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Title: Similar but different: revealing the relative roles of species-traits versus biome properties structuring genetic variation in South American marsh rats

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40

41 ABSTRACT

42 **Aim:** Wetland habitats, and the ecological restrictions imposed by them, structure patterns of
43 genetic variation in constituent taxa. As such, genetic variation may reflect properties of the
44 specific biomes species inhabit, or shared life history traits among species may result in
45 similar genetic structure. We evaluated these hypotheses jointly by quantifying the similarity
46 of genetic structure in three South American marsh rat species (*Holochilus*), and test how
47 genetic variation in each species relates to biome-specific environmental space and historical
48 stability.

49 **Location:** South America.

50 **Taxon:** Rodentia

51 **Methods:** Using complementary analyses (Mantel tests, dbRDA, Procrustes, covariance
52 structure of allele frequencies, and ENMs) with 8,000–32,000 SNPs per species, we
53 quantified the association between genomic variation and geographic and/or environmental
54 differences.

55 **Results:** Significant association between genetic variation and geography was identified for
56 all species. Similarity in the strength of the association suggests connectivity patterns dictated
57 by shared species-traits predominate at the biome scale. However, substantial amounts of
58 genetic variation are not explained by geography. Focusing on this portion of the variance,
59 we demonstrate a significant quantitative association between genetic variation and the
60 environmental space of a biome, and a qualitative association with varying regional stability.
61 Specifically, historically stable areas estimated from ecological niche models are correlated
62 with local levels of geographic structuring, suggesting that local biome-specific histories
63 affect population isolation/connectivity.

64 **Main conclusions:** These tests show that although species exhibit similar patterns of genetic
65 variation that are consistent with shared natural histories, irrespective of inhabiting different
66 wetland biomes, local biome-specific properties (i.e., varying environmental conditions and

67 historical stability) contribute to departures from equilibrium patterns of genetic variation
68 expected by isolation by geographic distance. The reflection of these biome-specific
69 properties in the genetic structure of the marsh rats provide a window into the differences
70 among South American wetlands with evolutionary consequences for their respective
71 constituent assemblages.

72 73 KEYWORDS

74 climatic changes, environment, phylogeography, rodent, next generation sequencing,
75 wetland.

76 77 78 79 **Introduction**

80 In the tradition of using concordance across taxa to infer the common effects of
81 abiotic factors in structuring genetic variation, comparative phylogeographic studies usually
82 focus on a specific geographic region with expanded taxonomic sampling. The role of abiotic
83 factors in structuring genetic variation can also be tested by controlling for the potentially
84 confounding influence of biotic factors, for example, by studying a subset of species with
85 similar biological traits, such as similar ecological or life history traits, within a given
86 geographic region (Papadopoulou & Knowles, 2015). However, genetic variation may be
87 shaped by species-specific traits in concert with the abiotic factors (Capurcho et al., 2013;
88 Massatti & Knowles, 2014; Choueri et al., 2017), or regional differences in abiotic factors
89 may mediate the effects of such factors on genetic variation (Papadopoulou & Knowles,
90 2016). For relatively understudied regions and taxa, accommodating these complexities
91 becomes an important part of the study design because tests focused on a single component
92 structuring genetic variation (i.e., abiotic as opposed to biotic factors) may be giving an
93 especially incomplete picture given the lack of background information for determining
94 appropriate hypotheses.

95 Here we test for the effects of both abiotic and biotic factors in structuring genetic
96 variation of three species of marsh rats (genus *Holochilus*: Cricetidae) that are restricted to
97 wetland habitats associated with different biomes throughout South America (Fig. 1). Marsh
98 rats are semi-aquatic, large bodied rodents with a herbivorous diet (Hershkovitz, 1955;
99 Gonçalves, Teta, & Bonvicino, 2015) that inhabit open and seasonally flooded grasslands.
100 They share a number of characters associated with specialization for aquatic life and an

101 herbivorous diet. These include not only morphological traits (fusiform body shape, webbed
102 and elongate hindfeet, loss of molars lophs; Hershkovitz, 1962; Stein, 1988; Dominguez-
103 Bello & Robinson, 1991), but also behavioural traits linked to foraging and how the
104 mammals seek shelter, as well as where they mate (Eisenberg, 1981). As a function of these
105 shared traits, they may also exhibit similar patterns of genetic variation – that is, biotic factors
106 related to being adapted to wetlands may play a role in the structuring of genetic variation
107 across the species (Avice, 2000). However, the wetlands they inhabit differ. For example, two
108 of the focal species (*Holochilus sciureus* and *Holochilus vulpinus*) inhabit patchy wetlands
109 across forested biomes, whereas the other taxa (*Holochilus chacarius*) lives in an open
110 contiguous wetland (Fig. 1). The forested wetlands include wetlands distributed along rivers
111 throughout the Amazon biome, and wetland patches throughout the Atlantic Forest, where *H.*
112 *sciureus* and *H. vulpinus* are distributed, respectively. However, *H. vulpinus* can also be
113 found in the wetlands of a non-forested biome, the Pampas (Fig. 1). Unlike the forest-
114 associated taxa, *H. chacarius* is distributed throughout the contiguous wetlands of
115 Chaco/Pantanal biomes (Fig.1). These South American wetlands oscillate between flooded
116 and unflooded phases, although the length, depth, frequency and timing of the phases vary
117 across biomes (Junk, 2013).

118 In addition, the history of the wetlands differs. For example, the Pantanal is a biome
119 whose formation is linked to precipitation changes since the Last Glacial Maximum (LGM;
120 McGlue et al., 2015). For the forest associated wetlands within the Amazon, while some
121 riverine forest persisted during more arid periods of the Pleistocene (Erwin & Adis, 1982),
122 *terra firme* vegetation expanded, replacing the flooded forests, and interrupting the
123 distribution and availability of flooded environments (Choueri et al., 2017; Thom et al.,
124 2018). Likewise, expansions and contractions of forested areas in association with climatic
125 change and sea level shifts no doubt impacted the wetlands of the Atlantic Forest, although
126 we lack information specific to the wetland habitats. With a history of climatic (or sea level)
127 driven change, the stability of wetland habitats varied not only across biomes, but also within
128 biomes (Bush, Silman & Listopad, 2007; Quattrocchio, Borromeia, Deschamps, Grill, &
129 Zavala, 2008; Whitney et al., 2011), as well as across biomes, during the Pleistocene. As
130 such, the genetic structure of the marsh rats may reflect biome-specific properties of the
131 different wetland habitats – that is, abiotic factors related to the wetlands themselves that are
132 extrinsic to the shared characteristics of the marsh rat taxa.

133 We approach the question of the effects of both abiotic and biotic factors in
134 structuring genetic variation of three species of South American marsh rats by quantitatively

135 assessing not only the similarity of genetic structure across species, but also the
136 correspondence between patterns of genomic variation and biome-specific aspects of the
137 wetland habitats. Specifically, using 8,000–32,000 SNPs per species, we quantify (i) the
138 similarity of genetic structure in three South American marsh rat species (*Holochilus*), and
139 (ii) test how genetic variation in each species relates to (a) environmental and (b) historical
140 stability differences across the biomes (i.e., biome-specific impacts on genetic structure of the
141 marsh rat species). Such tests involve an evaluation of whether the data conform to levels of
142 gene flow expected under an isolation by distance (IBD) model, where a similar
143 correspondence between geographic and genetic data would suggest a similar migration
144 history among the taxa reflecting the shared traits among the marsh rats (e.g., Peterson &
145 Denno, 1998). In addition, the degree to which the biome-specific environmental properties
146 and historical stability (as characterized by ecological niche models for the past and present)
147 contribute to genetic structure properties is evaluated by multiple procedures for testing for a
148 correspondence between the biome properties and deviations from IBD, including both
149 quantitative and qualitative measures of association (He, Edwards & Knowles, 2013;
150 Knowles, Massatti, He, Olson & Lanier, 2016).

151 Given the recent origin of the marsh rat taxa (Machado, Leite, Christoff, & Giuliano,
152 2013), these biome-specific properties, not more ancient Miocene and Pliocene climatic
153 changes (Hoorn et al., 2010), are a logical starting point for testing the potential effects of
154 biome-specific properties on genetic variation in constituent taxa. Moreover, with few
155 information on the physiogamy of regions and potential impact of Pleistocene climatic
156 changes on wetland habitats specifically (see Aleixo, 2006; McGlue et al., 2015; Ledru et al.,
157 2016; Leite et al. 2016; Thom et al., 2018), differences in the genetic structure of constituent
158 taxa across biomes, and any correspondence with regional properties can be mutually
159 informative (e.g., Ferreira et al., 2018; Thom et al., 2018). As one of the first (and to our
160 knowledge) only studies that brings the resolution of genomic data to these vast, but
161 relatively understudied, biomes, our findings provide insights about not only the history of
162 the taxa, but also the features of the different wetlands with evolutionary consequences for
163 their respective assemblages.

164

165

166 **Material and methods**

167 **DNA extraction, amplification, and sequencing**

168 Genomic data was generated for 26 individuals and 9 populations of *H. chacarius*, 24
169 individuals and 6 populations of *H. sciureus*, and 18 individuals and 6 populations of *H.*
170 *vulpinus*, sampled across their entire range (see Fig. 2, Table S1.1, Appendix S1 in
171 Supporting Information). Tissues were collected or requested from museums (see Appendix
172 S2 in Supporting Information). DNA was extracted from liver, muscle or skin using the
173 Qiagen DNeasy Blood and Tissue Kit.

