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Capuchin monkey biogeography : understanding Sapajus Pleistocene range expansion and the current sympatry between Cebus and Sapajus

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1 ORIGINAL ARTICLE

2 **Capuchin monkey biogeography: understanding *Sapajus* Pleistocene range**
3 **expansion and the current sympatry between *Cebus* and *Sapajus***

4

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39 **ABSTRACT**

40

41 **Aim**

42 Our aim was to examine gracile capuchin (*Cebus*) and robust capuchin monkey
43 (*Sapajus*) diversification, with a focus on recent *Sapajus* expansion within Amazonia.

44 We wanted to reconstruct the biogeographical history of the clade using statistical
45 methods that model lineages' occupation of different regions over time, to evaluate
46 recently proposed "Out of Amazonia" and "Reinvasion of Amazonia" hypotheses as
47 alternative explanations for the extensive geographic overlap between reciprocally
48 monophyletic gracile (*Cebus*) and robust (*Sapajus*) capuchin monkeys.

49

50 **Location**

51 Central and South America.

52

53 **Methods**

54 We reconstructed a time-calibrated molecular phylogeny for capuchins under Bayesian
55 inference from three mitochondrial genes. We then categorized 12 capuchin clades
56 across four Neotropical centres of endemism and reconstructed the biogeographic
57 history of the capuchin radiation using six models implemented in ‘BioGeoBEARS’.
58 We performed a phylogeographic analysis for a robust capuchin clade that spans the
59 Atlantic Forest, Cerrado, Caatinga and Amazonia.

60

61 **Results**

62 We find support for a late Miocene vicariant *Cebus-Sapajus* divergence and a
63 Pleistocene *Sapajus* invasion of Amazonia from the Atlantic Forest. Our new analyses
64 confirm *Sapajus* diversified first in the Atlantic Forest, with subsequent range
65 expansion into widespread sympatry with *Cebus* in Amazonia, as well as multiple
66 expansions into drier savanna-like habitats. We do not find mitochondrial molecular
67 congruence with morphological species distinctions for *Sapajus flavius*, *S. cay*, *S.*
68 *macrocephalus*, *S. libidinosus* and *S. apella*; instead, these five morphological types
69 together form a single widespread clade (Bayesian posterior probability = 1) with
70 geographic substructure and shared ancestry during the Pleistocene.

71

72 **Main conclusions**

73 Our results support vicariance dividing ancestral capuchin populations in Amazonia
74 versus the Atlantic Forest, and a Pleistocene “Amazonian invasion” by *Sapajus* to
75 explain the present day sympatry of *Cebus* and *Sapajus*.

76

77 **Keywords** Amazonia, Atlantic Forest, BEAST, phylogeography, Caatinga, *Cebus*
78 *kaapori*, Cerrado, RASP Biogeography, range expansion, *Sapajus flavius*

79

80 **INTRODUCTION**

81

82 Neotropical primates are the major diurnal mammalian radiation in the
83 Neotropics, and include over 140 species found across Amazonia, the Andes, the
84 Atlantic Forest, and even habitats such as the Llanos, Caatinga, Cerrado and Central
85 Grasslands that are too hot and dry to support many types of mammals. Neotropical
86 primate diversity is highest within the tropical rainforest habitat in Amazonia. Major
87 questions include whether Neotropical primates diversified first in Amazonia, and how
88 and when they spread throughout the various biomes they now inhabit.

89 The wide distribution of capuchin monkeys, with species ranging from Central
90 America to northern Argentina, makes them a potential model system for addressing
91 questions of Neotropical primate biogeography. Capuchins comprise two major forms,
92 the more lightly built gracile capuchin, *Cebus*, and the robust capuchin, *Sapajus*.
93 Although recent studies have confirmed the genetic distinctiveness of these two lineages
94 (Lynch Alfaro *et al.*, 2012a; Martins Jr. *et al.*, 2014), key questions remain regarding
95 their present day distribution, including the extensive sympatry between Amazonian
96 *Cebus* and *Sapajus* species.

97 Two competing hypotheses have been posited to explain current capuchin
98 distributions (Lynch Alfaro *et al.*, 2012a). The “Out of Amazonia” (OOA) hypothesis
99 places the ancestral range for all capuchins in Amazonia, an initial diversification of
100 both crown robust and crown gracile capuchins within Amazonia, and subsequent

101 independent and recent invasions of non-Amazonian regions by both gracile and robust
102 capuchins (Lynch Alfaro *et al.*, 2012a). The “Reinvasion of Amazonia” (ROA)
103 hypothesis proposes that gracile capuchins, *Cebus*, diverged from robust capuchins,
104 *Sapajus* early in capuchin evolutionary history; crown *Cebus* originated in Amazonia
105 and crown *Sapajus* in the Atlantic Forest; and the presence of *Sapajus* in the Amazonia
106 today reflects a recent invasion from the Atlantic Forest into *Cebus*-occupied territory
107 (Lynch Alfaro *et al.*, 2012a).

