 ±1.22	18.2	17.77±0.56	17.73±0.72	17.44±0.09
<b>LLM</b>	12.63	12.81 ±0.51	12.9	12.66±0.41	13.08±0.29	13.16±0.26
<b>JL</b>	45.28	39.29 ±2.05	46.71	45.71±1.42	44.27±1.89	44.46±1.02

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3 844 Table 4: Values obtained for the 10 principal components (PC) in the Principal  
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5 845 Component Analysis (PCA) using a matrix of variance-covariance for *Metachirus*  
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7 846 (new species), *M. nudicaudatus*, and *M. myosuuros* (sensu present study), with their  
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10 847 respective percentages of variation.

PC	Eigenvalue	% variance
1	25.171	67.076
2	6.38044	17.003
3	1.85989	4.9562
4	1.13538	3.0256
5	0.842989	2.2464
6	0.680801	1.8142
7	0.487185	1.2982
8	0.297252	0.79212
9	0.257846	0.68711
10	0.203593	0.54253

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852 Table 5: Summary of comparative diagnostic characters for the type species,  
 853 *Metachirus aritanai* new species, *M. nudicaudatus* and *M. myosuuros* (*sensu present*  
 854 *study*).

Characters	<i>Metachirus aritanai</i> new species	<i>Metachirus nudicaudatus</i>	<i>Metachirus myosuuros</i>
<b>Dorsal colouration</b>	Light brown speckled with gold	Brown speckled with black and light orange	Orange-brown to light brown
<b>Ventral colouration</b>	Pure cream	Yellowish-cream	Cream to yellowish
<b>Lateral body colouration</b>	Slightly lighter than the dorsum	Orange to brown, visibly lighter than the back, expressive presence of blackish and orange hairs	Yellowish with variations of light grey or light golden tones
<b>Supraocular spots colouration</b>	Usually pure cream	Cream to slightly orange, especially around the edges	Usually yellowish to cream
<b>Cheeks colouration</b>	Usually pure cream	Varies from cream to orange-cream	Usually yellowish
<b>Lighter hairs behind the base of the ears</b>	Yellowish and in small quantities	Rust colour and in large quantities	Different tones of yellow that vary in quantity
<b>Tarsal hair colouration</b>	Cream	Brownish orange	Variations of blackish to brown-greyish or golden hairs
<b>Skull</b>	Wider, inflated and more robust (between 15.79 mm and 16.91	Narrower, lowered and more delicate (between 12.95 mm and 13.16 mm)	Narrower, lowered and more delicate (between 12.07 mm and 12.48 mm)

		mm)		
	<b>Rostrum</b>	Wider and more robust	Narrower and more delicate	Wider and more robust
	<b>Interorbital crests</b>	Usually absent	Developed	Developed
	<b>Pre-maxillary region</b>	Wider, short and pointed	Narrower, long and rounded	Narrower, long and usually pointed
	<b>Frontal region</b>	More regular and rounded posterior limit, without the presence of a median protuberance	Irregular in the posterior portion, with a protuberance in the median region	Usually irregular in the posterior portion, with a protuberance in the median region
	<b>Lower molars</b>	Wide entoconids	Small entoconids	Small entoconids
	<b>Average degree of molecular divergence (Cyt b)</b>	8.20%	8.20%	14.60%
	<b>Estimated time of molecular divergence (Cyt b)</b>	3.3 mya (Early Pliocene)	3.3 mya (Early Pliocene)	3.5 mya (Early Pliocene)
	<b>Geographic distribution</b>	Xingu/Tocantins Interfluve (Figure 1)	Suriname, Guyana, French Guiana and the Brazilian portion of the Guiana shield, in the northern Pará and Amapá states (Figure 1)	Atlantic Forest in eastern Brazil and probably northern Argentina (Figure 1)

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856

857 Table 6: External morphological characters for the two subspecies that were  
 858 recognised by Gardner & Dagosto (2007) to occur within the Brazilian Amazon.  
 859 Note the degree of morphological variation, which was used by Voss et al. (2019)  
 860 and Voss (2022) in their delimitation of *M. myosuroides*. Morphological variation of the  
 861 populations from the eastern Amazonia, Tapajós basin, northern Mato Grosso State  
 862 (region of the Teles Pires and Juruena Rivers) and the Pantanal region, expand the  
 863 variation presented in this table for the two subspecies.

<b>Characters</b>	<i>Metachirus n. colombianus</i>	<i>Metachirus n. tschudii</i>
<b>Holotype</b>	AMNH 15448	AMNH 11815/10107
<b>Type-locality</b>	Donamo (Don Amo), Magdalena, Colombia	Guayabamba (Santa Rosa de Hayabamba, Peru)
<b>Dorsal colouration</b>	Dark yellowish brown, markedly varying with black, with a narrow-blackened stripe	Brown greyish yellow with a broad darker stripe that range from blackish to chestnut brown
<b>Sides of the body</b>	Yellowish brown	Yellowish brown with a noticeable tone of rust close to the tail
<b>Spots above the eyes</b>	Generally orange	Cream-orange
<b>Ventral colour</b>	Predominantly whitish	Generally cream-yellowish
<b>Hairs behind the ears</b>	Many to medium quantity, from yellowish to yellowish/reddish	Many to medium quantity, varying from yellowish to yellowish/reddish
<b>Colour at the base of the tail</b>	Chestnut-yellowish with a narrow-blackened stripe	Reddish with a blackish to chestnut stripe

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**Hair's length at the base of the tail**