174 Four reduced representation libraries were sequenced using ddRADseq method
175 (Peterson Weber, Kay, Fisher, & Hoekstra, 2012; Appendix S1). Briefly, DNA was double
176 digested with the restriction enzymes EcoR1 and MseI and 150 base pair, single-end reads
177 sequenced on four lanes of Illumina *HiSeq2000*, which produced 270 million raw reads at the
178 Centre for Applied Genomics, Canada.

179

180 **Processing of Illumina Data and generating summary statistics**

181 Raw sequence reads were processed separately for each species in Stacks v.1.35
182 (Catchen, Hohenlohe, Bassham, Amores, & Cresko, 2013). The reads were demultiplexed
183 and filtered using *process radtags*. Only reads with Phred score >10, unambiguous barcodes,
184 and individuals with more than 500,000 reads were retained (additional details are given in
185 Appendix S1). A *de novo* assembly of filtered reads with a minimum coverage depth of 6
186 were used to identify putative loci with the USTACKS. A catalogue of consensus loci was
187 constructed for each species with CSTACKS with a distance between individuals for a given
188 locus ≤ 2 . Alleles were called for loci using SSTACKS.

189 Characterizations of genetic variation and population summary statistics were
190 generated with the POPULATIONS program (Catchen et al., 2013). At this step, all loci
191 present in at least two populations were identified and exported in Variant Call Format (vcf).
192 This first dataset (hereafter referred as the full dataset) was used to calculate population
193 genetic diversity statistics, such as nucleotide diversity (π), major allele frequency, observed
194 heterozygosity (H_O), and Wright's inbreeding coefficient (F_{IS}) at each locus. Population-
195 level summaries of genetic diversity were also characterized (average π , H_O and F_{IS}), and a
196 one-way ANOVA was used to test for significant differences in genetic diversity between
197 species (conducted in R, Car package; R Core Team 2014). A second dataset with one
198 randomly chosen SNP and a maximum of 20% missing data was generated with the toolset
199 PLINK v.1.07 (Purcell et al., 2007) and used for all other analyses (hereafter referred to as
200 the putative unlinked-SNP dataset).

201 The full dataset consists of 359,728 SNPs and 26 individuals of *H. chacarius*, 357,050
202 SNPs and 24 individuals of *H. sciureus* and 160,879 SNPs and 18 individuals of *H. vulpinus*.
203 The unlinked-SNPs dataset consists of 32,210 SNPs in 25 individuals of *H. chacarius*, 17,513
204 SNPs in 20 individuals of *H. sciureus* and 8,035 SNPs in 15 individuals of *H. vulpinus* (Table
205 S1.2 in Appendix S1).

206

207 **Structuring of genetic variation**

208 A series of complementary approaches were used to test for similar structuring of
209 genetic variation across taxa and for a correspondence between properties of the wetland
210 habitats and genetic variation. These tests were selected because they differ in their
211 respective assumptions, and hence potential to capture different aspects of population genetic
212 structure.

213

214 *Procrustes analyses*. Tests of the similarity among taxa in the structuring of genetic variation,
215 including the fit to, but also the departures from, expectations of isolation by distance were
216 conducted using Procrustes analyses (Wang, Zöllner, & Rosenberg, 2012). Specifically, an
217 association between genetic variation and geography while retaining latitudinal and
218 longitudinal information for sampled individuals was quantified using the *procrustes* and
219 *protest* functions in VEGAN package (Oksanen et al., 2017). The strength of the association
220 was compared across taxa by the similarity statistic t_0 , which ranges from 0 to 1, and
221 provides a basis for investigating the history of migration.

222 The statistical technique, involves a transformation of a genetic map onto a
223 geographic map to maximize their similarity (i.e., minimize the sum of squared Euclidean
224 distances between the two maps), where a principle component analysis (PCA) of the genetic
225 data was used to generate individual-level coordinates of the first two components (PC1 and
226 PC2). The position of individuals in the genetic map can be visualized, highlighting whether
227 individuals depart from expected levels of gene flow (i.e., they are genetically too similar, or
228 less similar to other individuals sampled across the landscape than expected given their
229 geographic position). To explore how aspects of the migration history (i.e., departures from
230 expected patterns of gene flow under isolation by distance) reflect the wetlands, the
231 correspondence between the genetic map and the (i) environmental space and (ii) stability of
232 a biome were examined. To test for an association between biome-specific environmental
233 space and genetic variation, the residuals of the Procrustes analysis between genes and
234 geography for each species were paired with a matrix of extracted PCA data of

235 environmental variables (described below) for the sequenced individuals (e.g., Knowles et
236 al., 2016). For examining how the stability/instability of habitat within biomes might impact
237 the history of migration, we compared the general correspondence between the position of
238 individuals in genetic space and areas of projected stability; stability is inferred by comparing
239 ecological niche models for different periods of the past with the present, and are described
240 below. In particular, deviations from expected patterns of gene flow (i.e., departures from
241 IBD) was examined in relation to the mapped areas of historical habitat instability to assess
242 whether there was any qualitative correspondence of genetic variation with this biome-
243 specific property (see also Knowles et al., 2016).

244 The significance of the similarity statistic t_0 was evaluated for each species based on
245 10,000 permutations, where geographical locations were randomly permuted across the
246 different sample localities. In each species, the sensitivity of the similarity statistic t_0 to
247 particular populations was also evaluated by repeating the analyses excluding one population
248 at a time, with replacement (which is represented by the similarity statistic t' following
249 Wang et al., 2012). We also computed a similarity score (t' ; following Wang et al., 2012)
250 between PCA coordinates for the complete data set and those for data sets in which one
251 population was excluded to assess whether any populations had a disproportionate effect on
252 the relationship between genes and geography (Wang et al., 2012; Knowles et al., 2016).

253
254 *Covariance structure of allele frequencies.* Spatial patterns of genomic variation were also
255 examined based on an allele frequency covariance matrix using the program SpaceMix
256 (Bradburd, Ralph, & Coop, 2016); comparison with the results from the PCA provides a
257 measure of whether the PCAs, and hence Procrustes analyses, may be biased by sampling
258 effects (see Novembre & Stephens, 2008). Following the developer's recommendations, 10
259 "fast" independent chains were run for 5×10^6 MCMC iterations, without conditioning
260 populations on their locations and with no admixture. This was followed by a "long" run of
261 10^8 iterations, with parameters sampled every 10^2 iterations, in which population locations
262 were initiated at the origin (i.e., inferred from the "fast" runs), and all other parameters (α_0 ,
263 α_1 , α_2 , η , and w) were drawn randomly from their priors at the start of each chain.

264
265 *Mantel tests and dbRDA.* As a complementary test to the analyses described above, the
266 correlation between pairwise F_{ST} -values and Euclidean geographic distances among
267 populations, as well as associations between genetic distance and environmental resistance,
268 was examined using a Mantel, partial Mantel tests, and dbRDA (Legendre & Anderson,

1999) from the R package VEGAN (Oksanen et al., 2017). For the Mantel tests, a sequential population drop out procedure, in which the test was repeated excluding one population at time, was also conducted to confirm that the results were robust. For the partial Mantel test an environmental resistance matrix among populations was generated based on raster maps obtained with the ENMs using CIRCUITSCAPE v4.0 (Shah & McRae, 2008). The *capscale* R function was used in the dbRDA analysis to test for the relationship between pairwise genetic distances and corresponding climatic variables (represented by the PC1 of the 19 climate layers used in the ENMs), and removing the effect of geographic distance separating populations (He et al., 2013). In addition, a second partial Mantel test and dbRDA analyses were performed with the environmental variables extracted from the PCA data for the locations of sequenced populations (He et al., 2013) to examine whether the environment might make a significant contribution to patterns of genetic variation, after controlling for the effects of geography.

282

283 **Characterizing historical stability and environmental space across distributional areas**

284 To characterize historical stability and compare the environmental space of the
285 distributional areas of each *Holochilus* taxa, environmental niche models (ENMs) were
286 generated from the 19 bioclimatic variables for the present (Bioclim), Holocene (6 kya; MPI-
287 ESM-P; Bioclim) and the Last Glacial Maximum (LGM; 21 kya; MPI-ESM-P; Bioclim;
288 Hijmans, Cameron, Parra, Jones, & Jarvis, 2005) using Maxent 3.3.3k (Phillips, Anderson &
289 Schapire, 2006). Georeferenced occurrence data representative of the ranges of each species
290 was used; vetted data were obtained by direct examination of specimens (Appendix S2) or
291 from taxon-specific bibliographic sources (HersHKovitz, 1955; Pardiñas & Teta, 2011;
292 Pardiñas, Teta, Voglino, & Fernández, 2013; D'Elía, Hanson, Mauldin, Teta, & Pardiñas,
293 2015).

