

1 **Barcoding Brazilian mammals to monitor biological diversity and threats:**
2 **trends, perspectives, and knowledge gaps**

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33 **Abstract**

34 DNA barcoding and environmental DNA (eDNA) represent an important advance for
35 biomonitoring the world's biodiversity and its threats. However, these methods are highly
36 dependent on the presence of species sequences on molecular databases. Brazil is one of the
37 largest and most biologically diverse countries in the world. However, many knowledge gaps
38 still exist for the description, identification, and monitoring of its mammalian biodiversity using
39 molecular methods. We aimed at unraveling the patterns related to the presence of Brazilian
40 mammal species on molecular databases to improve our understanding on how effectively it
41 would be to monitor them using DNA barcoding and environmental DNA, and contribute to the
42 conservation of this flagship zoological group. We found that many gaps exist in molecular
43 databases, with many taxa being poorly represented, particularly from the Amazon biome, the
44 order Lagomorpha, and arboreal, gomivorous, near extinct, and illegally traded species.
45 Moreover, our analyses revealed that the year of species description was the most important
46 factor determining the probability of a species to have been sequenced. Primates are the group
47 with the highest number of species considered to be priority for sequencing due to their high
48 level of combined threats. We highlight where investments are needed to fill knowledge gaps
49 and increase the representativity of species on molecular databases to enable a better monitoring
50 ability of Brazilian mammals encompassing different traits using DNA barcoding and
51 environmental DNA.

52

53 **Keywords:** biomonitoring; conservation; environmental DNA; Mammalia; Neotropics.

54 **1. Introduction**

55 The fast pace of biodiversity decline worldwide demands effective and rapid
56 biomonitoring tools to evaluate species and population trends. However, most traditional
57 methods used in the inventory of terrestrial biodiversity are usually time-consuming either due to
58 the high effort to set the equipments in the field and capture the specimens or due to the time to
59 properly process the samples and identify species (Carl et al. 2020; Gogarten, 2020; Mena et al.
60 2021). Moreover, they can also be inaccurate in terms of species identification due to taxonomic
61 limitations related to morphologically identifying some taxa (Meek et al. 2013). These can be
62 particularly problematic for taxa and regions with high levels of cryptic species, such as the
63 Neotropical region (Clare et al. 2007; Domingos et al. 2014; Funk et al. 2012).

64 Morphological identification of mammals can be complex due to the need to evaluate
65 internal anatomical structures (such as skulls or teeth) (Galimberti et al. 2015), and requires
66 specialized taxonomic training and practice, and a comparative reference material in zoological
67 collections, which is often lacking (Francis et al. 2010; Pečnikar and Buzan, 2014), in particular
68 in vast unexplored regions of the Neotropics. However, molecular tools developed in the last
69 decades considerably increased the ability to overcome the need to evaluate morphological
70 characteristics for species identification and description (Schindel et al. 2005; Galimberti et al.
71 2015). The application of DNA barcoding to ecological studies have largely advanced in the last
72 two decades, enabling the genetic identification of mammal species from tissue samples and
73 DNA traces recovered from the environment (eDNA), greatly improving species detectability
74 (von Cräutlein et al. 2011; Ivanova et al. 2012; Lynggaard et al. 2023; Saranholi et al. 2023).
75 Moreover, it has also increased our ability to understand the impacts of anthropogenic pressures
76 on threatened species (Klippel et al. 2015; Guimarães-Costa et al. 2020). The application of

77 eDNA can yield comprehensive, cost-effective, and representative datasets for different
78 monitoring conditions and target species (Ji et al. 2013; Mena et al. 2021).

79 The International Barcode of Life (IBOL) initiative is considered to be one of the largest
80 biodiversity consortiums in the world, with a total of five billion dollars invested over the course
81 of ten years (Lewin et al. 2018). IBOL aims at sequencing all known multicellular species in the
82 world until 2030 (Lewin et al. 2018; Lewin et al. 2022), which will make it possible to monitor
83 Earth's biodiversity on an unprecedented scale. However, we are still far from achieving this goal
84 for some species groups and countries, hindering our ability to properly monitor the world's biota
85 ecological trends and dynamics. Reliable species identification based on DNA relies on the
86 presence of the species sequence on molecular databases (Kvist, 2013; Theissinger et al. 2023).
87 Thus, taxonomic groups that are poorly represented in molecular databases are generally harder
88 or impossible to be identified using molecular tools (Kvist, 2013; Theissinger et al. 2023),
89 making it also challenging for these taxa to be monitored and protected. Thus, identifying and
90 filling biodiversity gaps in molecular databases is crucial to properly monitor and protect the
91 world's biodiversity (Šmíd, 2022). Countries in tropical regions harbour a significant number of
92 non-sequenced vertebrate species (Šmíd, 2022), due to both challenges in collecting and
93 sequencing tissue samples, and the high biodiversity levels of the regions, making tropical
94 species more vulnerable to anthropogenic threats.

95 In addition to enhancing biodiversity monitoring, DNA barcoding can also improve
96 species identification for different threats, such as poaching, roadkill, and illegal wildlife trade
97 (Sanches et al. 2011; Klippel et al. 2015; Formentão et al. 2021), which are important threats to
98 the conservation of mammals (Costa et al. 2005; Ferreira, 2014; Pinto et al. 2022). One of the
99 main challenges to monitor the impact of these threats is that they often leave unidentifiable

100 traces of biological tissue for morphological identification (Santos et al. 2012; Klippel et al.
101 2015), including meat, nails or hair, making it difficult or even impossible for species
102 morphological detection and identification (Huffman and Wallace, 2012; Johnson et al., 2014).
103 However, animal remains can be used in the detection of particular species with the use of
104 environmental DNA, including airDNA or surface swabs (Bohmann et al. 2014; Clare et al.
105 2022; Drescher et al. 2022; Lynggaard et al. 2023; Gonçalves et al. 2015).

106 Brazil is the fifth largest country in the world and the largest tropical country, representing
107 almost half of the entire area of the Neotropical region (44%), which is one of the most diverse
108 biogeographic regions worldwide (Raven et al. 2020). Furthermore, the country hosts seven
109 biomes, with two biodiversity hotspots (Cerrado and Atlantic Rainforest), as well as the largest
110 and most diverse world's rainforest, the Amazon (Myers et al. 2000; Kintish, 2007). Brazil is
111 also a megadiverse country, with one of the richest mammalian fauna worldwide (775 mammal
112 species) (Mittermeier et al. 1997, Costa et al. 2005; Abreu et al. 2022), representing over 10% of
113 the mammal species on the planet (Mammal Diversity Database, 2023). Similar to other
114 megadiverse countries, many cryptic species and previously unknown taxa still remain to be
115 described in Brazil (Pinto et al. 2015; Domingos et al. 2017). On average, more than four new
116 Brazilian mammal species are described every year (Paglia et al. 2012), including recent
117 descriptions of species with a wide range of body sizes, such as bats, primates, and river dolphins
118 (Hrbek et al. 2014; Moratell and Dias, 2015; Boubli et al. 2019). Thus, Brazilian high cryptic
119 mammalian diversity and species richness, makes it difficult to taxonomically identify, describe,
120 and monitor mammalian biodiversity within the country, challenging the conservation of this
121 ecologically diverse group.

122 Our study aimed at unraveling the current ability to monitor Brazilian mammals using
123 DNA barcoding and environmental DNA (eDNA) based methods to improve our understanding
124 on how effectively it is possible to monitor biological trends and dynamics. More specifically,
125 our goals were threefold: 1) describe mammal species coverage on molecular databases based on
126 different ecological and conservation traits (distribution, conservation status, threat, order, diet,
127 habit); 2) understand what are the most important traits that predict the probability of a species to
128 be available at molecular databases; 3) create a priority list of threatened Brazilian mammal
129 species to be sequenced for DNA barcoding.

