1 **Title page**

2 **Potential mammalian species for investigating the past connections between Amazonia**
3 **and the Atlantic Forest**

4 **Short title: Potential mammals to investigate Amazonia and the Atlantic Forest past**
5 **connections**

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30 **Abstract**

31 Much evidence suggests that Amazonia and the Atlantic Forest were connected through at
32 least three dispersion routes in the past: the northeast route, the central route, and the
33 southeast-northwest route. According to previous studies, the southeast-northwest route
34 would have been the most frequently used. However, few studies have assessed the use of
35 these routes based on multiple species. Here we present a compilation of potential mammal
36 species that could have dispersed between the two forest regions to investigate these
37 connections. We evaluate the geographic distributions of mammals occurring in both
38 Amazonia and the Atlantic Forest and the likely connective routes between these forests.
39 We classified the species per habitat occupancy (strict forest specialists, species that prefer
40 forest, or generalists) and compiled the genetic data available for each species to evaluate
41 their potential for phylogeographic studies focusing on genetic exchange between the two
42 forest regions. We found 127 mammalian species occurring in both Amazonia and the
43 Atlantic Forest for which significant genetic data was available. Hence, highlighting their
44 potential for phylogeographic studies investigating the past connections between the two
45 forests. Differently from what was previously proposed, the northeast route showed
46 evidence of past use by more mammal species than the remaining two routes. The central
47 route would have been the second most important in terms of species. Our results show the
48 potential of using mammal species to investigate and bring new insights about the past
49 connections between Amazonia and the Atlantic Forest.

50 **Keywords:** Biogeography, Moist Tropical Rainforests, Neotropical Forests, Connective
51 routes, Dry diagonal, Mammals, Genetic data.

52 **Introduction**

53 Amazonia and the Atlantic Forest are among the most diverse forests in the world
54 [1, 2]. Biogeographical patterns of these megadiverse forests have been investigated since
55 the 19th century [3, 4]. Currently, these forests are separated by the 'dry diagonal'
56 comprising the Caatinga, the Cerrado and the Dry Chaco ecoregions. However, different
57 evidence sources including biogeographical [5-10], palynological [11-13], and geological
58 [14] show that these forests have been connected in the past.

59 Three routes have been suggested as past connections between Amazonia and the
60 Atlantic Forest, one through the forests of North-eastern Brazil (the northeast route [NE
61 route]), another through the gallery forest of the Brazilian Cerrado ecoregion (the central
62 route [CE route]) and a third through the forests of the Paraná Basin, the Moist Chaco, and
63 the Pantanal (the southeast-northwest route [SW route]) [5, 6, 15]. According to Por [5], the
64 SW route would have been the first to be formed and this connection would have occurred
65 more often over time, followed by the NE route.

66 In a study aimed to test Por's hypothesis [5], Ledo & Coli [10] reviewed the
67 literature for molecular evidence of connections between Amazonia and the Atlantic Forest
68 for ca. 60 vertebrates, including 10 mammals. They found more studies that evidenced
69 connections through the SW route compared to the NE route [10]. However, this result
70 could be biased due to the poor sampling in the northeast region [16]. It remains uncertain
71 whether the SW route was indeed the most frequently formed connection and thus the most
72 used route in the past.

73 There is a great number of recent sister species of birds in Amazonia and the
74 Atlantic Forest that may have resulted from the use of the NE route [8]. However, like Ledo

75 & Colli [10], these authors did not consider the CE route as independent but included it as
 76 part of the NE route. Yet, the CE route has been well documented as a separate migratory
 77 pathway in the literature for both animals and plants [5, 6, 15, 17, 18]. Moreover,
 78 investigating eight small mammals, Costa [6] found a larger number of small, related
 79 mammals occurring in Amazonia and the Atlantic Forest that could have come through the
 80 CE route. However, this study was limited for small mammals which have specific traits,
 81 such as limited dispersion ability. Considering more species with different traits and
 82 divergence time may further add evidence on the use of the CE connection, as well as for
 83 the other routes. In this context, ecological and genetic data may shed light on the use and
 84 frequency of these past connections.

85 Some phylogeographical studies investigate the role of the historical connections
 86 between Amazonia and the Atlantic Forest in terms of dispersion and diversification of
 87 several animal species, such as mammals [6, 19], birds [8], reptiles [20-22], amphibians
 88 [23] (for a literature revision of vertebrate evidence see [10]), and insects [24]. However,
 89 the totality of species that may evidence past connections between Amazonia and the
 90 Atlantic Forest have not been mapped and this information is particularly scarce for
 91 mammals.

92 There are currently several databases of species geographic and molecular
 93 distribution data that can be used to evaluate those past forest connections. A compilation
 94 of the data available, including geographic, ecological, and genetic data, for species that
 95 may show evidence of the past connections between Amazonia and the Atlantic Forest
 96 could thus be especially useful for testing the aforementioned hypotheses.