108 Phylogeographic analyses using pure dispersal models strongly support the
109 “Reinvasion of Amazonia” hypothesis (Lynch Alfaro *et al.*, 2012a), and as such have
110 transformed the interpretation of capuchin taxonomy, conservation biology,
111 morphology and ecology (see Lynch Alfaro *et al.*, 2012b, 2014, 2015b; Matthews,
112 2012; Rylands *et al.*, 2013; Martins Jr. *et al.*, 2014; Oliveira *et al.*, 2014; Boubli *et al.*,
113 2015; Schneider & Sampaio, 2015; Wright *et al.*, 2015). However, limitations of these
114 studies have left key predictions of the OOA hypothesis untested. For example, Lynch
115 Alfaro *et al.* (2012a) and Nascimento *et al.* (2015) provided limited sampling of eastern
116 Amazonia and Cerrado/Caatinga localities, which have the potential to strongly
117 corroborate the OOA scenarios. In addition, capuchin monkey biogeography has yet to
118 be interpreted in light of models that integrate processes beyond pure dispersal
119 (Ronquist, 1997; Ree *et al.*, 2005; Ree & Smith, 2008; Landis *et al.*, 2013).

120 Here we assemble an expanded sampling of capuchin monkeys across their
121 range and conduct new biogeographic analyses using recently developed statistical
122 approaches. Our overall goal is to test hypotheses explaining capuchin biogeography
123 (Boubli *et al.*, 2012; Lynch Alfaro *et al.*, 2012a; Nascimento *et al.*, 2015; Ruiz-Garcia *et*
124 *al.*, 2016). We thus assemble a new cytochrome *b* + D-loop + cytochrome oxidase I
125 matrix with three times the samples and more comprehensive spatial and taxonomic

126 sampling compared to Lynch Alfaro *et al.* (2012a). We produce a detailed phylogeny
127 for capuchin monkeys, with all major lineages represented, to confirm the monophyly
128 of *Cebus* and *Sapajus* and to recover major clades within the capuchin radiation. We
129 compare alternative biogeographical scenarios using a flexible framework for inferring
130 spatial histories that allows for a number of different mechanisms causing range
131 expansion or contraction. We assess whether the employment of a more complete
132 genetic dataset and statistical methods with expanded models increase support for
133 previous conclusions about capuchin biogeographical evolution or support new
134 scenarios altogether. Our sampling allows us to address two new biogeographic
135 hypotheses for this clade. The first hypothesis postulates vicariance between Amazonia
136 and the Atlantic Forest. Divergence between *Sapajus* and *Cebus* is caused by isolation
137 of ancestral capuchin populations in the Atlantic Forest versus Amazonian habitats,
138 respectively, with subsequent expansion of *Sapajus* into sympatry with Amazonian
139 *Cebus* (“Reinvasion of Amazonia” hypothesis). The second, opposite, hypothesis
140 postulates vicariance within Amazonia due to the formation of the Amazon River.
141 Capuchins arise in Amazonia, and subsequently *Cebus* is isolated north of the Amazon
142 River, with *Sapajus* isolated south of the Amazon River and expanding into the Atlantic
143 Forest. Sympatry occurs with subsequent crossing of Amazon River in both directions
144 (“Out of Amazonia” hypothesis).

145

146 **MATERIALS AND METHODS**

147

148 *DNA extraction, amplification, sequencing and alignment*

149

150 We sequenced three mitochondrial genes, cytochrome *b* (*Cyt b*, 1133 bp), D-loop
151 (1244 bp) and cytochrome oxidase I (COI, 673 bp), for 108 capuchin samples from
152 poorly known or previously unsampled localities and species (see Table S1 in Appendix
153 S1). For *Cyt b* and D-loop we used primers and PCR protocols from Lynch Alfaro *et al.*
154 (2012a) and Boubli *et al.* (2012). We adapted COI primers and protocols from Ward *et*
155 *al.* (2005) (see Appendix S2). We combined the 108 new samples with 40 samples
156 analysed previously by Lynch Alfaro *et al.* (2012a) and 13 new samples with reliable
157 species identification extracted from GenBank, totaling 161 capuchin samples. Note
158 that samples from Ruiz-Garcia *et al.* (2016) were not available for use in this study. We
159 used only sequences over 500 bp long to improve clade support (Nascimento *et al.*,
160 2015). We used the most recent comprehensive taxonomic review for capuchins
161 (Rylands *et al.*, 2013), in relation to specimen morphology and collection locality, to
162 assign species names to samples (locality data in Figure 1 and Table S1 in Appendix
163 S1). Although the limitations of mitochondrial DNA studies are well understood, *Cyt b*,
164 D-loop and COI have performed well in capturing phylogeny and biogeographical
165 history in a wide range of species (Tobe *et al.*, 2010; Zhang *et al.*, 2011; Robins *et al.*,
166 2014). In addition, mtDNA sequence can be reliably generated from museum “crusties”
167 and skins using now standard protocols (e.g., Lynch Alfaro *et al.*, 2012a, 2015a; Mercês
168 *et al.*, 2015).