Generally up to 15 mm

Generally over 20 mm

**Tail colour**

Dark brown toward the belly gradually lighter towards the tip

Brown or blackish towards the belly and whitish tip

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3 880 Figure Legends  
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6 881 Figure 1: Sampling localities of examined *Metachirus* specimens. Type-localities are  
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8 882 represented by white stars with their respective names. The type species *Metachirus*  
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10 883 *nudicaudatus* is represented by orange circles and *Metachirus myosuuros* from the  
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12 884 Atlantic Forest by blue circles. The localities of the new species described herein are  
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14 885 shown as red circles. Black circles represent taxa/or new species that are pending  
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16 886 systematic revision **for determining their specific status.**  
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18 887  
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20 888 Figure 2: Strict consensus tree of *Metachirus* based on mitochondrial cytochrome b  
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22 889 gene (801 bp). Values on the nodes refer to the Bootstrap and posterior probability.  
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26 891 Figure 3: Dated phylogenetic tree of *Metachirus* based on *cytochrome b* gene (*cytb*).  
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28 892 Colours: red = Guyana Shield, *M. nudicaudatus*; yellow = Xingu/Tocantins,  
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30 893 *Metachirus* **new species**; dark green = Central America and pink = Northwestern  
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32 894 Amazonia; orange = **Eastern Amazonia/Teles Pires River**; blue = Southeastern  
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34 895 Amazonia; and light green = Atlantic Forest, *M. myosuuros*; black = outgroup).  
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36 896 Values above nodes represent divergence time in million years and values below  
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38 897 nodes the posterior probability. Node bars represent the Highest Posterior Density  
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40 898 (HPD) interval of 95%. Time scale in million years.  
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44 900 Figure 4: Principal Component Analysis (PCA) performed for males and females of  
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46 901 *Metachirus nudicaudatus*, *M. myosuuros* and *M. aritanai* new species described here.  
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48 902 **Yellow square = *M. nudicaudatus* male specimen; green squares = *M. nudicaudatus***  
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50 903 **female specimens; pink triangles = *M. myosuuros* male specimens; blue triangles = *M.***  
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3 904 *mysuro*s female specimens; blue circle = *M. aritanai* new species, male specimen;  
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5 905 red circles = *M. aritanai* new species, female specimens.  
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10 907 Figure 5: View of the head of (A) *Metachirus nudicaudatus*, (B) *Metachirus*  
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12 908 *mysuro*s, (C) *Metachirus aritanai* new species. Note the differences in colour, the  
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14 909 overall lighter colouration of the new species, including on the nape, top of the head  
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16 910 and hairs at the base of the ear. Bar = 50 mm.  
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21 912 Figure 6: Dorsal and ventral views of *Metachirus nudicaudatus* (A), *Metachirus*  
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23 913 *mysuro*s (B) and *Metachirus aritanai* new species (C). Bar = 50 mm.  
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28 915 Figure 7: Dorsal and ventral view of the skull of *Metachirus nudicaudatus* (A)  
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30 916 *Metachirus mysuro*s (B) and *Metachirus aritanai* new species (C). Bar = 25 mm.  
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920 Figure 8: Adult individual of *Metachirus aritanai* new species from Carajás National  
921 Forest, Pará, Brazil. Note some diagnostic characters of external morphology,  
922 especially the overall light colouration. This new species presents the clearest  
923 individuals in relation to the other **amazonian** populations of jupatis examined (see  
924 list of examined material and original descriptions of the nominal taxa of  
925 *Metachirus*). Photo: Natalia Ardente.

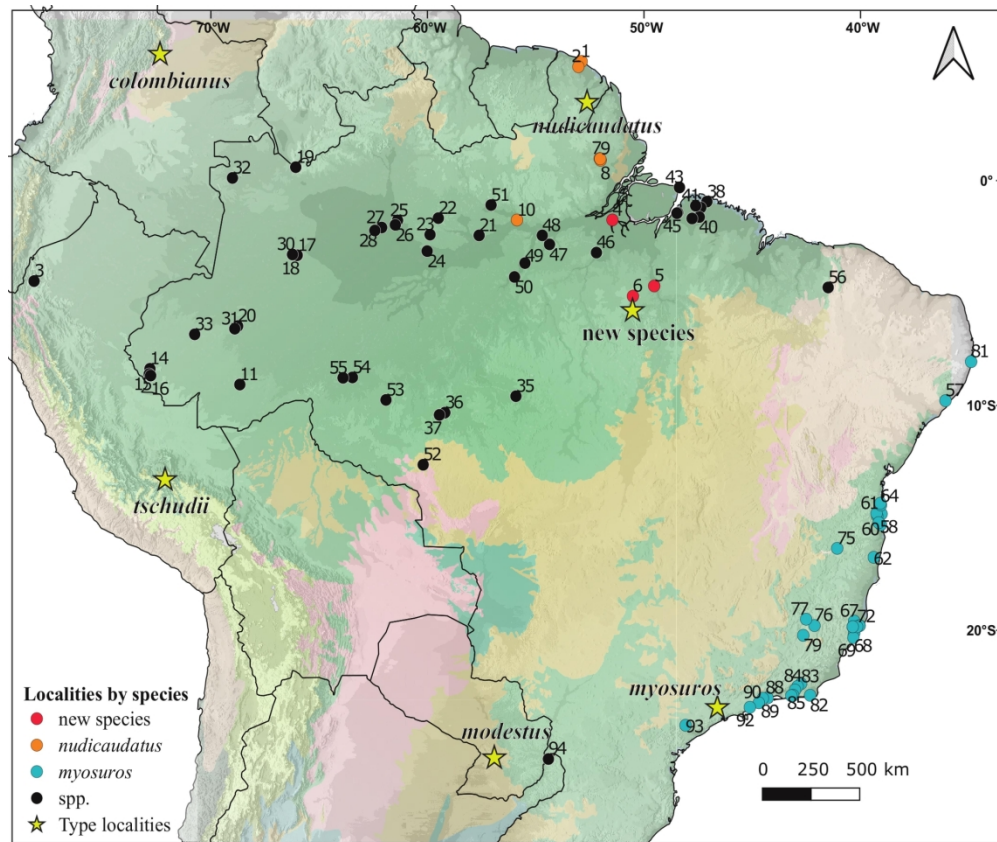


Figure 1: Sampling localities of examined *Metachirus* specimens. Type-localities are represented by white stars with their respective names. The type species *Metachirus nudicaudatus* is represented by orange circles and *Metachirus myosuroides* from the Atlantic Forest by blue circles. The localities of the new species described herein are shown as red circles. Black circles represent taxa/or new species that are pending systematic revision for determining their specific status.

180x152mm (300 x 300 DPI)



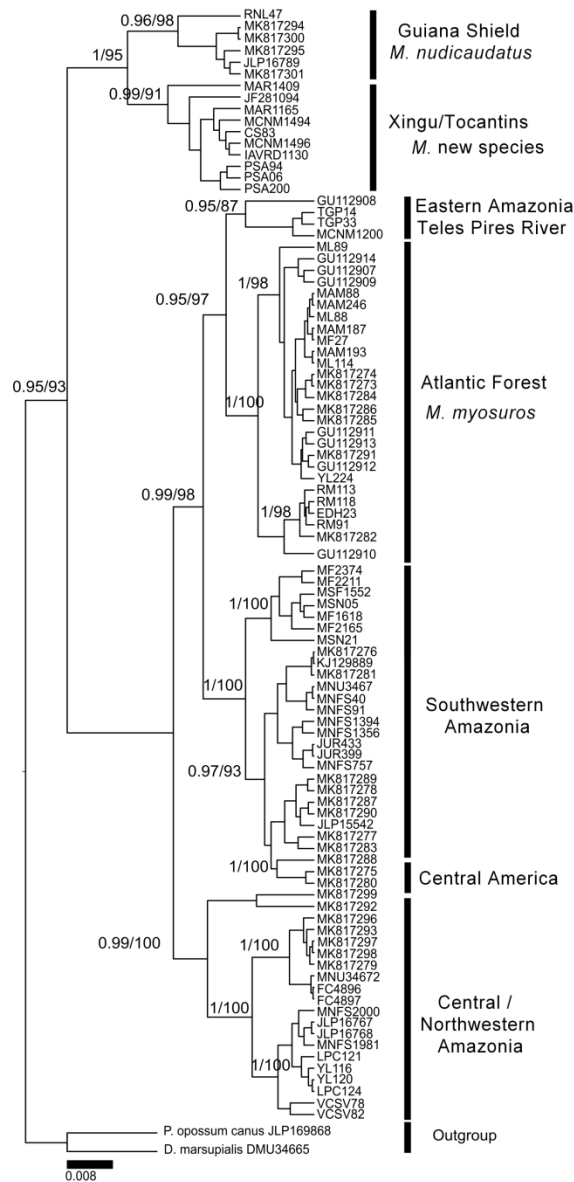


Figure 2: Strict consensus tree of *Metachirus* based on mitochondrial cytochrome b gene (801 bp). Values on the nodes refer to the Bootstrap and posterior probability.