130

131 **2. Methods**

132 *2.1. Dataset about Brazilian mammals*

133 Using a list of mammal species compiled by the Brazilian Mammalogical Society (Abreu
134 et al. 2022), we searched for the presence of available Cytochrome c oxidase subunit I (COI)
135 DNA barcode sequences for each species on the main molecular database responsible for storing
136 DNA barcodes (Bold Systems), and then categorized each species as being present or absent
137 from the database. Thus, for each Brazilian mammal species, we compiled a set of traits using
138 data from multiple references: species description year, biome of occurrence, diet, habitat,
139 endemism to Brazil, restricted distribution (occurring in an area of less than 20,000 km²), weight,
140 IUCN conservation status, population trend, and threats (poaching, roadkill, and illegal wildlife
141 trade) (Supplemental files).

142 We initially compiled data about biome, diet, habitat, endemic to Brazil, restricted
143 distribution, and weight, using the information available at Paglia et al. (2012). For species that
144 were only described or recognized to occur in Brazil after the publication of Paglia et al. (2012),

145 we complemented the information present on the database with additional literature searches and
146 consults to specialists on different mammal orders. For information about mammal species
147 conservation status and population trends we used IUCN (2021). To assess whether species are
148 known to be affected by roadkills and poaching, we used Grilo et al. (2018) and Praill et al.
149 (2022), and Ferreira (2014), respectively. Finally, we used the CITES database for the list of
150 mammal species that are threatened due to illegal wildlife trade (CITES, 2023).

151

152 *2.2. Statistical analysis*

153 To spatially represent species coverage on molecular databases, we considered the
154 distribution maps of 663 Brazilian mammal species that were available at Map of Life
155 (<https://mol.org/species> - Marsh et al. (2022)). We used grid cells of 50 km across the whole
156 extension of Brazil and plotted the density of mammal richness using the density analysis plugin
157 available at QGIS (<https://qgis.org>). We calculated density following two steps: initially, we
158 considered the 663 mammal species as the total species richness; afterwards, we focused on the
159 427 mammal species for which there were DNA sequences available. From this, we calculated
160 the relative density of species with DNA sequences available. We further repeated this analysis
161 for each individual mammal order (Figure S1).

162 To infer the influence of mammal species biological traits and other important historical,
163 ecological and conservation-related predictors on the probability of a species to have a DNA
164 barcode deposited in public database, we applied a classical machine-learning approach using
165 Classification and Regression Trees (CART, Breiman et al, 1984) implemented in the package
166 RPART (Therneau et al, 2022). The advantages of this algorithm over other machine-learning or
167 linear statistics alternatives is the straightforward use of both continuous and discrete data, and

168 the fact that it can be implemented when there are missing values (NAs) for some predictors
169 without incurring in the complete removal of the case (i.e., mammal species, in our dataset) as
170 would be necessary in linear regression approaches. As usually done for regression or
171 classification machine learning approaches, we partitioned the data into two sets, one for training
172 (70% of the cases) and another for testing (30% of the cases). This approach of training the
173 algorithm with one set of data and testing it with an independent set is a powerful approach that
174 allows for straightforward error estimation.

175 Hereinafter, we use the term “bold status” in reference to the data of species that either
176 have (bold status = yes) or do not have (bold status = no) a COI DNA barcode deposited in the
177 BOLD systems. We run two independent CART analyses, always using bold status as the
178 dependent variable: 1) using all compiled variables as independent variables; 2) excluding all
179 predictors for which the data reflects Brazilian-specific information, namely Biome distribution,
180 poaching, roadkill, and Brazilian endemism status (Figure S2 and Figure S3). After both
181 analyses, we calculated the accuracy of each classification tree based on the number of correctly
182 identified bold status in the testing set.

183 For the prioritization of the threatened species to be sequenced, we used the following
184 species traits: endemism to Brazil, restricted distribution (distribution within an area of less than
185 20,000 km²), conservation status (vulnerable, endangered or critically endangered), population
186 trend (decreasing), and threats (poaching, hunting, and illegal wildlife trade). Species that
187 matched five of these criteria were listed as extremely high priority, while species matching three
188 to four were listed as high priority, and one to two were considered as intermediate priority.
189 Furthermore, non-threatened species according to IUCN were not included in our list and were
190 considered to be low priority for sequencing in relation to conservation purposes.

191

192 3. Results

193 3.1. *Species spatial coverage on molecular databases*

194 In total, 482 Brazilian mammal species were present on the BOLD system database,
195 which represents 62.19% of Brazilian mammals (775 spp.). The Amazon Forest was the biome
196 with the highest number of species (453 spp.), but also with the highest number of missing
197 species on BOLD (181 spp. - 39.96%). On the opposite, the Pampas biome had the lowest
198 number of species (83 spp.), but with the highest proportion of species present on BOLD (71
199 spp. - 85.54%). In relation to the distribution of species coverage across the country, the northern
200 and southern portions of Brazil, located in the Amazon Forest and the Pampas had the lowest and
201 the highest coverage, respectively (Figure 1). The western part of Brazil, including mostly the
202 Amazon Forest, had the lowest species coverage, reaching values as low as 75%. Moreover,
203 regarding the representativity of each mammal order, only Chiroptera and Cetartiodactyla had
204 higher representativity of species on BOLD in western Amazon, but lower in the Pampas.

205

206 3.2. *Species taxonomic, ecological and conservation representation in molecular* 207 *databases*

208 In relation to the Brazilian mammal orders with at least 10 species, Cetartiodactyla (60
209 spp.) had the highest species coverage on BOLD (47 spp. - 78.33%) and Primates had the lowest
210 (131 spp. - 33.59%). Considering Brazilian mammal diets, insectivorous species were the most
211 common across Brazilian mammals (288 spp. - 37.35%), planctophagous species had the highest
212 species coverage (10 spp. - 90.00%), while gomivorous species had the lowest (22 spp. -
213 18.18%). Regarding species habits, arboreal habit was the most common across Brazilian

214 mammals (240 spp - 31.96%) and also had the lowest species coverage (111 spp. - 46.25%), with
215 aquatic species having the highest species coverage (46 spp. - 79.31%). In relation to the level of
216 threat, most Brazilian mammal species were listed as least concern (471 spp.), which was also
217 the group with the highest species coverage (356 spp. - 75.58%), while not evaluated (15 spp. -
218 15.79%) and critically endangered species (4 spp. - 28.57%) had the lowest coverage.

219

220 *3.3. Traits influencing species DNA barcoding*

221 In our first analysis, using all predictors, the species year of description and whether it
222 was listed as least concern according to the IUCN were the most important variables influencing
223 the decision tree algorithm and explaining bold status (Fig. S2). These analyses had an accuracy
224 of 76.7% (i.e., correctly identified bold status) based on the testing set, which is in line with
225 other studies using machine-learning approaches to classify biological data (Domingos et al.
226 2014, Scalon et al. 2020). In our second analysis, excluding Brazilian-specific information, we
227 found that species not evaluated by the IUCN and year of description were the most important
228 variables influencing the decision tree algorithm and explaining bold status (Fig. S2). These
229 analyses had an accuracy of 70.9% (i.e., correctly identified bold status) based on the testing set.

230

231 *3.4. Representativity of species under threat*

232 In relation to some of the main known threats for Brazilian mammals, roadkill (93 spp.)
233 was the one with the highest number of species coverage on molecular databases (84.54%);
234 followed by poaching (123 spp.) (84 spp. - 68.29%), and illegal wildlife trade (187 spp.) with the
235 lowest species coverage (60.43%). Regarding the species coverage for these threats in relation to
236 each mammal order (with more than one species), Pilosa, Cingulata and Chiroptera were the

237 ones with the highest species coverage (100% for all threats), while Cetartiodactyla, Primates,
238 Rodentia, and Sirenia showed the lowest species coverage (Figure 4).

239

240 3.5. Prioritization for species DNA barcoding

241 Forty five Brazilian mammal species are currently listed as threatened according to the
242 IUCN Red list, but still do not have publicly available DNA barcodes according to our results.
243 From those, we listed seven species as extremely high priority, 35 species as high priority and
244 three as moderate priority for DNA barcoding (Table S1). The most important species to be
245 sequenced (extremely high priority) due to their high level of threat coupled with their endemism
246 to Brazil and restricted distribution were: Marca's marmoset (*Mico marcai* - Primates), Rondon's
247 marmoset (*Mico rondoni* - Primates), Maranhão red-handed howler (*Alouatta ululata* - Primates),
248 Coimbra Filho's titi monkey (*Callicebus coimbrai* - Primates), black-faced lion tamarin
249 (*Leontopithecus caissara* - Primates), buffy-headed marmoset (*Callithrix flaviceps* - Primates),
250 and pied tamarin (*Saguinus bicolor* - Primates).