169

170 *Phylogenetic Reconstruction*

171

172 We used MRBAYES 3.2.6 (Ronquist *et al.*, 2012) to reconstruct the phylogenetic
173 relationships among capuchins, using *Saimiri* as an outgroup. We partitioned *Cyt b* and
174 COI by codon for the analysis and ran the Markov Chain Monte Carlo (MCMC) for 50

175 million generations. We used ‘sumt’ to calculate the Bayesian consensus tree. We then
176 used BEAST 1.8.1 (Drummond *et al.*, 2012) to reconstruct a time tree appropriate for
177 our biogeographical analyses by pruning the alignment to a single exemplar taxon for
178 each recovered well-supported clade. We considered branch lengths and recovered
179 polytomies in addition to posterior probabilities at nodes when distinguishing clades.
180 This resulted in a new, pruned alignment with 13 tips, representing 12 capuchin clades
181 and one outgroup. We specified a HKY + G model of nucleotide substitution and
182 partitioned by codon, using *Cyt b* only to avoid branch length bias due to missing data
183 in the other genes. We used *Saimiri* as an outgroup and the fossil *Neosaimiri* (Kay,
184 2015) to calibrate the split at 12.5 Ma (lognormal prior, offset=12.5, mean=0, SD=1)
185 between capuchins and squirrel monkeys for the time tree. We ran the MCMC analysis
186 for 100 million generations, sampling states every 10,000 generations with a Yule prior
187 on the diversification rate, and an uncorrelated, lognormal relaxed molecular clock.

188

189 *Biogeographical modelling*

190

191 *Biogeographical scenarios* In addition to testing for evidence for “Out of Amazonia”
192 versus “Reinvasion of Amazonia” hypotheses (see Introduction), we tested for
193 reciprocal monophyly for *Sapajus* in Amazonia (*S. macrocephalus* + *S. apella*); the
194 Cerrado + Caatinga + Central Grasslands (*S. cay* + *S. libidinosus*); and the Atlantic
195 Forest (*S. flavius* + *S. xanthosternos* + *S. robustus* + *S. nigritus*). Lack of reciprocal
196 monophyly across habitats would suggest multiple independent invasions and
197 convergent adaptations to habitat types by independent *Sapajus* lineages.

198

199 *Historical biogeography* To test alternative hypotheses of capuchin origins and
200 radiation we used the R-package ‘BioGeoBEARS’, a recently developed flexible
201 maximum likelihood framework for inferring spatial histories (Matzke, 2013, 2014; see
202 Buckner *et al.*, 2015 and Lynch Alfaro *et al.*, 2015a for its recent application to primate
203 taxa). ‘BioGeoBEARS’ software allows for a statistical comparison of a suite of models
204 that include alternative biogeographic processes related to cladogenesis and that allow
205 for species distributions across multiple regions (Matzke, 2014).

206 Our tree was pruned to include one representative tip for each ‘species’
207 recovered in the MRBAYES analysis. We lumped *Sapajus flavius*, *S. cay*, *S. libidinosus*,
208 *S. apella*, and *S. macrocephalus* (*Sapajus* clade 4, see below) as one species. This
209 resulted in a new, pruned alignment with 12 tips. We coded presence/absence in four
210 major geographic regions for each tip based on known species distribution using
211 Rylands *et al.* (2013): Atlantic Forest (AF), Cerrado/Caatinga/Central Grasslands (CC),
212 Amazonia (AM), and Central America/Andes (CA). We used the pruned, time-
213 calibrated tree with regional coding in ‘BioGeoBEARS’ (Matzke, 2013; Matzke, 2014).
214 Our candidate pool consisted of six models: DEC, DEC-J, DIVA, DIVA-J,
215 BAYAREA-LIKE, BAYAREALIKE-J and we used model weights calculated from
216 corrected Akaike information criterion (AICc) to identify the best model or set of
217 models for our data (Matzke, 2014). ‘BioGeoBEARS’ script is available in Appendix
218 S2.