142x299mm (300 x 300 DPI)

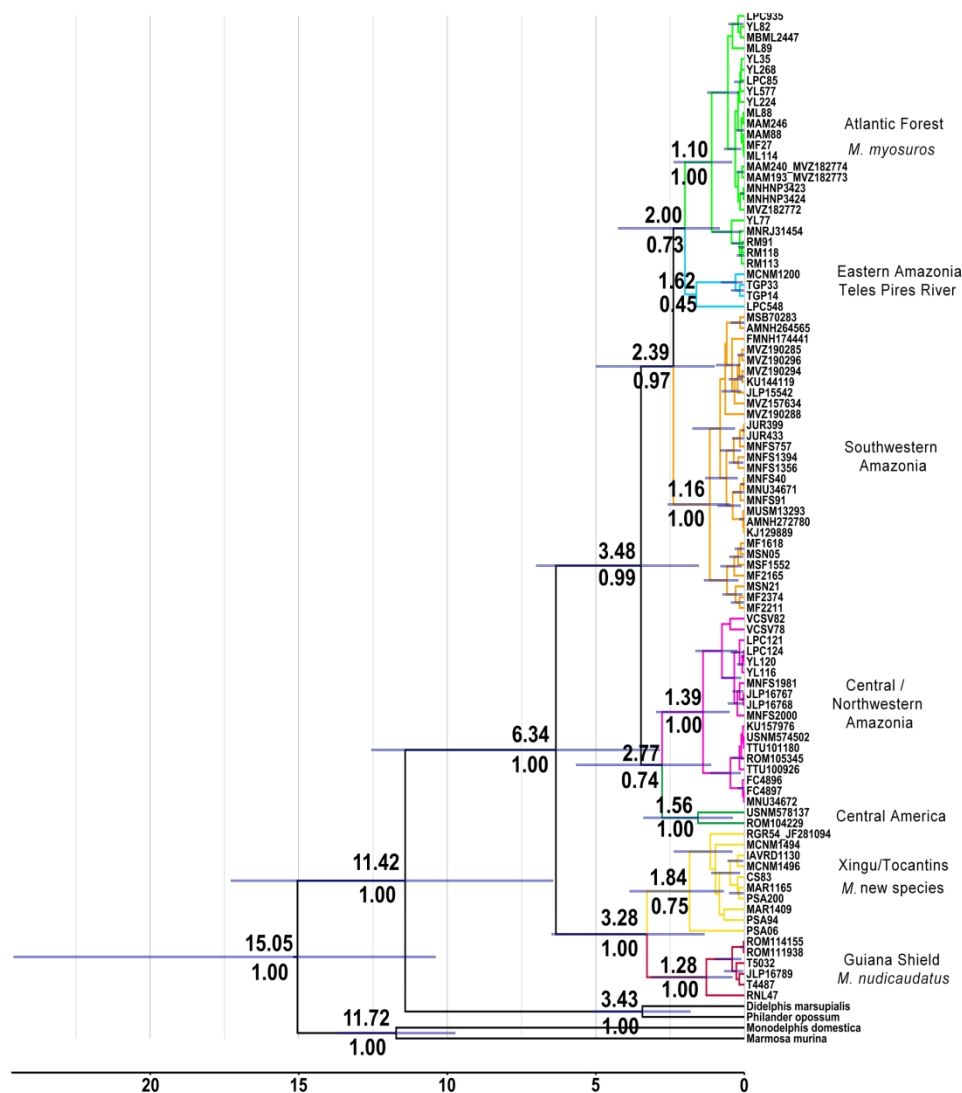


Figure 3: Dated phylogenetic tree of *Metachirus* based on cytochrome b gene (cytb). Colours: red = Guyana Shield, *M. nudicaudatus*; yellow = Xingu/Tocantins, *Metachirus* new species; dark green = Central America and pink = Northwestern Amazonia; orange = Eastern Amazonia/Teles Pires River; blue = Southeastern Amazonia; and light green = Atlantic Forest, *M. myosuroides*; black = outgroup). Values above nodes represent divergence time in million years and values below nodes the posterior probability. Node bars represent the Highest Posterior Density (HPD) interval of 95%. Time scale in million years.

225x248mm (300 x 300 DPI)

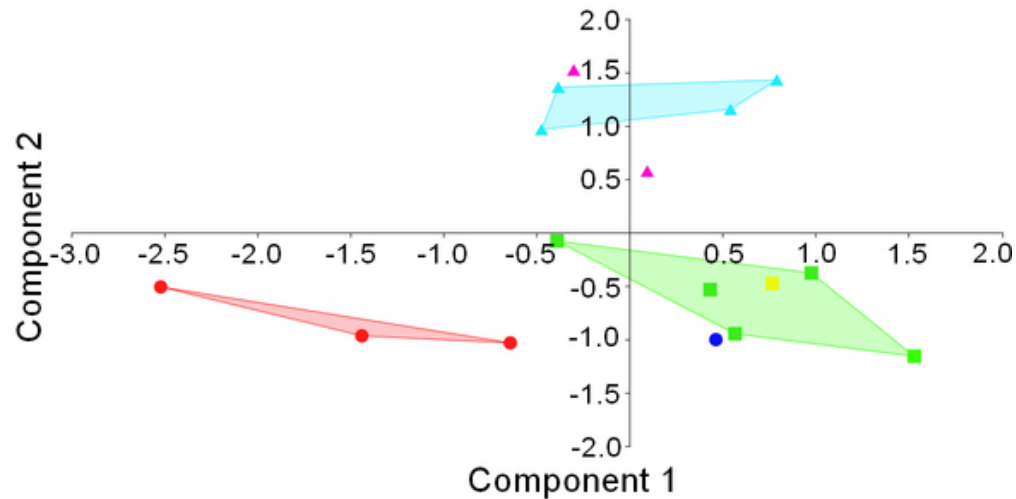


Figure 4: Principal Component Analysis (PCA) performed for males and females of *Metachirus nudicaudatus*, *M. myosuros* and *M. aritanai* new species described here. Yellow square = *M. nudicaudatus* male specimen; green squares = *M. nudicaudatus* female specimens; pink triangles = *M. myosuros* male specimens; blue triangles = *M. myosuros* female specimens; blue circle = *M. aritanai* new species, male specimen; red circles = *M. aritanai* new species, female specimens.

