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1           **How many Pygmy Marmoset (*Cebuella* Gray, 1870) species are there? A**  
2           **taxonomic re-appraisal based on new molecular evidence**

3

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25 **Abstract**

26

27 The pygmy marmoset, *Cebuella pygmaea*, the smallest of the New World  
28 monkeys, has one of the largest geographical distributions of the Amazonian  
29 primates. Two forms have been recognized: *Cebuella pygmaea pygmaea* (Spix,  
30 1823), and *C. p. niveiventris* Lönnberg, 1940. In this study, we investigated if the  
31 separation of pygmy marmosets into these two clades can be corroborated by  
32 molecular data. We also examine and compare coloration of the pelage in light of the  
33 new molecular results. We analyzed the mtDNA cytochrome *b* gene and, for the first  
34 time for any Neotropical primate, we used a reduced representation genome  
35 sequencing approach (ddRADseq) to obtain data for recently collected,  
36 geographically representative samples from the Rio Japurá, a northern tributary of the  
37 Rio Solimões and from the Javari, Jutaí, Juruá, Madeira and Purus river basins, all  
38 tributaries south of the Solimões. We estimated phylogenies and diversification times  
39 under both maximum likelihood and Bayesian inference criteria. Our analysis showed  
40 two highly supported clades, with intraclade divergences much smaller than interclade  
41 divergences, indicating two species of *Cebuella*: one from the Rio Japurá and one to  
42 the south of Solimões. The interpretation of our results in light of the current  
43 taxonomy is not trivial however. Lönnberg stated that the type of Spix's pygmy  
44 marmoset (type locality 'near Tabatinga') was obtained from the south of the  
45 Solimões, and his description of the distinct *niveiventris* from Lago Ipixuna, south of  
46 the Solimões and several kilometres east of Tabatinga, was based on a comparison  
47 with specimens that he determined as typical *pygmaea* that were from the upper Rio  
48 Juruá (south of the Solimões). As such it remains uncertain whether the name

49 *pygmaea* should be applicable to the pygmy marmosets north of the Rio Solimões  
50 (Tabatinga type locality) or south (near Tabatinga but across the Solimões). Finally,  
51 our analysis of pelage coloration revealed three phenotypic forms: 1) south of the Rio  
52 Solimoes, 2) Eirunepé-Acre, upper Juruá basin; and 3) Japurá. More samples from  
53 both sides of Solimões in the region of Tabatinga will be necessary to ascertain the  
54 exact type locality for Spix's *pygmaea* and to resolve the current uncertainties  
55 surrounding pygmy marmoset taxonomy.

56

57 **Key words:** Amazonia, *Cebuella*, phylogenomics, taxonomy

58 **1. Introduction**

59

60 Weighing around 119 g (range 85–140 g; Soini, 1993), the pygmy marmoset is  
61 the smallest of the New World monkeys. In spite of its diminutive size, it has one of  
62 the largest geographical distributions for a single Amazonian primate (Fig. 1). Pygmy  
63 marmosets are sister to the Amazonian marmosets (*Mico*). Molecular genetic data  
64 have indicated that the two genera diverged during the early Pliocene (Perelman et al.,  
65 2011; Schneider et al., 2012; Buckner et al., 2015), but, based on fossil material  
66 studied by Marivaux et al. (2016), the divergence would appear to have been earlier,  
67 in the early Late Miocene about 11 Ma. Pygmy marmosets are not sympatric with the  
68 Amazonian marmosets; the Rio Madeira, a right bank tributary of the Amazon,  
69 separates *Cebuella* from *Mico*, the latter restricted to the east and the former to the  
70 west of the river. Pygmy marmosets are small exudativore-insectivores, sharing with  
71 all other marmosets (*Mico* and *Callithrix*) dental adaptations to exploit tree gums; a  
72 food that makes up a significant portion of their diet (Kinzey et al., 1975; Ramirez et  
73 al., 1977; Soini, 1982).

74 The pygmy marmoset was first described by Johann Baptist von Spix in 1823,  
75 with the type locality “near Tabatinga, Rio Solimões, Amazonas, Brazil.” Tabatinga is  
76 a small town on the north (left) bank of the Rio Solimões (upper portion of the  
77 Amazon), near the Colombian border. In 1940, Einar Lönnberg described a  
78 subspecies from the Lago Ipixuna, on the south bank of the Rio Solimões, which he  
79 distinguished as having a paler and more greyish back than *C. p. pygmaea*, and  
80 underparts (chest, belly, and inner surface of arms and legs) that were entirely white.

81 Although the geographic ranges of the two subspecies were poorly defined—  
82 *niveiventris* was known only from its type locality—this taxonomy was subsequently  
83 recognized by Cruz Lima (1945), Cabrera (1957), Vieira (1955), Hill (1957), Napier  
84 and Napier (1967), and Napier (1976) (Fig. 1). Hershkovitz (1977), however, did not  
85 accept the validity of *C. p. niveiventris*, concluding that the color of the underparts is  
86 individually and locally variable. Hershkovitz's (1977) conclusion that *C. pygmaea*  
87 was, *contra* Lönnberg, monotypic was accepted by Groves (1989), but Van  
88 Roosmalen and Van Roosmalen (1997) argued again for the validity of Lönnberg's  
89 subspecies, based on pygmy marmosets with white underparts that they found in  
90 localities on the west bank of the lower Rio Madeira. Groves (2001, 2005) concluded  
91 that the color distinction of the underparts *was* consistent and sufficiently diagnostic  
92 to justify the separation. Based on this alone, Van Roosmalen and Van Roosmalen  
93 (1997) proposed that the Rio Solimões was a barrier to the dispersal of the two taxa,  
94 separating *pygmaea* to the north and *niveiventris* to the south. Rylands et al. (2009)  
95 pointed out, however, that the situation was more complicated than that. Lönnberg  
96 (1940) had distinguished *niveiventris* by comparing it to what he believed was typical  
97 *C. pygmaea* Spix, from the upper Rio Juruá, south, not north, of the Solimões.

98 In this study, we use a molecular phylogenetics approach to clarify the current  
99 taxonomy of pygmy marmosets, taking advantage of the availability of newly  
100 collected samples from the Japurá, Javari, Jutai, Juruá, Madeira and Purus river  
101 basins, and thus covering distant parts of the species' distribution in Brazil, allowing  
102 us to further assess the diversity of this lineage. We followed previous studies that  
103 have used mtDNA markers to investigate the relationships of pygmy marmosets but  
104 also took a genomic approach (ddRADseq); the first use of genomics to investigate

105 the phylogenetic relationships of a group of New World primates. We also examine  
106 and compare the pelage of these newly collected specimens with material studied by  
107 Lönnberg deposited in Stockholm's Natural History Museum.

108

## 109 **2. Methods**

110

111 For the molecular analysis we used recently collected, geographically and  
112 phenotypically representative samples of *C. pygmaea* from the Japurá, Javari, Jutai,  
113 Juruá, Madeira, and Purus river basins, totaling 14 individuals (Table 1). For  
114 outgroups, we used the genus *Aotus*, representing the monotypic Aotidae, and 12  
115 additional species representing all callitrichid genera except for the lion tamarins  
116 *Leontopithecus*. We chose outgroups to provide multiple calibration points, sister-  
117 species pairs, and species pairs that are separated by the Rio Amazonas-Solimões  
118 (Table 1). For all specimens, we generated new sequence data, except for *Callithrix*  
119 *jacchus* and *Aotus nancymae* where the complete mitochondrial cytochrome *b* gene  
120 and homologous genomic regions were extracted from the complete genomes  
121 deposited in Genbank.

122 Total genomic DNA was extracted from muscle tissues preserved in 95% ethanol  
123 using the standard phenol-chloroform extraction protocol of Sambrook et al. (1989).  
124 We amplified the complete mitochondrial cytochrome *b* (*cyt b*) gene by polymerase  
125 chain reaction (PCR) with the primers MonkeyGluF1 (5'-  
126 CCATGACTAATGATATGAAAARCC-3') and MonkeyProR1 (5'-  
127 AGAATSTCAGCTTTGGGTGTTG-3') developed for this study. PCR products were  
128 purified using ExoSap (Werle et al. 1994) and subjected to fluorescent dye-terminator

129 (ddNTP) sequencing following the manufacturer's recommended protocol for BigDye  
130 sequencing chemistry (Applied Biosystems) and using the primers MonkeyCytbF2  
131 (5'-GGATCAARYAAYCCRTCAGG-3'), MonkeyCytbR1 (5'-  
132 GCBCCTCAGAADGATATTTG-3') and MonkeyCytbR2 (5'-  
133 CGTAGRATTGCRTATGCRAA-3') developed for this study. Subsequent to the  
134 cycle sequencing reaction, the products were precipitated with 100% Ethanol / 125  
135 mM EDTA solution, re-suspended in Hi-Di formamide, and resolved on an ABI  
136 3130xl automatic sequencer (Applied Biosystems). Sequences were assembled,  
137 edited, aligned and trimmed using the software Geneious v8.1.8; alignment was done  
138 using the MUSCLE (Edgar, 2004) alignment plugin in Geneious v8.1.8 and  
139 confirmed by eye.

140 We also performed a partial representational genome sequencing using the double  
141 digest RAD sequencing protocol (ddRADseq) (Peterson *et al.*, 2012). This protocol  
142 was adapted to allow simultaneous digestion and adaptor ligation, and for use on the  
143 IonTorrent PGM (<https://github.com/legalLab>). Briefly, 200 ng of genomic DNA of  
144 each individual was digested with SdaI and Csp6I restriction enzymes (ThermoFisher)  
145 and the IonTorrent P and A adaptors were linked to the digested fragments, all in one  
146 step. The fragments were enriched via PCR. The A adaptor is a "Y divergent" (Coyne  
147 *et al.* 2004), resulting in the enrichment of only those ddRAD fragments with one P1  
148 and one A adaptor. Furthermore, the A adaptor contains a unique molecular barcode  
149 for identification of individuals. Following the PCR enrichment, we selected  
150 fragments in the range of 320 to 400 bp using the Pippin Prep (Sage Science). Based  
151 on the analysis of complete primate genomes deposited in Genbank, we expected to  
152 observe ~12,000 ddRAD fragments in the range of 320 to 400 bp. This information

153 was then used to optimize the number of individuals to be analyzed in one run of the  
154 IonTorrent PGM.

155 Finally, we developed a Python script to perform an *in silico* double digest of the  
156 complete genomes of *Callithrix jacchus* and *Aotus nancymae* available in Genbank.  
157 Using this script we extracted all fragments in the size range of 320 to 400 bp  
158 generated by *in silico* restricting the genomes with the SdaI and Csp6I restriction  
159 enzymes whereby each fragment had to have both restriction enzyme cuts. The  
160 complete ddRADseq protocol, scripts for estimating number of ddRAD fragments  
161 within a given size range, and scripts for extracting ddRAD fragments from published  
162 genomes are available on GitHub (<https://github.com/legalLab>).