251

252 4. Discussion

253 Many gaps still exist in the ability to identify and monitor Brazilian mammals and their
254 threats using DNA barcoding and environmental DNA, considering the available COI data in
255 public molecular databases. The Amazon biome (more specifically its western region), the order
256 Lagomorpha, arboreal, gomivorous, near extinct, and illegally traded species, are proportionally
257 the taxa with the lowest representativity in molecular databases. Furthermore, the most important
258 traits that define the likelihood of a taxa to have been sequenced are the year of description and
259 its conservation status on IUCN, with long-described species and of least concern having a

260 higher probability of being represented in DNA barcode libraries than others. Lastly, primates
261 were the most speciose threatened taxa that needs to be sequenced to increase species coverage
262 in molecular databases.

263

264 *4.1. Species spatial coverage on molecular databases*

265 Our results follow the general finding that 81% of mammal species worldwide have
266 genetic data available on molecular databases (Šmíd, 2022). The differences in species coverage
267 between biomes might be related with an effect of the number of species with a restricted
268 distribution (Šmíd, 2022), which would restrict certain species to one or few biomes, making it
269 also harder to acquire biological samples for sequencing. Furthermore, biomes with lower
270 species richness, such as the Pampas, require lower sampling and sequencing efforts to achieve a
271 higher species coverage in molecular databases.

272 The Amazon biome was not only the biome with the poorest species representation on
273 molecular databases, but also held the grid cells with the lowest species richness coverage across
274 Brazil. These could be related with the low accessibility of the Amazon, especially in its western
275 portion, which has limited access from roads (Grilo et al. 2021). Our results are partially
276 congruent with Šmíd (2022), which pointed out that the Northern Amazon is one of the best
277 represented portions of the world for mammals, but are discordant with the results in comparison
278 with the western Amazon, which was one of the poorest represented areas in our study. This
279 might be related with an increase in the availability of distribution maps for mammals and newly
280 described species (Marsh et al. 2022; Rengifo et al. 2022), which revealed more specific regional
281 patterns for species that were not considered in Šmíd (2022).

282 Chiroptera and Cetartiodactyla were relatively well represented in BOLD across the
283 western Amazon, which is the opposite trend compared to other mammalian orders, and might be
284 explained by species of both these orders having large distributions (Šmíd, 2022). Many species
285 within both orders have wide distributions covering large proportions of the Neotropical region
286 (Marsh et al. 2022), which would make it easier for biological samples of these species to be
287 collected and sequenced in other more accessible biomes or surrounding countries.

288

289 *4.2. Species taxonomic, ecological and conservation representation in molecular*
290 *databases*

291 Mammals are a highly diverse order, which includes species that have many different
292 diets, habits, and levels of threat (Paglia et al. 2012). In order for DNA barcoding to be an
293 effective tool for the detection of species with these characteristics, these species need to be
294 sequenced and deposited in molecular databases (Balint et al. 2018; Blaxter et al. 2022).
295 However, the molecular database that we evaluated is highly skewed towards species groups or
296 with specific traits (Cetartiodactyla, planctophagous, aquatic, and least concern species), which
297 hinders our ability to monitor taxa with different traits in the landscape as well as fully
298 understand ecosystem dynamics.

299 These molecular availability trends are probably related to the characteristics of the
300 species from each group. Arboreal species, for example, represent close to a third of Brazilian
301 mammal species, but have less than half of the total species present on molecular databases. This
302 could be related with the fact that canopies still remain an ecological frontier and one of the least
303 accessible places on land (Erwin, 1983; Nakamura et al. 2017). Moreover, many arboreal

304 mammals tend to be elusive and rarely come down to the ground (Eppley et al. 2022; Kaizer et
305 al. 2022), increasing the challenges to collect biological samples for further sequencing.

306 It is alarming for species conservation that there are still large gaps for threatened
307 Brazilian mammals on molecular databases, with their representativity ranging from 25% to
308 60%. The poor representation of these groups in relation to less threatened species can also be
309 related with shared traits among threatened species, such as small populations and geographic
310 ranges, which also makes it difficult for biological samples from these species to be collected for
311 sequencing. Furthermore, it is interesting to note that species that were not evaluated in relation
312 to their conservation status had the worst representativity (~10%), which might be further
313 leading to a low interest in studying them, and consequently creating a gap in their representation
314 on molecular databases. Alternatively, their lack of evaluation can also be related with species
315 that were only recently described and, thus, still do not have biological samples available for
316 sequencing.

317

318 *4.3. Traits influencing species sequencing*

319 Our CART results provide clear evidence that researchers were better able to obtain and
320 sequence samples from non-threatened species, and those who have been described for a long
321 time. We interpret this finding in two non-exclusive ways: over the years, it was easier for
322 researchers to obtain and sequence samples from species that are not in some important threat
323 category (more abundant and easier to obtain biological samples), and species that have been
324 taxonomically available for more time were also more prone to be sequenced. Moreover, it is
325 important to highlight that most Brazilian mammal species are not threatened (504 spp - 65.03%),
326 which is probably associated with the fact that most species have not been evaluated by the

327 IUCN. Although it is reasonable to hypothesize that some biological traits such as having a
328 widespread distribution and displaying larger sizes would influence the availability of specimens
329 for obtaining samples and sequencing, these had minor influences on the decision tree in relation
330 to the best predictors (Fig. S3). Nonetheless, supporting this hypothesis, the biological traits that
331 were important in explaining bold status were weight, and the distribution in the Atlantic Forest
332 and Marine biomes, indicating that these characteristics indeed influenced the availability of
333 specimens for researchers. In this regard, it is important to highlight that all major Brazilian
334 universities and research institutions are located in richer states within the Atlantic Forest biome
335 and in the southeast coast, mostly in the states of São Paulo and Rio de Janeiro.

336 The second analysis also reinforces the fact that year of description is an important
337 predictor of bold status, which could be related with researchers having additional time to get
338 biological samples and sequence earlier described species. Furthermore, the biological traits
339 weight, arboreal locomotion and gomivory were important predictors. Taken together, our
340 CART results support the idea that focus should be given to sequence more recently described
341 species. Also, as would be expected, at least one biological trait related to species body size (i.e.,
342 weight) is important in terms of researchers gaining access to samples, highlighting the need to
343 invest in smaller species.

344

345 *4.4. Molecular identification of species under threat*

346 Road mortality, poaching, and illegal wildlife trade are some of the main direct threats to
347 the Brazilian mammalian fauna (Galletti et al. 2021). We found that roadkills and poaching are
348 the threats more likely to be monitored using DNA barcoding, but illegal wildlife trade still
349 remains a challenge, with only 60% of species coverage of threatened species. Additionally,

350 primates and rodents are groups that need increased efforts for sequencing threatened species, as
351 they are still largely under-represented on BOLD.

352 Brazil has the fourth largest road network worldwide (DNIT, 2019), and it is essential
353 that a proper biomonitoring scheme is implemented to measure animal-vehicle collisions. In São
354 Paulo State alone, one out of 26 Brazilian states, it has been estimated that around 40,000
355 medium and large wild mammals are roadkilled each year, including a high number of casualties
356 of endangered species (Abra et al. 2021). Thus, roadkill potentially causes significant impacts on
357 mammal populations and even local species extinctions (Grilo et al. 2021), demanding further
358 investigations, where DNA barcoding can give a significant contribution. DNA barcoding is of
359 utmost importance in situations where roadkill monitoring is conducted by non-specialists, like
360 road maintenance personnel on private toll roads in Brazil, for instance. A study conducted by
361 Abra et al. (2018) revealed that non-specialists lack the capacity to identify rare or infrequently
362 observed species, those that closely resemble other species (such as small wild canids and
363 felids), or species that are not easily distinguishable. Consequently, these individuals often
364 misidentify, provide ambiguous descriptions, or fail to identify these species at all, resulting in
365 an unreliable roadkill database. One additional aspect that needs to be addressed is that
366 biological samples of fresh roadkilled animals can also be sequenced for high-quality reference
367 genomes (Allio et al. 2021), which could help to fill molecular databases and help monitoring
368 this threat.