49x27mm (300 x 300 DPI)

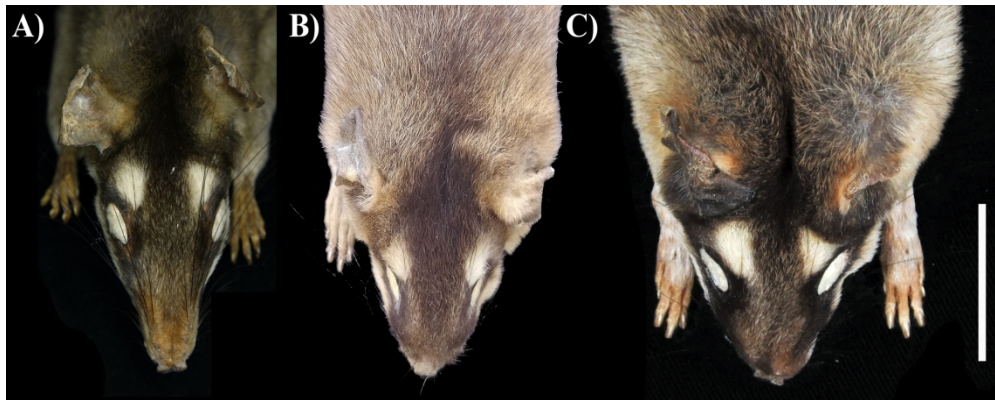


Figure 5: View of the head of (A) *Metachirus nudicaudatus*, (B) *Metachirus mysosuros*, (C) *Metachirus aritanai* new species. Note the differences in colour, the overall lighter colouration of the new species, including on the nape, top of the head and hairs at the base of the ear. Bar = 50 mm.

211x83mm (300 x 300 DPI)

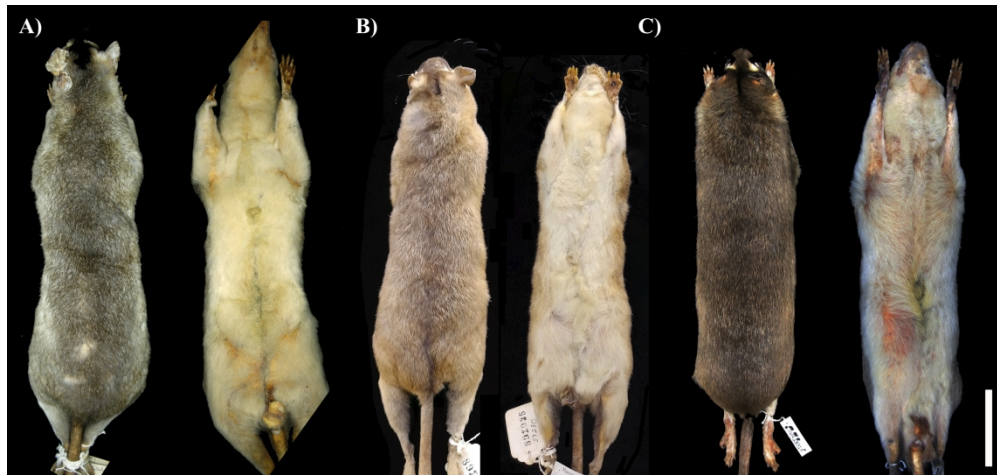


Figure 6: Dorsal and ventral views of *Metachirus nudicaudatus* (A), *Metachirus mysosuros* (B) and *Metachirus aritanai* new species (C). Bar = 50 mm.

259x121mm (300 x 300 DPI)

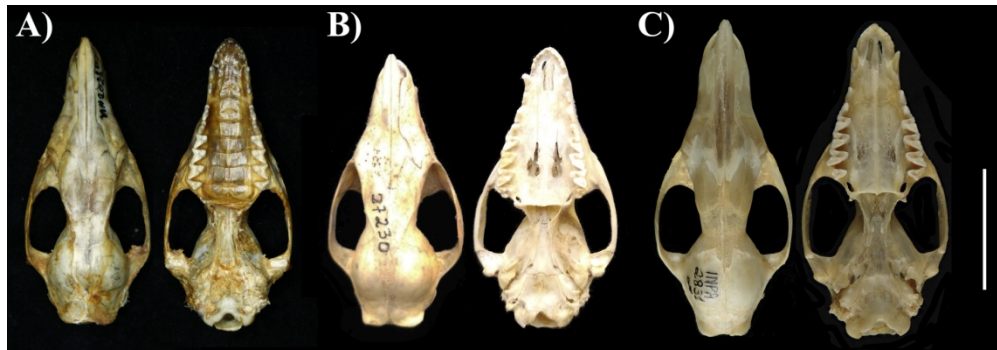


Figure 7: Dorsal and ventral view of the skull of *Metachirus nudicaudatus* (A) *Metachirus myosuroides* (B) and *Metachirus aritanai* new species (C). Bar = 25 mm.

166x57mm (300 x 300 DPI)

<b>ID present study</b>	<b>Voucher</b>	<b>Accession</b>	<b>Reference</b>
<i>Metachirus myosuros</i>	MAM187	KJ129967	Giarla & Jansa, 2014
<i>Metachirus myosuros</i>	MAM193	In submission	This study
<i>Metachirus myosuros</i>	MAM88	In submission	This study
<i>Metachirus myosuros</i>	ML88	In submission	This study
<i>Metachirus myosuros</i>	MAM246	In submission	This study
<i>Metachirus myosuros</i>	ML114	In submission	This study
<i>Metachirus myosuros</i>	MF27	In submission	This study
<i>Metachirus myosuros</i>	YL224	In submission	This study
<i>Metachirus myosuros</i>	YL268	GU112913	Agrizzi et al. 2013
<i>Metachirus myosuros</i>	YL35	GU112911	Agrizzi et al. 2013
<i>Metachirus myosuros</i>	YL577	GU112912	Agrizzi et al. 2013
<i>Metachirus myosuros</i>	YL82	GU112909	Agrizzi et al. 2013
<i>Metachirus myosuros</i>	LPC935	-	Patton et al. 2001
<i>Metachirus myosuros</i>	MBML2447	GU112907	Agrizzi et al. 2013
<i>Metachirus myosuros</i>	ML89	In submission	This study
<i>Metachirus myosuros</i>	EDH23	MK817282	Voss et al. 2019
<i>Metachirus myosuros</i>	YL77	GU112910	Agrizzi et al. 2013
<i>Metachirus myosuros</i>	RM113	In submission	This study
<i>Metachirus myosuros</i>	RM118	In submission	This study
<i>Metachirus myosuros</i>	RM91	In submission	This study
<i>Metachirus</i> sp.	MCNM1200	In submission	This study