163 Sequencing reads were processed using the pyRAD pipeline (Eaton, 2014).  
164 During *de novo* assembly, we used a minimum coverage of 6x per locus/allele,  
165 assembling all fragments in the 320 to 400 bp range. Following demultiplexing and  
166 extraction of loci using the above criteria (steps 1–2 of the pyRAD pipeline), we  
167 included the ddRAD loci of *Callithrix jacchus* and *Aotus nancymae* extracted from  
168 complete genomes, and proceeded with clustering of loci across individuals, and the  
169 generation of datasets for analyses (pyRAD steps 3–7). In the final datasets a locus  
170 was included only if it was present in at least 50% of individuals.

171 We jointly estimated phylogeny and diversification times under an uncorrelated  
172 lognormal relaxed clock model implemented in the program BEAST v1.8.2 using soft  
173 calibrations obtained from Perelman et al. (2011). We used normal priors with  
174 standard deviations corresponding to those reported in Table 2 of Perelman et al.  
175 (2011). Additionally, we cross-validated our topology under both the maximum  
176 likelihood and Bayesian inference criteria in RAxML (Stamatakis, 2014) and

177 ExaBayes (Aberer et al., 2014), respectively. Robustness of the RAxML analyses was  
178 accessed via 1000 bootstrap replicates, while in the ExaBayes analyses we ran two  
179 independent chains each 3 million generations long, sampling 9000 topologies  
180 following burnin of first 10% of the trees. We also cross-validated our divergence  
181 time estimates using the *chronos* function in the R package APE (Paradis et al., 2004),  
182 using the mean calibration points from Perelman et al. (2011), and the 9000  
183 topologies generated in ExaBayes.

184 We examined the coloration pattern of the pelage of all specimens used in this  
185 study and compared them with photos of the specimens used by Lönnberg to describe  
186 *C. p. niveiventris* as distinct from *C. pygmaea pygmaea*.

187 This research adhered to Brazilian laws that govern primate research as well as the  
188 American Society of Primatologists' principles for the ethical treatment of primates.  
189 Research permits were granted through relevant Brazilian authorities (FUNAI and  
190 IBAMA/ICMBio, and institutional IACUC committees—permit numbers are listed in  
191 the acknowledgments).

192

### 193 **3. Results**

194

#### 195 *3.1. Phylogenetic study*

196 We sequenced the complete mitochondrial cytochrome *b* gene (1,148 bp) from 24  
197 specimens, and extracted from Genbank the complete cytochrome *b* gene of *Callithrix*  
198 *jacchus* and *Aotus nancymaae*. Next generation sequencing on the IonTorrent PGM  
199 produced between 30 and 50 thousand usable reads per species after processing in  
200 pyRAD (Eaton, 2014). On average, between 7.5 and 14.3 thousand unique loci with

201 an average of 3.5x coverage were observed in each individual in the 320–400 bp  
202 range; the number of loci obtained by data-mining the genomes of *Callithrix jacchus*  
203 and *Aotus nancymae* was 14170 and 14201, respectively. Filtering alleles and loci to  
204 a minimum 5x coverage, between 850 and 1400 loci with an average coverage of 7.3–  
205 8.3x coverage were retained. The probability of observing a spurious allele was less  
206 than  $1e^{-21}$ . In the final dataset, where a locus had to be present in at least 50% of the  
207 individuals (Streicher et al., 2016), 954 loci were retained. These 954 loci represented  
208 954 unlinked SNPs and had 20891 variable sites of which 9764 were parsimony  
209 informative. Total alignment length was 307673 bp.

210 Analysis in BEAST of the cytochrome *b* as well as the ddRADseq alignments  
211 resulted in robustly supported phylogenetic hypotheses (Figs. 2 and 3) that were  
212 identical except for phylogenetic relationships among *Cebuella p. pygmaea* and *C. p.*  
213 *niveiventris* individuals (Figs. 2 and 3). Divergence time estimates were also similar  
214 in the mtDNA and ddRAD phylogenies, however, in general, intermediate divergence  
215 times were older in the mtDNA vs. the ddRAD phylogenies. Phylogenies generated  
216 under the maximum likelihood and Bayesian inference paradigms implemented in  
217 RAxML and ExaBayes, respectively, were identical, and were identical to those  
218 inferred from BEAST (Drumond et al 2012) analyses. Divergence time estimates  
219 inferred in BEAST and using the *chronos* function in the R package APE (Paradis et  
220 al., 2004) was also comparable.

221 Notably, all phylogenetic analyses showed a split between pygmy marmosets  
222 from the Japurá and those from the south bank of the Rio Amazonas-Solimões. These  
223 two clades were highly supported with BI = 1.0 and ML bootstrap = 100. The  
224 estimated divergence time between these two clades was 2.2452 (95% HPD 1.7059–

225 2.9606) MYA, while the divergence time between the sister taxa *Saguinus bicolor* and  
226 *S. martinsi* was estimated at 0.8191 (95% HPD 0.5240–1.1150) MYA. Since some  
227 authors consider *Saguinus martinsi* to be a subspecies of *S. bicolor*, we also estimated  
228 the divergence of *Saguinus bicolor* clade from the *Saguinus midas* sister clade at  
229 1.6319 (95% HPD 1.1964–2.2265) MYA. Although they are not necessarily sister  
230 taxa (Buckner et al., 2014), the estimated time of divergence between *Mico*  
231 *humeralifer* and *M. argentatus* distributed on the left and right banks of the Rio  
232 Tapajós, respectively, was estimated at 1.1889 (95% HPD 0.8142–1.6892) MYA. We  
233 also included night monkeys of the genus *Aotus*. The genus is composed of two main  
234 clades, the gray- and the red-necked species occurring north and south of the Rio  
235 Amazonas-Solimões, respectively. The distribution of these groups parallels that of  
236 the supposed (see below) distribution of *Cebuella* in having distinct forms either side  
237 of the Rio Amazonas-Solimões. The divergence of the *Aotus vociferans* clade from  
238 the *A. nigriceps/nancymae* sister clades found north and south of the Rio Amazonas-  
239 Solimões, respectively, was estimated at 2.2584 (95% HPD 1.6370–3.0906) MYA.

240

### 241 3.2. Pelage coloration

242 With regard to overall pelage coloration, we identified three broad patterns for  
243 specimens in the Brazilian Amazon: 1) south of the Rio Solimões, 2) Eirunepé-Acre,  
244 upper Juruá basin, southwestern Brazilian Amazon; and 3) the Japurá basin, north of  
245 the Rio Solimões (see Fig. 4 and Table 2 for full descriptions). To summarize, the  
246 first, along the south bank of the Rio Solimões-Amazonas has white, pale, buff to  
247 whitish undersides, including the inner arms and legs. The throat and chest are  
248 ochraceous orange, but in some the throat is buffy to whitish in the centre. We are

249 provisionally referring to them as *C. cf. niveiventris* Lönnberg (Figs. 5 and 6). In the  
250 second pattern, from the upper Juruá extending west into Peru and Ecuador, the  
251 underparts are darker, dull yellowish-brown tawny, with a dull ochraceous orange to  
252 brownish throat (Fig. 7). Lönnberg (1940) believed that these pygmy marmosets were  
253 typical *pygmaea*. Our current molecular data suggest however, that they are  
254 phylogenetically part of the same large clade containing all the remaining white or  
255 pale chested specimens from south of the Solimões included in our analysis (although  
256 they do form a separate subclade in the ddRAD tree, see Figs. 2 and 3). We are  
257 provisionally referring to them as *C. cf. pygmaea* (Spix). The third pattern, from the  
258 middle Japurá, north of the Rio Solimões in Brazil, have dark, orangey brown  
259 underparts, and we are provisionally referring to them as *C. aff. pygmaea* (Spix)  
260 (Figs. 5, 8 and 9).

261

## 262 **4. Discussion**

263

### 264 *4.1. Phylogenetic study*

265 The results of both our time trees showed two distinct, reciprocally monophyletic  
266 groups of *Cebuella*; one on the right bank of the Japurá and the other south of the Rio  
267 Amazonas-Solimões. They diverged from one another in the Pliocene/Pleistocene  
268 boundary (2.2452 [95% HPD 1.7059–2.9606] MYA). Our time trees also show  
269 divergence times of selected outgroup sister taxa or clades, helping put the split  
270 between the two *Cebuella* clades into a broader perspective within the platyrrhines.  
271 We recovered a more recent divergence time between the sister taxa *Saguinus bicolor*  
272 and *S. martinsi* (Middle Pleistocene – 0.8191 [95% HPD 0.5240–1.1150] MA

273 divergence) and between *Mico argentatus* and *M. humeralifer* found on the opposite  
274 banks of the Rio Tapajós (Middle to Early Pleistocene – 1.1889 [95% HPD 0.8142–  
275 1.6892] MYA). *Mico argentatus* and *M. humeralifer* are unlikely to be sister taxa  
276 (Buckner et al. 2014), and thus, sister taxa divergences within the genus *Mico* are  
277 likely to be even more recent. The two *Cebuella* clades diverged at around the same  
278 time as the dwarf marmoset *Callibella humilis* from the Amazonian marmosets of the  
279 genus *Mico*. The divergence between the two *Cebuella* sister clades is older than that  
280 of any other callitrichid sister-species pairs (see also Buckner et al., 2015). Last but  
281 not least, the separation and timing of the separation of the *Cebuella* sister clades is  
282 paralleled by species of the genus *Aotus* where the grey- and the red-necked species  
283 clades occur north and south of the Solimões-Amazonas, respectively. These clades  
284 also diverged at the Pliocene/ Pleistocene boundary (2.2584 MA; 95% HPD 1.6370–  
285 3.0906 MA), and thus it is highly probable that the same historical event, such as the  
286 formation of the modern course of the Rio Solimões-Amazonas, resulted in the  
287 isolation of primate populations on either side of the river, eventually leading to  
288 vicariant speciation.

289

#### 290 4.2 Pelage coloration

291

292 Hershkovitz (1977) concluded that variation in pelage coloration did not allow for  
293 the identification of more than one taxon of the pygmy marmoset, a conclusion that  
294 was unsettling considering its very small size and its very large geographic  
295 distribution. As is frequently the case, Hershkovitz was hampered by too few  
296 specimens (many of them of unknown or indefinite provenance), making geographic

297 patterns difficult or impossible to detect. This is exacerbated in the case of *Cebuella*  
298 by the uncertainty concerning the type locality of Spix's *pygmaea* (in particular  
299 regarding from which side of the Rio Solimões the type was collected), and the fact  
300 that it is a mounted individual in poor condition, hindering its comparison with other  
301 specimens (Elliot, 1913; and Fig. 8).

302 The type locality is "vicinity of Tabatinga on the banks of the River Solimões,"  
303 Tabatinga is a town on the northern (left) bank of the Solimões (locality 3, Fig. 1),  
304 and Cruz Lima (1945) complemented the type locality description pointing this out.  
305 The south bank of the Solimões is also, however, in the vicinity of Tabatinga, and  
306 Lönnberg (1940, pp.21–22) stated that "The type locality of the main species is  
307 indicated by Spix to be Tabatinga, on the southern side of Rio Solimões near the  
308 junction with Rio Javary", presumably near the town of Benjamin Constant.  
309 Unfortunately, Lönnberg did not say where Spix provided this more precise type  
310 locality. We checked the account of the Spix and Martius expedition (Spix and  
311 Martius, 1938), but found no more specific reference to the type locality. The travel  
312 diary refers mostly to Spix's interaction with the local Tikuna tribe and the time spent  
313 with them. Given it is common for indigenous peoples to keep pygmy marmoset as  
314 pets, it is likely that the type of *pygmaea* was a pet living in one to the villages visited  
315 by Spix. In this case, determining the precise location where the type specimen  
316 originally came from will be difficult, although Lönnberg must, of course, have had  
317 access to a written source.