369 Furthermore, Brazilian mammals can be heavily impacted by poaching, with over 15% of
370 mammal species (123 spp.) impacted by this threat, which represents a significant proportion of
371 the total number of species threatened by poaching worldwide (301 spp) (Ripple et al. 2016).
372 Moreover, poaching can lead to severe mammal population declines or even local species

373 extinctions (Jorge et al. 2013; Galetti et al. 2016). However, it is a difficult threat to monitor as
374 several species are reduced to unidentifiable parts after being hunted (remains of bones, organs,
375 hair, meat and skin), making it often almost impossible to morphologically identify them. In
376 addition, poaching is a crime according to the Brazilian environmental law since 1967, and
377 suspects may face detention and financial penalties, but that needs evidence for legal actions to
378 be properly conducted. Thus, DNA barcoding can significantly help to fight this threat and
379 legally prosecute poachers, as it can allow species identification from biological remains.
380 Moreover, if an endangered species is proven to have been killed, the punishment can be more
381 severe under Brazilian laws (Di Rocco and Anello, 2021).

382 Finally, illegal wildlife trade can be an important source of introduction not only of
383 invasive mammal species, but also different pathogens (Rosa et al. 2018), and exert important
384 pressures that might lead to populations extinction (Scheffers et al. 2019), which requires an
385 intensive biomonitoring program. Tackling illegal wildlife trade, which threatens around one
386 fourth of mammals in Brazil and globally, demands the identification of where and which
387 species are being traded (Scheffers et al. 2019). However, this is the threat where Brazilian
388 mammals are less represented on molecular databases (~60%), limiting actions to fight against it.
389 Thus, more investments are needed to increase the representativity of Brazilian mammal species
390 threatened due to illegal wildlife trade on molecular databases. Particular attention needs to be
391 given for species inhabiting the Atlantic Rainforest, the Amazon Forest, and the Cerrado, as
392 these biomes account for hotspots of illegally traded mammal species (Scheffers et al. 2019).
393 Moreover, since traded wildlife is often transported on hidden compartments, which are difficult
394 to detect and monitor, environmental DNA from the air and swabs coupled with portable DNA
395 sequencers (e.g., MinION, Oxford Nanopore Technologies) could help tackling this threat in a

396 more efficient way (Clare et al. 2022; Lynggaard et al. 2023). This is particularly important
397 because, even though Neotropical mammals are mostly traded as pets, trading for products, such
398 as clothing, medicines or religious purposes, can also represent an important issue, especially in
399 the western Amazon (Scheffers et al. 2019).

400

401 *4.5. Prioritization of species sequencing*

402 Primates represent the third most speciose mammal order in Brazil, representing almost
403 one sixth of the total Brazilian mammal species richness (16.90%). Nonetheless, over two thirds
404 of priority Brazilian mammal species to be sequenced are primates (31 spp. - 68.89%). Many
405 neotropical primate species not only have a small distribution (Marsh et al. 2022), but are also
406 elusive due to their arboreal habits, which makes them more susceptible to extinction due to
407 habitat loss, and also harder to collect biological samples for sequencing. Moreover, the Amazon
408 is the most speciose Brazilian biome for primates (Paglia et al. 2012), but many places are hard
409 to access due to the lack of roads or other types of access through parts of intact vegetation
410 (Grilo et al. 2021). Primates are highly susceptible to deforestation as many species cannot move
411 very far on a deforested landscape or survive in the absence of trees (Boyle and Smith, 2010;
412 Mendes-Oliveira et al. 2017). Altogether, these factors are likely to explain the dominance of
413 primates as high priority for sequencing in Brazil.

414 Sequencing priority species is an important task to be conducted at a fast pace, as it will
415 enable them to be properly detected and their populations monitored across the landscape
416 (Monchamp et al. 2023). Moreover, it can make a significant difference in terms of increasing
417 endangered species detection to combat poaching and wildlife trade, but also improving species
418 identification after roadkills, which can help in elaborating strategies for preventing these

419 accidents. This would be an important step to promote the conservation of the species on this list,
420 but it is also necessary to promote the use of DNA sequences to monitor Brazilian wildlife across
421 the country. This methodology is still not widely used to monitor wildlife in Brazil due to its
422 high costs and lack of sequencing facilities in some parts of the country.

423

424 *4.6. Perspectives and challenges for sequencing Brazilian mammals*

425 Around 3,000,000 dollars have been invested by the Brazilian Barcode of Life initiative for
426 sequencing Brazilian biodiversity during the period of 2010-2014, which involved over 100
427 participating groups and 500 people from different institutions around the country (Eizirik,
428 2015). This initiative has initially led to the generation of barcodes from 2,122 specimens
429 belonging to 344 mammal species, which represents ~45% of the current number of mammal
430 species in Brazil (Eizirik, 2015; Abreu et al. 2022). However, progress towards achieving a
431 comprehensive understanding of Brazilian mammal genetic diversity is still far from complete,
432 with less than 65% of Brazilian mammal species currently barcoded. Clearly, more investments
433 are needed to speed up this process and achieve the goals of the Earth Biogenome project, a
434 difficulty shared with other similar initiatives, and in line with the results they have reached so
435 far (Threlfall and Blaxter, 2021; Darwin Tree of Life Project Consortium, 2022). The new
436 barcoding phase may be viewed as more challenging since most of the target species remaining
437 are primarily found in the Amazon biome, and the majority exhibit arboreal behavior, making
438 them less susceptible to being roadkilled, poached, or trafficked.

439 Even though Brazil generally has a better infrastructure for processing biological samples
440 and sequencing species in comparison to other countries in the Global South (Colella et al.
441 2023), this infrastructure is still lacking in some regions. In remote areas of Brazil, such as the

442 western Amazon, where there is a lack of access to roads, electricity, and research facilities,
443 sequencing technologies that are less dependent on electricity and internet access, and also
444 lighter and more mobile are needed (Ebenezer et al. 2022). Thus, some parts of the country still
445 need more investments for building sequencing facilities for biodiversity description and
446 monitoring. This has also led to large knowledge gaps regarding the presence of some taxonomic
447 groups on molecular databases (Eizirik, 2015). Furthermore, sequencing costs remain
448 comparatively high in Brazil after taking into account the conversion to the Brazilian currency,
449 which hinders large country efforts to locally sequence Brazilian species at a fast pace.
450 Moreover, for biological samples that were collected in Brazil but sequenced elsewhere, there is
451 a need to make an equal share of the benefits according to the Nagoya Protocol (Colella et al.
452 2023). This is an important part of the discussion on sequencing tropical biodiversity in relation
453 to the debate about the agreement of the Kunming-Montreal Global Biodiversity Framework
454 (KM-GBF) (Hughes, 2023). Thus, it is important that at least part of the benefits from Digital
455 Sequencing Information (DSI) that were sequenced outside the tropics, but which the biological
456 samples were originally collected in tropical countries, is reinvested in the countries of origin to
457 increase their infrastructure and capacity building.

458 There are still many challenges for DNA barcoding to be properly used to describe new
459 species and on mammal biodiversity monitoring programs in developing countries, such as the
460 lack of financial investments, capacity building, and infrastructure (Eizirik, 2015; Paknia et al.,
461 2015). The lack of taxonomists, the high prices of sequencing in the country, the lack of
462 infrastructure together with its large territorial area and high mammalian cryptic diversity are
463 among the main challenges that limit the advance of using DNA barcoding to study Brazilian
464 mammal diversity. Furthermore, Brazilian researchers face a highly beaurocratic process in order

465 to export biological samples to be sequenced outside the country (Colella et al. 2023). However,
466 advancing our knowledge regarding these gaps in megadiverse countries will significantly
467 increase our capacity to monitor biodiversity and fill the Linnean, Wallacean and Darwinian
468 shortfalls in some of the most biodiversity rich parts of the planet (Hortal et al. 2015; Šmíd,
469 2022; Vergara-Asenjo et al. 2023).