219

220 *Phylogeographical reconstruction of widespread Sapajus* To examine phylogeographic
221 patterns for a widespread robust capuchin group recovered in the MRBAYES tree, we
222 performed both symmetric and asymmetric discrete states phylogeographic analyses in
223 BEAST under a constant size coalescent prior. These analyses allowed us to take into

224 consideration the uncertainty in the relationships by simultaneously estimating the
225 topology and the distribution history. To compare the asymmetric and symmetric
226 analyses for their fit to our data, we performed marginal likelihood estimation (MLE)
227 using path sampling (PS) and stepping stone (SS) sampling (Beale, 2012, 2013). We
228 used the default priors for the MLE. We categorized the locality of each tip for the
229 widespread *Sapajus* clade in the Bayesian consensus tree as occurring in one
230 biogeographical ‘region’ (Figure 2). We divided Amazonia into four regions using the
231 Amazon River and two major tributaries, Negro and Madeira, as described by Wallace
232 (1852) and later Cracraft (1985): Guianas (GU), Negro (NE), Inambari (IN), R ndonia
233 (RO). We also separated the drier open ecosystems Central Grasslands, Cerrado and
234 Caatinga (CC), from the Atlantic Forest (AF). These regions were modified from
235 Jameson Kiesling *et al.* (2015) and Cracraft (1985). As mtDNA is inherited maternally,
236 it tracks the movement of female lineages over time. Capuchin monkeys are female
237 philopatric with male-biased dispersal (Lynch Alfaro *et al.*, 2014), so tracing female
238 lineage was a conservative test for the amount of movement over time in this taxon.

239 Within Amazonia, we tested for isolation by river barriers for widespread
240 *Sapajus*. If gene flow within Amazonian capuchin populations was restricted to major
241 interfluves, we expected a pattern of closely related subclades within, not across, four
242 major Amazonian regions delineated by the Amazon River and its major tributaries.
243 Evidence for the river barrier hypothesis was found for the sister group to capuchins, the
244 Amazonian squirrel monkeys (Lynch Alfaro *et al.*, 2015a), as well as for marmosets and
245 tamarins (Buckner *et al.*, 2015). Robust capuchin lineages with clades spanning
246 multiple interfluves would provide evidence against rivers as significant isolating
247 mechanisms.

248

249 **RESULTS**

250

251 *Capuchin Phylogenetics*

252

253 We find strong support for reciprocally monophyletic clades of gracile and
254 robust capuchins in the phylogenetic reconstruction from MRBAYES (Figure 3a). Our
255 truncated species time tree from BEAST (Figure S1 in Appendix S3) estimates the
256 mean divergence time between *Sapajus* and *Cebus* at 5.8 Ma [95% higher posterior
257 density (HPD) = 2.24 - 10.94] (late Miocene).

258 For robust capuchins we recover strong support for the clades *S. xanthosternos*
259 (posterior probability [pp] = 1) and *S. nigritus* (pp = 1), but little evidence for
260 reciprocally monophyletic ‘species’ clades for the rest of *Sapajus*. Two *S. robustus*
261 samples form a weakly supported clade (pp = 0.74). All other robust capuchins form
262 one large widely distributed clade (‘*Sapajus* CLADE 4,’ pp = 1), comprising *S. apella*,
263 *S. macrocephalus*, *S. cay*, *S. libidinosus*, and *S. flavius*. Subclades within this group are
264 not always congruent with morphological hypotheses about robust capuchin species, a
265 result concordant with the mitochondrial results from Ruiz-García and colleagues
266 (2012, 2016). Most strikingly we find *S. apella sensu stricto* to be extensively
267 paraphyletic with respect to other *Sapajus* species. Within the widespread clade, we
268 recover several geographical subclades: (1) *S. flavius* (pp = 0.95); (2) *S. libidinosus*
269 together with eastern *S. apella* (pp = 0.80); (3) *S. cay* from Paraguay and Rondônia/Mato
270 Grosso plus *S. apella* from Guaporé and Apuí (pp = 0.81); (4) *S. cay* from Chapada dos
271 Guimarães and *S. apella* from Alta Floresta, both in Mato Grosso, Brazil (pp = 1); (5) *S.*
272 *apella/macrocephalus* (pp = 1) from north of the Amazon and Solimões rivers, as well
273 as from regions just south of the Amazon River near the Xingu and Tapajos rivers, and

274 just south of Manaus, together reaching all four Amazonian quadrants, as far northwest
275 as Colombia; (6) *S. apella/macrocephalus* (pp = 0.95) from Brazil and Peru. Minimum
276 clade ranges for each of the six subclades of the widespread *Sapajus* clade are
277 geographically coherent though several do not match current morphological hypotheses
278 about robust capuchin taxonomy (Figure 3c).