318 Van Roosmalen and Van Roosmalen (1997) attributed all pygmy marmosets north  
319 of the Rio Solimões to *C. p. pygmaea*, even though Lönnberg (1940) in describing  
320 *niveiventris* as a distinct subspecies had compared it with six specimens that he

321 deemed typical *C. pygmaea* from the upper Rio Juruá. south of the Rio Solimões.  
322 They were collected by A. M. Olalla from João Pessoa, Rio Juruá, in 1936 (one  
323 female collected on July 15, and two males and three females collected on July 24,  
324 1936); today in the Natural History Museum, Stockholm. João Pessoa, also listed by  
325 Cruz Lima (1945), Vieira (1955), Hill (1957) and Cabrera (1957), is today called  
326 Eirunepé, and is on the left bank of the upper Rio Juruá 6°39'36"S, 69°52'26"W),  
327 opposite its confluence with the Rio Tarauacá (Paynter and Traylor, 1991) (see Fig. 1,  
328 locality 17). Lönnberg (1940) believed that the six specimens from there conformed  
329 with the description by Spix (1823) of *Cebuella pygmaea*:

330 “The colour of these specimens agrees with the original description given  
331 by Spix, and repeated by later authors. The lower side is in these  
332 specimens dirty yellowish brown, sometimes with an irregular and narrow  
333 stripe of greyish or even dirty whitish extending some way along the  
334 middle of the belly” (pp.20–21).

335 Lago Ipixuna, the type locality of *niveiventris*, is approximately 700 km north-east  
336 of Eirunepé, on the south bank of the Rio Solimões, just west of the Rio Coarí, and  
337 about midway between the rios Tefé and Purus. Hence the vernacular names of  
338 western (*pygmaea*) and eastern (*niveiventris*) pygmy marmosets used by Hill (1957)  
339 and Napier (1976).

340 Van Roosmalen and Van Roosmalen (1997) attributed all pygmy marmosets south  
341 of the Rio Solimões to *niveiventris* based on five localities along the west (left) bank  
342 of the lower Rio Madeira (four sightings in the wild and a pet):

343 “All animals observed in the wild, as well as one live specimen obtained  
344 in the community of Democracia, showed the typical characters of the  
345 *niveiventris* subspecies” (p.4).

346 Van Roosmalen and Van Roosmalen’s (1997) “typical characters”, were “sharply  
347 contrasting whitish chest, belly and inner surface of arms and legs.” (p.4).

348 Hershkovitz (1977, p.464) reported on pygmy marmosets captured “near  
349 Tabatinga” in 1963 that were received by the California National Primate Research  
350 Center, Davis. The marmosets were short-lived, and their skins and skulls are now in  
351 the U. S. National Museum (Smithsonian Natural History Museum), Washington, DC.  
352 Twenty-four individuals (NMNH 336302-339325) caught from 23 February to 8 April  
353 1963 mostly had buffy underparts, but the color varied from nearly white to orange. In  
354 fact, all but two of the specimens are very similar in pelage coloration; all have buffy  
355 (greyish or off white) underparts and distinct black speckling on the back and rump,  
356 and a reddish-brown tail with black rings (see Fig. 10). Specimen 336302 (female)  
357 was noticeably whiter on the chest and abdomen and inner legs and arms than the  
358 others (Hershkovitz’s nearly white underparts) (Fig. 11). The dorsum of specimen  
359 336304 (female) is more uniform brownish with little black speckling, and the nape  
360 and mantle were the same colour as the back to the rump. Its ventrum is closer to a  
361 pale ochraceous (Hershkovitz’s orange underparts) (Fig. 12). This “batch” may well  
362 have originated from three localities (336302 and 336304 perhaps as pets purchased  
363 in Tabatinga), but where exactly, and which side of the Rio Solimões, has not been  
364 recorded.

365 A second batch of 12, captured in June and July 1963 (NMNH 337319-337330),  
366 were all similar, with underparts that were mainly tawny but some more pale buff and

367 orange colored (Fig. 13). Hershkovitz observed that “the difference in color of  
368 underparts between the two batches is striking” (p.464). Hershkovitz suggested that  
369 the “near Tabatinga” series was made up of specimens from several localities; that the  
370 January, pale-bellied individuals (Fig. 10) may have originated from a single locality,  
371 ‘perhaps’ (1977, p. 464) on the southern bank of the Amazon, and that the saturate-  
372 bellied June–July specimens (Fig. 12) ‘likely’ (1977, p. 464) came from the opposite,  
373 north, Tabatinga bank.

374       According to Hershkovitz (1977), two of three specimens collected by C.  
375 Kalinowski in 1957 from Santa Cecília on the Río Manítí, a small tributary south of  
376 the Río Amazonas just below Iquitos and above the mouth of the Río Napo in Peru,  
377 are similar to *niveiventris* (locality 35, Fig. 1). Of three specimens from the Río  
378 Yaquerana (locality 40, Fig. 1) and upper Javará, one has a white ventrum like  
379 *niveiventris*, a second likewise, but with more grey on the undersurface of the limbs,  
380 and the ventrum of the third was dominantly buffy. Specimens from Apayacu, north  
381 of the Río Amazonas, below the mouth of the Río Napo (locality 36, Fig. 1), Intillama  
382 (close to San Pablo, Río Aguarico, locality 27), and Boca Río Curaray (locality 32) a  
383 right bank tributary of the Río Napo have underparts that conform to Lönnberg’s  
384 *pygmaea*. Specimens from the Rio Copataza (locality 30) and Montalvo (locality 31)  
385 on the Río Bobonaza (localities in the Pastaza basin, north of the Río Marañón) are  
386 intermediate, with greyish and buffy underparts.

387       The specimens we examined in this study also conform with Hershkovitz’ (1977)  
388 findings with respect to specimens found along both banks of the Solimões, i.e., there  
389 are some variability on the coloration of the underparts going from predominantly  
390 white to orange or grey irrespective of the river bank. In fact, our specimen CCM19,

391 collected in Benjamin Constant, right across the river from Tabatinga on the mouth of  
392 the Javari and on the south bank of the Solimões shows some orange pelage in its  
393 underparts (Fig. 14a). Conversely, an individual we examined at the Museu Paraense  
394 Emílio Goeldi in Belém, Brazil, collected in the Mamirauá Sustainable Development  
395 Reserve on the left bank of the Solimões, opposite the town of Tefé, shows very white  
396 pelage in its underparts (Fig. 14b) (unfortunately, no tissue from this Mamirauá  
397 specimen was available for genetic analysis).

398       Regarding the Juruá specimens that Lönnberg (1940) found to be distinct from  
399 *niveiventris*, believing they were typical *pygmaea*, Carvalho (1957) examined a  
400 specimen from Seringal Oriente, on the right bank of the upper Rio Juruá in the state  
401 of Acre, Brazil (locality 19, Fig. 1), and recorded that the color of the ventrum had a  
402 thin, whitish median band on the underside, with the hairs on the ventrum changing to  
403 yellowish and black towards the flanks; a pattern also found in the Olalla specimens  
404 from João Pessoa (Eirunepé) (Fig. 7). Lönnberg (p.22) observed that “[O.] Thomas  
405 has also recorded the typical species [*pygmaea*] as far west as Pebas, Peru.” (locality  
406 37, Fig. 1). Specimens NMNH 16610 from Sarayacu, Peru (locality 41, Fig. 1) and  
407 NMNH 267506 from Huachi, Oriente, Río Pastaza, Ecuador (exact locality unknown,  
408 but evidently somewhere in the region of localities 30 and 31, Fig. 1) are similar to  
409 the João Pessoa (Eirunepé) specimens in Stockholm.

410       The phenotype typified in the Eirunepé specimens collected by A. M. Olalla,  
411 evidently occurs, therefore, in other localities on the upper Juruá (Seringal Oriente  
412 [locality 19] and Ocidente [locality18]), besides Pebas (Río Napo, Peru [37]),  
413 Sarayacu (41) and the Río Pastaza, Ecuador. This group is distinct from specimens  
414 north of the Solimões and perhaps Napo, and may be a distinct taxon, but although the

415 three Juruá specimens are grouped in the molecular phylogenetic analyses, their  
416 separation from other lineages south of the Solimões could be very recent.

417

#### 418 *4.3. Distribution and taxonomy of the pygmy marmoset*

419 *Cebuella* occurs south of the Rio Japurá-Caquetá, in Colombia, extending north to  
420 the right bank of the Río Orteguzza, a left bank tributary of the upper Caquetá (Izawa,  
421 1979; Defler, 2004). In Ecuador, it occurs throughout the eastern tropical Amazon  
422 lowlands, in the basins of the Marañón tributaries, the rios Tigre, Pastaza, Napo and  
423 Aguarico. In Peru, it occurs east from the Rio Santiago and the Río Cumbaza, and  
424 from the east bank of the Rio Huallaga, above the mouth of the Cumbaza, to the  
425 Ucayali valley, and the ríos Pachitea, Urubamba, and Manu of the upper reaches of  
426 the Madre de Dios (Aquino and Encarnación, 1994). In Bolivia, it has been recorded  
427 just south of the Rio Muyumanu (Buchanan-Smith et al., 2000), but it is otherwise  
428 believed to be restricted to the north of the Río Tahuamanu in the western Pando and  
429 the Rio Abunã along the northern border of Bolivia to the east. (Izawa, 1979; Izawa  
430 and Bejarano, 1981; Rylands et al., 1993; Buchanan-Smith et al., 2000; Porcel et al.,  
431 2010). In Brazil, it is restricted to the left banks of the rios Abunã and Madeira, and  
432 south of the rios Solimões-Amazonas and Japurá (Rylands et al., 1993; Bicca-  
433 Marques and Calegari-Marques, 1995; Van Roosmalen and Van Roosmalen, 1997;  
434 Messias et al. 2011).

435 Defler (2004) and Van Roosmalen and Van Roosmalen (1997, 2016) indicated  
436 that *pygmaea* was the form occurring north of the rios Marañón and Amazonas-  
437 Solimões, east from the mouth of the Rio Japurá, south of the ríos Japurá-Caquetá and  
438 Caguán, and west as far as the Río Pastaza, and that *niveiventris* was the form south of

439 the Rio Amazonas-Solimões, west from the Rio Madeira to the lower Rio Huallaga  
440 and the Rio Ucayali in Peru, and north of the Río Tahuamanu into Bolivia. Our  
441 genetic analyses confirm the notion of two distinct taxa occupying these general range  
442 limits with their lineages separating in the late Pliocene or early Pleistocene.  
443 However, with the data currently available we cannot say *pygmaea* is a valid name for  
444 animals found either in the north or in the south of the Solimões for all the reasons  
445 already outlined above.