294 A Principal Component Analysis (PCA) was used to identify a subset of
295 environmental variables with < 0.7 of correlation based on analyses from the *prcomp*
296 function in R; among correlated variables, the variable with the highest score was retained.
297 Occurrence data were rarefied using SDMTToolBox at a resolution of 10 km to reduce spatial
298 autocorrelation. To avoid overfitting, and considering the semi-aquatic habit of *Holochilus*,
299 the geographical extent of the environmental layers used as a mask corresponded to a 100km
300 buffer surrounding the respective river basins where the species are distributed. To achieve a
301 balance between goodness-of-fit and model complexity, we used the *ENMevaluate* function
302 from ENMeval package (Muscarella et al., 2014), and we tested models over combinations of

303 regularization parameters from 0.5 to 3 in intervals of 0.5 and combinations of features
304 parameters (Auto, Linear, Quadratic, Hinge, Linear + Quadratic and Linear + Quadratic +
305 Hinge, according to Maxent recommendations). Regularization and features parameters were
306 chosen using Akaike Information Criterion (AIC; Warren & Seifert, 2011) and Area Under
307 the receiver-operator Curve (AUC; Swets, 1988). Each model parameter class was replicated
308 10 times for cross-validation. For each model we extracted binary predictions, where suitable
309 habitat presence was inferred according to the significance threshold for each model from
310 Maxent.

311 Climatically stable areas were inferred from the intersection of the binary predictions
312 under current and past climate scenarios (see Table S3.1, Fig. S3.1, Appendix S3 in
313 Supporting Information for additional details). Variation in current environmental conditions
314 across each biome was quantified with a PCA of the 19 Bioclim variables using the
315 *rasterPCA* function from the DISMO package in R (Hijmans, Phillips, Leathwick, & Elith,
316 2016), with input variables rescaled from 0 to 1 (so that the PCs are not sensitive to
317 differences in the units). For a graphical presentation of this variation across the landscape,
318 the *ggRGB* function from RStoolbox package in R (Leutner & Horning, 2016) was used to
319 construct maps, with the red layer corresponding to PC1, green layer corresponding to PC2
320 and the blue layer corresponding to PC3. To visualize how the position of sampling localities
321 spans the environmental space, the environmental dispersion of sequenced individuals
322 relative to the total environmental space for each species was mapped using the
323 environmental data (PC1 and PC2 of the PCA performed with the bioclimatic variables) and
324 the occurrence points of sequenced individuals (see Lanier, Massatti, He, Olson, & Knowles,
325 2015).

326

327 **Results**

328 **Population genetic summary statistics**

329 Values of population genetic summary statistics were broadly overlapping among
330 taxa (Table S4.1, Fig. S4.1, Appendix S4 in Supporting Information), except for a statistically
331 significant difference in H_{obs} , with higher values in *H. vulpinus* compared with the other
332 species. Genetic diversity, as measured by π , was similar among taxa, varying from 0.1 to
333 0.2. Inbreeding coefficients, F_{is} , were also similar across taxa with the exception of two
334 populations; specifically, S_MAM in *H. sciureus* and YAC in *H. chacarius* had substantially
335 higher inbreeding coefficients, although neither exhibited reduced genetic diversity.

336

337 **Historical stability and environmental space across distributional areas**

338 The current environmental characteristics of the biomes inhabited by the three species
339 clearly differ (Fig. 3). The environmental space of the Atlantic Forest and Pampas area
340 inhabited by *H. vulpinus* differs the most from the other regions. There is some resemblance
341 between the environmental space occupied by *H. vulpinus* and the north-western region of *H.*
342 *sciureus*. However, these two areas are separated by a large region with different
343 environmental characteristics (see the two bluish areas are separated by a large green area;
344 Fig. 3). Comparison of the environmental conditions where populations were sampled to the
345 rest of biome shows that the sites where genetic samples were collected are generally
346 representative of the environmental range encompassed by the species' distributions,
347 spanning the entire dispersion of environmental values of the PCA (Fig. 4).

348 Estimates of habitat stability based on similarity in spatial distribution of suitable
349 habitat from the LGM, the Holocene and the present, show differences in the stability of the
350 habitat across biomes, as well as within each biome. Specifically, the habitat across most of
351 the distributional range of *H. vulpinus* is projected to be stable, whereas only the
352 southwestern Amazon is predicted to be stable for *H. sciureus*, and only small and patchy
353 areas were inferred to be stable for *H. chacarius* (Fig. 2).

354

355 **Structuring of genetic variation**

356 Regarding the geographic distribution of genetic variation within each species, several
357 patterns are reinforced across methods. For example, when the position of individuals along
358 PC1 and PC2 from a genetic PCA is projected onto the geographic distribution of sampled
359 individuals (Fig. 5), it highlights how similarity in the multidimensional genetic space is
360 related to where an individual occurs geographically, confirming the Procrustes results (Figs.
361 2 and 6). Likewise, this similarity is also evident when comparing the results from the spatial
362 covariance of alleles analyses (SpaceMix results) with those from a PCA of genetic variation
363 (Fig. S4.2 in Appendix 4). This general correspondence suggests that the PCAs (and
364 therefore the Procrustes analyses) are not subject to biases that can be introduced from the
365 sampling distribution of individuals (Novembre & Stephens, 2008). However, the position of
366 some individuals of *H. chacarius* in multivariate genetic space differs somewhat from the
367 spatial covariance of alleles. Specifically, we note that the GOL population occupies a more
368 distinctive position relative to other populations in the geogenetic map of Spacemix
369 compared with its overlap with other populations in the PCA (Fig. S4.2 in Appendix 4).
370 However, results from the sequential population drop-out procedure in the Procrustes

371 analysis showed that exclusion of the GOL population neither produced large changes in the
372 strength of the association between genes and geography, nor resulted in a significant
373 distortion of the genetic PC map (Figs. 5 and 6). We also note that the sampling gap in *H.*
374 *sciureus*, in which central Amazonian samples were not available (Fig. 2), does not appear to
375 biased the position of these individuals in PC space (Bradburd et al., 2016) given the
376 similarity between the two approaches (Fig. S4.2 in Appendix 4). Likewise, it is also
377 noteworthy that inclusion of the geographically disjunct GUI population in *H. sciureus* also
378 did not strongly affect the influence the PCA of genetic variation genetic.

379 The strength of the association between genes and geography varied substantially
380 when individual populations were sequentially excluded; within a species, the shift from the
381 t_0 -value (t_0-t') was as high as 0.15 with the exclusion of specific populations (Fig. 6, Table
382 S4.2 in Appendix 4). The differences in the strength of the association between genes and
383 geography does not appear to be attributable to the undue influence of any single population
384 on the PCA of genetic variation in any of the species (i.e., t' -values were > 0.91 in all but
385 four cases; see Table S4.2 in Appendix 4). However, we note that in both *H. vulpinus* and *H.*
386 *chacarius*, but not *H. sciureus*, the same populations that resulted in higher association
387 between genes and geography when excluded had a disproportionate influence on the PCA of
388 genetic variation (the URU population in *H. vulpinus*; the YAC and BOL populations in *H.*
389 *chacarius*; in contrast to the GUI population in *H. sciureus*).

390 Results of Mantel, partial Mantel tests and dbRDA based on geography and
391 environment were not significant for any taxa (Tables S4.4 and S4.5 in Appendix 4),
392 including the Mantel tests with sequential removal of individual populations. The only
393 exception was a significant association between environment and genetic variation based on
394 dbRDA test only in *H. chacarius*; however, the association was not significant after
395 controlling for geography.

396 The lack of significance based on Mantel tests and dbRDAs contrasts with a
397 significant association between geographic and genetic variation in each species detected by
398 the Procrustes analysis, which unlike the former tests retains the geographic information
399 about latitudinal and longitudinal position of populations relative to patterns of genetic
400 differentiation. The strength of association between genes and geography (t_0) was quite
401 similar across all species (when the outlier population GUI in *H. sciureus* was excluded),
402 ranging from 0.64 to 0.67, out of a maximum of 1, meaning there is also a substantial amount
403 of variation not explained by the geographic position of populations in each species.

404

405

406 **Genetic deviations/displacements in geographic and environmental space**

407 In *H. vulpinus*, when we consider the deviations of individuals in genetic space from
408 expectations based on their geographic location (i.e., the length of the lines connecting a
409 sampled population to a sequenced individual; Fig. 2), the deviations primarily vary along a
410 latitudinal axis. Moreover, the individuals tend to occupy the central area of the species
411 distribution and the position of these individuals in the PC genetic space show a strong
412 correspondence with areas of stability (Fig. 2), with the exception of one population in the
413 southeast (BAR; Fig. 2) whose individuals deviate in a southerly direction.

414 In contrast, the directions of departures in genetic space relative to where individuals
415 of *H. sciureus* were sampled geographically tend to follow a longitudinal axis (Fig. 2).
416 Nevertheless, this species also shows some correspondence between the position of
417 individuals in genetic space and areas of projected stability like *H. vulpinus*. Likewise, the
418 deviations of individuals sampled in what is projected to be stable areas historically
419 (N_MAM and S_MAM) tend to show relatively small departures from geographic
420 expectations.

421 Lastly, in *H. chacarius* the displacement of many individuals to the central part of the
422 species range was observed even though the sampled populations correspond to areas of
423 projected stability (Fig. 2). Only some of the northern populations (PNP and COR, but not
424 POC) are somewhat distinct from this general cluster.

425 Tests of a correlation between the residuals from the Procrustes analysis of genes and
426 geography with the environmental distance among populations suggests that environmental
427 differences contribute to some of the genetic differences observed among individuals in each
428 species (Table S4.3 in Appendix 4). As with the tests of isolation by distance that did not
429 retain the relative position of sampled populations in geographic space, neither the partial
430 Mantel nor dbRDA detected significant contribution of environmental differences to genetic
431 distances (Tables S4.4 and S4.5 in Appendix 4).

