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1 **Influence of environmental heterogeneity on the distribution and persistence of a**
2 **subterranean rodent in a highly unstable landscape**

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28 Abstract

29 In this study we combine information from landscape characteristics, demographic inference
30 and species distribution modelling to identify environmental factors that shape the genetic distribution
31 of the fossorial rodent *Ctenomys*. We sequenced the mtDNA control region and amplified 12
32 microsatellites from 27 populations distributed across the Iberá wetland ecosystem. Hierarchical
33 Bayesian modelling was used to construct phylogenies and estimate divergence times. We
34 developed species distribution models to determine what climatic variables and soil parameters
35 predicted species presence by comparing the current to the historic and predicted future distribution
36 of the species. Finally, we explore the impact of environmental variables on the genetic structure of
37 *Ctenomys* based on current and past species distributions. The variables that consistently correlated
38 with the predicted distribution of the species and explained the observed genetic differentiation
39 among populations included the distribution of well-drained sandy soils and temperature seasonality.
40 A core region of stable suitable habitat was identified from the Last Interglacial, which is projected to
41 remain stable into the future. This region is also the most genetically diverse and is currently under
42 strong anthropogenic pressure. Results reveal complex demographic dynamics, which have been in
43 constant change in both time and space, and are likely linked to the evolution of the Paraná River.
44 We suggest that any alteration of soil properties (climatic or anthropic) may significantly impact the
45 availability of suitable habitat and consequently the ability of individuals to disperse. The protection of
46 this core stable habitat is of prime importance given the increasing levels of human disturbance
47 across this wetland system and the threat of climate change.

48 Keywords: *Ctenomys*, habitat fragmentation, Iberá Wetland, metapopulation, population
49 genetics, species distribution modelling.

50 Introduction

51 An understanding of the relationship between the genetic structure of populations and the
52 landscape they occupy plays an important role in the study of metapopulation dynamics. Determining
53 the effect of landscape characteristics on the genetic connectivity of populations is fundamental for
54 understanding potential drivers of spatial population, genetic differentiation structure and ultimately
55 speciation (Manel *et al.* 2003, Storfer *et al.* 2010).

56 A landscape consists of a mosaic of patches of suitable habitat characterized by different
57 environmental features within a matrix of unsuitable habitat (Fahrig and Merriam 1985). The ability of
58 an animal to use resources within a landscape patch is determined not only by the distance between
59 patches but also by the nature of the routes connecting them (Taylor *et al.* 1993). Consequently, the
60 degree of isolation between subpopulations depends on the actual capacity of movement of the
61 species in relation to the landscape heterogeneity (Bowne & Bowers 2004; Anderson *et al.* 2015).

62 When trying to characterize the relationship between the landscape and the genetic structure
63 of a population, it may also be important to consider the influence that past demography and
64 historical environmental conditions may have had on the current distribution of genetic variability.
65 Data on past climatic conditions can provide useful information on long-term environmental
66 fluctuations; and thus, the projection of the environmental niche of extant populations back in time
67 allows the identification of environmentally stable areas through time. These stable areas are
68 expected to show higher genetic diversity than environmentally variable regions, since they were able
69 to sustain populations over long time periods, and favour their diversification (Carnaval *et al.* 2009;
70 Rodriguez-Robles *et al.* 2010). When a projection to the future of the environmental niche is also
71 available, locating these climatically stable and genetically variable areas becomes essential for

72 conservation, as these areas could become diversity hotspots where conservation efforts can be
73 centered.

74 The Iberá Wetlands, located in the Province of Corrientes in north-eastern Argentina,
75 constitute a highly unstable environment, both spatially and temporally. This region is part of the
76 Guaraní Aquifer, one of the largest groundwater reservoirs covering about 1,100,000 km² across the
77 territories of Argentina, Brazil, Paraguay and Uruguay (Amore 2011). The area and depth of marshes
78 and lagoons vary according to the state of local rivers and the frequency of rainfall across seasons.
79 The increase of human activity in this area, especially from agriculture and forestry, has led to further
80 fragmentation and loss of habitat. Human disturbance is currently considered one of the major
81 causes of the decline in biodiversity, impacting on dispersion and gene flow (Frankham *et al.* 2010).
82 The species inhabiting this wetland have not only been impacted by the extreme climatic changes of
83 the Late Quaternary but also by short-term climatic fluctuations due to recent trends in global change.
84 Therefore, climatic change at both short and long-term time-scales could have influenced the
85 population genetic structure of species that currently inhabit this region (Stevaux 2000, Iriondo 2003).