446       Unfortunately, in this study we had no tissue samples available for animals found  
447 on the immediate vicinity of the left (north) bank of the Solimões and near Tabatinga.  
448 Determining the phylogenetic relationship between these animals and our existing  
449 samples would help shed light on the current uncertainties surrounding the taxonomy  
450 of *Cebuella*.

451       One possibility we envisage, and that could complicate matters further, is that we  
452 find that pygmy marmosets inhabiting forests adjacent to both banks of the Solimões  
453 are genetically similar. This would support Hershkovitz' (1977) findings and our own  
454 observations that there is great phenotypic variability and overlap in color morphs in  
455 individuals collected in this area of Amazonia.

456       Such findings would appear surprising in light of what we know about Amazonian  
457 biogeography and the role of large Amazonian rivers as important biogeographical  
458 barriers to primates (Boubli et al., 2015). On the other hand, meandering, white-water  
459 rivers such as the Solimões are known to be dynamic and to change their course often,  
460 forming oxbow lakes and, in the process, transferring pieces of land from one side of  
461 the river to the opposite bank (Ayres & Clutton-Brock, 1999, Gascon et al., 2000).  
462 Animals that happen to be present on these pieces of land, in particular animals with

463 small home ranges such as pygmy marmosets, can be passively transferred from one  
464 bank of the river to the other. Such river dynamics would have favored intermittent  
465 contact between pygmy marmosets populations on opposite banks of the Solimões,  
466 potentially allowing them to interbreed. Only further sampling along the Solimões and  
467 further inland will help clarify this issue.

468

## 469 **Conclusions**

470 In this study, we generated phylogenetic hypotheses and calibrated time trees with  
471 very tight HPD intervals. This was possible due to the use of ddRADseq loci.  
472 Although ddRADseq and RADseq loci are generally considered more appropriate for  
473 population level questions than for phylogenetic reconstruction (Leaché et al., 2015),  
474 all principal types of genomic markers currently used in phylogenomic analyses are  
475 appropriate and informative for reconstructing phylogenetic relationships of groups  
476 that diverged during the Cenozoic (Collins and Hrbek, 2015; see also Eaton et al.,  
477 (2017). The ddRADseq and RADseq loci are particularly suitable for more recent  
478 divergences since their information content peaks at 20 million years of divergence,  
479 and at even more recent divergence times their information content is greater than that  
480 of UCE or Exon loci (Collins and Hrbek, 2017). Thus it should not be surprising that  
481 in our study we were able to generate a robust phylogenetic hypothesis, since this  
482 hypothesis was based on 954 loci (with an average of 7.3–8.3x coverage) with 20588  
483 variable sites of which 9628 were parsimony informative, assuming no more than  
484 50% missing data which has empirically been shown to maximize phylogenetic  
485 informativeness and support (Streicher et al., 2016). ddRADseq data are easy to

486 generate (<https://github.com/legalLab>), and thus we hope primate phylogenomic  
487 analyses will become the norm in the near future.

488 The phylogenetic study strongly suggests that there are two distinct species of  
489 pygmy marmoset in the Brazilian Amazon, one in the Japurá basin and one south of  
490 the Solimões. The identification of further taxa will depend on a more complete and  
491 exhaustive molecular phylogenetic analysis, and corresponding analyses of phenotype  
492 variation throughout the vast range of the pygmy marmoset in Bolivia, Brazil,  
493 Colombia, Ecuador and Peru. Needed are genetic analyses and a thorough review of  
494 the pelage variation in specimens throughout the pygmy marmoset's broad  
495 distribution (Buchanan-Smith et al., 2000).

496

#### 497 **Gazetteer**

498

499 Numbered localities are shown in Figure 1.

500

#### 501 **Bolivia**

502

- 503 1. Ponton, Río Muyumanu, south bank, right bank tributary of the Río  
504 Tahuamanu, Pando, Bolivia, 11°31'S, 69°03'W. Field survey 1997.  
505 Buchanan-Smith et al. (2000).

506

#### 507 **Brazil**

508

- 509 2. Japurá, Rio, right bank, middle, near Vila Bittencourt, Amazonas, Brazil,  
510 1°50'32.9"S, 69°01'12"W. Specimens UFAM / CTGA-M720/M723/M724.
- 511 3. Tabatinga, vicinity of, town, north bank of the Rio Solimões, Amazonas,  
512 Brazil, 4°14'S, 69°56'W. Type locality of *Cebuella pygmaea* (Spix, 1823) in  
513 near this town, but not established as whether it is north of the Rio Solimões.  
514 Hershkovitz (1977; locality 171).
- 515 4. Javari, Rio, mouth, south bank of the Rio Solimões, near Tabatinga,  
516 Amazonas, Brazil, 4°21'7.4", 70°2'18.4" (S4.352070, W70.038446). Type  
517 locality of *Cebuella pygmaea* (Spix), as restricted by Lönnberg (1940).
- 518 5. Benjamin Constant, Rio Solimões, right bank, Amazonas, Brazil, 4°19'48"S,  
519 69°46'12". Specimen INPA 4041 [CCM19].
- 520 6. Pati, Rio, left bank, Jutai-Solimões Ecological Station, Amazonas, Brazil,  
521 3°18'36"S, 67°31'48"W. Specimen JT079.
- 522 7. Jutai, Rio, Extractivist Reserve, Amazonas, Brazil. 3°13'12"S, 67°19'48"W.  
523 Specimen JT057.
- 524 8. Jutai, Rio, Extractivist Reserve, left bank, Amazonas, Brazil. 3°44'24"S,  
525 67°28'12"W (S3.74, W67.47). Specimen JT095.
- 526 9. Barroso, Comunidade, municipality of Uarini, Reserva de Desenvolvimento  
527 Sustentável Mamirauá, left bank of middle Rio Solimões, Amazonas, Brazil,  
528 8°33'58"S, 72°47'W. Specimen RDSM323/MPEG37114.
- 529 10. Tefé (= Ega), Lago de, south bank of the Rio Solimões, Amazonas, Brazil,  
530 3°27'S, 64°27'W, near sea level. Castelnau. Hershkovitz (1977; locality  
531 182).

- 532 11. Ipixuna, Lago do, south of the Rio Solimões, Amazonas, Brazil, 3°52'S,  
533 63°52'W. Hershkovitz (1977, locality 183). Type locality of *Cebuella*  
534 *pygmaea niveiventris* Lönnberg, 1940. Swedish Museum of Natural History.  
535 Allotype specimens NRM A59.4504.
- 536 12. Jacinto, Igarapé do, Rio Purus, right bank, 10 km south of Tapauá,  
537 Amazonas, Brazil, 5°42'S, 63°12'W. Specimen UFAM / CTGA-M170.
- 538 13. Bonfim, Rio Madeira, west (left) bank, opposite the town of Borba,  
539 Amazonas, Brazil, 4°20'S, 59°40'W. Sighting. M. G. M. Van Roosmalen and  
540 T. van Roosmalen, July, August 1996. Identified as *C. p. niveiventris*. Van  
541 Roosmalen and Van Roosmalen (1997).
- 542 14. Xadá, Lago, Rio Madeira, west (left) bank, Amazonas, Brazil, 5°15'36"S,  
543 60°43'12"W. Specimen INPA / FR20 [INPA 5677]. [Vencedor, Rio Madeira,  
544 west (left) bank, Amazonas, Brazil, 5°20'S, 60°45'W. Sighting. M. G. M.  
545 Van Roosmalen and T. van Roosmalen, July, August 1996. Identified as *C.*  
546 *p. niveiventris*. Van Roosmalen and Van Roosmalen (1997).]
- 547 15. Matupiri, Lago, Santa Maria, Rio Madeira, west (left) bank, Amazonas,  
548 Brazil, 5°33'15"S, 61°15'47"W. Sighting. M. G. M. Van Roosmalen and T.  
549 van Roosmalen, July, August 1996. Identified as *C. p. niveiventris*. Van  
550 Roosmalen and Van Roosmalen (1997). Specimen. Female  
551 CCM251. [Matupirizinho, Lago, Novo Jerusalem, Amazonas, Brazil,  
552 5°33'28"S, 61°07'20"W. Sighting. M. G. M. Van Roosmalen and T. van  
553 Roosmalen, July, August, 1996. Identified as *C. p. niveiventris*. Van  
554 Roosmalen and Van Roosmalen (1997).]

- 555 16. Democracia, Rio Madeira, west (left) bank, 15 km south of Manicoré,  
556 Amazonas, Brazil, 5°48'S, 61°26'W. Specimen INPA 4119 (CCM94). M. G.  
557 M. Van Roosmalen and T. van Roosmalen, July, August 1996. Identified as  
558 *C. p. niveiventris*. Van Roosmalen and Van Roosmalen (1997).
- 559 17. João Pessoa (= Eirunepé), left bank of the upper Rio Juruá, 6°50'30"S,  
560 70°14'27"W, opposite its confluence with the Rio Tarauacá, Brazil (Paynter  
561 and Traylor, 1991). Collected by A. M. Olalla. 1936. Cited by Lönnberg  
562 (1940), who considered it to be *C. p. pygmaea*. Hershkovitz (1977; locality  
563 176). Swedish Museum of Natural History. Specimens NRM: A61.2170,  
564 A61.2171, A61.2172, A61.2173, A61.2176, A61.2127, A61.3310.
- 565 18. Ocidente, Rio Juruá, right bank, Acre, Brazil, 8°33'58"S, 72°47'W (S8.566,  
566 W72.8). Specimens INPA / MNFS1019/1020/1361.
- 567 19. Seringal Oriente, right bank of upper Rio Juruá, Acre, Brazil, 8°48'S,  
568 72°46'W, 100–200 m above sea level. Collected by F. C. Novaes and M.  
569 Moreira in 1951. Museu Paraense Emílio Goeldi (MPEG), Belém, Pará.  
570 Cited by Carvalho (1957) who indicated its similarity to the nominotypical  
571 *Cebuella pygmaea*, following Lönnberg (19430). Hershkovitz (1977;  
572 locality 89).
- 573 20. Santo Antônio, cachoeira, Rio Madeira, approximately 9 km upstream of  
574 Porto Velho, Rondônia, Brazil (08°46'46.4"S, 63°58'14.0"W). Specimen,  
575 Scientific Collection of the Federal University of Rondônia. UFROM 175,  
576 adult male, skull, skin and skeleton. Collected by Juliano Coragem and  
577 Ivonete Santa Rosa Gomes, October 10, 2009. Messias et al. (2011).