470 Finally, one last challenge that needs to be addressed is that the presence of a species
471 sequence on a molecular database does not fully guarantee its future identification, because of
472 the high genetic divergence of some populations (Gaubert et al. 2014; Galimberti et al. 2015). In
473 these cases, sequences from multiple biological samples spanning different geographic locations
474 are needed to properly identify these species (Gaubert et al. 2014; Galimberti et al. 2015). Thus,
475 more efforts are needed to evaluate the genetic variability representation of these species on
476 molecular databases, and additional targeted sampling efforts to increase genetic variability on
477 molecular databases.

478

479 **5. Conclusion**

480 Even though Brazil is a large and highly species diverse country that requires a high
481 investment to enable sequencing its biodiversity, large advances have been made for Brazilian
482 mammal species in the last 15 years, which enabled a significant increase in monitoring capacity
483 using DNA barcoding and environmental DNA. However, many sequencing gaps still exist,
484 especially in relation to specific biomes, traits, diets, taxonomic groups, and threats. Even though
485 time since species description has been the most important factor to determine the sequencing
486 probability of a Brazilian mammal species, now that the gaps for species in molecular databases
487 have been identified, more targeted efforts are needed to increase species representativity and

488 genetic variability to enable the use of DNA techniques to sample and monitor Brazilian
489 mammal biodiversity.

490 Given that DNA barcoding is a standardized, reliable and precise method, this should
491 facilitate biodiversity assessment, enhance species identification, especially detection of cryptic
492 species (e.g. arboreal mammals), wildlife forensics, conservation genetics and the conservation
493 of endangered species. These efforts will also help building important prospects for the study and
494 understanding of Neotropical mammals ecology and evolution.

495 **Competing interests statement**

496 The authors have no competing interests to declare in the publication of the current study.

497

498

499 **Declaration of Generative AI and AI-assisted technologies in the writing process**

500 The authors would like to disclose that they have not used any form of artificial
501 intelligence for writing the current article.

502

503 **Authors contributions**

504 The roles are: Conceptualization: HFMO; GBF; JMS and FMCBD; Data curation: HFMO;
505 GBFreire-Jr.; VAM; FA; NFC; LGAG; GRL; Formal analysis: DCS, FMCBD; Funding
506 acquisition: HFMO; GRC; FMCBD; Investigation: HFMO; Methodology: HFMO and FMCBD;
507 Project administration: HFMO and FMCBD; Supervision: FMCBD; Roles/Writing - original
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517

518 **Data statement**

519 By the time the article is accepted, the dataset regarding this study will be made available

520 at: https://github.com/Hernanif/Data_accessibility-

521 [Barcodeing_mammals/tree/10e0b215055f2af27091338e7141dfb3246cb81f](https://github.com/Hernanif/Data_accessibility-Barcodeing_mammals/tree/10e0b215055f2af27091338e7141dfb3246cb81f)

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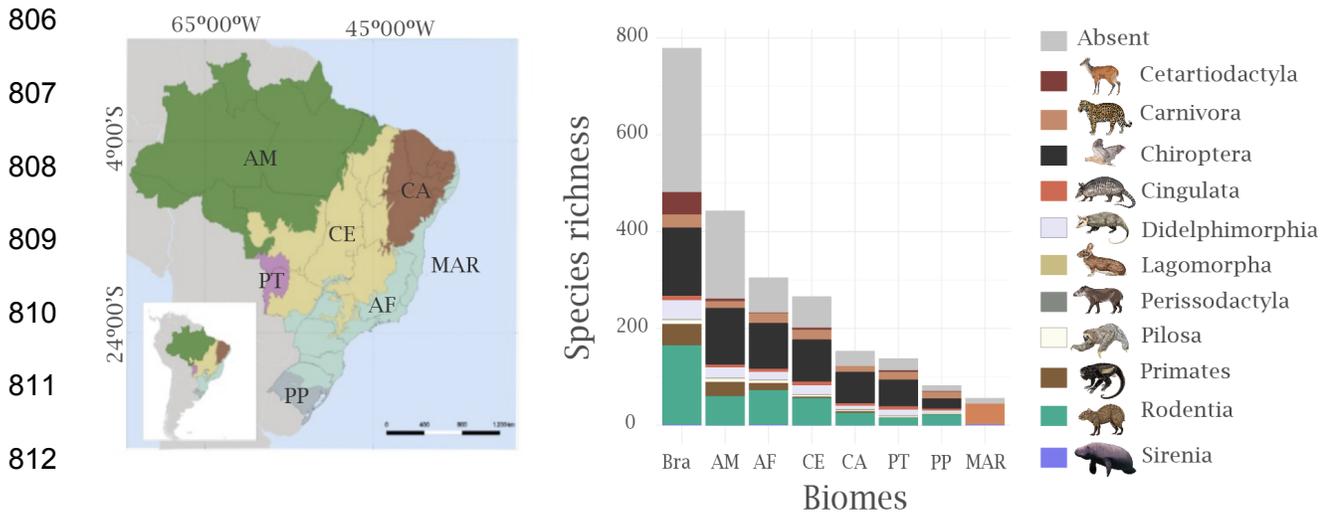
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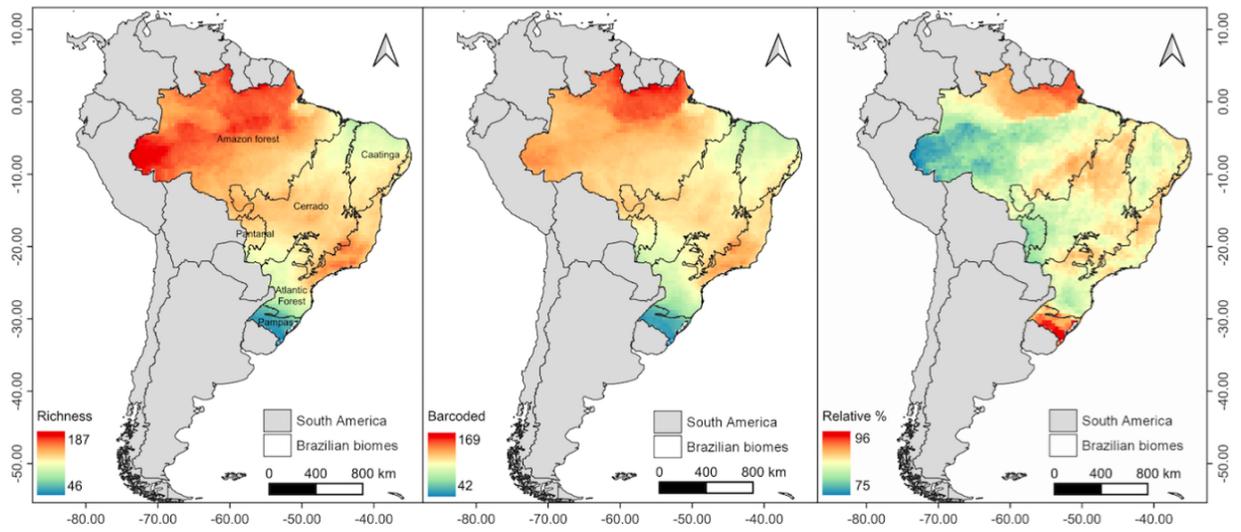
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805 **Figures**



813 **Figure 1.** Occurrences of DNA samples from Brazilian mammal species that are represented on
 814 Bold Systems and the representativity of the mammal species that have been sequenced per
 815 biome. AM= Amazon Forest, AF= Atlantic Forest, CE= Cerrado, PT= Pantanal, PP= Pampas,
 816 MAR= Marine.