86 Subterranean rodents of the genus *Ctenomys* have specific habitat requirements and
87 restricted dispersal capacity, promoting the establishment of a strong genetic structure, which can
88 result in spatial patterns where geographically close populations become genetically distinct (Reig
89 and Kiblicky 1969, Reig *et al.* 1990, Wlasiuk *et al.* 2003, Kittlein & Gaggiotti, 2008; Fernández *et al.*
90 2012). Populations of *Ctenomys* living around the Iberá Wetlands belong to the *torquatus* species
91 group, which has been extensively studied during the past few years (Giménez *et al.* 2002; Mirol *et*
92 *al.* 2010; Fernández *et al.* 2012). Although three nominal species (*C. roigi*, *C. perrensis* and *C.*
93 *dorbingyi*) had been previously described inhabiting this area, a recent study based on microsatellite

94 data has found that all the populations are part of a larger metapopulation of six distinct evolutionary
95 lineages with different degrees of genetic isolation (*Fig. 1*, Fernández *et al.* 2012). Although these
96 lineages are currently described as discrete genetic clusters, their future structure will depend on the
97 temporal dynamics of their unstable habitat; i.e., these lineages may become distinct species or
98 continue to evolve as a single metapopulation.

99 In this study we examine the potential role of various environmental factors in shaping the
100 genetic differentiation of *Ctenomys* populations distributed around and across the Iberá Wetlands.
101 We combined information from landscape genetic analyses, demographic inference and species
102 distribution modelling (SDM), in order to determine what factors best predict the distribution of
103 populations, and which variables best explain the observed genetic differentiation among
104 subpopulations at the local level and across the metapopulation as a whole. This information is
105 important not only for explaining the current population dynamics of this group in this wetland system,
106 but also for understanding how past changes in geology and climate patterns may have shaped this
107 metapopulation. Comparing the species' distribution under current climatic conditions, to those
108 inferred for the Last Glacial Maxima (21,000 years BP), and conditions in the Last Inter-Glacial
109 period (120,000 – 140,000 years BP) as well as in scenarios predicted by models for the year 2060,
110 we aimed at predicting areas of stability that may defied conservation hotspots of biodiversity.

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112 Materials and Methods

113 *Sampling and Genetic Data*

114 The sampling of *Ctenomys* populations covered 27 localities throughout Corrientes Province,
115 and denotes a representative sample of sites with known favourable habitat characteristics for this
116 group in the area. Genetic data used in this study consisted of 354 individuals genotyped for 12
117 microsatellite loci (Lacey *et al.* 1999; Lacey 2001), of which 195 were also sequenced for a DNA
118 fragment of 374 bp of the mitochondrial (mtDNA) control region. These data were generated by our
119 lab and previously reported in Fernández *et al.* (2012 *Fig. 1*, Table 1, JQ686014-JQ686050) and
120 Mirol *et al.* (2010 *Fig. 1*, Table 1), except for three new sequences from sampling site Ea. Tacuaritas,
121 which did not render any new haplotype. We also obtained 41 additional control region sequences
122 and 67 cytochrome b sequences (426 bp) of related species from Genbank (species and Genbank
123 accession numbers are reported as Supplementary Information, Table S1 and in Giménez *et al.* 2002
124 Genbank: AF500038-AF500070). This species group of *Ctenomys*, identified as the Corrientes's
125 group, has been the subject of previous studies (Giménez *et al.* 2002; Mirol *et al.* 2010; Fernández *et*
126 *al.* 2012) where historical and recent gene flow has been found between genetically independent
127 groups. Therefore, the Corrientes group can be considered a metapopulation of multiple groups
128 interacting through gene flow.