- 578 21. Antimari, Rio, west (left) bank tributary of the Rio Acre, Antimari State  
579 Forest (66,168 ha), traversed by the municipalities of Bujari, and Sena  
580 Madureira, Acre, Brazil, 9°01'15"-9°11'41"S, 68°00'19"-68°01'45"W,  
581 250–300 m above sea level. Sighting. Calouro et al. (1991; see Bicca  
582 Marques and Calegaro-Marques, 1995).
- 583 22. Acre, Federal University of, Zoobotanical Park, urban district of Rio Branco,  
584 municipality of Rio Branco, Acre, Brazil. 9°56'30"-9°57'19"S,  
585 67°52'08"-67°53'00"W, 155 m above sea level. Sighting. Bicca Marques  
586 and Calegaro-Marques (1995).
- 587 23. Rôla, Rio do, basin, municipalities of Rio Branco and Xapuri, 10°–11°S,  
588 68°– 69°W, 216–260 m above sea level. Sighting. Brazil, IMAC (1993; see  
589 Marques and Calegaro-Marques, 1995).
- 590 43. Feijó, Acre, Brazil. Museu Paraense Emílio Goeldi, Belém, Pará, Brazil.  
591 8°9'50"S, 70°21'14"W. MPEG 21854.44. Purus, Floresta Nacional, left bank  
592 of the Rio Pauini, Amazonas, Brazil. Approx. 7°42'S, 67°06'W. Instituto  
593 Nacional de Pesquisas da Amazônia, Manaus, Amazonas. INPA 7256 (field  
594 #RS44).
- 595
- 596 **Colombia**
- 597
- 598 24. Letícia, Rio Solimões, Colombia, 4°15'S, 69°56'W, 100 m above sea level.  
599 P. Hershkovitz, June 1952. Hershkovitz (1977; locality 58e).
- 600 25. Puerto Limón, municipality of San Miguel de Mocoa, department of  
601 Putumayo, Colombia, 1°01'57.6"S, 76°26'42.2"W. Sighting. Izawa (1979).

602

603 **Ecuador**

604

605 26. La Coca, Río Napo, Ecuador, 0°28'S, 76°58'W, 258 m above sea level.

606 Jiménez de la Espada, 1865. Hershkovitz (1977; locality 63). [San Pablo,

607 Río Aguarico, right bank, Ecuador, 0°16'27"S, 76°25'29"W. Field research

608 and genetic study 2001–2003. Yépez et al. (2005) and Monteros et al.

609 (2011).]

610 27. Intillama (Indillama), Río Napo, left bank, Ecuador, 0°27'S, 76°31'W, 250 m

611 above sea level. P. Hershkovitz, June 1936. Hershkovitz (1977; locality 65).

612 [Sacha, Río Napo, north bank, 30 km south of San Pablo (Río Aguarico),

613 Ecuador, 0°28'49.5"S, 76°27'43.4"W (UTM, 18 Zone Datum PSA 56

614 337296E, 9946861N). Field research 2001–2003. Yépez et al. (2005).]

615 28. Zancudo Cocha, Laguna, Río Aguarico, right bank, approximately 150 km

616 west of San Pablo (Río Aguarico), border of Cuyabeno National Park,

617 Ecuador, 0°35'56.9"S, 75°29'24.6"W (UTM, 18 Zone Datum PSA 56

618 445459E, 9933749N. Field research 1997. Yépez et al. (2005).

619 29. Amazonico, Río Arajuno, south bank, approximately 150 km west of Sacha

620 (Río Napo) and San Pablo (Río Aguarico), 1°03'1.8"S, 77°31'20.2"W

621 (UTM, 18 Zone Datum PSA 56 219290E, 9883728N). Field research 2003.

622 Yépez et al. (2005). [Yasuní National Park, at km 47 of the Pompeya Sur-Iro

623 road to Maxus Ecuador, Inc., southeastern Ecuador, 0°42'01"S, 76°28'05"W.

624 Field research 1995. Youlatos (1999).]

- 625 30. Copataza, Río, Río Pastaza, Ecuador, 2°07'S, 77°27'W, 450 m above sea  
626 level. R. Olalla, April 1939, C. Buckley, 1877-1878. Hershkovitz (1977,  
627 locality 74).
- 628 31. Montalvo, Río Bobonaza, Río Pastaza, Ecuador, 2°04'S, 76°58'W, 314 m  
629 above sea level. R. Olalla, February 1932. Hershkovitz (1977; locality 75a).  
630
- 631 **Peru**
- 632
- 633 32. Río Curaray, mouth, right bank tributary of the Río Napo, Peru, 2°22'S,  
634 74°05'W, 140 m above sea level. Olalla Bros. October-December 1925,  
635 March, 1926. Hershkovitz (1977, locality 80).
- 636 33. Mishana, Río Nanay, left bank tributary of the Río Amazonas, Peru, 3°45'S,  
637 73°35'W. Field research 1974. Kinzey *et al.* (1975). Hershkovitz (1977;  
638 locality 94b).
- 639 34. Iquitos, Río Amazonas (or Marañón), left bank, Peru, 3°44'S, 73°15'W, 106  
640 m above sea level. Hershkovitz (1977; locality 92).
- 641 35. Santa Cecília, Río Manítí, a small tributary south of the Río Amazonas just  
642 below Iquitos and above the mouth of the Río Napo, Peru, 3°26'S, 72°46'W,  
643 110 m above sea level. C. Kalinowski, January 1957. Hershkovitz (1977;  
644 locality 89).
- 645 36. Apayacu, Río, north of the Río Amazonas, Peru, 3°21'S, 72°07'W, about 100  
646 m above sea level. Hershkovitz (1977; locality 86).
- 647 37. Pebas, Río Marañón, Peru, 3°10'S, 71°48'W, 100 m above sea level. R. W.  
648 Hendee, January-February 1928. Hershkovitz (1977; locality 85).

- 649 38. Chimbote, Río Marañón, Peru, 3°49'S, 70°41'W. Locality of Cruz Lima  
650 (1945). Hershkovitz (1977; locality 83).
- 651 39. Tapiche, Río, right bank, just below mouth of its tributary, the Rio Blanco,  
652 Loreto, Peru, 5°35'S, 73°53'W. Field research. Soini (1987).
- 653 40. Yaquerana, Río, mouth, left bank, Peru, 5°43'S, 72°58'W. C. Kalinowski,  
654 September 1957. Hershkovitz (1977; locality 118).
- 655 41. Sarayacu, Río Ucayali, Peru, 6°44'S, 75°07'W. Olalla Bros. July, March  
656 April 1927. Castelnau and Deville, 1847, presumably on right bank.  
657 Hershkovitz (1977; locality 102).
- 658 42. Manu National Park, Cocha Cashu Biological Station, Rio Madre de Dios,  
659 Peru, 11°54'S, 71°22'W, elevation about 400 m). Field research. Terborgh  
660 (1983).

661

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663

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677

678

## 679 **References**

680

681 Aberer, A.J., Kobert, K., Stamatakis, A., 2014. ExaBayes: massively parallel  
682 Bayesian tree inference for the whole-genome era. *Mol. Biol. Evol.* 31, 1–8. DOI:  
683 10.1093/molbev/msu236.

684

685 Aquino, R, Encarnación, F., 1994. Primates of Peru / Los Primates del Perú. *Prim.*  
686 *Rep.* 40, 1–130.

687

688 Ayres, J. M. and Clutton-Brock, T. H., 1992. River Boundaries and Species Range  
689 Size in Amazonian Primates. *Am. Nat.* 140, 531-537.

690

691 Bicca-Marques, J.C., Calegari-Marques, C., 1995. Updating the known distribution  
692 of the pygmy marmoset (*Cebuella pygmaea*) in the state of Acre, Brazil. *Neotrop.*  
693 *Primates* 3, 48–49.

694

695 Boubli, J.P., Ribas, C., Lynch, J. W., Alfaro, M. E., Nazareth, M., Silva, F., ... Farias,  
696 I.P., 2015. Spatial and temporal patterns of diversification on the Amazon : A test

697 of the riverine hypothesis for all diurnal primates of Rio Negro and Rio Branco in  
698 Brazil. *Mol. Phylogenet. Evol.*, 82, 400–412.  
699 <http://doi.org/10.1016/j.ympev.2014.09.005>  
700  
701 Brazil, IMAC., 1993. Relatório de Caracterização Preliminar da Bacia Hidrográfica  
702 do Riozinho do Rolâ. Report, Instituto de Meio Ambiente do Acre (IMAC), Rio  
703 Branco, Acre.  
704  
705 Buchanan-Smith, H.M., Hardie, S.M., Caceres, C., Prescott, M.J., 2000. Distribution  
706 and forest utilization of *Saguinus* and other Primates of the Pando Department,  
707 northern Bolivia. *Int. J. Primatol.* 21, 353–379.  
708  
709 Buckner, J.C., Lynch Alfaro, J., Rylands, A.B., Alfaro, M.E., 2015. Biogeography of  
710 the marmosets and tamarins (Callitrichidae). *Mol. Phylogenet. Evol.* 82(B),  
711 413–425.  
712  
713 Cabrera, A., 1957. Catalogo de los mamíferos de América del Sur. *Rev. Mus.*  
714 *Argentino de Cienc. Nat. “Bernardino Rivadavia”* 4, 1–307.  
715  
716 Carvalho, A. 1957. Alguns Mamíferos do Acre Ocidental. *Boletim do Museu*  
717 *Paraense Emílio Goeldi* 6, 1-9.  
718 Collins, R.A., Hrbek, T. 2017. An in silico comparison of protocols for dated  
719 phylogenomics. *Systematic Biology*, in press

720 Coyne, K.J., Burkholder, J.M., Feldman, R.A., Hutchins, D.A., Cary, S.C., 2004.  
721 Modified serial analysis of gene expression method for construction of gene  
722 expression profiles of microbial eukaryotic species. *Appl. Environ. Microbiol.* 70,  
723 5298-5304. DOI: 10.1128/AEM.70.9.5298  
724  
725 Cruz Lima, E. da., 1945. *Mammals of Amazônia, Vol. 1. General Introduction and*  
726 *Primates. Contribuições do Museu Paraense Emílio Goeldi de História Natural e*  
727 *Etnografia, Belém do Pará.*  
728  
729 Defler, T. R. 2004. *Conservation International Tropical Field Guide Series: Primates*  
730 *of Colombia. Conservation International, Bogotá, Colombia, 550 pp.*  
731  
732 Drummond, A.J., Suchard, M.A., Xie D., Rambaut, A., 2012. Bayesian phylogenetics  
733 with BEAUti and the BEAST 1.7 *Mol. Biol. Evol.* 29, 1969–1973.  
734  
735 Eaton, D.A., 2014. PyRAD: assembly of de novo RADseq loci for phylogenetic  
736 analyses. *Bioinformatics.* 30, 1844–9  
737  
738 Eaton, D.A., Spriggs, E.L., Park, B., Donoghue, M.J., 2017. Misconceptions on  
739 missing data in RAD-seq phylogenetics with a deep-scale example from  
740 flowering plants. *Systematic Biology* 66, 399–412. doi: 10.1093/sysbio/syw092  
741  
742 Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and  
743 high throughput. *Nucleic Acids Res.* 32, 1792–1797

744

745 Elliot, D.G., 1913. A Review of the Primates. 3 vols. American Museum of Natural  
746 History, New York.

747

748 Gascon, C., Malcolm, J. R., Patton, J. L., da Silva, M. N. F., Bogart, J. P., Loughed,  
749 S. C., ... Boag, P. T., 2000. Riverine barriers and the geographic distribution of  
750 Amazonian species. PNAS 97, 13672–13677.

751 <http://doi.org/10.1073/pnas.230136397>

752

753 Groves, C. P., 1989. A Theory of Human and Primate Evolution. Oxford Science  
754 Publication, Oxford.

755

756 Groves, C. P., 2001. Primate Taxonomy. Smithsonian Institution Press, Washington,  
757 DC.

758

759 Groves, C. P., 2005. Order Primates. In: Wilson, D.E., Reeder D. M. (eds), Mammal  
760 Species of the World. A Taxonomic and Geographic Reference. Third edition.  
761 Johns Hopkins University Press, Baltimore, MD, pp. 111–184.