432

433 **Discussion**

434 Similarity in the structuring of population genetic variation (Fig. 2) and population
435 genetic diversities (see Table S4.1, Fig. S4.1 in Appendix 4) highlight the contribution of
436 common natural history traits to the similar migration histories of the taxa. However, biome-
437 specific effects on genetic structure explain a substantial amount of genetic variation not
438 explained solely by gene flow associated with shared traits among the taxa. Specifically,

439 quantitative and qualitative analyses (Figs. 2 and 3) show that variation of the environment
440 and historical stability within biomes contribute to patterns of genetic structure.

441 Below we discuss the insights these findings offer about the South American wetlands
442 and their constituent taxa as one of the first, and to our knowledge, only study that brings the
443 resolution of data from next-generation sequence technologies to these vast, but relatively
444 understudied, landscapes. In addition, we highlight the niche our work fills in comparative
445 phylogeography: the study of ecologically similar taxa from different regions, as opposed to a
446 tradition of comparing taxa (often with differing ecologies) from a single region, to make
447 inferences about the processes underlying observed patterns of genetic variation (Avice,
448 2000; Knowles, 2009; Hickerson et al., 2010).

449

450 **Insights from genomic analyses about South American biomes**

451 Despite differences in the distribution of wetland habitats across the biomes inhabited
452 by the taxa (e.g., patches of wetlands versus large contiguous wetlands), the migration history
453 of the species is similar. Specifically, the strength of the association between genetic
454 variation and geography was similar across the biomes (when the outlier population GUI in
455 *H. sciureus* was excluded). Moreover, even when considering all populations, the Procrustes
456 analyses indicated the genetic isolation between geographically distant populations in the
457 species inhabiting the two biomes of forest-based patches of wetlands was not more similar
458 to each other relative to the species in the contiguous wetlands. Instead, the strength of the
459 association between genes and geography was most similar in *H. chacarius*, which inhabits
460 the large contiguous wetlands, and *H. vulpinus*, which inhabits patches of wetlands in the
461 Atlantic Forest and in the open vegetation of the Pampas. Together, these results emphasize
462 that gene flow decreases with geographic distance similarly across taxa, irrespective of
463 differences across biomes. This similar migration history suggests that shared characters of
464 the marsh rats, that is biotic factors, play a key role in structuring genetic variation when
465 genetic variation is measured at the scale of the species entire distribution. It may be that the
466 effect of the current configuration of habitats (contiguous versus patchy) may be discernible
467 at different spatial scales, such as at a local landscape level. Such scale-dependencies of
468 biotic traits in migration history is something we would like to investigate in the future;
469 however, we cannot address this question with the available sampling.

470 Notwithstanding the similar migration histories suggested by the similar strength of
471 the genes and geography association across taxa, the association between biome-specific
472 properties with deviations from expectations under isolation by distance point to potential

473 hypotheses about how the biomes may impact genetic structure. In particular, the magnitude
474 of deviations from a pattern of isolation by distance differs across the landscape in each
475 taxon, suggesting localized differences in population connectivity and/or persistence. The
476 most dramatic effect is across Amazonia, where the more northern population of *H. sciureus*
477 tend to be genetically quite similar despite fairly large distances separating the sampled
478 individuals (Fig. 2). This geographic area that individuals map based on their genetic makeup
479 is consistent with an estimated area of habitat stability based on ENMs for the past and
480 present (Fig. 2), which suggests a potential role of climatic shifts, and coincidentally is
481 estimated to be less stable relative to the southern sampled populations (Mayle, Burbridge, &
482 Killeen, 2000; Urrego, Silman, & Bush, 2005; Bush et al., 2007; Arruda et al., 2017). In
483 contrast, in southern regions of the distribution of *H. sciureus*, there is a strong
484 correspondence between the genetic and geographic position of individuals (Fig. 2), and their
485 general genetic distinctiveness relative to the more northern populations suggests a history of
486 relative regional isolation within the biome (e.g., Knowles et al. 2016). Differences in fit of
487 northern and southern regions to expected patterns of gene flow under an isolation by
488 distance model, in concert with corresponding differences in duration of stability inferred
489 from the ENMs (Fig. 2), suggests variation in the historical stability within a biome may be
490 contributing to genetic structure of constituent taxa. Specifically, our genetic analyses suggest
491 a dynamic of recent expansion may characterize the wetlands from the northern part of the
492 Amazonian biome, in contrast to relative population persistence within the southern region.
493 This pattern agrees with some, but not all, aspects of inferences about past regional stability
494 from other studies. For example, the area corresponding to our LOR, JUR, and MAD
495 populations (Fig. 2) corresponds to an area that is hypothesized to have been associated with
496 a dynamic geological history (Aleixo & Rossetti, 2007; Leite & Rogers, 2013), and stable
497 past hydroclimate variation (see Cheng et al., 2013). Although the sampling in the east is
498 limited, the genetic clustering of the GUI population with other western Amazonian sampled
499 populations suggests however that the wetlands in the eastern area (or at least the north-east;
500 Fig 2) is also relatively unstable, unlike the results from some landscape genetic and geologic
501 studies (Aleixo & Rossetti, 2007; Leite & Rogers, 2013).

502 Although less dramatic, patterns of genetic structure in *H. chacarius* show a
503 somewhat similar pattern with populations from the northern part of their range exhibiting the
504 greatest deviations under isolation by distance (individuals from the north tend to be
505 genetically more similar to individuals from the southern than expected), with most of the
506 estimated regions of stability located in the south (Fig. 2). This complex history suggests the

507 expansion of wetland across this biome may be fairly recent (see Bezerra & Mozeto, 2008),
508 with more sparse vegetation and intermittent torrential flows along the alluvial fans in the past
509 (Whitney et al., 2011; Assine et al., 2015). In contrast, while *H. vulpinus* shows the larger
510 continuous areas of stability among all species (see Arruda et al., 2017 for proposed
511 expansion scenarios), it nonetheless shows a correspondence between genes and geography
512 that is similar to the other two species (i.e. the t_0 of *H. vulpinus* is 0.69, while the t_0 of *H.*
513 *sciureus* and *H. chacarius* is 0.52 and 0.64, respectively). Such similarity again emphasizes
514 that differences in stability by itself, or continuity of wetlands (as opposed to being patchy),
515 is not sufficient to override patterns of genetic variation within each species that are
516 consistent with migration history where gene flow decreases with distance (Fig. 2).

517 In addition to the potential effect of historical stability, multiple lines of evidence
518 point to other characteristics of the biomes that may affect patterns of genetic variation, albeit
519 at a local level (as opposed to the regional scale of an entire biome, given similar degrees in
520 the strength in the association between genes and geography). Environmental differences
521 across the landscape show a significant effect on the position of individuals in genetic space,
522 after controlling for the effect of geography. However, it is noteworthy that this effect, as
523 with test of a general correspondence between genes and geography, is only detectable when
524 retaining the relative distance of populations latitudinally and longitudinally (i.e., with
525 Procrustes analyses). The lack of significance when reducing the geographic separation of
526 populations to a one-dimensional axis (Mantel and dbRDA analyses; Table S4.4 and S4.5 in
527 Appendix 4) highlights how connectivity patterns do not mirror expectations based on
528 random diffusion across a landscape (Excoffier, Foll, & Petit, 2009). That is, a longitudinal
529 distance versus a latitudinal distance is not equivalent in terms of the impact on population
530 differences. As such, our work highlights that within each of the biomes, connectivity
531 patterns vary locally in each species.

532 Patterns of environmental heterogeneity across the landscape (Fig. 3) and/or shifts in
533 habitat stability over time (Fig. 2) can cause populations separated by similar geographic
534 distances to differ in levels of connectivity (McRae, 2006; He et al., 2013). Although our
535 tests are insightful in that they identify patterns suggestive of environmental factors
536 influencing connectivity, as correlative analyses they do not provide tests of the process itself
537 (Knowles et al., 2016). Determining which of the dynamics might produce genetic variation
538 consistent with what is observed (i.e., applying a model selection framework to distinguish
539 between the geographic distribution of wetland habitats versus shifting climates; see He et al.,
540 2013; Knowles & Massatti, 2017), is beyond the scope of this study without additional

541 sampling for conducting spatially explicit model-based analyses. However, as with the
542 guiding theme of this work, tests to identify concordant processes across species will be
543 especially exciting to understand the extent to which genetic variation in the taxa might arise
544 from common processes, despite differences in the biomes themselves.

545

546 **Similar taxa, but different biomes, in comparative phylogeography.**

547 Although the study of multiple taxa across different regions predominates in historical
548 biogeography and macroecology (Leite et al., 2014; Arregoitia, Fisher, & Schweizer, 2017),
549 comparative phylogeographic analyses have focused primarily on co-distributed taxa from a
550 single area (Avice, 1992; Knowles, 2009; Hickerson et al., 2010). There are several potential
551 explanations for this tradition and one is related to sampling efforts for phylogeographic
552 inference: population sampling poses logistical constraints such that a more circumscribed
553 geographic region is simply more feasible. The focus on more circumscribed region also
554 reflects the traditional motivation behind the comparative phylogeography – making
555 inferences about the history of a region. It is through tests of concordance across taxa from a
556 particular region that specific biogeographic barriers (Avice, 1992) or areas of long-term
557 stability (Carnaval, Hickerson, Haddad, Rodrigues, & Moritz, 2009) might be inferred. In
558 fact, such tests of concordance across taxa with differing ecologies have been used to identify
559 how abiotic factors might supersede any ecological differences among taxa (Naka, Bechtoldt,
560 Henriques, & Brumfield, 2012) or reject the role of historical barriers in driving divergence
561 patterns (Smith et al., 2014).