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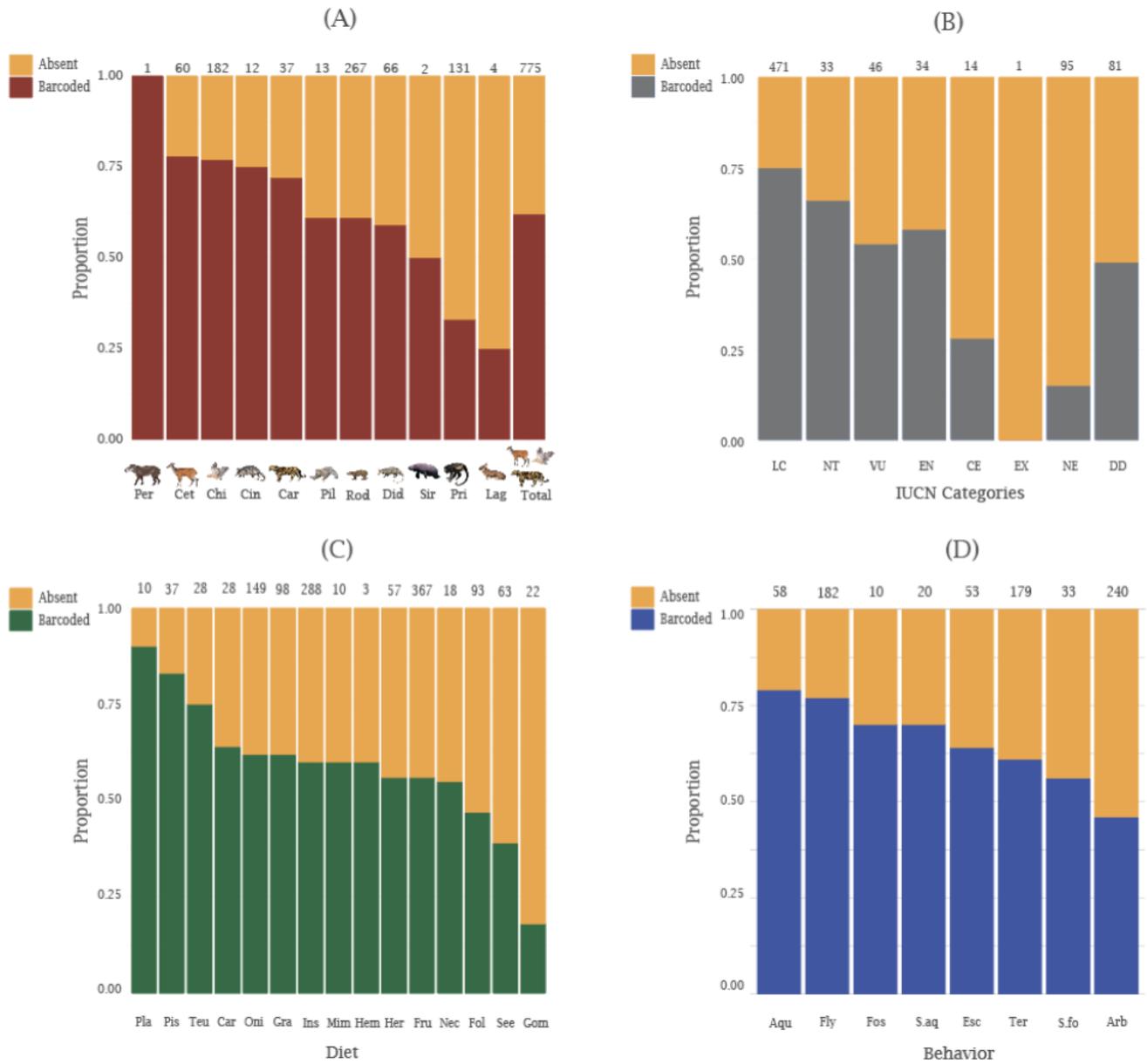
818 **Figure 2.** Distribution of mammal species richness in Brazil (left), distribution of Brazilian
 819 mammal species richness with available sequences of DNA barcoding (center), and proportion of
 820 Brazilian mammal species with available sequences of DNA barcoding in relation to the total
 821 species richness of Brazilian mammals within an area (right).

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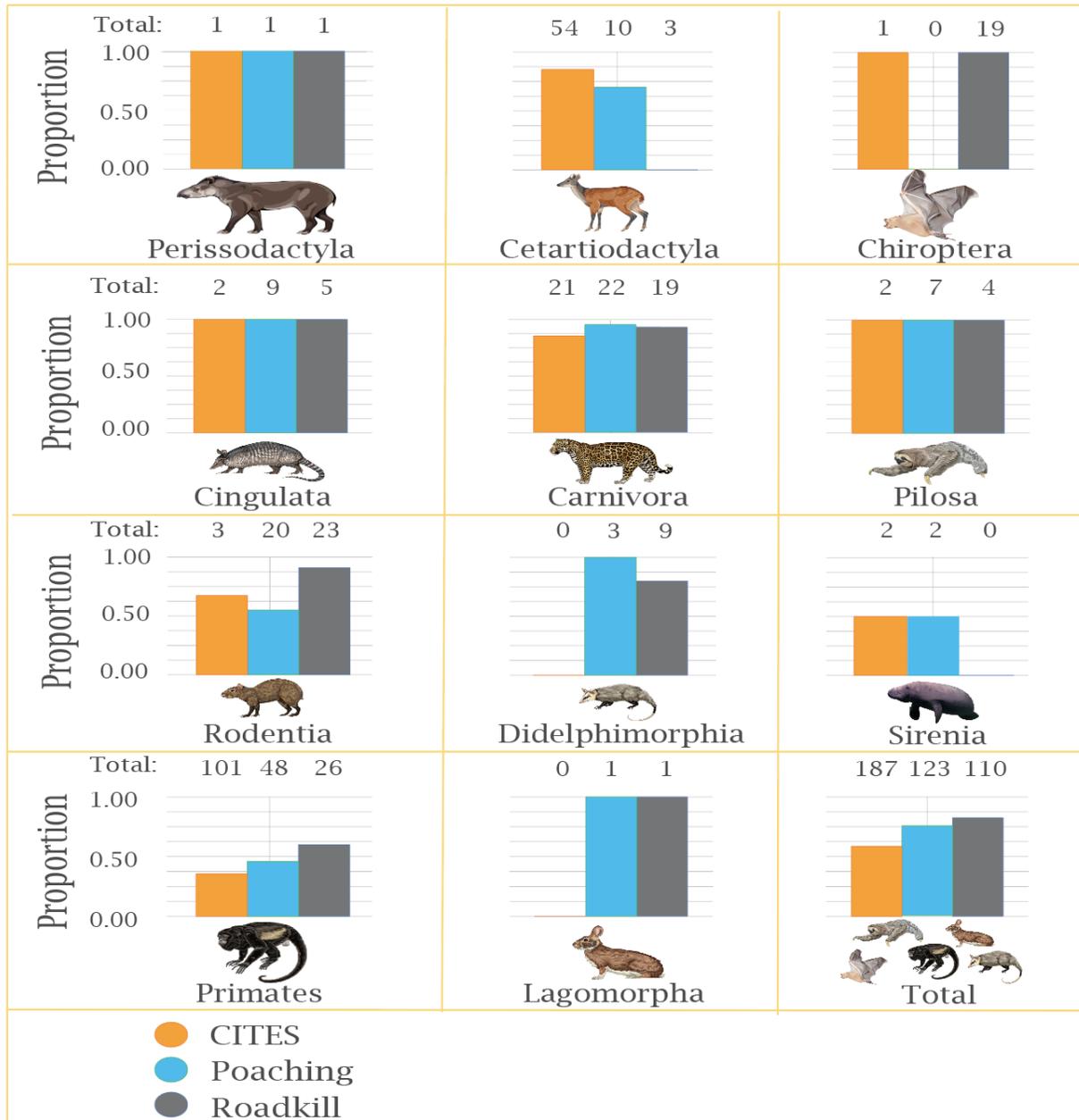
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827 **Figure 3.** Proportion of Brazilian mammals species represented on molecular databases

828 according to: A) species richness within each mammal order, B) conservation status, C) diet and