762

763 Hershkovitz, P., 1977. Living New World Monkeys (Platyrrhini) with an Introduction  
764 to Primates, Vol. 1. The Chicago University Press, Chicago, IL.

765

- 766 Hill, W.C.O., 1957., Primates. Comparative Anatomy and Taxonomy III. Pithecoidea  
767 Platyrrhini (Families Hapalidae and Callimiconidae). Edinburgh University Press,  
768 Edinburgh.
- 769
- 770 Izawa, K., 1979. Studies on peculiar distributio pattern of *Callimico*. Kyoto  
771 University Overseas Research. Reports of NewWorld Monkeys, 1–19.
- 772
- 773 Izawa, K., Bejarano, G., 1981. Distribution ranges and patterns of nonhuman primates  
774 in western Pando, Bolivia. Kyoto University Overseas Research. Reports of  
775 NewWorld Monkeys, 1–12.
- 776
- 777 Kinzey, W.G., Rosenberger A.L., Ramirez, M., 1975. Vertical clinging and leaping in  
778 a Neotropical anthropoid. *Nature* 255, 327–328.
- 779
- 780 Leaché, A.D., Chavez, A.S., Jones, L.N., Grummer, J.A., Gottscho, A.D., Linkem,  
781 C.W., 2015. Phylogenomics of phrynosomatid lizards: Conflicting signals from  
782 sequence capture versus restriction site associated DNA sequencing. *Genome*  
783 *Biology and Evolution* 7, 706–719. doi: 10.1093/gbe/evv026
- 784
- 785 Lönnberg, E., 1940. Notes on marmosets. *Ark. Zool.*, Stockholm 32A(10), 1–22.
- 786
- 787 Marivaux, L., Adnet, L., Altamirano-Sierra, A.J., Pujos, F., Ramdarshan, A., Salas-  
788 Gismondi, R., Tejada-Lara, J.V., Antoie, P.-O., 2016. Dental remains of cebid  
789 platyrrhines from the earliest late Miocene of western Amazonia, Peru:

790 macroevolutionary implications on the extant capuchin and marmoset lineages.  
791 Am. J. Phys. Anthropol. 161, 478-493. DOI 10.1002/ajpa.23052.  
792  
793 Messias, M.R., Coragem, J.T., Gomes, I.S.R., Oliveira, M.A., Bonavigo, P. H.,  
794 Nienow, S. dos S., Souza, E.S. de., 2011. Southern extension of the geographical  
795 range of the pygmy marmoset *Cebuella pygmaea niveiventris* (Lönnerberg, 1940)  
796 in the southwestern Amazon basin, state of Rondônia, Brazil. Neotrop. Primates  
797 18, 30–31.  
798  
799 Napier, J.R. and Napier, P.H., 1967. A Handbook of Living Primates. Academic  
800 Press, London.  
801  
802 Napier, P.H., 1976. Catalogue of Primates in the British Museum (Natural History).  
803 Part 1: Families Callitrichidae and Cebidae. British Museum (Natural History),  
804 London.  
805  
806 Paradis, E., Claude, J., Strimmer, K., 2004. APE: analyses of phylogenetics and  
807 evolution in R language. Bioinformatics 20, 289–290. DOI:  
808 10.1093/bioinformatics/btg412  
809  
810 Paynter Jr., R.A. and Traylor Jr., M.A., 1991. Ornithological Gazetteer of Brazil.  
811 A–M. President and Fellows of Harvard College Cambridge, MT.  
812

813 Perelman P., Johnson, W.E., Roos, C., Seuánez, H.N., Horvath, J.E., Moreira, M.A.,  
814 Kessing, B., Pontius, J., Roelke, M., Rumpler, Y., Schneider, M.P.C., Silva, A.,  
815 O'Brien, S.J., Pecon-Slattery, J., 2011. A molecular phylogeny of living primates.  
816 PLoS Genet. 7, e1001342.  
817  
818 Peterson, B.K., Weber, J.N., Kay, E.H., Fisher, H.S., Hoekstra, H.E., 2012., Double  
819 digest RADseq: an inexpensive method for de novo SNP discovery and  
820 genotyping in model and non-model species. PLoS One 7, e37135. DOI:  
821 10.1371/journal.pone.0037135  
822  
823 Ramirez, M., Freese, C.H., Revilla, J., 1977. Feeding ecology of the pygmy  
824 marmoset, *Cebuella pygmaea*, in northeastern Peru. In: Kleiman, D.G. (ed.), The  
825 Biology and Conservation of the Callitrichidae. Smithsonian Institution Press,  
826 Washington, DC, pp.91–104.  
827  
828 Rylands, A.B., Coimbra-Filho, A.F., Mittermeier, R.A., 1993. Systematics,  
829 distributions and some notes on the conservation status of the Callitrichidae. In:  
830 Rylands, A.B. (ed.), Marmosets and Tamarins: Systematics, Behaviour, and  
831 Ecology, Oxford University Press, Oxford, pp.11–77.  
832  
833 Rylands, A.B., Coimbra-Filho, A.F., Mittermeier, R.A. 2009. The systematics and  
834 distributions of the marmosets (*Callithrix*, *Callibella*, *Cebuella*, and *Mico*) and  
835 callimico (*Callimico*) (Callitrichidae, Primates). In: Ford, S. M. L. Porter, L.,

- 836 Davis, L. C. (eds.), *The Smallest Anthropoids: The Marmoset/Callimico*  
837 *Radiation*, Springer, New York, pp.25–61.
- 838
- 839 Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. *Molecular Cloning: A Laboratory*  
840 *Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- 841
- 842 Soini, P., 1982. Ecology and population dynamics of the pygmy marmoset, *Cebuella*  
843 *pygmaea*. *Folia Primatol.* 39, 1–21.
- 844
- 845 Soini, P., 1993. The ecology of the pygmy marmoset, *Cebuella pygmaea*: some  
846 comparisons with two sympatric tamarins. In: Rylands, A.B. (Ed.), *Marmosets*  
847 *and Tamarins: Systematics, Behaviour and Ecology*. Oxford University Press,  
848 Oxford, pp.257–261.
- 849
- 850 Soini, P., 1987. Sociosexual behavior of a free-ranging *Cebuella pygmaea*  
851 (Callitrichidae, Platyrrhini) troop during postpartum estrus of its reproductive  
852 female. *Am. J. Primatol.* 13, 223–230.
- 853
- 854 Spix, J.B. von, 1823. *Simiarum et Verspertilionum Brasiliensium Species Novae*. F.S.  
855 Hübschmann, Munich.
- 856
- 857 Spix, J. B. von and Martius. C. F. von, 1938. *Viagem pelo Brasil*. Translation of Spix,  
858 J. B, von and Martius. C. F. von, 1831. *Reise in Brasilien auf Behehl Sr. Majestat*  
859 *Maximilian Joseph I., Königs von Baiern, in den Jahren 1817 bis 1820*. Volume

860 3. M. Lindauer, Munich. Translation by L.F. Lahmeyer. Instituto Histórico e  
861 Geográfico Brasileiro, Imprensa Nacional, Rio de Janeiro.  
862

863 Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-  
864 analysis of large phylogenies. *Bioinformatics* 30, 1312–1313. DOI:  
865 10.1093/bioinformatics/btu033  
866

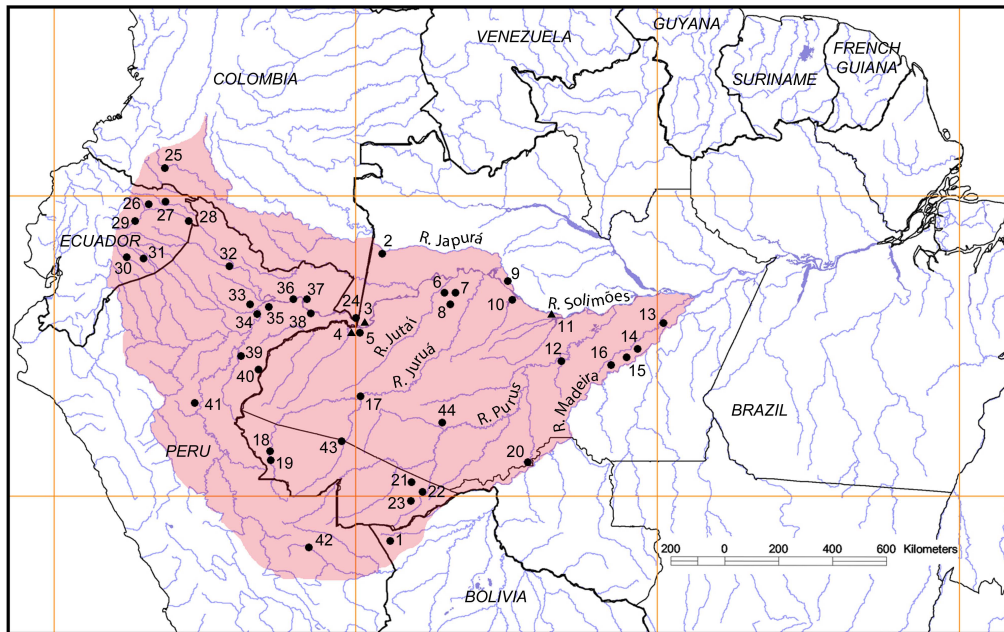
867 Streicher, J.W., Schulte, II J.A., Wiens, J.J., 2016. How should genes and taxa be  
868 sampled for phylogenomic analyses with missing data? An empirical study in  
869 iguanian lizards. *Systematic Biology* 65, 128–145. doi: 10.1093/sysbio/syv058  
870

871 Van Roosmalen, M.G.M., Van Roosmalen, T., 1997. An eastern extension of the  
872 geographical range of the pygmy marmoset, *Cebuella pygmaea*. *Neotrop.*  
873 *Primates* 5, 3–6.  
874