562 As a complement to the study of co-distributed taxa, there is a precedent for studying
563 taxa from different regions in comparative phylogeography. For example, analysis of
564 evolutionarily independent regions can be considered as natural replicates and can be used for
565 making generalizations about geologic events that have broadly affected landscape histories
566 (Bermingham & Moritz, 1998), while controlling for discord that may arise from differences
567 in species-specific traits to mitigate poor predictive power when concordance across regions
568 is not supported (see Papadopoulou & Knowles, 2016). Here we adopted a strategy of similar
569 species, but different regions as an inferential framework. Moreover, with a complementary
570 set of tests and the genetic resolution provided by thousands of loci, we address how genetic
571 patterns vary (i.e., we move beyond concordant versus discordant binary). As such, we were
572 able to identify concordance in genetic variation (similar strength in the association between
573 genes and geography, despite differences in the wetlands from the different biomes) that
574 suggests a highly specialized semi-aquatic life may result in similar genetic isolation by

575 distance. However, inspection of local deviations from isolation by distance identified
576 additional biome-specific effects related to environmental differences and historical stability.
577 That is, rather than biotic versus abiotic factors predominating on the genetic structure, our
578 framework identifies how both components can influence genetic variation, as well as their
579 respective geographic scales of influence. Regarding this latter point, the biotic factors
580 predominate at the regional scale (at the level of genetic structure across the entire biome),
581 whereas abiotic factors appear to contribute to local departures from isolation by distance
582 (see Fig. 2). These scale-specific effects are especially interesting considering the qualitative
583 correspondence between regions of instability and departures from isolation by distance,
584 which may be suggestive of particular populations that might have been vulnerable to
585 environmental change (Rocha et al. 2014; Choueri et al. 2017; Harvey, Aleixo, Ribas, &
586 Brumfield, 2017).

587 With very few genetic studies on organisms that inhabits the flood-dominated
588 landscapes in South America, the present work adds to our knowledge, but there is
589 admittedly much that remains unknown. For example, the effect on terrestrial organisms (as
590 opposed to aquatic or semi-aquatic species) may not show the same relative role of biotic and
591 abiotic factors or geographic scale at which they might predominate, given that flooding and
592 rivers may act as barriers in terrestrial organisms rather than as routes for connectivity (Lima,
593 Lima-Ribeiro, Tinoco, Terribile, & Collevatti, 2014; Rocha et al. 2014). This suggests the
594 importance of a nuanced view of interpreting concordance (or the lack thereof) across taxa.
595 Specifically, taxa may show opposing patterns of connectivity and yet this discord could arise
596 from deterministic processes (i.e., not the idiosyncrasies of history; see Massatti & Knowles,
597 2014) because what constitutes routes of connectivity in a given wetland biome depends upon
598 species-specific traits (e.g., for terrestrial versus aquatic taxa).

599

600 **FIGURE LEGENDS**

601 Figure 1. Examples of the open biomes of the central, southern and northern region of South
602 America inhabited by *Holochilus* (see Fig. 2 for distributional map), such as (a) Poconé and
603 (b) Miranda, in Pantanal biome, both habitats occupied by *H. chacarius*; (c) Caçapava do Sul
604 in Pampa biome, within the range of *H. sciureus*, and (d) São José do Norte in Atlantic Forest
605 biome, within the range of *H. vulpinus*; (e) lower Xingú River, and (f) Japurá River both in
606 Amazon biome, inhabited by *H. sciureus*; (g) *H. chacarius*.

607

608 Figure 2. Map of the distributions with sampled populations marked for each of the three
609 marsh rats species, with each species color coded (*Holochilus chacarius*, *Holochilus*
610 *sciureus*, and *Holochilus vulpinus* are shown in black, green, and orange, respectively);
611 sampling locations span each species' range, which are largely non-overlapping with each
612 taxa occupying different biomes. On the right plots of Procrustes-transformed PCA's of
613 genomic variation with each individual mapped in the genomic PC-space (marked by circles;
614 note the genetic position of some individuals are largely overlapping in some cases) relative
615 to the geographical location of sampled populations (marked by triangles) showing the
616 deviation in the genomic PC-space from the expected pattern of genetic variation based on
617 geography (i.e., the length of the line connecting individuals to their geographical location
618 represents the magnitude of the deviation), as well as how the association between genes and
619 geography differed among species, with $t_0 = 0.69$, 0.64 , and 0.52 for *H. vulpinus*, *H.*
620 *chacarius*, and *H. sciureus*, respectively. Additionally, the plots for each taxon are shown on
621 the projected stability of each region, where stability is defined as areas that remained
622 suitable overtime (see Fig S3.1 in Appendix 3 for distributional maps from ENMs for each
623 geologic period). Stable areas since LGM, 21 kya, are marked in orange, yellow marks areas
624 that have been stable since Holocene, 6 kya, relative to the unstable areas marked in green
625 (i.e., projected suitable areas for the present, but not the past).

626
627 Figure 3. Map of the environmental variation across the region where the three species are
628 distributed (orange dots represent *H. vulpinus*, black dots *H. chacarius*, and green dots *H.*
629 *sciureus*), where differences in color depict geographic regions that differ the most from each
630 other. Specifically, PC1, PC2 and PC3 of bioclimatic variables across the landscape were
631 rescaled between 0 and 1, and the RGB color composite was calculated and plotted in the
632 map with PC1 set as the red scale, PC2 as the green scale, and PC3 as the blue scale. Colored
633 dots correspond to the populations presented in Fig. 2. Note that the border used to
634 characterize the relative difference in environment corresponded to the same area used to
635 generate the ENMs in each species (see Fig. 2) and does not include the Andes.

636
637 Figure 4. Dispersion in environmental space of the sampled populations used in our genetic
638 analyses (marked as colored dots) relative to the PC values for *Holochilus* sampling locations
639 used in the ENMs. For *H. sciureus* PC1 is strongly positively correlated with Minimum
640 Temperature of Coldest Month (Bio6) and explains most of the variation among populations
641 (i.e., 55.49%), whereas PC2 explains relatively little variation among populations (19.66%).

642 For *H. vulpinus* PC1 is strongly positively correlated with the Annual Precipitation (Bio12)
643 and explains most of the variation among populations (48.56%), whereas PC2 explains
644 24.26% of the variation among populations. For *H. chacarius* PC1 is strongly positively
645 correlated with the Minimum Temperature of Coldest Month (Bio6) and explains most of the
646 variation among populations (54.4%), whereas PC2 explains 21.8% of variation among
647 populations.

648

649 Figure 5. Distribution of individuals along PC1 and PC2 of genomic variation color coded,
650 and the percentage of variance explained by each PC are shown on a map (i.e., different
651 colors correspond to individuals with the greatest genomic difference along PCs) for each
652 species. Elevation differences are shown in grey scale on each map as well.

653

654 Figure 6. Comparison of the changes in the strength of the association between genes and
655 geography with the exclusion of individual populations (i.e. t'') relative to when all
656 populations are analyzed (i.e. t_0). Values for each species are standardized by t_0 (i.e. 0 on y-
657 axis corresponds to t_0) such that positive values indicate a stronger association between genes
658 and geography when a population is excluded, whereas negative values indicate a weaker
659 association. Bar colors represent sampling populations following the colored names of
660 populations in Fig. 2.