829 D, behavior.

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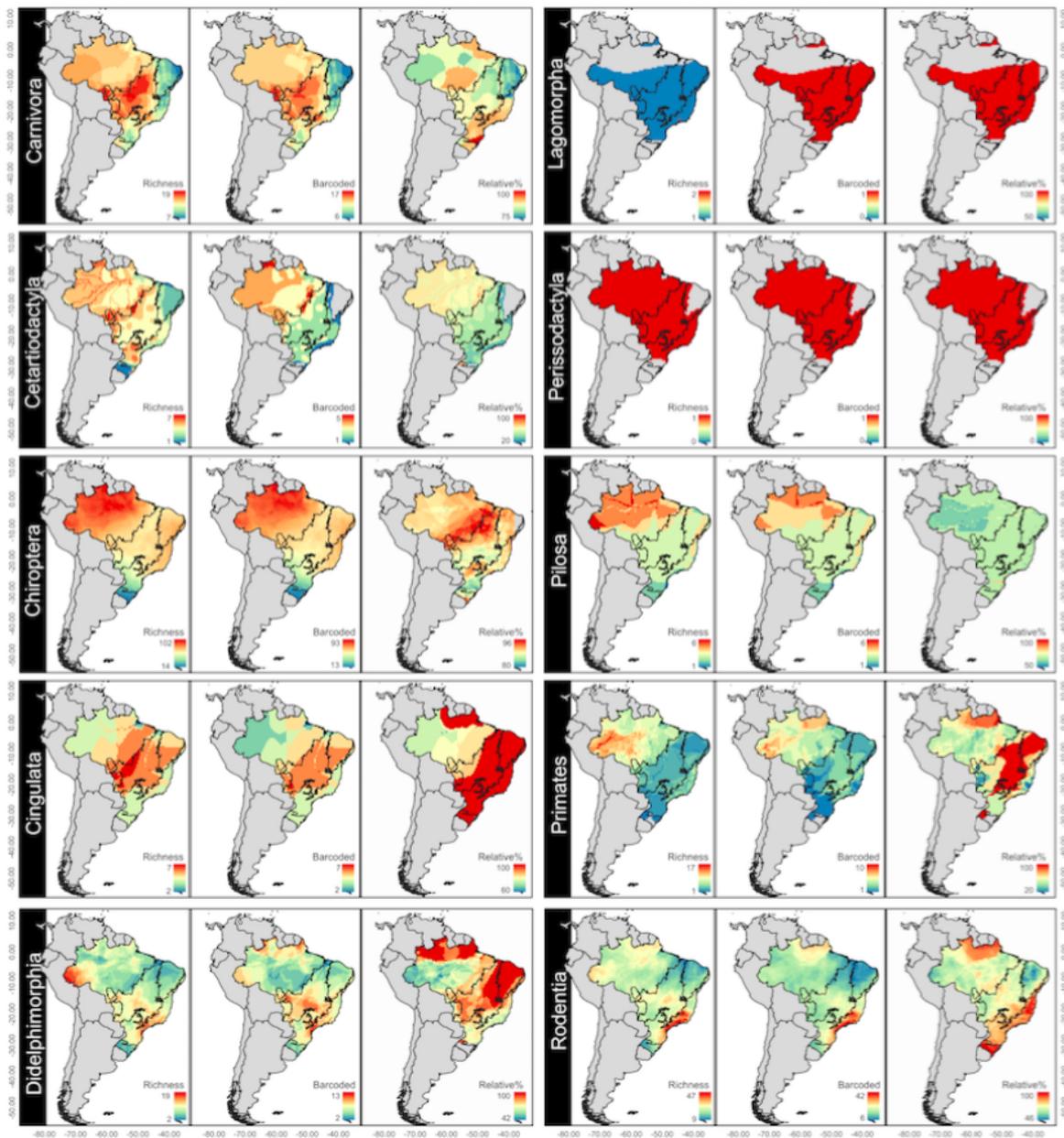
831

832 **Figure 4.** Proportion of Brazilian mammals species represented on molecular databases

833 according to their taxonomic order and type of threat (poaching, roadkill, and illegal wildlife

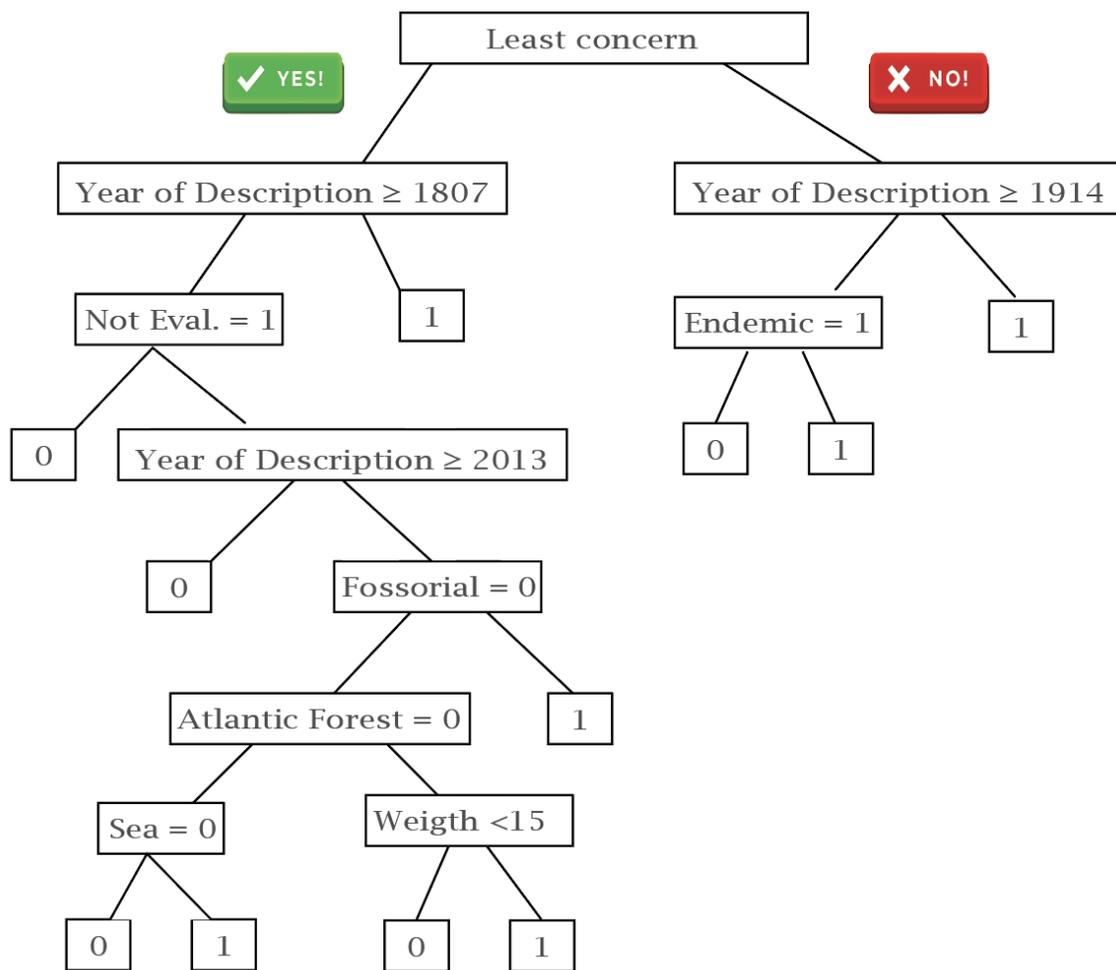
834 trade).

835 **Supplementary material**



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837 **Figure S1.** Distribution of mammal species richness of each order in Brazil (left panels),
 838 distribution of Brazilian mammal species richness with available sequences of DNA barcoding
 839 for each order (panels in the center), and proportion of Brazilian mammal species with available
 840 sequences of DNA barcoding in relation to the total species richness of Brazilian mammals
 841 within an area for each order (right panels).



842

843 **Figure S2.** Results of Classification and Regression Trees analysis (CART analysis) to evaluate

844 the most important traits of Brazilian mammals that predict their probability of being sequenced

845 for the COI DNA barcode and deposited in BOLD systems.