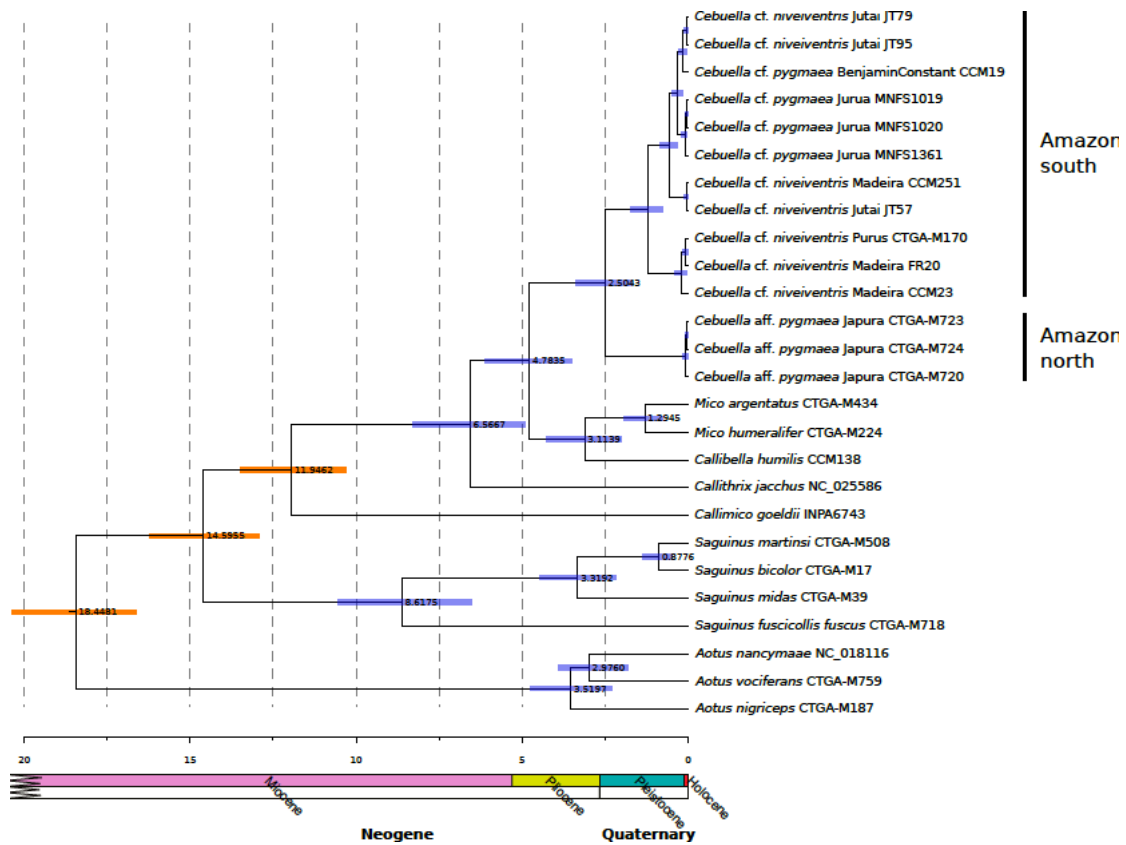
875 Van Roosmalen, M.G.M, Van Roosmalen, T., 2016. On the origin of allopatric  
876 primate species. *Biodiv. J.* 7, 117–198.  
877

878 Vieira, C. da C., 1955. Lista remissiva dos mamíferos do Brasil. *Arq. Zool., São*  
879 *Paulo* 8(11), 341–374.  
880

881 Werle, E., Schneider, C., Renner, M., Völker, M., Fiehn, W., 1994. Convenient  
882 single-step, one tube purification of PCR products for direct sequencing. *Nucleic*  
883 *Acids Res.* 22, 4354–4355.

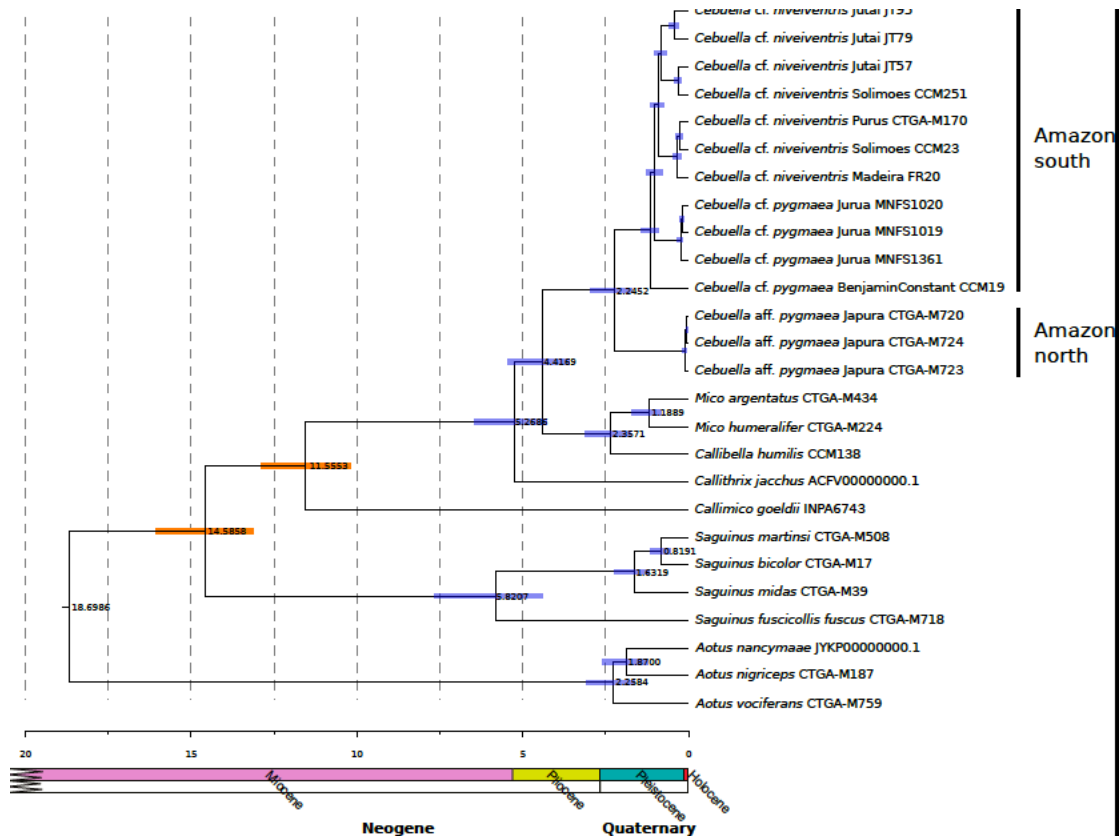


887 **Fig. 1.** Distribution of the pygmy marmoset (*Cebuella*). Numbers correspond to the  
 888 gazetteer. The black triangles are type localities. Localities of the samples used in the  
 889 molecular analysis of this study: **2.** Japurá (CTGA-M720, 723, 724); **5.** Benjamin  
 890 Constant, upper Rio Solimões (CCM19); **6.** Rio Jutai, Rio Patiá, (JT079, JT095); **8.**  
 891 Rio Jutai, Extractivist Reserve (JT057); **12.** Jacinto, Rio Purus (CTGA-M170); **14.**  
 892 Lago Xada, Rio Madeira (FR 20); **18.** Ocidente, upper Rio Juruá (MNFS 1019, 1020,  
 893 1361).



895

896 **Fig. 2.** BEAST cytochrome *b* time tree for 26 primate samples, including 14 pygmy  
 897 marmosets - including 3 *Cebuella aff. pygmaea* (CTGA-M 720, 723, 724), 4 *Cebuella*  
 898 *cf. pygmaea* (MNFS 1019, 1020, 1361; CCM 19) and 7 *Cebuella cf. niveiventris* (JT  
 899 57, 95, 79; CTGA-M170; FR 20; CCM 251, 23) – and 12 other taxa for comparative  
 900 purposes. Numbers in nodes correspond to time in millions of years and error bars  
 901 represent 95% HPD intervals. Divergences represented by orange error bars were  
 902 used as calibration points. Bayesian posterior probabilities are all equal to 1, except  
 903 for the sister taxon relationship between sample MNFS 1019 and 1020 supported by  
 904 0.95, sample FR 20 and CTGA-M170 supported by 0.94, sample CTGA-M724 and  
 905 723 supported by 0.55, and in the outgroups *Aotus nancymae* was sister taxon of  
 906 *Aotus vociferans* with  $pp = 0.77$ . See supplementary materials, Figure 1, for a map  
 907 showing the localities for all specimens used in this phylogenetic analysis



908

909 **Fig. 3.** BEAST ddRAD time tree for the same taxa as in Figure 3. Numbers at nodes  
 910 correspond to time in millions of years and error bars represent 95% HPD intervals.

911 Divergences represented by orange error bars were used as calibration points.

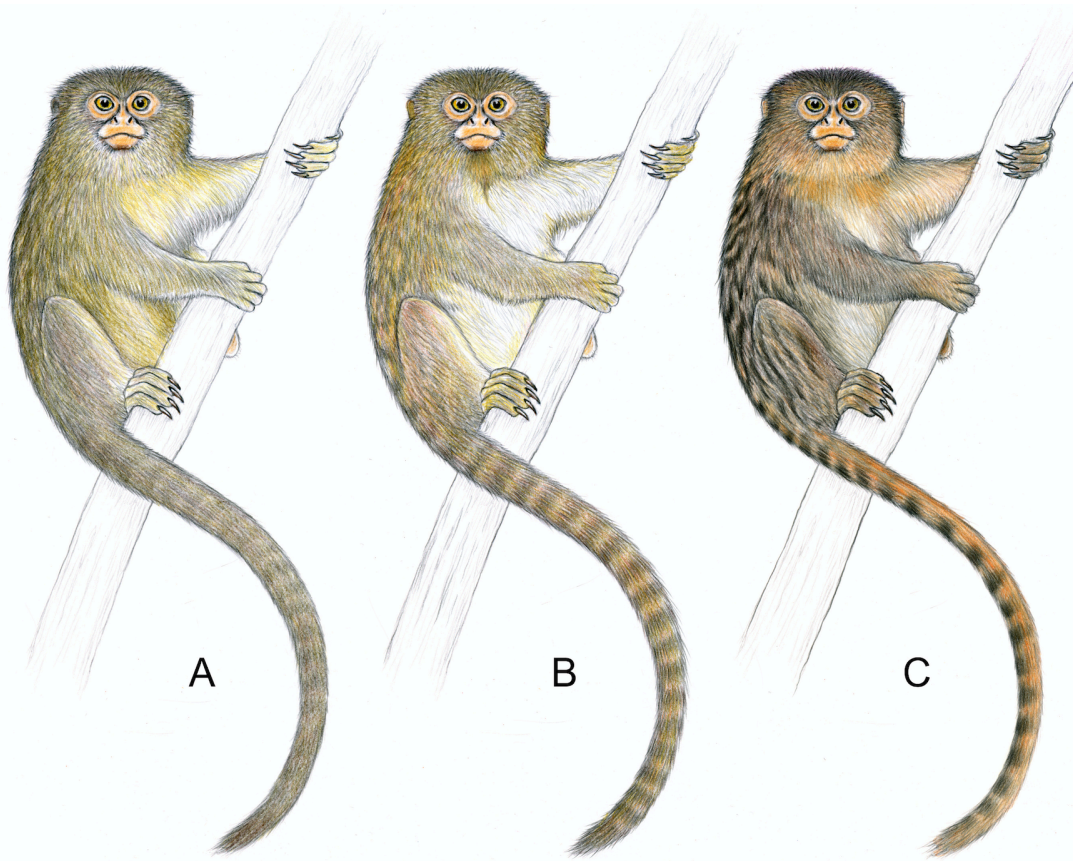
912 Bayesian posterior probabilities are all equal to 1, except for the sister taxon

913 relationship between sample MNFS 1019 and 1020 supported by 0.87 and sample

914 CCM 23 and CTGA-M170 supported by 0.76. See supplementary materials, Figure 1,

915 for a map showing all localities for the specimens used in this phylogenetic analysis.