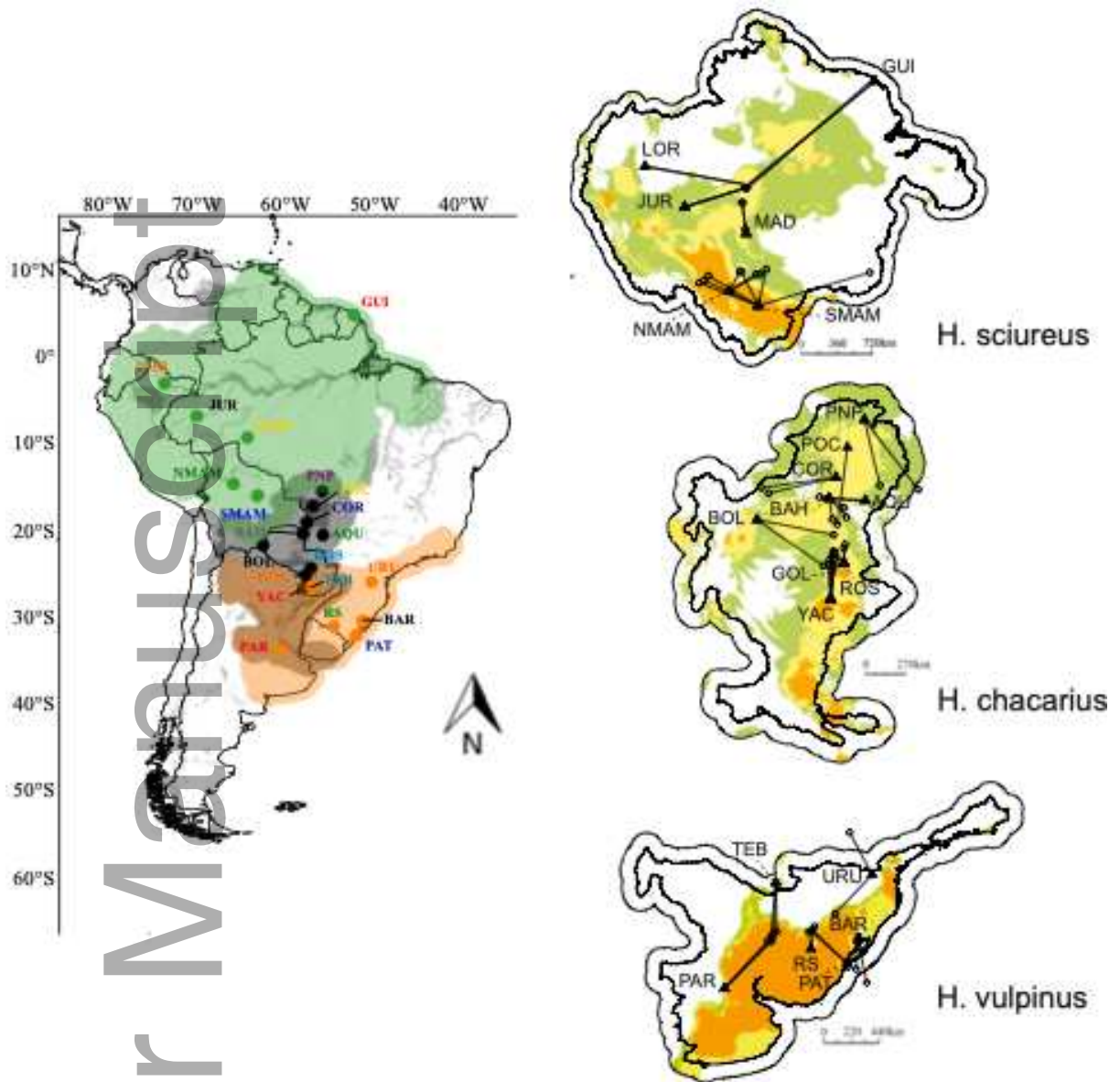
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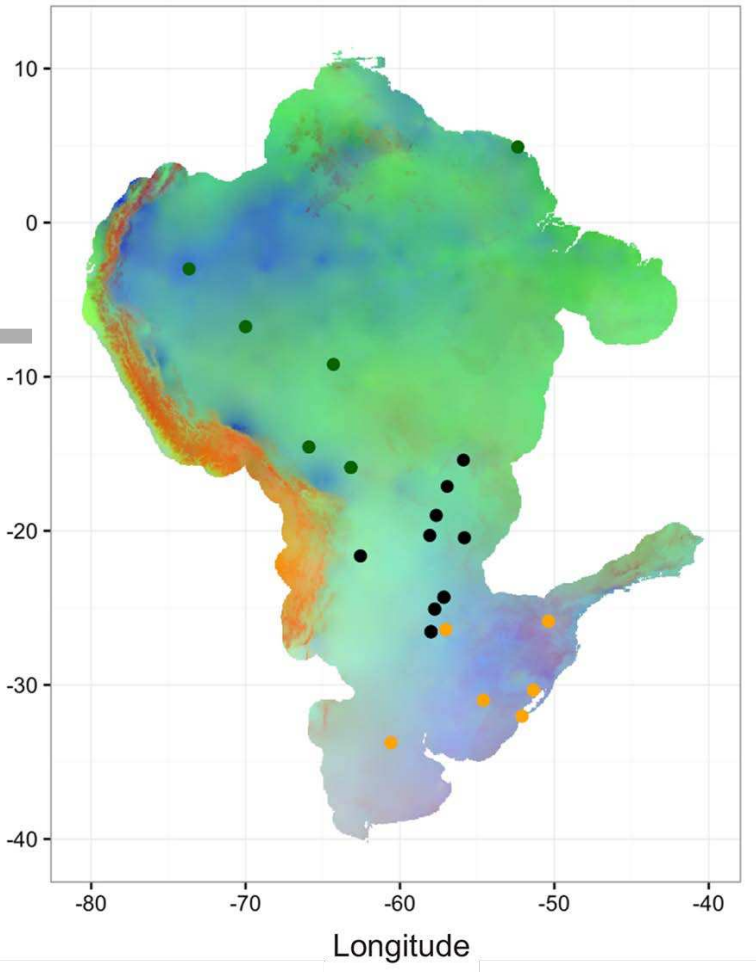


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Fig 1

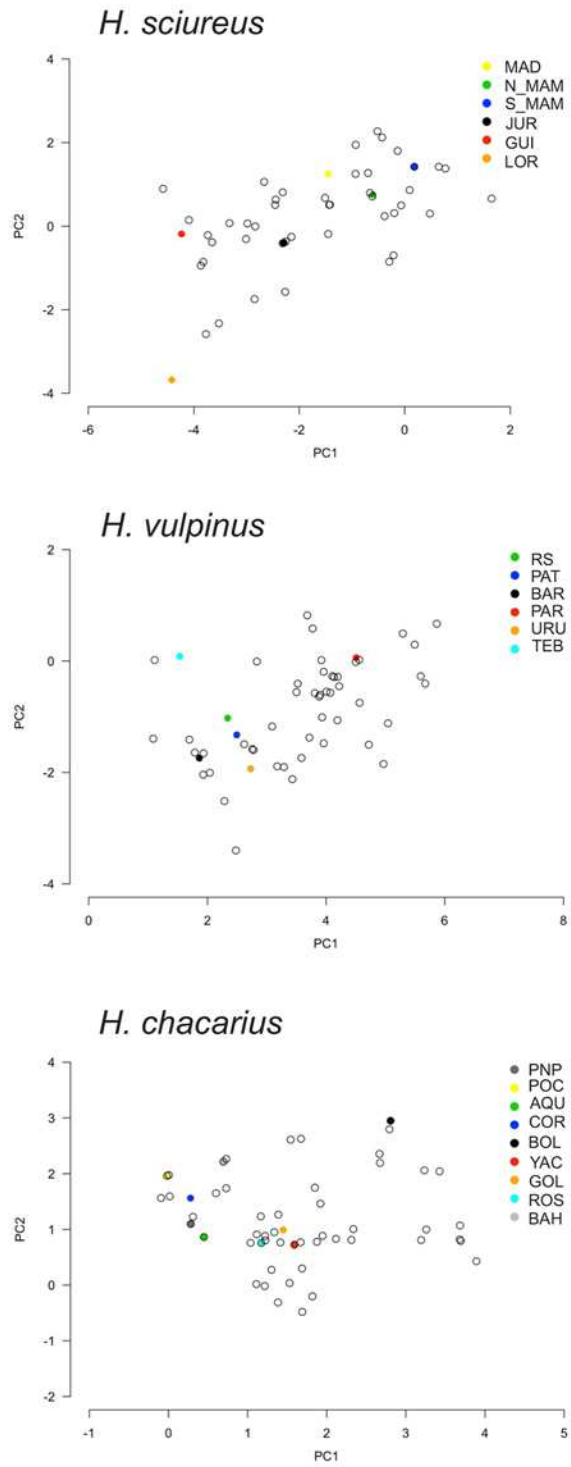


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 667 **Fig 2**
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671 **Fig 3**

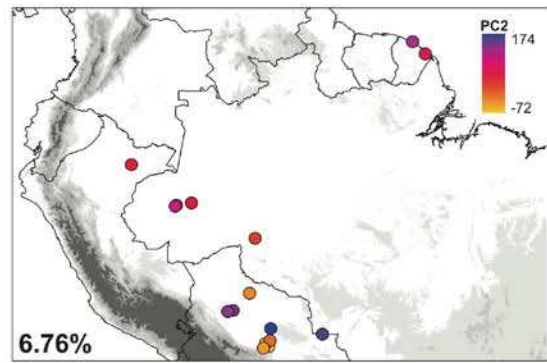
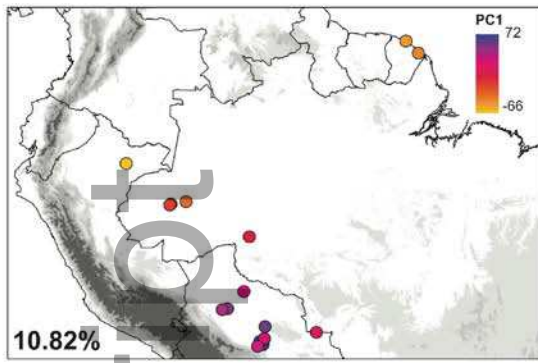


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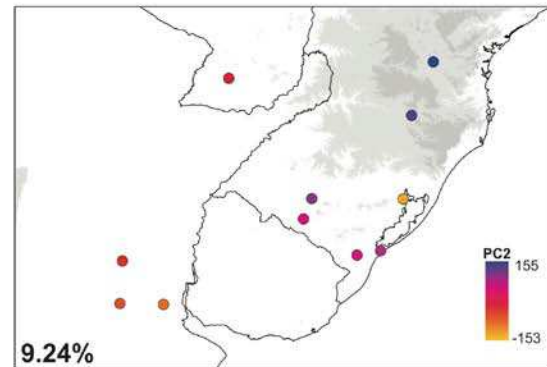
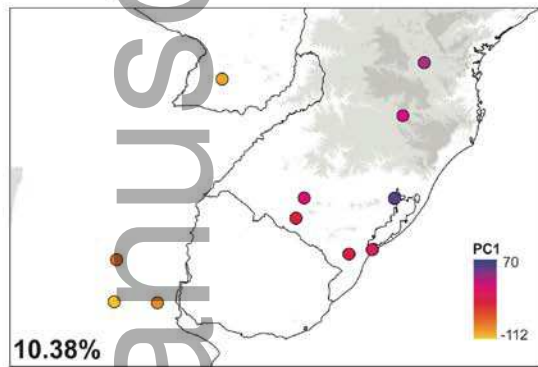
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Fig 4