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3	<i>Metachirus</i> sp.	TGP14	In submission	This study
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5				
6	<i>Metachirus</i> sp.	TGP33	In submission	This study
7				
8	<i>Metachirus</i> sp.	LPC548	GU112908	Agrizzi et al. 2013
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11	<i>Metachirus</i> sp.	MF1618	In submission	This study
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14	<i>Metachirus</i> sp.	UFMT1353	In submission	This study
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16				
17	<i>Metachirus</i> sp.	MSF1552	In submission	This study
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19				
20	<i>Metachirus</i> sp.	MF2165	In submission	This study
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23	<i>Metachirus</i> sp.	MF2374	In submission	This study
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26				
27	<i>Metachirus</i> sp.	MF2211	In submission	This study
28				
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30	<i>Metachirus</i> sp.	UFMT1357	In submission	This study
31				
32	<i>Metachirus</i> sp.	MVZ190290	-	Patton et al. 2001
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35	<i>Metachirus</i> sp.	INPA2984 or MVZ193511	-	Patton et al. 2001
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39	<i>Metachirus</i> sp.	MNSF1394	In submission	This study
40				
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42	<i>Metachirus</i> sp.	MNFS40	In submission	This study
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44				
45	<i>Metachirus</i> sp.	MNFS91	In submission	This study
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49	<i>Metachirus</i> sp.	INPA2994 or MVZ193533	-	Patton et al. 2001
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52	<i>Metachirus</i> sp.	MVZ190298	-	Patton et al. 2001
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54	<i>Metachirus</i> sp.	INPA2983	-	Patton et al. 2001
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57	<i>Metachirus</i> sp.	FC4896	In submission	This study
58				
59	<i>Metachirus</i> sp.	INPA2516	-	Patton et al. 2001
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2	<i>Metachirus</i> sp.	INPA2517	-	Patton et al. 2001
3				
4	<i>Metachirus</i> sp.	LPC121	In submission	This study
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8	<i>Metachirus</i> sp.	LPC124	In submission	This study
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10	<i>Metachirus</i> sp.	INPA4377	-	Patton et al. 2001
11				
12	<i>Metachirus</i> sp.	INPA4378	-	Patton et al. 2001
13				
14	<i>Metachirus</i> sp.	INPA4160	-	Patton et al. 2001
15				
16	<i>Metachirus</i> sp.	INPA4159	-	Patton et al. 2001
17				
18	<i>Metachirus</i> sp.	INPA4493	-	Patton et al. 2001
19				
20	<i>Metachirus</i> sp.	INPA4494	-	Patton et al. 2001
21				
22	<i>Metachirus</i> sp.	INPA4528	-	Patton et al. 2001
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24				
25	<i>Metachirus nudicaudatus</i>	RNL47	In submission	This study
26				
27				
28	<i>Metachirus nudicaudatus</i>	MAR1409	In submission	This study
29				
30				
31	<i>Metachirus nudicaudatus</i>	MCNM1494	In submission	This study
32				
33	<i>Metachirus nudicaudatus</i>	PSA06	In submission	This study
34				
35	<i>Metachirus new species</i>	PSA94	In submission	This study
36				
37				
38	<i>Metachirus new species</i>	MAR1165	In submission	This study
39				
40				
41	<i>Metachirus new species</i>	PSA200	In submission	This study
42				
43				
44	<i>Metachirus new species</i>	CS83	In submission	This study
45				
46				
47	<i>Metachirus new species</i>	MPEG1130	In submission	This study
48				
49				
50	<i>Metachirus new species</i>	MCNM1496	In submission	This study
51				
52				
53	<i>Didelphis marsupialis</i>	DMU34665	U34665	Patton et al. 2001
54				
55	<i>Philander opossum canus</i>	JLP169868	JQ778972	Patton et al. 2001
56				
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Clades	1	2	3	4	5
1. Xingu/Tocantins	0.02				
2. Guiana Shield	<b>8.2</b>	0.01			
3. Northwestern Amazonia	<b>16.1</b>	<b>14.9</b>	0.01		
4. Central America	<b>16.1</b>	<b>14.7</b>	<b>9.6</b>	0	
5. Southwestern Amazonia	<b>14.7</b>	<b>13.1</b>	<b>9.5</b>	<b>9.6</b>	0.01
6. Eastern Amazonia	<b>14.1</b>	<b>12.9</b>	<b>9.8</b>	<b>9.9</b>	<b>6.5</b>
7. Atlantic Forest	<b>14.4</b>	<b>11.6</b>	<b>8.1</b>	<b>8.3</b>	<b>5.3</b>

For Review Only

6	7	Intra clade
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0.01		1
<b>5.6</b>	0.01	1

For Review Only

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	<i>Metachirus new species</i>		<i>M. nudicaudatus</i>		<i>M. myosuroides</i>	
	♂ (N= 1)	♀ (N= 3)	♂ (N= 1)	♀ (N= 5)	♂ (N= 4)	♀ (N= 2)
<b>MCC</b>	59.35	53.40 ±3.22	59.91	59.75±2.56	56.73±2.28	55.92±1.47
<b>WZ</b>	29.13	25.87±1.86	29.53	29.66±2.48	27.77±0.70	27.31±0.84
<b>NAS</b>	16.57	14.93±0.81	15.8	16.37±0.64	17.53±0.74	16.97±0.11
<b>RWJ</b>	11.02	9.88±0.70	11	10.85±0.31	11.47±1.08	12.06±0.25
<b>RWF</b>	8.12	7.62±0.52	8.4	8.33±0.47	7.65±1.21	7.48±0.37
<b>NW</b>	11.71	11.42 ±0.84	11.68	12.09±0.39	14.49±0.98	12.69±0.52
<b>CI</b>	8.42	8.5 ±0.49	8.32	8.99±0.30	9.61±0.30	9.56±0.14
<b>PC</b>	28.49	25.51 ±1.06	28.16	27.32±0.97	29.14±1.42	28.52±0.32
<b>GCW</b>	16.91	15.79 ±0.71	12.95	13.16±0.51	12.48±0.53	12.07±0.26
<b>MAX</b>	25.47	23.02 ±1.29	24.72	25.28±0.73	24.27±0.50	23.85±0.19
<b>LUM</b>	11.93	10.98 ±1.15	11.93	11.79±0.42	11.54±0.55	10.77±0.26
<b>CAN</b>	8.98	7.70 ±0.65	8.41	8.12±0.40	9.47±0.71	9.14±0.08
<b>WPP</b>	10.7	9.51 ±0.09	10.75	10.40±0.86	11.56±0.49	11.29±0.26
<b>WM</b>	17.14	15.76 ±1.22	18.2	17.77±0.56	17.73±0.72	17.44±0.09
<b>LLM</b>	12.63	12.81 ±0.51	12.9	12.66±0.41	13.08±0.29	13.16±0.26
<b>JL</b>	45.28	39.29 ±2.05	46.71	45.71±1.42	44.27±1.89	44.46±1.02