129 *Species tree*

130 We used the hierarchical Bayesian model implemented in *BEAST v. 1.7.1 (Heled &
131 Drummond 2010) to test species trees for the six lineages delimited in Fernández *et al.* (2012)
132 belonging to the Corrientes group, along with the other five species of the torquatus group, using both

133 mtDNA fragments (236 control region and 67 cytochrome b sequences). BEAST estimates a
134 multispecies coalescent based on multiple gene trees embedded in a shared species tree, along with
135 the effective population size of both extant and ancestral species (Heled & Drummond 2010). For
136 gene tree inferences, we used JMODELTEST (Posada 2008) to select the nucleotide substitution
137 model that best fitted the data and a Yule speciation process. With the species tree we also
138 estimated the dates of the appearance of *Ctenomys* in the Province of Corrientes and its separation
139 from the rest of the torquatus group. We used two mutation rates previously estimated for the
140 *Ctenomys* mtDNA control region: 4.42 % (Mora *et al.* 2013) and 2.96% (Roratto *et al.* 2015) per
141 million years. The analysis was run twice, with 2.5×10^8 MCMC iterations, a sample size of 25×10^3
142 and with the first 20% excluded as burn-in. Both runs were combined into a single chain with the
143 program LOG COMBINER 1.7.1 (Drummond & Rambaut 2007). Convergence was evaluated using
144 the program TRACER (Rambaut & Drummond 2007) and the cladogram was created from the
145 posterior distribution of species trees with Fig Tree v 1.4.0 (Rambaut 2012).

146 *Impact of environmental factors on genetic structure and genetic diversity*

147 To examine the impact of environmental variables on the genetic structure of *Ctenomys* we
148 used the Hierarchical Bayesian method developed by Foll & Gaggiotti (2006), implemented in the
149 program GESTE (version 2.0). The environmental variables were obtained from the analysis of
150 Landsat images (<http://glovis.usgs.gov/>, see Supplementary Data -Environmental variables), and
151 from other sources including the Project Soils of Argentina (Cruzate *et al.* 2006-2009; SAGyP -
152 INTA); the National Weather Service; the Meteorological Information Centre and the Hydrological
153 Integrated Data Base of the Ministry of Water Resources (Table 1).

154 Using environmental data for each sampling site, we performed the analysis according to two
155 spatial scales: sample locality (SLoc) on one hand and the 12 genetic clusters (GC) inferred using
156 TESS in Fernández *et al.* (2012) (see *Fig. 1*) on the other. The idea behind this two-level analysis
157 was to evaluate what environmental variables influence the genetic structure of the *Ctenomys* group
158 at different spatial scales. In the GC case, environmental values corresponding to each cluster were
159 the average value across the sample localities included in each group. To verify the robustness of the
160 estimated parameters we performed three independent runs. We used 10 pilot runs of 10^3 iterations,
161 followed by an additional burn-in period of 5×10^6 iterations. The thinning interval was 50, and the
162 sample size used for posterior distribution estimates was 6×10^4 .

163 We conducted a first analysis with all nine environmental variables available (Table 1). This
164 run produced a total of $2^9=512$ alternative models. With this many alternative models the
165 interpretation of the posterior probabilities becomes difficult, since there is always a fraction of the
166 probability distribution that is allocated to models that do not explain the observed pattern. Although
167 each alternative non-explanatory model can have a negligible probability (i.e. less than 0.001) the
168 sum of all of them is not. Therefore, we carried out analyses including the five top factors to obtain a
169 more robust posterior distribution estimates of the regression parameters.

170 *Species Distribution Modelling*

171 To determine which environmental factors best predicted the probability of presence of
172 *Ctenomys*, SDMs were developed using maximum entropy in MaxENT v3.3.3 (Phillips *et al.* 2006)
173 based on the current distribution of this species in Corrientes and Entre Ríos (N=54). Details of the
174 climatic variables and methods used to model the current, past and future distribution of *Ctenomys*,
175 are found in Supplementary Data. Multivariate Environmental Similarity Surfaces (MESS) analysis

176 was undertaken to determine whether climatic conditions in the predicted range of the past and future
177 differed significantly from those in the current species distribution (Elith *et al.* 2010).