279 Within *Cebus*, we recover eight strongly supported clades: *Cebus versicolor*
280 forms a clade (pp = 1) with *C. cesarae* within the Magdalena river valley in the
281 Colombian Andes, as part of a larger clade including *C. capucinus* in Panama and
282 Colombia (pp = 1), and *C. imitator* in Central America (pp = 1). In the eastern Andes,
283 *C. brunneus* (pp = 1) is sister to *C. leucocephalus* (pp = 1). Within Amazonia, there is
284 strong support for a *C. unicolor* + *C. albifrons* clade (pp = 1). *C. yuracus* forms a clade
285 with one *C. unicolor* sample (pp = 1) in western Brazil and Ecuador, and *C. o. olivaceus*
286 + *C. o. castaneus* + *C. kaapori* form a strongly supported clade (pp = 1). Our *C. kaapori*
287 sample is from the holotype, collected by Queiroz (1992), from the type locality Chega-
288 Tudo, in Carutapera Municipality, Maranhão State, Brazil. Here we provide a correction
289 to the original Queiroz (1992) for the coordinates of that locality, 02°20'S 46°05'W
290 (Table S1 in Appendix S1).

291

292 *Reconstruction of Capuchin Biogeographical History*

293

294 Model weights based on AICc favoured the DEC model (Table 1, Figure 4).
295 Under this scenario the ancestral capuchin population is inferred to have had a wide
296 range that included both Amazonia and the Atlantic Forest. The divergence between
297 *Sapajus* and *Cebus* is a vicariance event, where *Sapajus* is isolated in the Atlantic
298 Forest, while the lineage leading to gracile *Cebus* is isolated in Amazonia. Crown

299 *Sapajus* is recovered with a distribution restricted to the Atlantic Forest, with
300 subsequent rapid expansion to the Caatinga/Cerrado/Central Grasslands and Amazonia
301 regions at about 500 ka. This means that *Cebus*, established in Amazonia at
302 approximately 2.4 Ma, diversified for about 2 million years before the appearance of
303 any *Sapajus* clade in that region.

304 For crown *Cebus*, a single expansion event to the Central America/Andes region
305 occurred at ~2.2 Ma, with subsequent range contractions to just Central America/Andes
306 for the lineage leading to *C. capucinus*, *C. imitator* and *C. versicolor*, and to Amazonia
307 only for both the lineage leading to *C. brunneus* (note that the “Amazonia” region
308 extends to the northern Venezuelan coast; see Figure 4), and for the lineage leading to
309 *C. unicolor*.

310

311 *Reconstruction of widespread Sapajus radiation*

312

313 We do not find any statistical difference in support for the symmetric versus the
314 asymmetric analyses ($K \approx 0$). Both the phylogeographical reconstructions detail the
315 explosive Pleistocene range expansion throughout Amazonia, Cerrado, Caatinga and
316 southern grasslands for *Sapajus* (Figure 2 and Figure S2 in Appendix S3). In the
317 asymmetric reconstruction, robust capuchins enter Amazonia by a single expansion
318 from AF to RO at around 300-500 ka, with a second unique expansion across the
319 Amazon River to GU at 200 ka, and at least two independent expansions across the
320 Madeira River from RO to IN. There is also evidence for expansion from GU to IN, and
321 IN to GU, as well as back-crossing from both IN and GU to RO. No strong
322 geographical pattern demarcates rivers as firm barriers separating the four Amazonian
323 regions; instead, there has been significant and recurrent recent movement by *Sapajus*

324 across the Amazon, Solimões, Madeira and Negro rivers. *Sapajus* also spread from RO
325 three times into the Cerrado/Caatinga (CC) region, all within the last 200 ka.

326 The addition of a distribution partition to our BEAST analysis provides support
327 for the monophyly of *Sapajus robustus* (0.93) and its position as the sister group to a
328 widespread Amazonian-Grasslands-Atlantic Forest clade (pp = 1.0). *Sapajus robustus* is
329 restricted to the Atlantic Forest, making it clear that the explosive radiation of robust
330 capuchin monkeys began with a recent migration into Amazonia and grasslands.

331 A significant difference between our asymmetric and symmetric BEAST
332 analyses is related to *S. flavius*. In the symmetric BEAST phylogeography analysis, *S.*
333 *flavius* is recovered as the sister group to *Sapajus* CLADE 4 (Figure S2 in Appendix
334 S3). On the other hand, *S. flavius* is embedded within the widespread clade in the
335 asymmetric analysis (Figure 2), suggesting an expansion back into the Atlantic Forest
336 from the Amazonia/Grasslands regions.