PC	Eigenvalue	% variance
1	25.171	67.076
2	6.38044	17.003
3	1.85989	4.9562
4	1.13538	3.0256
5	0.842989	2.2464
6	0.680801	1.8142
7	0.487185	1.2982
8	0.297252	0.79212
9	0.257846	0.68711
10	0.203593	0.54253

For Review Only

<b>Characters</b>	<i>Metachirus aritanai</i> new species	<i>Metachirus nudicaudatus</i>	<i>Metachirus mysosuros</i>
<b>Dorsal colouration</b>	Light brown speckled with gold	Brown speckled with black and light orange	Orange-brown to light brown
<b>Ventral colouration</b>	Pure cream	Yellowish-cream	Cream to yellowish
<b>Lateral body colouration</b>	Slightly lighter than the dorsum	Orange to brown, visibly lighter than the back, expressive presence of blackish and orange hairs	Yellowish with variations of light grey or light golden tones
<b>Supraocular spots colouration</b>	Usually pure cream	Cream to slightly orange, especially around the edges	Usually yellowish to cream
<b>Cheeks colouration</b>	Usually pure cream	Varies from cream to orange-cream	Usually yellowish
<b>Lighter hairs behind the base of the ears</b>	Yellowish and in small quantities	Rust colour and in large quantities	Different tones of yellow that vary in quantity
<b>Tarsal hair colouration</b>	Cream	Brownish orange	Variations of blackish to brown-greyish or golden hairs
<b>Skull</b>	Wider, inflated and more robust (between 15.79 mm and 16.91 mm)	Narrower, lowered and more delicate (between 12.95 mm and 13.16 mm)	Narrower, lowered and more delicate (between 12.07 mm and 12.48 mm)
<b>Rostrum</b>	Wider and more robust	Narrower and more delicate	Wider and more robust
<b>Interorbital crests</b>	Usually absent	Developed	Developed

<b>Pre-maxillary region</b>	Wider, short and pointed	Narrower, long and rounded	Narrower, long and usually pointed
<b>Frontal region</b>	More regular and rounded posterior limit, without the presence of a median protuberance	Irregular in the posterior portion, with a protuberance in the median region	Usually irregular in the posterior portion, with a protuberance in the median region
<b>Lower molars</b>	Wide entoconids	Small entoconids	Small entoconids
<b>Average degree of molecular divergence (<i>Cyt b</i>)</b>	8.20%	8.20%	14.60%
<b>Estimated time of molecular divergence (<i>Cyt b</i>)</b>	3.3 mya (Early Pliocene)	3.3 mya (Early Pliocene)	3.5 mya (Early Pliocene)
<b>Geographic distribution</b>	Xingu/Tocantins Interfluve (Figure 1)	Suriname, Guyana, French Guiana and the Brazilian portion of the Guiana shield, in the northern Pará and Amapá states (Figure 1)	Atlantic Forest in eastern Brazil and probably northern Argentina (Figure 1)

1		
2	<b>Characters</b>	<i>Metachirus n. colombianus</i>
3	<b>Holotype</b>	AMNH 15448
4	<b>Type-locality</b>	Donamo (Don Amo), Magdalena, Colombia
5		
6	<b>Dorsal colouration</b>	Dark yellowish brown, markedly varying with black,
7		with a narrow-blackened stripe
8		
9	<b>Sides of the body</b>	Yellowish brown
10	<b>Spots above the eyes</b>	Generally orange
11	<b>Ventral colour</b>	Predominantly whitish
12		
13	<b>Hairs behind the ears</b>	Many to medium quantity, from yellowish to
14		yellowish/reddish
15	<b>Colour at the base of the tail</b>	Chestnut-yellowish with a narrow-blackened stripe
16	<b>Hair's length at the base of</b>	
17	<b>the tail</b>	Generally up to 15 mm
18		
19	<b>Tail colour</b>	Dark brown toward the belly gradually lighter towards
20		the tip
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*Metachirus n. tschudii*

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AMNH 11815/10107

Guayabamba (Santa Rosa de Hayabamba, Peru)

Brown greyish yellow with a broad darker stripe that range  
from blackish to chestnut brown

Yellowish brown with a noticeable tone of rust close to the tail

Cream-orange

Generally cream-yellowish

Many to medium quantity, varying from yellowish to  
yellowish/reddish

Reddish with a blackish to chestnut stripe

Generally over 20 mm

Brown or blackish towards the belly and whitish tip

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## 1 Appendix 1

2 List of material examined, except *Metachirus aritanai* new species which was  
3 presented in the formal description:

4 *Metachirus nudicaudatus*

5 BRAZIL - **Amapá**: Municipality of Serra do Navio, Igarapé do Ouro MN 20229; Serra  
6 do Navio MN 20234, 20142, 20204; 20206; Teresinha, Amapari River MN 20203,  
7 20235, 20230, 20232, 20233, 20202, 20207, 20228; 20231, 20205, MPEG 12922,  
8 12926. **Pará**: Sacarazinho River, Oriximiná MPEG 10441, 10105, 10440, 10104,  
9 10439, 10443, 10442. FRENCH GUYANNA. **Paracou**: AMNH 266451, 267009,  
10 267010 (photography).

11 *Metachirus myosuroides* (*sensu* present study)