178 *Niche Stability*

179 In order to assess whether the current genetic structure is better explained by past or current
180 climatic conditions, we performed a General Linear Model within GESTE using three explanatory
181 variables: 1- the probability of *Ctenomys* presence during the Last Glacial Maximum (LGM)
182 approximately 21,000 years ago; 2- the probability of current presence (Current) and 3- the niche
183 stability (N_S) since the Last Inter-Glacial (LIG). N_S was estimated following Ortego *et al.* (2012)
184 calculations as: i) $1 - |N_{CURRENT} - N_{LGM}|$, where N_S is the niche stability between LGM-Current and ii) $1 -$
185 $|N_{LGM} - N_{LIG}|$ where N_S is the niche stability between LIG-LGM. Since all three variables are highly
186 correlated, we performed a separate run for each of them and used the variance left unexplained by
187 each model as the criterion to choose the best model (i.e., the lower the variance left unexplained,
188 the better the fit).

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196 Results

197 *Estimation of species tree*

198 The species trees based on mutation rates of 2.96% and 4.42% /Mya are shown in *Fig. S1a*
199 and *Fig. S1b*, respectively. The divergence time estimated between the ingroup and the rest of the
200 *torquatus* group which includes species from Brazil and Uruguay, was between 0.508 Mya (95% HPD
201 0.31-0.71 Mya, mutation rate 2.96%) and 0.341 Mya (95% HPD: 0.20-0.47 Mya, mutation rate
202 4.42%). The estimated tMRCA for the ingroup ranged from 0.079 Mya (95% HPD 0.05-0.12 Mya) to
203 0.119 Mya (95% HPD 0.07-0.018 Mya)

204 *Impact of environmental factors on the genetic structure*

205 The first run of the GESTE analysis with 9 variables yielded very similar results for both
206 clustering levels (Table S2). The models with the highest posterior probability (SLoc: $p=0.299$, GC:
207 $p=0.104$) in both cases included Distance (D) and Drainage class (Dc). In the case of SLoc the model
208 also incorporates Relative Bulk density (RBd) while for GC the model included Ndvi. Table 2 shows
209 the explanatory power of each variable calculated as the sum of posterior probabilities across all
210 models. For SLoc, Dc and RBd have equally explanatory power (0.997), closely followed by Distance
211 (0.993). For populations, D has the highest explanatory power (0.852) closely followed by Dc (0.823).

212 We then carried out a second GESTE analysis with the five variables that had the highest
213 explanatory power in the previous analysis: E, Dc, RBd, D and PC1 for SLoc and Dc, Ndvi, HtPr, D
214 and PC1 for GC. The results are shown in Table 3 a, where the three top models are displayed. As
215 expected, the posterior probabilities of the best models increased, as the number of variables
216 considered decreased, although the most probable models remained the same.

217 In order to infer the importance of the effect of each variable, we used the estimates of the
218 regression coefficients of the best model (Table 3, b). Their sign indicates whether the factor
219 increases or decreases with the genetic differentiation, and its absolute value shows the magnitude
220 of the effect (Foll & Gaggiotti 2006). For both clustering levels, drainage (Dc) shows the highest
221 absolute value and a positive relationship with genetic differentiation measured through local F_{ST} .
222 This means that the most poorly drained localities revealed the highest differentiation with the rest of
223 the metapopulation. For clustering level SLoc, the second and third most influential variables were
224 RBd ($\alpha_2=-0.91$) and D ($\alpha_4=0.57$), with a negative and positive relationship with genetic differentiation
225 respectively. For clustering level GC two other factors included in the best model increased genetic
226 differentiation, D ($\alpha_5=0.94$) and Ndvi ($\alpha_3=0.85$).

227 *Species Distribution Modelling*

228 All models had high predictive probability for *Ctenomys* presence and did not overfit the
229 presence data. The SDM for the current distribution containing all variables at a resolution of 30 arc
230 seconds (Fig. 2) had a model average AUC > 0.90. Distribution models with variables used for past
231 and future projections with current presence data all had model averages AUC >0.85. The variables
232 that contributed the most and positively, to the current SDM were soil characteristics, followed by a
233 negative correlation with temperature seasonality and precipitation in the wettest month. Soil
234 characteristics which positively predicted the probability of presence in the model, included soils with
235 low flood risk, good drainage, and 'Sandy' and 'Sand-Silt' soil types. On the other hand, 'Silt-Sand'
236 had a negative correlation with probability of presence. Lastly, soil types susceptible to erosion and
237 those with low fertility in the upper layer were also important in the model. When the current
238 distribution was modelled at the lower resolution, the SDM demonstrated lower probability of

239 *Ctenomys* presence in the region, with the same three variables contributing most to the model (Fig.
240 2).