97 Here we aim to identify potential mammal species useful for investigating the past
 98 connections between Amazonia and the Atlantic Forest through geographical distribution

99 patterns, habitat preference, and genetic data. Furthermore, we aim to identify the potential
100 connective routes previously proposed in the literature. We believe that our results may
101 serve as basis for future biogeographic studies considering different mammalian taxonomic
102 groups.

103 **Material and Methods**

104 Species data

105 We considered mammal species of interest for investigating the past connections
106 between Amazonia and the Atlantic Forest to fulfil the following three criteria: 1) species
107 occurring in both Amazonia and the Atlantic Forest, 2) species that use forests, and 3)
108 species with genetic data available on GenBank [25]. Additionally, we identified potential
109 past connective routes between Amazonia and the Atlantic Forest by investigating the
110 distribution maps for each of these species.

111 Geographical data

112 Geographical distribution maps of forest mammalian species from Amazonia and
113 the Atlantic Forest were compiled from the IUCN - International Union for Conservation of
114 Nature [26]. To identify the mammalian species that occur the two regions, Amazonia and
115 Atlantic Forest, the IUCN maps were overlaid on the Ecoregion maps [27] using the
116 Amazonian and the Atlantic Forest limits, through the *gIntersection* function of the R
117 package ‘rgeos’ v. 0.5.5 [28] in R v. 3.6.3 [29]. Subsequently, only species with
118 occurrences in both Amazonia and the Atlantic Forest were selected. The predefined
119 identifications based on the overlaid IUCN occurrence maps were revised using the

120 annotated list of mammals in Brazil of Paglia et al. [30] since this reference agree with
121 current geographical and genetic data available in the databases used in this study.

122 Habitat classification

123 We selected solely forest species by accessing the IUCN information on species
124 habitat use through the *rl_habitats* function of the R package ‘rredlist’ v. 0.6.0 [31].
125 Species that were exclusive to open and/or aquatic habitats were not considered for this
126 study. We generated a scale of habitat preference for each species from forest specialist to
127 generalist, as this is key information for studies about the connective routes between
128 Amazonia and the Atlantic Forest. We based this scale of habitat preference on the detailed
129 text about species' habitat and ecology, available in the IUCN database [25] and additional
130 literature reviews [e.g., 33-38]. The criteria used for classifying the species according to
131 habitat were as follows: 1) Strict forest specialists (SF), encompassing species that only
132 occur in forests, 2) Species that prefer forest (PF), encompassing species that use not only
133 forested habitats but prefer these environments, or 3) Generalists (G) encompassing species
134 that use both forests and open environments (Table 1). Then, we used a Pearson's Chi-
135 squared test to assess the relationship between the species' habitat preferences and the
136 routes used through the *chisq.test* function in the R v. 3.6.3 basic package ‘stats’ [28].

137 Genetic data

138 We compiled genetic data for each species from the Genbank database [25]. Data
139 compilation was done during January 2020, by registering the amount of molecular data
140 available (nucleotide sequences) for each species. The genetic data was used to assess the
141 taxonomic representativeness (i.e., which taxonomic groups represent the highest
142 availability of published genetic data) and, consequently, their potential usefulness in

143 evaluating the past existence and use of connections between Amazonia and the Atlantic
144 Forest.

145 We created categories for the availability of genetic data to assess the potential
146 usefulness of the mammal species. These categories were defined using the quantile
147 function of the R package 'stats' [28]. We considered species with low availability of
148 genetic data those with zero to 22 nucleotide sequences in the database: species with sparse
149 genetic data availability those with 23 to 74 sequences, species with regular availability
150 those with 75 to 225 sequences in the database and species with high data availability those
151 with 226 to over 1000 sequences. We also calculated the average of sequences by species
152 for each taxonomic order to compare the availability of genetic data for these different
153 taxonomic groups.

154 Identification of potential connective routes

155 To identify the potential connective routes between Amazonian and Atlantic forests
156 used by mammals, the geographical areas of these connections on ecoregion maps were
157 first delimited using Olson's ecoregion polygons [27]. The NE route area was delimited
158 using the boundaries of the Caatinga ecoregion, the transition areas between the Caatinga,
159 the northern Cerrado and eastern Amazonia, the Babaçu Forest, and adjacent dry forests,
160 which represent transition areas between Amazonia and the Atlantic Forest (Fig 1; route 1).
161 The area of the CE route was selected using the limits of the Cerrado ecoregion (excluding
162 the northern part, which was selected for the NE route; Fig 1; route 2). The area of the SW
163 route was delimited using the boundaries of the Pantanal and the Chaco ecoregions, the
164 transition areas between Amazonia and the Pantanal (such as Chiquitano Dry Forests), the
165 southern Cerrado and the southwestern Atlantic Forest (Fig 1; route 3).