916



917

918 **Fig. 4.** From left to right, A) *Cebuella* aff. *pygmaea* from the upper Rio Japurá, Brazil  
919 (based on specimens from locality 2, Figure 1), B) *Cebuella* cf. *niveiventris* from the  
920 south of the Solimões (based on specimens from localities 6, 7, 8, 12, 14, 15, 16 and  
921 44 in Figure 1), C) *Cebuella* cf. *pygmaea* from the upper Rio Juruá (based on  
922 specimens from locality 18, Figure 1). Illustrations copyright Stephen D.  
923 Nash/Conservation International.



924

925 **Fig. 5.** A) Dorsal and B) ventral views of pygmy marmosets. Largest individual is  
 926 *Cebuella cf. niveiventris* from the Rio Purus (CTGA-M170), locality 12 in Figure 1.  
 927 Three small individuals are from the middle Rio Japurá, Brazil (CTGA-M720, 723  
 928 and 724), locality 2 in Figure 1. Photo by Jean P Boubli.

929

930 **Fig. 6.** The mounted type specimen of *Cebuella niveiventris* Lönnberg, Department of  
 931 Zoology, Swedish Museum of Natural History, Stockholm. Specimen A61.330, Lago  
 932 do Ipixuna, south of the Rio Solimões, Amazonas, Brazil, 3°52'S, 63°52'W. Locality  
 933 11 in Figure 1. Photo courtesy of the Department of Zoology, Swedish Museum of  
 934 Natural History, Stockholm.

935

936 **Fig. 7.** Ventral view of specimen NRM 61.2127. Swedish Museum of Natural  
 937 History, Stockholm. João Pessoa (= Eirunepé), left bank of the upper Rio Juruá,  
 938 6°50'30"S, 70°14'27"W, opposite its confluence with the Rio Tarauacá, Brazil.

939 Locality 17 in Figure 1. Photo courtesy of the Zoology Department of the Swedish  
940 Museum of Natural History, Stockholm.

941

942 **Figure 8.** Mounted type specimen, *Cebuella pygmaea* Spix, Zoologische  
943 Staatssammlung München Museum. Vicinity of Tabatinga, north bank of Rio  
944 Solimões, western Amazonas at Colombian Frontier, Brazil. Locality 3 in Figure 1.

945 ([http://www.zsm.mwn.de/mam/i/Jacchus\\_pygmaeus\\_20\\_D.jpg](http://www.zsm.mwn.de/mam/i/Jacchus_pygmaeus_20_D.jpg) )

946

947 **Figure 9.** Ventral view of specimens UFAM / CTGA-M720/M723/724, Japurá, Rio,  
948 right bank, middle, near Vila Bitencourt, Amazonas, Brazil, 1°50'32.9"S,  
949 69°01'12"W. Locality 2 in Figure 1. Photo by Ingrid Macedo

950

951 **Figure 10.** Ventral view of specimens NMNH 336309 and 336312 from near  
952 Tabatinga, Amazonas (Locality Captured in January 1963 on behalf of the NIH  
953 California National Primate Research Center, San Diego, California, and sent to the  
954 NMNH in February, March and April 1963. National Museum of Natural History,  
955 Smithsonian Institution, Washington DC. Photo by Anthony B. Rylands.

956

957 **Figure 11.** Ventral view of specimen NMNH 336302, female, from near Tabatinga,  
958 Amazonas. Captured in January 1963 on behalf of the NIH California National  
959 Primate Research Center, San Diego, California. National Museum of Natural  
960 History, Smithsonian Institution, Washington, DC. Photo by Anthony B. Rylands.

961

962 **Figure 12.** Ventral view of specimen NMNH 336304, female, from near Tabatinga,  
 963 Amazonas. Captured in January 1963 on behalf of the NIH California National  
 964 Primate Research Center, San Diego, California. National Museum of Natural  
 965 History, Smithsonian Institution, Washington, DC. Photo by Anthony B. Rylands.  
 966

967 **Figure 13.** Ventral views of specimens NMNH 337322, 337325, 337323, 337329,  
 968 from near Tabatinga, Amazonas. Captured in August 1963 on behalf of the NIH  
 969 California National Primate Research Center, San Diego, California. National  
 970 Museum of Natural History, Smithsonian Institution, Washington, DC. Photo by  
 971 Anthony B. Rylands.  
 972

973 **Figure 14. A)** Specimen CCM19 of *C. cf. pygmaea* from Benjamin Constant, Brazil  
 974 **B)** Specimen MPEG 37114 of *C. cf. pygmaea* from the Reserva do Desenvolvimento  
 975 Sustentável, Mamirauá, Brazil.  
 976

977 **TABLES**

978

979 **Table 1**

980 List of voucher specimens and tissue samples used in this study and their localities.

Museum #	Original #	Institution / Tissue #	Species	Locality
RDSM no#	JT057	JT057	<i>Cebuella p. pygmaea</i>	Brazil, Amazonas: RESEX dc
RDSM no#	JT079	JT079	<i>Cebuella p. pygmaea</i>	Brazil, Amazonas: ESEC Juta
RDSM no#	JT095	JT095	<i>Cebuella p. pygmaea</i>	Brazil, Amazonas: RESEX dc
MPEG22954	MNFS1019	INPA / MNFS1019	<i>Cebuella p. pygmaea</i>	Brazil, Acre: Ocidente, right l
MPEG22953	MNFS1020	INPA / MNFS1020	<i>Cebuella p. pygmaea</i>	Brazil, Acre: Ocidente, right l
MPEG22952	MNFS1361	INPA / MNFS1361	<i>Cebuella p. pygmaea</i>	Brazil, Acre: Ocidente, righ b
INPA4041	MvR30=CCM19	INPA / MvR30=CCM19	<i>Cebuella p. pygmaea</i>	Brazil, Amazonas: Benjamin
INPA5677	FR20	INPA / FR20	<i>Cebuella p. niveiventris</i>	Brazil, Amazonas; left bank F

INPA7250	CCM23	INPA / CCM23	<i>Cebuella p. niveiventris</i>	Unknown locality
INPA7252	SISPUR-M170	UFAM / CTGA-M170	<i>Cebuella p. niveiventris</i>	Brazil, Amazonas: Igarapé do
INPA7254	SISJAP-M723	UFAM / CTGA-M723	<i>Cebuella nv sp</i>	Brazil, Amazonas: right bank
INPA7253	SISJAP-M720	UFAM / CTGA-M720	<i>Cebuella nv sp</i>	Brazil, Amazonas: right bank
		Genbank	<i>Aotus nancymae</i>	Not available
INPA no#	SISPUR-M187	UFAM / CTGA-M187	<i>Aotus nigriceps</i>	Brazil, Amazonas, Jacinto, ric
INPA no#	SISJAP-M759	UFAM / CTGA-M759	<i>Aotus vociferans</i>	Brazil, Amazonas, Taboca, ric
INPA4090	CCM 138	INPA / CCM 138	<i>Callibella humilis</i>	Brazil, Mato Grosso, Aripuan
RDSM no#	HGLN 02	INPA / HGLN 02	<i>Callibella humilis</i>	Aripuana river
INPA6743	RS 34	INPA / RS 34	<i>Callimico goeldii</i>	Not available
		Genbank	<i>Callithrix jacchus</i>	Not available
INPA no#	SISTAP-M364	UFAM / CTGA-M434	<i>Mico argentata</i>	Brazil, Pará, Itapuama, Tapaj
INPA no#	SISTAP-M224	UFAM / CTGA-M224	<i>Mico humeralifer</i>	Brazil, Pará, Cameta, Tapajos
RDSM no#	FES 09	INPA / FES 09	<i>Mico marcai</i>	Brazil, Mato Grosso, Aripuan
UFAM no#	SISIS 17	UFAM / CTGA-M17	<i>Saguinus bicolor</i>	Brazil, Amazonas, Manaus
INPA no#	SISTRO-M508	UFAM / CTGA-M508	<i>Saguinus martinsi</i>	Brazil, Pará, Saracá-Taquera,
INPA no#	SISJAT-M39	UFAM / CTGA-M39	<i>Saguinus midas</i>	Brazil, Amazonas, Jatapu

981

982 **Table 2**

983 Morphological comparisons of the underside pelage coloration of *Cebuella* specimens

984 in the Brazilian Amazon: 1) south of the Rio Solimões, 2) Eirunepé-Acre, upper Juruá

985 basin, southwestern Brazilian Amazon

	<b>South Solimoës</b>	<b>Juruá Eirunepé/Acre</b>
General ventral appearance	Light-colored, buff to whitish undersides; throat and chest ochraceous orange, sometimes throat is buffy to whitish in the center; the ochraceous orange throat and upper chest contrast with a pure white chest; undersides may be more greyish towards the abdomen (specially laterally) and legs	Dark-colored (dull yellowish brown) undersides; dull ochraceous orange to brownish throat, sometimes throat is whitish in the center; the ochraceous brownish coloration is limited mostly to the base of the neck, slightly contrasting the whitish to yellowish brown chest aspect of chest varies from white to light tawny (yellowish-brown); yellowish black hairs may cover the entire abdomen, penetrate the abdominal region forming a lateral line that marginate a whitish or wide median band along the entire underside
Throat	ochraceous orange, sometimes buffy to whitish in the center;	dull ochraceous orange to brownish, sometimes buffy to whitish in the center

Chest	ochraceous orange upper chest contrast with a whitish chest and belly	varies from white to buff to tawny (y brown)
Belly	buff to whitish, may be more greyish towards the abdomen (specially laterally) and legs	yellowish and black hairs may cover entire abdomen or penetrate the abd region forming a lateral line that may whitish narrow or wide median band
Limbs	inner surface of arms and legs varying from modified agouti to dominantly yellowish or grayish, general coloration of tights similar to belly	inner surface of arms dominantly yellow and of legs modified agouti to entirely or whitish
Tail	tail modified agouti with upper surface barred black, under surface brownish with banding shadowy; dorsal darker bands 20+ in number	tail modified agouti with upper surface black, under surface evenly ochraceous general aspect of no banding

986

987 **SUPPLEMENTARY MATERIALS**

988

989 **Figure 1.** Localities for the pygmy marmoset specimens used in our phylogenetic

990 analysis, numbered as in the gazetteer in the main text. 2 – CTGA M720, 723 and

991 724, right bank the Rio Japurá; 5 – CCM19, Benjamin Constant; 6 – JT059, ESEC

992 Jutai-Solimões, left bank of the Rio Jutai; 7 – JT057, RESEX do Rio Jutai, right bank

993 of the Rio Jutai; 8 – JT095, RESEX do Rio Jutai, left bank of Rio Jutai; 12 - CTGA

994 M170 AM, Igarapé do Jacinto, right bank of the Rio Purus, Tapauá; 14 – FR20, left

995 bank of the Rio Madeira, Lago Xadá; 15 – CCM251, Lago Matupiri, Rio Madeira; 18

996 – MNFS1019, 1020, and 1361 Ocidente, right bank of the Rio Juruá, Acre. Shaded

997 yellow is the hypothetical distribution of *Cebuella* aff. *pygmaea*. Shaded pink is the

998 hypothetical distribution of *C. cf. pygmaea* + *C. cf. niveiventris* (original shapes

999 downloaded from IUCN (International Union for Conservation of Nature) 2008.

1000 *Cebuella pygmaea*. The IUCN Red List of Threatened Species. Version 2017-2 (as

1001 per Van Roosmalen and Van Roosmalen, 1997).

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