241 We included soil characteristics in the SDM's projected during the LGM, the LIG and for the
242 year 2060 (Fig. 3), in order to account for their potential influence on species distribution. A negative
243 correlation with temperature seasonality, followed by precipitation in the wettest month, were the
244 most important projected variables. MESS analysis showed that climatic conditions during the LGM
245 had parallels with today's conditions, with few areas in the core of the species current range
246 predicted to have had conditions very different from the present day. However, the most suitable
247 habitat for *Ctenomys* based on these climatic features showed a significant shift northward. MESS
248 analyses indicate that temperature seasonality during the LIG, among other climatic variables, might
249 have extended outside their present range of values. Predictions of habitat suitability for the LIG
250 should thus be treated with caution as the model trained with present conditions included a narrower
251 range of values (Elith *et al.* 2010). Similarly, MESS analysis of predicted habitat suitability for the
252 year 2060 suggested that several key habitats for *Ctenomys* will experience a very different climate
253 from that known today. In particular areas to the north and west are predicted to experience
254 maximum temperatures above those recorded today, and potentially higher than those estimated
255 since the LGM.

256 *Niche Stability*

257 Regarding the relative importance of recent and historical events on the genetic structure we
258 found that for genetic clusters (GC), the genetic structure is best explained by *Ctenomys* recent
259 demographic events. First, the best model that includes the SDM probabilities of presence for the
260 Current distribution results in $p=1.00$ (regression coefficients $\alpha_1=-0.472$ 95%CI [-1.270; 0.299];

261 $\sigma^2=1.280$, 95%CI [0.540; 3.73]), whereas in the run with SDM probability of presence for the LGM
262 distribution the model with the highest probability was the null model that did not include any variable.
263 Second, when studying the possible influence of climatic stability we found that the model that best
264 explains the genetic differentiation includes the niche stability obtained between the LGM and current
265 conditions ($p=0.674$; regression coefficients $\alpha_1=0.69$, 95%CI [0.145; 1.26]; $\sigma^2=1.06$, 95%CI [0.465;
266 2.73]), while for the niche stability between LGM-LIG the model with the highest probability was the
267 null one. Comparing the regression parameters of both analyses (probabilities for Current distribution
268 and niche stability LGM-Current), the model with niche stability had the lowest residual variance, and
269 therefore, was the one that best explains genetic structure of the *Ctenomys* metapopulation (i.e., the
270 higher the niche stability, the weaker the effect of genetic drift).

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Discussion

281 The study of the effects that landscape features have on the distribution and connectivity of
282 populations can increase our understanding of the fundamental biological processes driving
283 metapopulation dynamics (Manel *et al.* 2003). Importantly, it may also help identify anthropogenic
284 pressures operating upon the landscape. This information is essential for any attempt to restore
285 natural connectivity among elements of a metapopulation (Lada *et al.* 2008), and thus, assist in the
286 development of conservation strategies for species and the habitat on which they depend. In this
287 study, we investigated how various environmental factors have shaped, and continue to affect, the
288 genetic structure of the *Ctenomys* metapopulation in Corrientes Province in an attempt to identify
289 areas that may be of particular importance for future conservation of the species.

290 We first explored what variables best explained the observed pattern of genetic differentiation
291 among subpopulations at the local level and across the metapopulation as a whole. Of all the
292 variables studied, two of them, distance between habitat patches and soil drainage ('Distance' and
293 'Drainage class') were most strongly associated with genetic structure at both spatial scales (locality
294 and genetic cluster). Given the low mobility of *Ctenomys*, the strong positive association of distance
295 with genetic structure is not surprising. In a previous study, Fernández *et al.* (2012) reported that the
296 genetic structure was consistent with a model of isolation by distance only across short distances.
297 This result is in agreement with a study on the Brazilian species *C. flamarioni* (Stoltz 2006), which
298 reported movements of approximately 0.25 km per year in continuous habitats. In the case of the
299 *Corrientes* group, where the landscape presents natural as well as rapidly increasing artificial
300 discontinuities and thus less connectivity throughout the system as a whole, the movement of the
301 individuals could be even more restricted. In our study, the shortest average distance between

302 localities was approximately 78.02 km, which is approximately ~300 times longer than their natural
303 annual dispersal distance based on estimates reported by Stoltz (2006).