166 To explore how many species might have used each connective route, the delimited
167 area for each route (NE, CE, and SW) was intersected with the species distribution maps
168 using the function *gIntersection* in the R package ‘rgeos’ v. 0.5-5 [27]. Thereby, we
169 quantified the total number of species associated with each route and the number of routes
170 associated with each species. To visualize this result in the geographic space, we calculated
171 the sum of rasters using the R package ‘raster’ v. 3.3 [39].

172 **Results**

173 We compiled geographic distribution maps, information about habitat preferences,
174 and genetic data for 127 mammal species occurring in both Amazonia and Atlantic Forest,
175 following nine taxonomic orders: Didelphimorphia (7), Pilosa (3), Cingulata (4),
176 Perissodactyla (1), Cetartiodactyla (3), Primates (3), Carnivora (12), Chiroptera (85) and
177 Rodentia (9) (Table 1). According to the IUCN, the geographic distribution of 113 of those
178 species appear to be continuous between Amazonia and the Atlantic Forest, whereas the
179 remaining 14 species present disjunct distributions (S1 Table).

180 In terms of habitat use, 17 species were classified as strict forest specialists (SF), 23
181 species with preference for forests (PF) and 88 species as generalists (G) (Fig 2; Table 1).
182 For the NE route, two species were SF, three PF and four were G. For the CE route, two
183 species were SF, two PF and none were G. For the SW route, one species was SF, no one
184 PF and two species were G (Fig 2; Table 1). For the NE and CE routes together, 10 species
185 were SF, seven PF and 20 were G. For the three connective routes combined, one species
186 was SF, four PF and 49 were G (Fig 2; Table 1). The result of the chi-square test for the
187 habitat preference was significant ($\chi^2 = 35.904$, $df = 12$, $p\text{-value} = 0.0003$).

188 Thus, we reject the null hypothesis, which states that habitat preference is independent from
189 choice of connective route.

190 Most of the species identified in this study as potentially useful for assessing the
191 connections between Amazonia and the Atlantic Forest have large amounts of genetic data
192 available in the investigated database, including different molecular markers (Fig 3; Table
193 1; Genbank access link for details on available molecular data, in S1 Table). Thirty of the
194 investigated species showed Low availability of genetic data in Genbank, 32 species show
195 Sparse genetic data availability, 33 Regular and 32 High availability of genetic data (Fig 3;
196 Table 1). The Cingulata and Chiroptera contained most of the available genetic data (with
197 averaging about 1,000 and 590 sequences per species, respectively; Fig 4), followed by the
198 Pilosa, Primates, Cetartiodactyla, Perissodactyla (averaging about 330, 300, 220, 200 and
199 190 sequences per species respectively; Fig 4). The Didelphimorphia and Rodentia also
200 showed a considerable number of available nucleotide sequences (averaging about 115 and
201 93 sequences per species, respectively; Fig 4).

202 As evidenced by the IUCN geographic distribution maps, 52 (41.6%) of the 127
203 evaluated species of mammals potentially dispersed between Amazonia and the Atlantic
204 Forest using all three connective routes (NE, CE, and SW). Thirty-eight of the 127 species
205 (30.4%) potentially used both the NE and the CE routes, whereas 14 species (11.2%) may
206 have potentially used the CE and the SW routes. Nine species (7.2%) are expected to have
207 used the NE route exclusively, five species (4%) the CE route exclusively, and four species
208 (3.2%) the SW route exclusively. Only three species show potential to have used a
209 combination of the NE and the SW routes (2.4%) (Fig 5). Hence, the potentially most used
210 connections were the NE route followed by the CE route (Fig 1, Fig 5, Fig 6). For two bat
211 species *Diclidurus scutatus* and *Micronycteris hirsute* it was not possible to attribute a

212 potential connective route, as these present extremely disjunct distributions between
213 Amazonia and Atlantic Forest (Table 1).

214 **Discussion**

215 Here we present an unprecedented list of potential mammal species for investigating
216 the past connections between Amazonia and the Atlantic Forest. Previous studies, including
217 data compilations, analysed a limited number of species of mammals. Here we were able to
218 include 127 species of mammals occurring in in both Amazonia and the Atlantic Forest to
219 add information about the potential past connections between the two biomes. We compiled
220 information about the species' distributions, habitat preferences, and the quantity of genetic
221 data available. We also showed the possible routes used by these mammals, and the
222 association between habitat use and their potential connection route between the two
223 rainforest regions.

224 According to Por [5], the SW connection between Amazonia and the Atlantic Forest
225 was the most used route. The connection through the NE was the second most common
226 route, whereas the route through the riverine forests in the interior of the Cerrado (CE
227 route) was the least used. This hypothesis has been confirmed by a review of vertebrate
228 molecular data [10]. The single previous study compiling data for multiple species of
229 mammals evaluating these connective routes was Costa [6]. This author investigated the
230 phylogeography of small mammals and found that the CE route was potentially the most
231 used route. However, our results show that, differently from these previous studies, the NE
232 route would have been the most common route. The CE route was the second most
233 common and the SW route was the least used. These contrasting results highlight the need
234 for further studies including multiple taxa, ecological traits, and evolutionary data. The list

235 we compiled here has the potential to subsidize other mammal phylogeographic studies and
236 shed light on the temporal and spatial use of the connections between Amazonia and the
237 Atlantic forest in relation to South American mammals' ecology and evolution.