12 BRAZIL - **Alagoas**: Usina Caeté, São Miguel dos Campos MN 30571, MN 30573;  
13 MN 17182, MN 30572. **Bahia**: Fazenda Brejo Grande, Itabuna MN 31454;  
14 Municipality of Ilhéus MN 11370, MN 11354, MN 11367, MN 11380; Ribeirão da  
15 Fortuna, Buerarema MN 11375, MN 11361, MN 11364, MN17196, MN11360, MN  
16 1365, MN 11369, MN 11376. **Espírito Santo**: Estação Biológica de Santa Lúcia,  
17 Santa Teresa MN 31425, MN 25992; Morro da Argola, Vila Velha MN 3985, MN  
18 5579, MN 5580, MN 5581; Santa Ana, Lagoa Juparanã, Linhares MN 1214; Serra MN  
19 11713; MN 11670, MN 11714. **Pernambuco**: Parque Dois Irmãos, Recife MN 8281,  
20 MN 8201, MN 8200. **Piauí**: Fazenda Nazareth, José de Freitas MPEG 36872. **Rio de**  
21 **Janeiro**: Angra dos Reis MN 1213, MN 1220; Araruama MN 25991; Município de  
22 Magé MN 42791; Mambucaba, Angra dos Reis MN 42772, MN 42773, MN 42774;  
23 Parati MN 62192; Pendotiba, Niterói MN 29951; Rio de Janeiro MN 5603, MN 5612,  
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3 25 MN 5806, MN 10246, MN 10253, MN 10254, MN 25993, MN 10247, MN 1218, MN  
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5 26 5805, MN 10245, MN 10249, MN 10251; Serra dos Órgãos MN 42792. **São Paulo:**  
6  
7 27 Fazenda Intervales MZUSP 27230; Fazenda Capricórnio, Ubatuba MZUSP 29193;  
8  
9 28 Bertioga Praia do Iporanga MZUSP 9865; Bertioga MZUSP 10204, 10205; Bauru  
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11 29 MZUSP 471; Reserva Florestal de Caraguatatuba MZUSP 9785; Itapura MZUSP  
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13 30 1874.  
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19 32 *Metachirus* spp. (new species or taxa to be revalidated pending comprehensive  
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21 33 systematic review)  
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24 34 **BRAZIL - Acre:** Fazenda Santa Fé, left bank of the Juruá River MPEG 28075; Igarapé  
25  
26 35 Porongaba, right bank of the Juruá River MPEG 28076, MPEG 28077, MPEG 28079,  
27  
28 36 MPEG 28080, MPEG 28078; Nova Vida, right bank of the Juruá River, INPA 2981,  
29  
30 37 INPA 2987; Sena Madureira: MPEG 10713, MPEG 10714, MPEG 10719, MPEG  
31  
32 38 10722, MPEG 10721, MPEG 10572, MPEG 10710, MPEG 10723, MPEG 10574,  
33  
34 39 MPEG 10712, MPEG 12924, MPEG 10571, MPEG 10573, MPEG 10709, MPEG  
35  
36 40 10711, MPEG 10715, MPEG 10716, MPEG 10717, MPEG 10718, MPEG 10720,  
37  
38 41 MPEG 10724, MPEG 12920; Sobral, left bank of the Juruá River INPA 2982, INPA  
39  
40 42 2986. **Amazonas:** Altamira, right bank of the Juruá River INPA 2980, MPEG 28081;  
41  
42 43 Barro Vermelho, left bank of the Juruá River INPA 2995, INPA 2994; Colocação Vira-  
43  
44 44 Volta, left bank of the Juruá River. INPA 2983; MPEG 28073, MPEG 28074; Ilha  
45  
46 45 Paxiuba, right bank of the Juruá River MPEG 28069; Lago Vai QuemQuer, right bank  
47  
48 46 of the Juruá River MPEG 28070; Penedo, right bank of the Juruá River MPEG 28071,  
49  
50 47 INPA 2993, INPA 2990, INPA 2991, INPA 2988, INPA 2989, INPA 2992; MPEG  
51  
52 48 28072; Macaco, left bank of the Jaú River INPA 4309, INPA 4377, INPA 4378, INPA  
53  
54 49 4429, INPA 4310, INPA 4311, INPA 4312, INPA 4431; Right bank of the Jaú River,  
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3 50 above the mouth INPA 4162, INPA 4313, INPA 4379, INPA 4493, INPA 4161, INPA  
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5 51 4433, INPA 4494; Parque Nacional do Pico da Neblina, São Gabriel da Cachoeira  
6  
7 52 INPA 2516, INPA 2517; Tambor, left bank of the Jaú River INPA 4159; INPA 4160.  
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10 53 Igarapé Caititu, right bank of the Uatumã River INPA 647, INPA 740; Lago  
11  
12 54 Meduinim, left bank of the Negro River INPA 4528; Municipality of Manaus INPA  
13  
14 55 178, INPA 179, INPA 193; Rodovia AM 10 (Manaus- Itacotiara), KM 46. MPEG  
15  
16 56 7163, MPEG 7164; Projeto de Dinâmica Biológica em Fragmentos Florestais  
17  
18 57 (PDBFF), Manaus INPA 1782, INPA 1781, INPA 1784, INPA 1785; Uatumã River,  
19  
20 58 Usina Hidrelétrica de Balbina MPEG 22140, MN 26926. **Mato Grosso:** Reserva  
21  
22 59 Ecológica Cristalino, Alta Floresta UFMG 2657 (photography); Humboldt, Aripuanã  
23  
24 60 River MPEG 10175, MPEG 10176. **Pará:** Itaituba MPEG 8094, MPEG 15243, MPEG  
25  
26 61 8096, MPEG 15242. **Rondônia:** BR 364, KM 54, Porto Velho INPA 174, INPA 175;  
27  
28 62 Cachoeira Nazaré MPEG 20752, MPEG 20753; Pirocoluína MN 1212; Usina  
29  
30 63 Hidrelétrica Samuel MPEG 22555, MPEG 22563. BR 14, KM 94, Capim MPEG 8098,  
31  
32 64 8099, 15260, 8100, 15240, 15241; Curuá-una MPEG 8975, 8976, 8978, 15431, 15432,  
33  
34 65 15463, 8977, 15429, 15430; Fordlândia MN 4793; Igarapé Açú MPEG 2556; Ilha do  
35  
36 66 Arapiranga Primavera MN 42976, MN 42977; Jatobal, Marabá MPEG 8979, MPEG  
37  
38 67 8980, MPEG 8981; município de Belém MPEG 12921, MPEG 12925, MNRJ 1217,  
39  
40 68 MPEG 12923; BR 316 Belém – Brasília, KM 92; MPEG 1758; Utinga. MPEG 2664,  
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42 69 MPEG 2557, MPEG 1068, MPEG 2555, MPEG 2559; MPEG 2554, MPEG 2560,  
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44 70 MPEG 2553, MPEG 2558; Nova Timboteua MN 26889, MN 26890, MN 26891;  
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46 71 Outeiro, Maguari MPEG 25401; Santarém MPEG 3380, MPEG 8093, MPEG 10152,  
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48 72 MPEG 8095; São Miguel do Guamá MPEG 1590; Usina Hidrelétrica Cachoeira  
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50 73 Porteira INPA 399, INPA 406; INPA 651.  
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## Rebuttal letter

Dear Dr. Josef Bryja

Associate Editor

*Mammalia*

We have now completed the revision of our manuscript Mammalia.2021.0176. We are glad to hear that you found our submission of the manuscript entitled: "A NEW SPECIES OF JUPATI, GENUS *Metachirus* Burmeister 1854 (DIDELPHIMORPHIA, DIDELPHIDAE), FOR THE BRAZILIAN AMAZONIA" by Miranda, C.L. and co-authors to be of interest, and we are grateful for the comments and suggestions put forward during the review process. Below we provide detailed responses (in red) to each point raised by the editor and the reviewers (in black).

On behalf of all co-authors,  
C.L. Miranda

### Point-by-point

Comments to the Author

[Author's reply/Action taken]:

Reviewer(s)' Comments to Author:

Reviewer: 1

The authors present the description of a new species of *Metachirus*. Although the proposal is correct and the evidence supports their conclusions, I believe that there are a number of aspects that the authors should consider:

1) The authors minimize the review by Voss et al. (2019), to the point that throughout the manuscript they indicate that *Metachirus* only contains one species and that it is a genus that has not been reviewed. In turn, Voss et al. (2019) provided detailed descriptions, measurements, and figures both for nudicaudatus and myosuros.