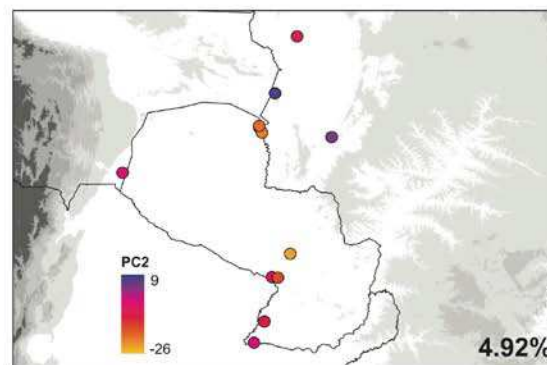
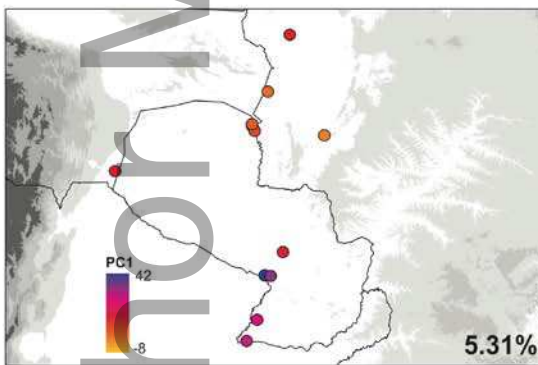
a) *H. sciureus*



b) *H. vulpinus*

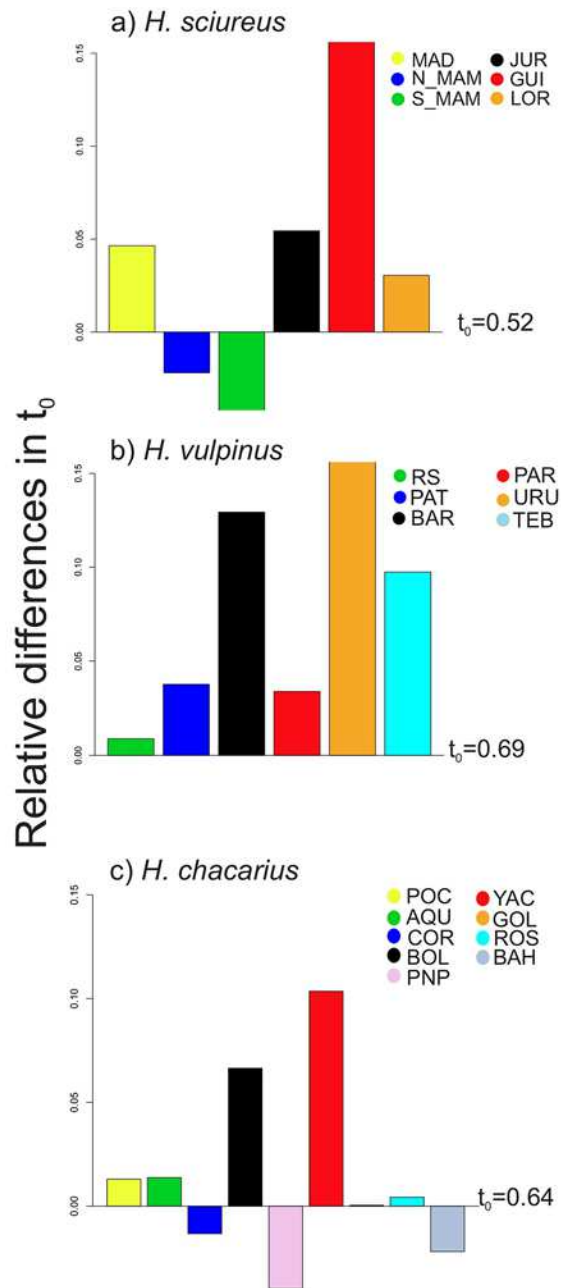


c) *H. chacarius*



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Fig 5



678

679 **Fig 6**

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916

917 BIOSKETCHES

918 The authors share an interest in the study of processes that structure the genetic variation
919 among taxa and across geography. LLK, JRP and ARP conceived the idea; JRP collected the

920 genomic data; JRP and ATT processed the genomic data; JRP, LLK and ATT performed the
921 analyses; all authors worked on the writing.

922

923 **SUPPORTING INFORMATION**

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

925 **Appendix 1** Summaries of geographical information and genomic sampling

926 **Appendix 2** Summaries of samples collected and scientific collections

927 **Appendix 3** Summaries of ENM settings and projections of current and LGM distributions

928 **Appendix 4** PC maps of genetic variation and summaries of genetic variation

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a)

b)