238 In fact, studies on paleo vegetation, pollen data and biogeographic approaches have
239 shown evidence of past connections between the northern Atlantic Forest and the eastern
240 Amazonia through the NE route [7, 12-14, 20, 40-42). Even so, no had directly shown the
241 potential importance of this route in connecting Amazonia and the Atlantic Forest. Also,
242 several species showed evidence of having used more than one route in the past. Most
243 species potentially used both the NE and CE routes or even the whole of the three routes.

244 Previous studies underestimated the number of mammalian species that could have
245 dispersed between Amazonia and the Atlantic Forest, especially for the NE route, but even
246 our study is likely to underestimate this number. For example, sampling artefacts
247 deficiencies in the dry diagonal of the northeast, in addition to the non-inclusion of point
248 records published for the northeast or available from specimens deposited in mammal
249 collections [16] may have led us to underestimate the number of potential taxa that could
250 have dispersed through the NE route. Additionally, many species showing disjunct
251 distributions, probably represent either sampling deficiencies, such as the two bat species
252 *Diclidurus scutatus* and *Micronycteris hirsute*, and also the huge extension of range of
253 *Promops centralis* after using complementary sampling schemes or cryptic diversity [43].

254 Examples of mammal species need to be reassessed regarding their past occurrences
255 along the NE route, include the anteater *Cyclopes didactylus*, the Kinkajou *Potos flavus* and
256 the squirrel *Guerlinguetus brasiliensis* [44-46, respectively]. The taxonomic status of the
257 guariba *Alouatta ululata*, previously a subspecies of *A. belzebul*, has been elevated to the
258 species category [47] and requires a revaluation through molecular and cytogenetic

approaches, as suggested by Viana et al. [48]. Miranda et al. [49-50] presented new records of common forest species for the NE route, such as the marsupials *Marmosa demerarae*, *Metachirus nudicaudatus* and *Didelphis marsupialis* (personal obs. C.L. Miranda). The rodent *Oecomys catherinae*, considered restricted to the Atlantic Forest, has been recorded along the NE route [51, 52]. The black-eared opossum *D. marsupialis* presents disjunct distribution, but recent records point to no disjunction along the NE route [e.g., 49, 50, 53]. The three-striped short-tailed opossum, *Monodelphis americana*, is also present in the eastern Amazonia and in the northeastern Atlantic Forest, with the populations south of the São Francisco River belonging to another species of the genus *Monodelphis* [54]. Hence, updating the known distribution of these species and others would certainly increase the number of species showing evidence of the past connections between Amazonia and the Atlantic Forests, mainly through the NE route. Additionally, as new records appear with increased sampling and systematic biogeographic studies from the NE region, future reassessments will certainly find further species showing evidence of past dispersions along the NE route [e.g., 47, 48, 53].

As highlighted by Costa [6], the forest environments in the Cerrado ecoregion should function as ecological [55] and historical corridors, allowing forest species to be present in the region. Thus, these forests form a connection that until our study had not been specifically recognized; here we call it the CE route, although it had already been considered a potentially independent route in other studies [5, 6, 15, 17]. Besides those riparian forests in the northeast dry diagonal, along the NE route, there is a marked presence of other types of forested habitats that may also serve the same function. Some examples include the Babaçu forests, the semi-deciduous forests, the mangroves, and the “boqueirões” (humid forests that occur in patches in the Caatinga, which some authors

283 consider remnants of the Atlantic Forest, while others consider relic environments with
284 biogeographical composition and affinities yet to be properly understood) and the “brejos
285 de altitude” or Altitude Swamps [49]. To evaluate this hypothesis would, however, require
286 specific studies that focus on the species that show evidence of the NE route to better map
287 their past dispersion routes.

288 According to the IUCN species distribution maps, many mammalian species seem
289 to have continuous distributions between Amazonia and the Atlantic Forest through
290 deciduous and semi-deciduous forests in the interior of the dry diagonal (composed by the
291 Caatinga, the Cerrado and the Chaco). However, many phylogeographic studies reveal that
292 some of these species, with seemingly continuous distributions, consist of currently isolated
293 populations [e.g., 56]. Moreover, many isolated populations show evolutionary well-
294 structured lineages with significant genetic divergence, so their taxonomic status is
295 probably in need of revision as they could represent species complexes [e.g., 6, 34, 56, 58].
296 Hence, further phylogeographic studies are necessary if we are to reveal whether these
297 mammalian populations are indeed connected or isolated in function of the current
298 fragmentation pattern of the South American forests, particularly that of anthropic origin so
299 evident in the Atlantic Forest remnants and in the arc of deforestation of Amazonia. Indeed,
300 the landscape becomes increasingly fragmented as we continue to lose forest habitat, the
301 environmental protection system fails to connect the ecoregions between Amazonia and the
302 Atlantic Forest [59], and under the complete laxation of the Brazilian environmental laws
303 under the current federal government [60]. Given the relationship that we observed between
304 species’ habitat preferences and their potential connection routes in the past, such forest
305 loss and habitat fragmentation could have dire consequences for the mammal populations
306 along these dispersal pathways.