We respectfully do not agree with the perception that we diminished the work of Voss et al. (2019). In Science, to disagree is not to diminish or belittle a work. We didn't consider the work of Voss et al. (2019) a systematic review of *Metachirus* because the part there is a preliminary taxonomic approach. Due to the greater competence of the authors and expertise in the taxonomy of marsupials, we can have a critical view and respectfully disagree when we deem it convenient, pointing out the reasons in a polite way. Then, we revised the text to make our vision clear by considering this study as a partial taxonomic review of *Metachirus*. Below, we outline for reviews points by which we consider a partial taxonomic review of *Metachirus*.

Voss et al. (2019) present a series of taxonomic decisions without the following essential parameters for a review: broader geographic coverage, sufficient numbers of

specimens examined for regions where subspecies and synonyms are recognized and need to have their status verified (see Figure 16, page 47 and Gardner & Dagosto, 2007), analyses (their phylogeny of cyt b precisely suggests the need for further study) and evidence for the presentation of table 17, page 49 for a very high number of synonyms. In addition, Voss et al. (2019) explicitly lack an examination of original descriptions, groups, and topotype samples of all available nominal taxa for *Metachirus*.

We clearly agree with the delimitation of the type species, *M. nudicaudatus* by Voss et al. (2019) for which the author had access to a good number of specimens, type and demonstrates this in the article through tables and figures. As for *M. myosuuros*, whose type-locality is Ipanema, São Paulo, we found the number of specimens examined to be low and we did not see support for delimitation in the topology presented for cit b. The delimitation hypothesis of *M. myosuuros* is very extensive and not adequately supported. Therefore, we considered sparingly even more in-depth studies *M. myosuuros* with occurrence from the Atlantic Forest to the northeast of Argentina.

2) The authors wrote that: "Some craniodental characters presented as stable by Voss et al. (2019) have since been shown to vary when a larger number of specimens were examined in Brazilian collections (C. L. Miranda, personal observation)." However, they do not provide any discussion of this aspect, nor do they show evidence to support their claims.

We removed personal observation and included topotypical samples of *M. myosuuros*. In addition, we have now included more comprehensive morphological data of *M. myosuuros* in the manuscript and in the Table 6 with external morphological characters of two subspecies and synonyms for Amazonia from the original descriptions and by Miranda Ribeiro (1936). Although our focus is on the Xingu/Tocantins interfluve species, we thought it pertinent to include this data in the sampling, including in the analyzes and figures of the specimens that have now been updated.

3) The authors should indicate, minimally, how is the morphology of the specimens they examined, especially for the Amazon, where Voss et al did not study specimens. By this I mean whether these specimens respond to the myosuuros or nudicaudatus morphotype, in the scheme of Voss et al.

*M. myosuuros* is only from the Atlantic Forest and Cerrado Atlantic Forest transition. *M. nudicaudatus* is from Guiana Shield, in agreement with the delimitation of Voss et al. (2019). In the manuscript we highlight which specimens refer to the type species, *M. nudicaudatus* and the new species (see Fig 1, list of specimens of the new species in the description and of the other two species in the list of examined specimens, appendix 1). The focus of the present study does not cover a genus review.

4) The authors compare the new species only with *M. nudicaudatus*. Although this is partially correct, given the phylogenetic results, a better contextualization of the findings would be desirable, especially a comparison with other geographically close

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2  
3 forms, such as myosuros. This is important because the morphology of the new  
4 species is reminiscent of that of myosuros.  
5

6 We fully agree. Therefore, we now include descriptions of *M. nudicaudatus* and *M.*  
7 *mysourus*, as described above. Whereas we do not go into extensive descriptions: we  
8 provide amended diagnosis and insertion in the comparative table and boards.  
9

10  
11  
12 5) Please include a mention to the book: Marsupiais do Brasil: Guia de identificação  
13 com base em caracteres morfológicos externos e cranianos, by Michel Barros Faria,  
14 Rayque de Oliveira Lanes and Cibele Rodrigues Bonvicino. Amélie Press, São  
15 Caetano do Sul. 2019. 84 pp. ISBN (Brazil) 978-85-53082-14-8  
16

17 We consider this book in Portuguese to be very important for beginning  
18 mastozoologists and that doesn't detract from its merit in any way. Your approach is  
19 targeted to this public. In addition, we avoid using non-English works other than original  
20 descriptions. We have other more in-depth chapters in Portuguese for Brazilian  
21 marsupials (e.g., Rossi et al., 2011, 2012, 2015). Another relevant point is that the  
22 delimitation of *M. nudicaudatus* in this book differs from that presented in this article  
23 and from Voss et al. (2019) without any justification for delimiting the type species for  
24 regions south of the Amazon River (Figure 38, page 48). We're not belittling the book,  
25 but we just don't think it's convenient to use it in this formal description article.  
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31 6) other comments were made directly on the manuscript (attached).  
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33 We review all suggestions. Many thanks.  
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37 Reviewer: 2

38 Comments to the Author

39 This work partially contributes to the knowledge of an apparent complex of species. I  
40 consider it correct to consider this population as a new species based on genetic  
41 divergence, and the few morphological characters. I also consider it important to add  
42 specimens in future reviews of the Amazonian *Metachirus*. The consideration of  
43 myosuros in the Atlantic forest (and possibly northeast Argentina) is also correct.  
44 Finally, I congratulate the authors for the chosen etymology.  
45  
46

47 Thank you!  
48  
49  
50

51 Associate Editor's Comments to Author:

52 Associate Editor: Bryja, Josef

53 Dear Authors,

54 I received two reviews of your manuscript. Both of them acknowledge the value of your  
55 work, but at the same time they provide a relatively high number of critical comments  
56 (see also attached files with the comments). Please consider all of them during the  
57 revision, I am sure you will find the suggestions useful. The revised version will be very  
58 likely sent again to both reviewers, so please address all comments carefully.  
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4 Josef Bryja, Associate Editor  
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8 \*\*\* AUTHOR REVISION CHECKLIST: IMPORTANT NOTES BY THE EDITORIAL  
9 OFFICE TO BE CHECKED PRIOR TO RETURNING THE REVISED MANUSCRIPT:  
10 1) File format of title page, main textbody, references list, and figure captions: WORD.  
11

12 **We have reviewed these points.**  
13  
14

15 2) File format of Tables: WORD or, if necessary, in ECXEL format.  
16

17 **We have included the tables in excel.**  
18  
19

20 3) File format of Figures: Graphics fomate (e.g., jpg, eps or tif format); line drawings with  
21 a resolution of 1200 dpi; photos with a resolution of 300 dpi.  
22

23 **We have reviewed these points.**  
24  
25

26 4) Decimal values: Must be given with decimal points (example: 1.2, not: 1,2).  
27

28 **We did not find any comma values in the manuscript, but we format all decimal  
29 numbers by also standardizing the number of digits after the comma.**  
30  
31

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38 **All the Figures are from our own authorship.**  
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42 captions and in the references list the Harvard style as per attached sample sheet.  
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48 before the References section.  
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50 Thank you.  
51

52 **We include a Statement on ethics before the reference list. Thank you so much for your  
53 attention.**  
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