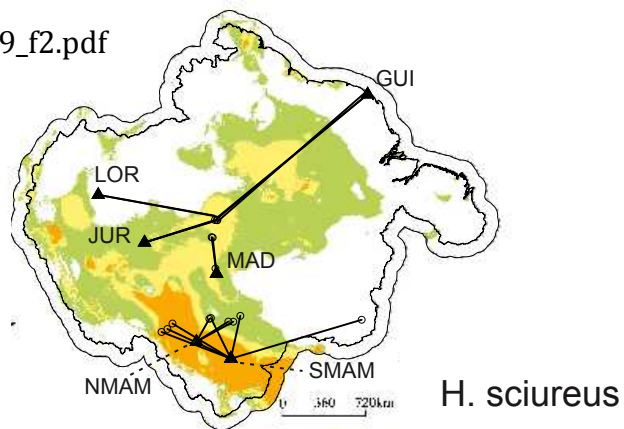
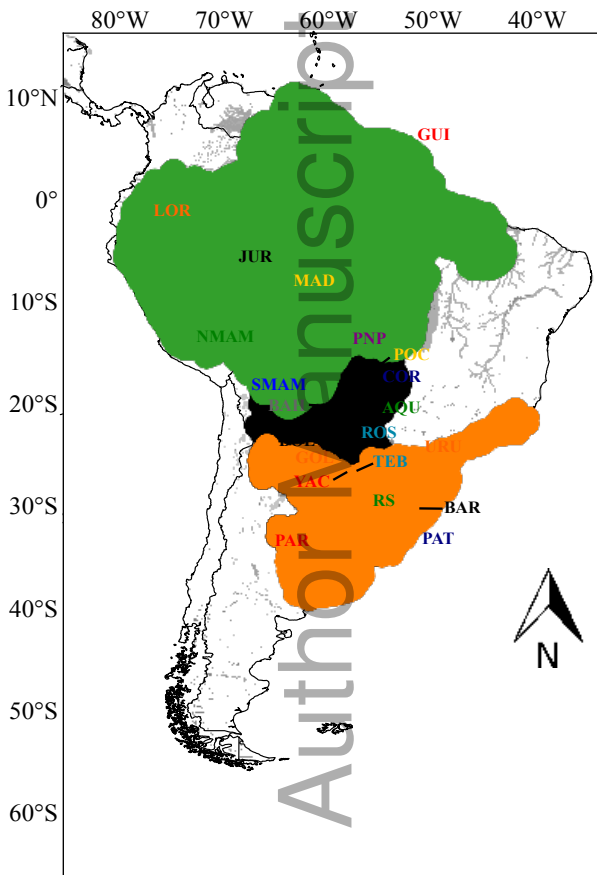
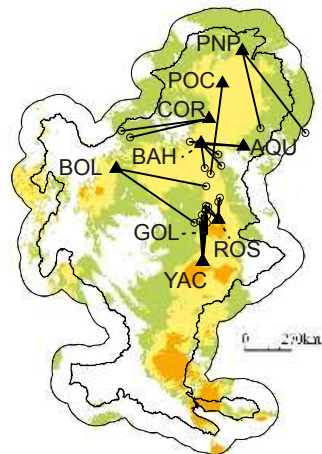
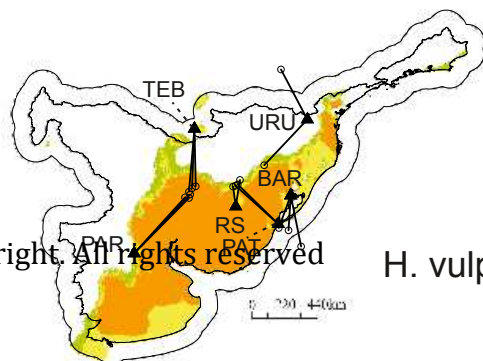
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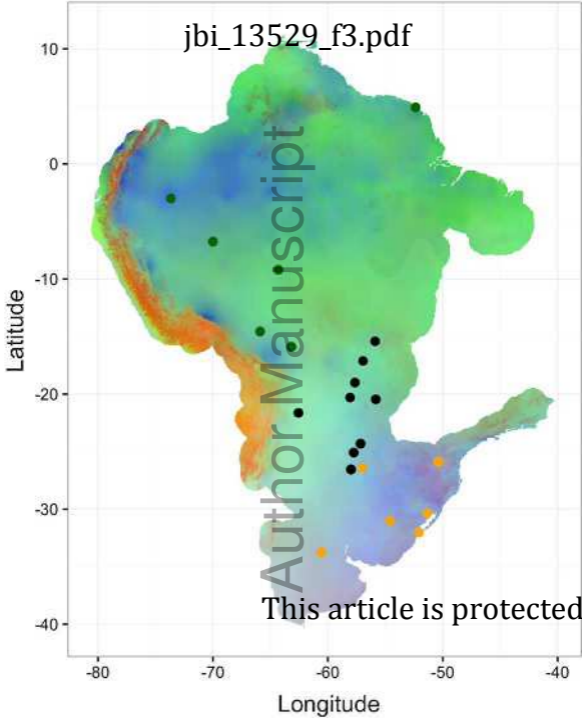
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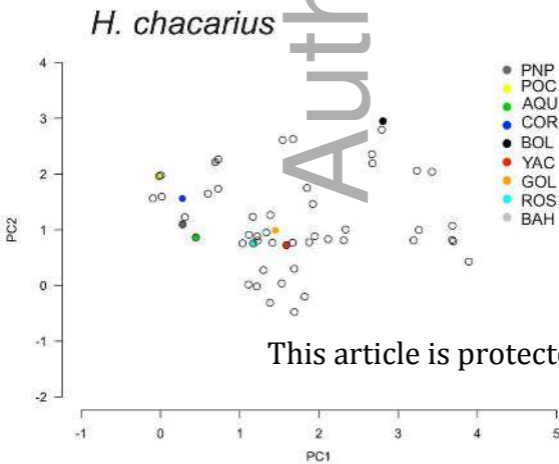
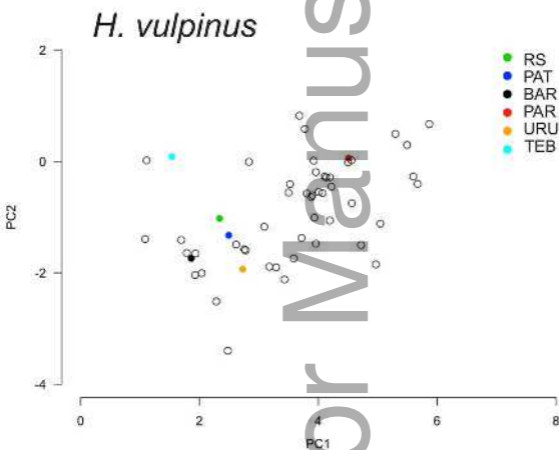
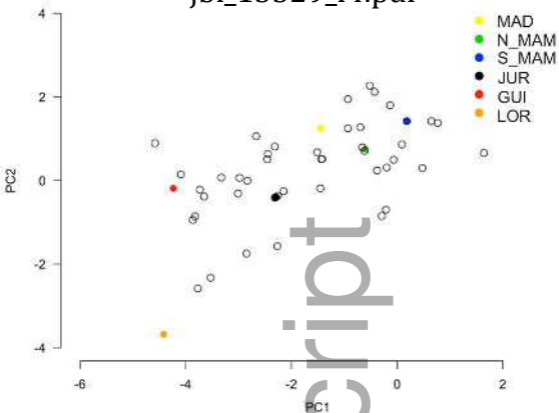
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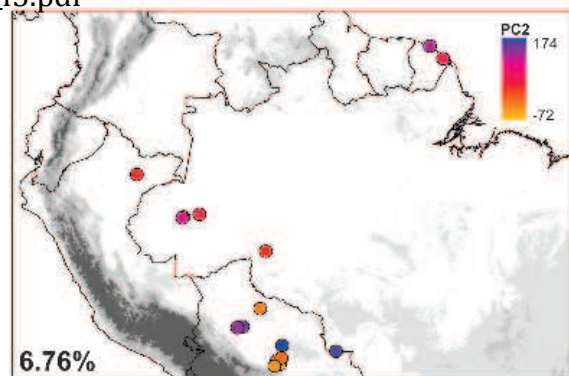
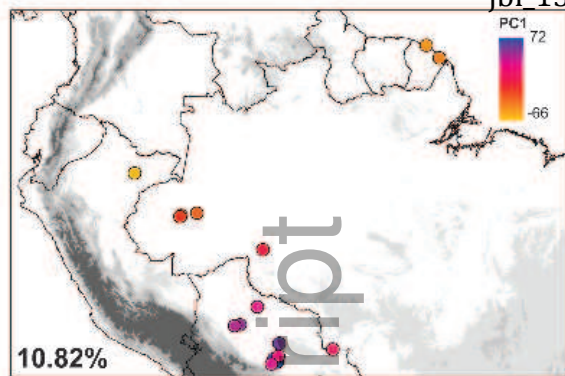
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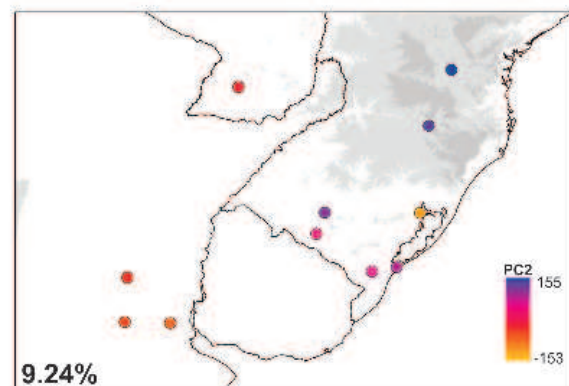
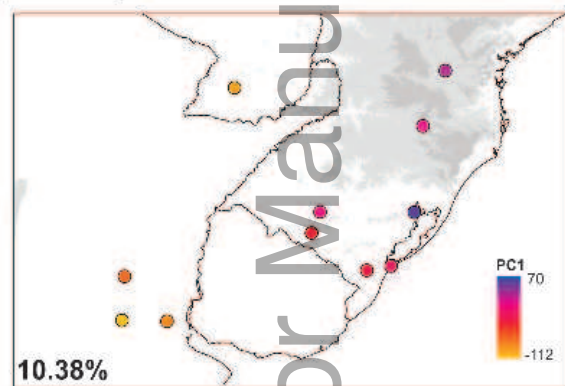


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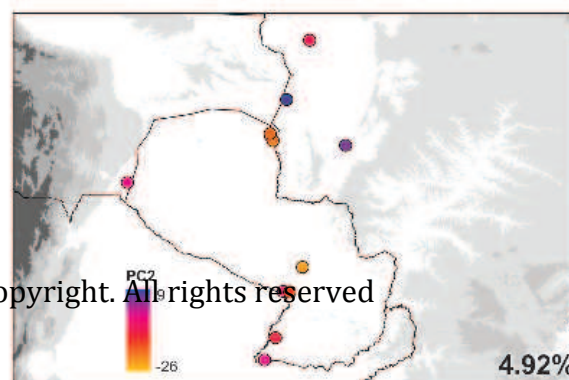
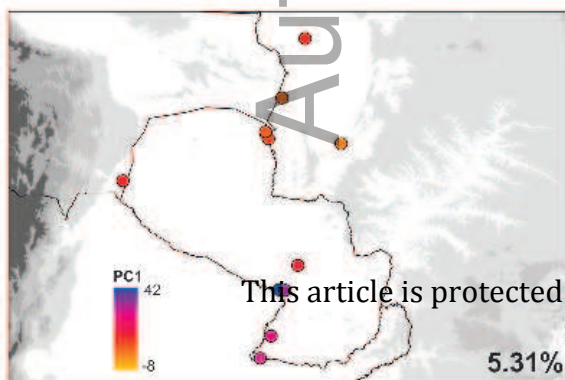
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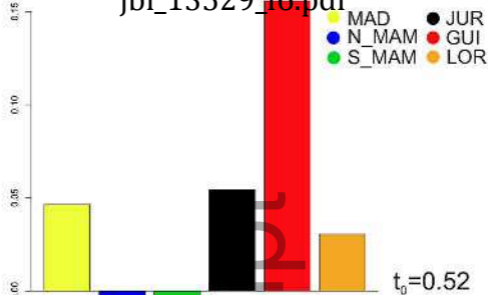


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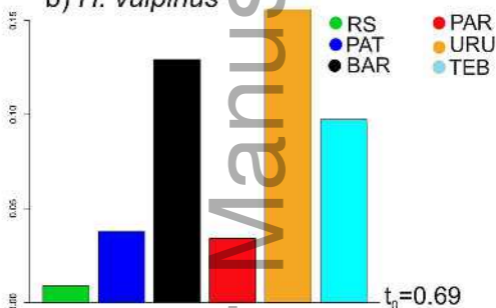


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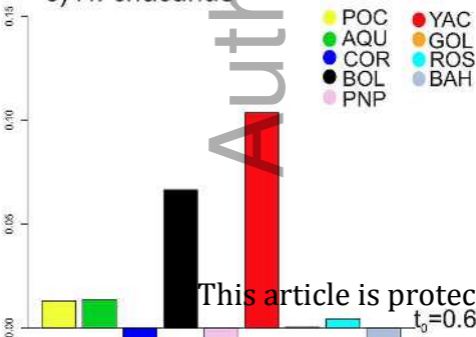
a) *H. sciureus*
jbi_13529_f6.pdf



b) *H. vulpinus*



c) *H. chacarius*



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