Whereas the CE route was associated with forest specialists or species mostly with forest habits, most routes between Amazonia and the Atlantic Forest were associated with habitat generalists. The NE route, for example, passed through the Caatinga or the Cerrado/Caatinga transition, which is a region of high environmental heterogeneity [61, 62]. Thus, here, we found species with a greater variety of habitat preferences. Subsequently, we believe that the inclusion of species with a wider range of habitat preferences (i.e. not only forest specialists) could be decisive for the identification and quantification of the past use of connective routes between Amazonia and the Atlantic Forest; not least because the past use of the connective routes seems related to the environmental heterogeneity of each region and environmental changes in the past [11, 12, 13]. Therefore, it would be interesting to make use of the 127 species that we identified, to evaluate the past forest connections in relation to different sampling methods and the differences in ecological characteristics among taxonomic groups.

The availability of genetic data for our 127 candidate species, revealed that many of them would serve for assessing the existence and importance of the past connections between Amazonia and the Atlantic Forest. Also, although we only show the total amount of available nucleotide sequences for each species, this initial compilation can be extremely useful in facilitating more detailed evaluations for future phylogeographic simulations and to further explore the past connections between Amazonia and the Atlantic Forest. However, it is important to highlight that, although many of these species have a large amount of available genetic data, it is common for many of the nucleotide sequences to contain missing information, which could render them unfit for use. Even so, many recent studies provide complete genetic data for mammals favouring the development of phylogeographic studies [57, 58, 63, 64]. Therefore, our results, not only inform us about

the dispersal history of the 127 mammalian species among the humid Neotropical forests, but our results also bring new insights about the connective routes that may have once existed between Amazonia and the Atlantic Forest.

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Authors contribution

AFM, LD and MJRP designed the study. AFM compiled the data and CLM and MJRP revised and complement it. AFM and CDR analyses the data. AFM, wrote the manuscript with contribution of LD, CDR, CLM and MJRP.

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540 **Tables**

541 **Table 1.** Potential mammal species for investigating the past connections between
 542 Amazonia and the Atlantic Forest. H = habitat preference (SF = Strict forest specialist; PF
 543 = Species that prefer forest; G = Generalists); R = Connective routes between Amazonia
 544 and the Atlantic Forest based on the IUCN expert species distribution maps (0 =
 545 Unidentified route; 1 = Northeast route [NE]; 2 = Central route [CE]; 3 = Southwest route
 546 [SW]); Potential = quantity of availability of genetic data based on the total number of
 547 nucleotide DNA sequences available on Genbank online database. The categories run from
 548 low (0-22 DNA sequences), to sparse (23-74 DNA sequences), satisfactory (75-225 DNA
 549 sequences), and high (226 to >1000 DNA sequences).

Species	Family	Order	H	R	Potential
<i>Tapirus terrestris</i>	Tapiriidae	Perissodactyla	G	1, 2, 3	Regular
<i>Mazama americana</i>	Cervidae	Cetartiodactyla	PF	2, 3	Regular
<i>Pecari tajacu</i>	Tayassuidae	Cetartiodactyla	G	1, 2, 3	High
<i>Tayassu pecari</i>	Tayassuidae	Cetartiodactyla	G	1, 2, 3	Sparse
<i>Bradypus variegatus</i>	Bradypodidae	Pilosa	SF	1, 2	High
<i>Cyclopes didactylus</i>	Cyclopedidae	Pilosa	PF	1	Regular
<i>Tamandua tetradactyla</i>	Myrmecophagidae	Pilosa	G	1, 2, 3	Regular
<i>Cabassous unicinctus</i>	Chlamyphoridae	Cingulata	G	1, 2, 3	Sparse
<i>Dasyopus novemcinctus</i>	Chlamyphoridae	Cingulata	G	1, 2, 3	High
<i>Dasyopus septemcinctus</i>	Chlamyphoridae	Cingulata	G	1, 2, 3	Sparse
<i>Myrmecophaga tridactyla</i>	Myrmecophagidae	Cingulata	G	1, 2, 3	Regular
<i>Alouatta belzebul</i>	Atelidae	Primates	SF	1, 2	High
<i>Alouatta caraya</i>	Atelidae	Primates	PF	2, 3	High
<i>Sapajus libidinosus</i>	Cebidae	Primates	G	1, 2	Regular
<i>Cerdocyon thous</i>	Canidae	Carnivora	G	1, 2, 3	Regular