337

338 **DISCUSSION**

339

340 Our expanded sample set with comprehensive representation of capuchin species
341 provides further support for the monophyly of robust and gracile capuchins with a split
342 at 5.8 Ma. A late Miocene *Sapajus-Cebus* split was previously supported using
343 combined nuclear and mitochondrial data in Perelman *et al.* (2011) (6.0 Ma, 95%
344 confidence interval 3.13-9.35 Ma) and Springer *et al.* (2012) (5.7 Ma average, 3.55-8.33
345 Ma composite 95% min-max). However, these time trees did not include all capuchin
346 species; here we add *Cebus kaapori*, *C. versicolor*, and *Sapajus flavius*. One exception
347 to monophyly of *Cebus* and *Sapajus* has been recently reported in Ruiz-Garcia *et al.*
348 (2016), where some *Cebus albifrons sensu lato* individuals from the Colombian Eastern

349 Llanos and Ecuadorian Amazonia displayed *Sapajus* mitochondrial haplotypes,
350 suggesting the possibility of localized past or present introgression across these two
351 genera. Unfortunately, neither sequences nor morphological data are publicly available
352 for any of these individuals at this time.

353 Our biogeographical analyses reconstruct a widespread capuchin ancestral
354 distribution across forested areas of South America, from Amazonia to the Atlantic
355 Forest, supporting Amazonia versus Atlantic Forest vicariant origin for *Cebus* and
356 *Sapajus*. The distribution of *Sapajus* exclusively in the Atlantic Forest until recently,
357 and *Cebus* exclusively in the Amazonia and Central America/Andes regions from early
358 on, suggests a cladogenetic event (vicariance) led to their initial diversification in
359 isolation.

360 Throughout capuchin evolution, the Brazilian Shield (within AF, CC, RO
361 Regions) and Guiana Shield (within GU) have been constant highland regions
362 undergoing few physical changes in configuration but perhaps experiencing changes in
363 floral assemblages related to climate (Aleixo & Rossetti, 2007). The divergence
364 between *Sapajus* and *Cebus*, dated consistently at approximately 6 Ma, coincides with
365 floral assembly of the Cerrado (4-8 Ma), as indicated by the onset of diversification of
366 many Cerrado plant lineages (Simon *et al.*, 2009). The coincidence in timing of the
367 divergence between robust and gracile capuchins and of the Cerrado assemblage
368 suggests that the development of the Cerrado may have acted as a vicariant agent,
369 especially given the reconstructed distributions of ancestral *Sapajus* (Atlantic Forest)
370 and *Cebus* (Amazonia). The only extant capuchins distributed in CC belong to our
371 widespread *Sapajus* CLADE 4 - which invaded those biomes only in the last 500 kyr,
372 and have recent cranial and post-cranial adaptations to drier habitats (Wright *et al.*,
373 2015).

374 Combining information from our biogeographical and phylogeographical
375 analyses, we find strong evidence that all of the Atlantic Forest robust capuchin species
376 are monophyletic clades, with the exception perhaps of *S. flavius*, which was found as
377 part of the widespread *Sapajus* ‘clade 4’ in our MRBAYES phylogeny. Major Amazonian
378 rivers do not appear to be strong barriers for dispersal or gene flow for *Sapajus*, with up
379 to twelve examples of dispersal across significant Amazonian rivers demonstrated
380 through our BEAST analysis by just the sampling included in this study.

381

382 *Gracile capuchin biogeography and phylogenetics*

383

384 In our analysis, *Cebus* shows strong geographical divisions, suggesting a
385 widespread ancestor diversified in Amazonia and was split by range expansion and
386 subsequent range contraction in the Andes and Central America. The Amazonian group
387 (*C. yuracus*, *C. unicolor*, *C. albifrons*, *C. olivaceus* and *C. kaapori*) is about the same
388 age as the CA group (*C. capucinus*, *C. imitator*, *C. versicolor*, *C. cesarae*, *C.*
389 *leucocephalus* and *C. brunneus*). The recently discovered *Cebus kaapori* was initially
390 considered to be a subspecies of *C. olivaceus* (Harada & Ferrari, 1996), but other
391 morphological taxonomists have argued that it is a unique species (Groves 2001, 2005;
392 Silva-Júnior, 2001), with some morphological characteristics more similar to *C.*
393 *albifrons sensu lato* (Masterson, 1995). In our MRBAYES analysis, *Cebus kaapori* is
394 recovered within the strongly supported *C. olivaceus* clade. A close relationship
395 between *C. kaapori* and *C. olivaceus* makes sense, as *C. o. castaneus* is the gracile
396 capuchin geographically most proximate to *C. kaapori*. *Cebus versicolor* is recovered as
397 sister to *C. cesarae*. This is concordant with geographical distribution as both species
398 are found in isolated pockets of the Magdalena Valley in Colombia. MtDNA COII

399 analysis of *Cebus albifrons sensu lato* by Ruiz-García *et al.* (2010) found *C. cesarae*
400 and *C. pleei* as sister to *C. versicolor*.