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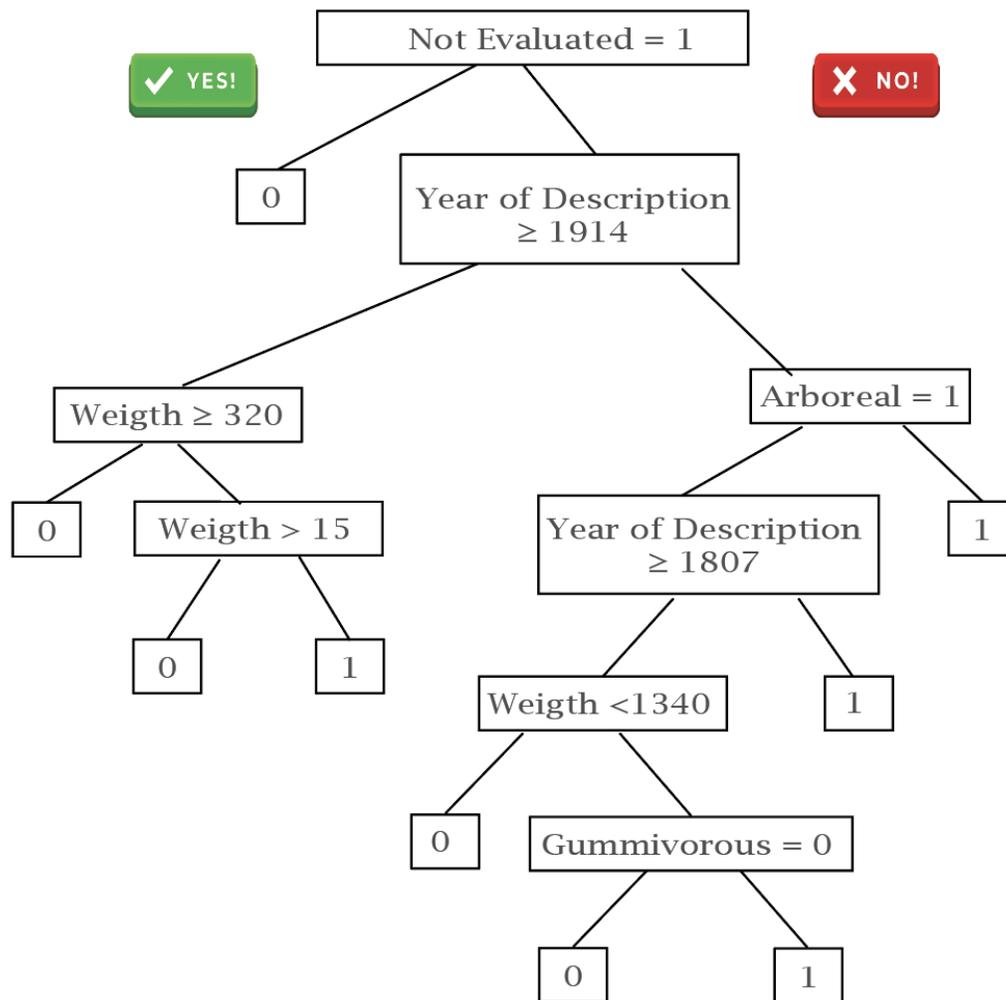
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853 **Figure S3.** Results of Classification and Regression Trees analysis (CART analysis) to evaluate
 854 the most important traits of Brazilian mammals that predict their probability of being sequenced
 855 for the COI DNA barcode and deposited in BOLD systems.

856

857 **Table SI.** Prioritization for threatened Brazilian mammal species to be sequenced for DNA
 858 barcoding.

Priority	Order	Species	Endemic	Restricted distribution	Conservation status	Population trend	Threat
EHP	Primates	<i>Mico marcai</i>	Yes	Yes	Vulnerable	Decreasing	IWT
EHP	Primates	<i>Mico rondoni</i>	Yes	Yes	Vulnerable	Decreasing	IWT
EHP	Primates	<i>Alouatta ululata</i>	Yes	Yes	Endangered	Decreasing	P
EHP	Primates	<i>Callicebus coimbrai</i>	Yes	Yes	Endangered	Decreasing	IWT
EHP	Primates	<i>Leontopithecus caissara</i>	Yes	Yes	Endangered	Decreasing	P, IWT
EHP	Primates	<i>Callithrix flaviceps</i>	Yes	Yes	Critically endangered	Decreasing	P, IWT
EHP	Primates	<i>Saguinus bicolor</i>	Yes	Yes	Critically endangered	Decreasing	P, R, IWT

HP	Primates	<i>Alouatta belzebu</i>	Yes	No	Vulnerable	Decreasing	P, R, IWT
HP	Primates	<i>Alouatta discolor</i>	Yes	No	Vulnerable	Decreasing	None
HP	Primates	<i>Alouatta guariba</i>	No	No	Vulnerable	Decreasing	P, R, IWT
HP	Primates	<i>Alouatta puruensis</i>	No	No	Vulnerable	Decreasing	P
HP	Primates	<i>Callicebus melanochir</i>	Yes	No	Vulnerable	Decreasing	P, IWT
HP	Primates	<i>Callicebus brunneus</i>	No	No	Vulnerable	Decreasing	P, IWT
HP	Primates	<i>Cebuella pygmaea</i>	No	No	Vulnerable	Decreasing	P, WT
HP	Primates	<i>Chiropotes utahickae</i>	Yes	No	Vulnerable	Decreasing	P, IWT
HP	Rodentia	<i>Euryoryzo- mys lamia</i>	Yes	Yes	Vulnerable	Decreasing	None
HP	Primates	<i>Lagothrix lagothrix</i>	No	No	Vulnerable	Decreasing	P

HP	Didelphi- morphia	<i>Marmosa phaea</i>	No	No	Vulnerable	Decreasing	None
HP	Cetartio- dactyla	<i>Mazama nana</i>	Yes	No	Vulnerable	Unknown	P, R
HP	Primates	<i>Mico munduruku</i>	Yes	No	Vulnerable	Decreasing	None
HP	Primates	<i>Saguinus ursula</i>	Yes	No	Vulnerable	Decreasing	None
HP	Primates	<i>Sapajus cay</i>	No	No	Vulnerable	Decreasing	P
HP	Lagomor- pha	<i>Sylvilagus tapetillus</i>	Yes	Yes	Vulnerable	Unknown	None
HP	Sirenia	<i>Trichechus inunguis</i>	No	No	Vulnerable	Decreasing	P, IWT
HP	Primates	<i>Ateles marginatus</i>	Yes	No	Endangered	Decreasing	P, R, IWT
HP	Primates	<i>Callithrix aurita</i>	Yes	No	Endangered	Decreasing	R, IWT
HP	Primates	<i>Chiropotes satanas</i>	Yes	No	Endangered	Decreasing	P, IWT

HP	Rodentia	<i>Coendou speratus</i>	Yes	Yes	Endangered	Decreasing	None
HP	Primates	<i>Lagothrix poepigii</i>	No	No	Endangered	Decreasing	P, IWT
HP	Chiropte- ra	<i>Lonchophylla bokermanni</i>	Yes	Yes	Endangered	Unknown	None
HP	Chiropte- ra	<i>Lonchophylla dekeysery</i>	Yes	Yes	Endangered	Decreasing	None
HP	Primates	<i>Mico schneideri</i>	Yes	No	Endangered	Decreasing	None
HP	Rodentia	<i>Microako- dontomys transitorius</i>	Yes	Yes	Endangered	Decreasing	None
HP	Rodentia	<i>Phyllomys thomasi</i>	Yes	Yes	Endangered	Decreasing	None
HP	Primates	<i>Sapajus robustus</i>	Yes	No	Endangered	Decreasing	R
HP	Primates	<i>Brachyteles hypoxanthus</i>	Yes	No	Critically endangered	Decreasing	P, IWT

HP	Primates	<i>Callicebus barbarabrownae</i>	Yes	No	Critically endangered	Decreasing	P, IWT
HP	Primates	<i>Callicebus grovesi</i>	Yes	No	Critically endangered	Decreasing	None
HP	Primates	<i>Callicebus vieirai</i>	Yes	No	Critically endangered	Decreasing	IWT
HP	Rodentia	<i>Cavia intermedia</i>	Yes	Yes	Critically endangered	Decreasing	None
HP	Didelphimorphia	<i>Monodelphis unistriata</i>	Yes	Yes	Critically endangered	Unknown	None
HP	Rodentia	<i>Phyllomys unicolor</i>	Yes	Yes	Critically endangered	Decreasing	None
IP	Primates	<i>Cebuella niveiventris</i>	No	No	Vulnerable	Decreasing	None
IP	Primates	<i>Cebus unicolor</i>	No	No	Vulnerable	Decreasing	None
IP	Rodentia	<i>Gyldenstolpia fronto</i>	No	No	Critically endangered	Unknown	None

859 *P (poaching), R (roadkill), IWT (illegal wildlife trade).

860 ****EHP (Extremely high priority), HP (high priority), IP (intermediate priority).**

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