<i>Eira barbara</i>	Mustelidae	Carnivora	PF	2, 3	Sparse
<i>Galictis vittata</i>	Mustelidae	Carnivora	G	1, 2	Sparse
<i>Leopardus pardalis</i>	Felidae	Carnivora	G	1, 2, 3	High
<i>Leopardus tigrinus</i>	Felidae	Carnivora	G	1, 2	High
<i>Leopardus wiedii</i>	Felidae	Carnivora	PF	2, 3	High
<i>Nasua nasua</i>	Procyonidae	Carnivora	G	2, 3	Regular
<i>Panthera onca</i>	Felidae	Carnivora	G	1, 2, 3	High
<i>Potos flavus</i>	Procyonidae	Carnivora	SF	1, 2	Regular
<i>Procyon cancrivorus</i>	Procyonidae	Carnivora	G	1, 2, 3	Sparse
<i>Puma yagouaroundi</i>	Felidae	Carnivora	G	1, 2, 3	Regular
<i>Speothos venaticus</i>	Canidae	Carnivora	G	2, 3	Regular
<i>Anoura caudifer</i>	Phyllostomidae	Chiroptera	G	2, 3	Regular
<i>Anoura geoffroyi</i>	Phyllostomidae	Chiroptera	G	1, 2, 3	High
<i>Artibeus concolor</i>	Phyllostomidae	Chiroptera	G	1, 2	Regular
<i>Artibeus lituratus</i>	Phyllostomidae	Chiroptera	G	1, 2, 3	High
<i>Artibeus obscurus</i>	Phyllostomidae	Chiroptera	G	1, 2, 3	High
<i>Artibeus planirostris</i>	Phyllostomidae	Chiroptera	G	1, 3	High
<i>Carollia brevicauda</i>	Phyllostomidae	Chiroptera	G	1, 2	High
<i>Carollia perspicillata</i>	Phyllostomidae	Chiroptera	G	1, 2, 3	High
<i>Centronycteris maximiliani</i>	Emballonuridae	Chiroptera	SF	1, 2	Low
<i>Choeroniscus minor</i>	Phyllostomidae	Chiroptera	G	1	Sparse
<i>Chrotopterus auritus</i>	Phyllostomidae	Chiroptera	PF	1, 2, 3	Regular
<i>Cynomops abrasus</i>	Molossidae	Chiroptera	SF	2	Sparse
<i>Cynomops greenhalli</i>	Molossidae	Chiroptera	PF	1	Low
<i>Cynomops planirostris</i>	Molossidae	Chiroptera	PF	1, 2, 3	Sparse
<i>Dermanura cinerea</i>	Phyllostomidae	Chiroptera	G	1, 2	High
<i>Dermanura gnomia</i>	Phyllostomidae	Chiroptera	G	1, 2, 3	Sparse
<i>Diaemus youngi</i>	Phyllostomidae	Chiroptera	G	1, 2, 3	Sparse

<i>Diclidurus albus</i>	Emballonuridae	Chiroptera	G	1, 2, 3	Low
<i>Diclidurus scutatus</i>	Emballonuridae	Chiroptera	PF	0	Low
<i>Diphylla ecaudata</i>	Phyllostomidae	Chiroptera	G	1, 2	Sparse
<i>Eptesicus brasiliensis</i>	Vespertilionidae	Chiroptera	G	1, 2, 3	Low
<i>Eptesicus diminutus</i>	Vespertilionidae	Chiroptera	G	1, 2, 3	Low
<i>Eptesicus furinalis</i>	Vespertilionidae	Chiroptera	G	1, 2, 3	Regular
<i>Eumops auripendulus</i>	Molossidae	Chiroptera	G	1, 2, 3	High
<i>Eumops delticus</i>	Molossidae	Chiroptera	G	1, 2	Low
<i>Eumops glaucinus</i>	Molossidae	Chiroptera	G	2, 3	Regular
<i>Eumops perotis</i>	Molossidae	Chiroptera	G	1, 2, 3	Sparse
<i>Furipterus horrens</i>	Furipteridae	Chiroptera	PF	1, 2	Sparse
<i>Gardnerycteris crenulatum</i>	Phyllostomidae	Chiroptera	G	1, 2	Regular
<i>Glossophaga soricina</i>	Phyllostomidae	Chiroptera	G	1, 2	High
<i>Glyphoncteris sylvestris</i>	Phyllostomidae	Chiroptera	PF	2	Low
<i>Histiotus velatus</i>	Vespertilionidae	Chiroptera	G	1, 2, 3	Low
<i>Lasiurus blossevillii</i>	Vespertilionidae	Chiroptera	G	1, 2, 3	Sparse
<i>Lasiurus cinereus</i>	Vespertilionidae	Chiroptera	G	2, 3	High
<i>Lasiurus ega</i>	Vespertilionidae	Chiroptera	G	1, 2, 3	Sparse
<i>Lasiurus egregius</i>	Vespertilionidae	Chiroptera	SF	3	Low
<i>Lichonycteris degener</i>	Phyllostomidae	Chiroptera	SF	1	Low
<i>Lonchorhina aurita</i>	Phyllostomidae	Chiroptera	PF	1, 2	Low
<i>Lophostoma brasiliense</i>	Phyllostomidae	Chiroptera	G	1	Sparse
<i>Lophostoma silvicolum</i>	Phyllostomidae	Chiroptera	G	1, 3	High
<i>Macrophyllum macrophyllum</i>	Phyllostomidae	Chiroptera	G	1, 2, 3	Sparse
<i>Micronycteris hirsuta</i>	Phyllostomidae	Chiroptera	G	0	Sparse
<i>Micronycteris megalotis</i>	Phyllostomidae	Chiroptera	G	1, 2, 3	Regular
<i>Micronycteris minuta</i>	Phyllostomidae	Chiroptera	G	1, 2, 3	Sparse
<i>Micronycteris schmidtorum</i>	Phyllostomidae	Chiroptera	G	1	Low