401

402 *Robust capuchin biogeography and phylogenetics*

403

404 Our phylogeographic analysis suggests robust capuchins were able to colonize
405 an array of divergent habitat types all within a very short time period, in the last 1 Myr.
406 Robust capuchins show evidence of multiple moves within and between all regions,
407 except that they are absent from the Central American and Andean region. This may be
408 explained because their arrival at the foot of the eastern Andes was so recent that the
409 mountain range was already fully formed and impassable (Lynch Alfaro *et al.*, 2012a).

410 Consistent with this recent explosive radiation, we find little support for the
411 internal topology of a morphologically diverse and geographically expansive clade of
412 robust capuchins. Morphological species contained in this group include *S. libidinosus*,
413 *S. cay*, *S. flavius*, *S. macrocephalus*, and *S. apella*. While there is significant individual
414 variation within *Sapajus* populations, these five morphological species each display
415 distinct morphological patterns with clear geographic correspondence (Silva-Júnior,
416 2001), possibly as a consequence of repeated founder effects. While the recent
417 divergence times among all the Amazonian and open region grasslands capuchins (all
418 under 400 Ka) suggest there probably has not been time enough for speciation among
419 these morphological types, our data do point to geographical clades that deserve further
420 study for their morphological and behavioural population characteristics. For example,
421 morphological differences between *S. macrocephalus* north and south of the Amazon
422 River have already been described (Rylands *et al.*, 2013); our analysis here provides
423 new hypotheses for geographic boundaries between major population centers within

424 Amazonian *Sapajus*, and suggests the need for new morphological and morphometric
425 analyses for undersampled regions. We suggest nuclear genomic methods will be
426 necessary to provide a complementary perspective to help resolve the relationships
427 within this clade.

428 Rapid Pleistocene expansion throughout Amazonia has been recovered for the
429 sister taxon to capuchin monkeys, the squirrel monkeys (Chiou *et al.*, 2011; Lynch
430 Alfaro *et al.*, 2015a). *Sapajus* presence today in western Amazonia is explained in the
431 BEAST phylogeography analysis as resulting from two distinct pathways: one was
432 expanding from RO west across the Madeira River, into IN, and then spreading north
433 into NE. The second was a move first from RO across the Amazon River into GU, then
434 expansion west across the Negro River into NE, and from there south to IN. The exact
435 distribution of *S. macrocephalus* in western Amazonia is contested (Groves, 2001,
436 2005; Silva-Junior, 2001). Rylands *et al.* (2013) depict *S. macrocephalus* morphological
437 diversity with Brazilian, Peruvian, Bolivian and Colombian forms; one explanation for
438 this variation congruent with our results is that western Amazonia has been colonized
439 multiple times from different *Sapajus* seed populations from different parts of
440 Amazonia. In contrast, most of *Sapajus* diversity in eastern Amazonia can be explained
441 by a single founder event from AF into RO, and another single founder event from RO
442 into GU, with some later expansions back to RO from GU.

443 *Sapajus cay* has been the subject of controversy within robust capuchin taxonomy,
444 and two major authorities (Groves, 2001, 2005; Silva-Junior, 2001) disagree
445 significantly about its distribution, in part due to differences in the localities of samples
446 available in each of their studies. *Sapajus cay* (*sensu* Rylands *et al.*, 2013) is recovered
447 here as paraphyletic, with evidence for at least two distinct *Sapajus* populations moving
448 independently into gallery forests in more open habitat types. *Sapajus cay* samples from

449 Paraguay, Rondônia, and Mato Grosso cluster with *S. apella* from Rondônia; while *S.*
450 *cay* from northern central Mato Grosso forms a clade with nearby *S. apella* from Alta
451 Floresta. These two populations have apparently converged morphologically to a lighter
452 pelage phenotype in the drier, open regions. *Sapajus libidinosus* (*sensu* Rylands *et al.*,
453 2013) marks a third expansion into CC from Amazonian *Sapajus*, with a resultant
454 lighter pelage (Figure 2). All expansions into drier habitats appear to have occurred in
455 the last 200-100 kyr, similar to findings for other Neotropical primates (Lynch Alfaro *et*
456 *al.*, 2015b).

457 We expected to recover *Sapajus flavius* as sister to *S. xanthosternos* because of
458 geographical proximity or sister to *S. libidinosus* based on a gradation of morphology
459 between the two morphotypes (Silva, 2010); or possibly sister to all other *Sapajus*
460 species, based on its unusual morphology (Lynch Alfaro *et al.*, 2012a). In our BEAST
461 phylogeography and MRBAYES analyses, *Sapajus flavius* is recovered as part of the
462 widespread *Sapajus* CLADE 4 (Figure 2 and 3b). However, in the symmetric BEAST
463 phylogeography analysis, *S. flavius* is recovered as the sister group to *Sapajus* CLADE
464 4 (Figure S2 in Appendix S3), suggesting all four Atlantic Forest *Sapajus* taxa may
465 have diverged from each other first prior to *Sapajus* expansion into other regions. Given
466 the difference in topology and the range of support values among analyses, the
467 phylogenetic placement of *S. flavius* remains uncertain, though close phylogenetic
468 affinity to *Sapajus* CLADE 4 is clear.