<i>Mimon bennettii</i>	Phyllostomidae	Chiroptera	G	1, 2	Low
<i>Molossops mattogrossensis</i>	Molossidae	Chiroptera	G	1, 2	Low
<i>Molossops neglectus</i>	Molossidae	Chiroptera	G	3	Low
<i>Molossops temminckii</i>	Molossidae	Chiroptera	G	1, 2, 3	Low
<i>Molossus currentium</i>	Molossidae	Chiroptera	G	3	Low
<i>Molossus molossus</i>	Molossidae	Chiroptera	G	1, 2, 3	High
<i>Molossus pretiosus</i>	Molossidae	Chiroptera	G	2, 3	Low
<i>Molossus rufus</i>	Molossidae	Chiroptera	G	1, 2, 3	Regular
<i>Myotis albescens</i>	Vespertilionidae	Chiroptera	G	1, 2, 3	Sparse
<i>Myotis nigricans</i>	Vespertilionidae	Chiroptera	G	1, 2, 3	Sparse
<i>Myotis riparius</i>	Vespertilionidae	Chiroptera	G	1, 2, 3	Regular
<i>Myotis simus</i>	Vespertilionidae	Chiroptera	G	1, 3	Low
<i>Noctilio albiventris</i>	Noctilionidae	Chiroptera	G	1, 2, 3	High
<i>Noctilio leporinus</i>	Noctilionidae	Chiroptera	G	1, 2, 3	High
<i>Nyctinomops aurispinosus</i>	Molossidae	Chiroptera	G	1, 2	Sparse
<i>Nyctinomops laticaudatus</i>	Molossidae	Chiroptera	G	1, 2, 3	Sparse
<i>Nyctinomops macrotis</i>	Molossidae	Chiroptera	G	1, 2, 3	Sparse
<i>Peropteryx kappleri</i>	Emballonuridae	Chiroptera	G	1, 2	Low
<i>Peropteryx leucoptera</i>	Emballonuridae	Chiroptera	G	1	Low
<i>Peropteryx macrotis</i>	Emballonuridae	Chiroptera	G	1, 2, 3	Sparse
<i>Phylloderma stenops</i>	Phyllostomidae	Chiroptera	G	1, 2	Sparse
<i>Phyllostomus discolor</i>	Phyllostomidae	Chiroptera	G	1, 2, 3	High
<i>Phyllostomus elongatus</i>	Phyllostomidae	Chiroptera	SF	1, 2	Regular
<i>Phyllostomus hastatus</i>	Phyllostomidae	Chiroptera	G	1, 2, 3	Regular
<i>Platyrrhinus lineatus</i>	Phyllostomidae	Chiroptera	G	1, 2, 3	Sparse
<i>Promops centralis</i>	Molossidae	Chiroptera	G	3	Sparse
<i>Promops nasutus</i>	Molossidae	Chiroptera	G	2, 3	Low
<i>Pteronotus personatus</i>	Mormoopidae	Chiroptera	G	1, 2	High

<i>Pygoderma bilabiatum</i>	Phyllostomidae	Chiroptera	SF	2, 3	Low
<i>Rhinophylla pumilio</i>	Phyllostomidae	Chiroptera	SF	1, 2	High
<i>Rhogeessa hussoni</i>	Vespertilionidae	Chiroptera	SF	1, 2	Low
<i>Rhogeessa io</i>	Vespertilionidae	Chiroptera	G	1, 2	Sparse
<i>Rhynchonycteris naso</i>	Emballonuridae	Chiroptera	PF	1, 2, 3	Regular
<i>Saccopteryx bilineata</i>	Emballonuridae	Chiroptera	G	1, 2	High
<i>Saccopteryx leptura</i>	Emballonuridae	Chiroptera	G	1, 2, 3	Regular
<i>Sturnira tildae</i>	Phyllostomidae	Chiroptera	PF	1, 2	High
<i>Thyroptera tricolor</i>	Thyropteridae	Chiroptera	SF	1	High
<i>Trachops cirrhosus</i>	Phyllostomidae	Chiroptera	PF	1, 2	High
<i>Uroderma bilobatum</i>	Phyllostomidae	Chiroptera	G	1, 2	High
<i>Uroderma magnirostrum</i>	Phyllostomidae	Chiroptera	PF	1, 2	Low
<i>Caluromys lanatus</i>	Didelphidae	Didelphimorphia	PF	2, 3	Sparse
<i>Caluromys philander</i>	Didelphidae	Didelphimorphia	PF	1, 2	Regular
<i>Chironectes minimus</i>	Didelphidae	Didelphimorphia	PF	2	Sparse
<i>Marmosa demerarae</i>	Didelphidae	Didelphimorphia	PF	1, 2	Regular
<i>Marmosa murina</i>	Didelphidae	Didelphimorphia	PF	1	Regular
<i>Metachirus nudicaudatus</i>	Didelphidae	Didelphimorphia	PF	2, 3	Regular
<i>Monodelphis americana</i>	Didelphidae	Didelphimorphia	SF	1, 2	Regular
<i>Coendou prehensilis</i>	Erethizontidae	Rodentia	PF	1, 2, 3	Sparse
<i>Cuniculus paca</i>	Ctenomyidae	Rodentia	SF	1, 2, 3	Regular
<i>Dasyprocta leporina</i>	Dasyproctidae	Rodentia	PF	2	Regular
<i>Dasyprocta prymnolopha</i>	Dasyproctidae	Rodentia	G	1, 2	Low
<i>Guerlinguetus aestuans</i>	Sciuridae	Rodentia	SF	1, 2	Low
<i>Hydrochoerus hydrochaeris</i>	Caviidae	Rodentia	G	1, 2, 3	Regular
<i>Hylaeamys megacephalus</i>	Cricetidae	Rodentia	SF	2	High
<i>Nectomys rattus</i>	Cricetidae	Rodentia	G	1, 2	Low
<i>Oecomys trinitatis</i>	Cricetidae	Rodentia	SF	1, 2	Low

550 **Figures legends:**

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552

553 **Figure 1.** Distribution of Tropical Moist Forests in South America in green emphasizing
554 Amazonia and the Atlantic Forest. Internal arrows represent connective routes between
555 these forests through the Northeast/NE route (1), the Central/CE route (2) and the

556 Southeast-Northwest/SW route (3). The width of the arrows represents the potential
557 frequency that we found by which the routes have existed in the past based on distribution
558 polygons of mammal species.

559

560

561 **Figure 2.** Number of mammalian species assumed to have dispersed by each of the
562 connective routes between Amazonia and the Atlantic Forest, as evidenced by the species
563 geographical distributions. The routes potentially used by mammalian species are presented
564 along the x-axis: 1) NE = Northeast route, 2) CE = Central route, 3) SW = Southeast -
565 Northwest route, and combinations of routes (1 + 2, 1 + 3, 2 + 3, and “All” for 1 + 2 + 3).
566 The grayscale represents species habitat preferences where SF = Strict forest specialists, PF
567 = Species that prefer forests and G = Generalists.

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570 **Figure 3.** Availability of genetic data for mammalian species that occur in both Amazonia
571 and the Atlantic Forest divided into the categories: Low = species with low availability of
572 genetic data (from 0 to 22 sequences), Sparse = species with sparse availability of genetic
573 data (from 23 to 74 sequences), Regular = species with satisfactory availability of genetic
574 data (from 75 to 225 sequences), and High = species with high availability of genetic data
575 (from 226 to over 1000 sequences).

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578 **Figure 4.** Availability of genetic data (Average of sequences per species) by orders for
 579 species of forest mammals that occur in both Amazonia and the Atlantic Forest.

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582 **Figure 5.** Venn diagram showing the past connective routes between Amazonia and the
 583 Atlantic Forest as evidenced by the IUCN geographical distribution maps for mammalian
 584 species that occur in both biomes.

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587 **Figure 6.** The overlaid distribution maps of potential mammal species for investigating the
588 connections between Amazonia and the Atlantic Forest show most connections for the
589 Northeast route (NE route), followed by the Central route (CE route) and finally the
590 smallest number of species were associated with the Southeast - Northwest route (SW
591 route). The colour scale represents the number of species per pixel on the map. A) Overlap
592 in the species distribution maps for all mammal species sampled in this study; B) Overlap
593 in species distribution maps for species associated with the NE route; C) Overlap in species
594 distribution maps for species associated with the CE route; D) Overlap in species
595 distribution maps for species associated with the SW route.