469
470 *Sympatry between robust and gracile capuchins*

471

472 Overall, our data and analyses support the "Reinvasion of Amazonia" hypothesis
473 (Lynch Alfaro *et al.*, 2012a) by *Sapajus* as indicated by the ancestral reconstructions of

474 the crown *Cebus* and crown *Sapajus* clades. The two capuchin genera diversify in
475 isolation, with *Sapajus* species only later invading a *Cebus*-occupied Amazonia at about
476 500 kya. The BEAST phylogeography analysis shows *Sapajus* invasion of Amazonia
477 occurring first in Rondonia (south-western Amazon) and quickly spreading to all four
478 quadrants of Amazonia. The recovered timing of entry into sympatry for gracile and
479 robust capuchins indicate an event in Amazonia less than 1 Ma, as a result of *Sapajus*
480 counter-invasion into a region already occupied by *Cebus*.

481

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497

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668

669 SUPPORTING INFORMATION

670 Additional Supporting Information may be found in the online version of this article:

671 **Appendix S1** Details of individual and GenBank accession numbers.

672 **Appendix S2** Supplementary details of methods.

673 **Appendix S3** Supporting results.

674

675 **BIOSKETCH:**

676

677 **Marcela G. M. Lima** is a biologist interested in biogeography, phylogenetics and

678 population genetics, with a focus on Neotropical vertebrates. Author Contributions:

679 M.G.M.L., J.C.B., M.E.A. and J.W.L.A. designed and ran phylogenetic and

680 biogeographic analyses and developed the article; M.G.M.L., J.S.S.J., A.M., A.L.,

681 I.P.F., J.P.B., F.R., H.Q., M.N.S., A.D.F. and J.W.L.A. collected and curated sample

682 material; M.G.M.L., A.M. and I.P.F. generated new genetic sequences; K.C.,

683 M.G.M.L., J.C.B. and J.L.A. designed maps and figures; and all authors contributed to

684 the writing of the article.

685

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687

688 **Tables**

689

690 **Table 1** Comparison between models estimated in ‘BioGeoBEARS’. For each model

691 implemented in the analysis are shown: values of log-likelihood (LnL), numbers of

692 parameters and Akaike’s information criteria (AIC, Δ AIC and AICc Weight).

693

Model	LnL	No. Parameters	AICc	ΔAICc	AICc Weight
DEC	-17.29	2	39.92	0	0.68
DIVALIKE	-18.9	2	43.14	3.22	0.14
BAYAREALIKE	-21.41	3	51.81	11.9	0
DEC+J	-17.19	3	43.38	3.46	0.12
DIVALIKE+J	-18.61	3	46.22	6.3	0.03
BAYAREALIKE+J	-18.61	3	46.22	6.3	0.03

694 **Figure Legends**

695

696 **Figure 1** Maps of capuchin monkey sample provenance: (a) *Cebus*, (b) *Sapajus*.

697 **Figure 2** Asymmetric discrete states time tree for the widespread clade of robust
698 capuchin monkeys with ancestral state reconstruction for regions of ancestral nodes and

699 map delineating centres of endemism used for the BEAST phylogeography analyses:

700 GU: Guianas; NE: Negro; RO: Rondonia; IN: Inambari; CC: Caatinga, Cerrado and

701 Central Grasslands; AF: Atlantic Forest. Branches are colour-coded to regions from

702 inset map. Posterior probabilities are shown as asterisks for above 0.95 and as circles

703 for between 0.70 and 0.95.

704 **Figure 3** Bayesian consensus tree for capuchin phylogeny: (a) *Cebus* and *Sapajus*

705 phylogeny and (b) details of *Sapajus* CLADE 4; (c) map with minimum convex

706 polygons to show geographic distribution of major subclades within the widespread

707 *Sapajus* clade. Subclades are presented as minimum convex polygons that connect

708 sample localities. Branches are colour-coded to regions from polygons map. Posterior

709 probabilities are shown as asterisks for above 0.95 and as circles for between 0.70 and

710 0.95. Sample species ID and number correspond to Table S1 in Appendix S1.

711 **Figure 4** 'BioGeoBEARS' ancestral range reconstruction of capuchin monkeys using

712 the DEC model. CA = Central America/Andes; AM = Amazonia; CC = Caatinga,

713 Cerrado and Central Grasslands; AF = Atlantic Forest. Inset map for four regions of

714 analysis.

715