304 The north-western half of the Corrientes Province consists of hills of fine to medium
305 sandstone, diagonally crossing the area, forming a fan-shaped landscape with the apex to the north.
306 These geographic features constitute evidence of major streams that crossed the territory now
307 occupied by the Iberá Wetland (Orfeo 2005). It has been proposed that the specific habitat
308 requirements of most subterranean rodents (well-drained and aerated soils) reflect the physical and
309 energetic limitations involved in digging through wet soil, plus the associated high CO₂ and low O₂
310 pressure found in the burrows (Buffenstein 2000). As expected, the size, shape and arrangements of
311 solids and voids in the soil have a direct effect on the movement of air and water. Larger pores are
312 commonly occupied by air; therefore they offer good aeration but poor water retention (Lal R., 1991).
313 They also present more rapid oxygen diffusion and carbon dioxide movement out of the soil. Sandy
314 soils like those present in the hills of the Corrientes Province have a preponderance of large pores
315 and little organic matter; consequently, they conduct water more rapidly presenting good drainage,
316 which, as indicated by our results, has a positive relation to the observed genetic structure. This is
317 consistent with the SDM results, which suggested that sandy or sandy-silty soils with good drainage
318 positively predicted the presence of *Ctenomys*. At a local level, 'Relative Bulk' density was also
319 included in the most probable models, showing a negative relationship with the genetic structure.
320 Relative Bulk density considers both the pore space and the solid particles of soil, thus a soil with a
321 higher 'Relative Bulk' density, drains water faster during wet periods, which for *Ctenomys* relates to
322 the positive correlation to soil drainage (Lal R., 1991).

323 When examining the impact of environmental variables on the metapopulation as a whole
324 (i.e., on the 12 genetic clusters inferred in Fernández *et al.* 2012), in addition to 'Distance' and
325 'Drainage class', 'Vegetation index' (Ndvi) becomes important, showing a positive relationship with
326 genetic structure. In a study on the landscape ecology of *Ctenomys porteousi*, Mapelli & Kittlein
327 (2009) found four habitat variables explaining patch occupancy, which included habitat quality in the
328 matrix surrounding the patch, average vegetation cover (Ndvi) in the patch, minimum vegetation
329 cover in the matrix surrounding the patch, and area of the nearest neighbor patch. Regarding Ndvi,
330 Mapelli & Kittlein (2009) found that low values in the habitat patch as well as in the area surrounding
331 habitat patches, corresponding to poorly vegetated soils, were positively correlated with occupancy.
332 Our data suggests that Ndvi, besides its influence on occupancy, is also an important factor
333 determining genetic structure as higher values of vegetation cover correlate with higher values of F_{ST} .
334 Both results are expected given that vegetation cover favors soil development, and enlargement of
335 the root system increases soil compactness, where burrowing becomes energetically more expensive
336 (Mapelli & Kittlein 2009). The fact that we found Ndvi as a factor in the model explaining genetic
337 structure only at the metapopulation level, probably indicates its influence not only on habitat patches
338 but also on their surrounding areas.

339 We also assessed environmental factors that predicted the distribution of populations of the
340 *Corrientes* group. The SDMs models demonstrated that the probability of *Ctenomys* presence was
341 higher in areas with a small range in seasonal temperature and low levels of precipitation in the
342 wettest month. However, in all SDMs, soil characteristics and particularly sandy soils with good
343 drainage, clearly played a major role in accurately predicting species presence. Given the
344 underground life style of these rodents, it is clear that subterranean burrows play an important role in
345 their interactions with the environment. Burrowing requires 360 - 3,400 times as much energy as

346 moving the same distance across the surface (Jarvis & Bennet 1991), so the distribution of
347 subterranean life will be strongly limited to habitats where tunnel excavation is energetically feasible
348 (Busch *et al.* 2000).

349 Furthermore, the genetic differentiation among groups of *Ctenomys* found in Corrientes
350 Province seems to be intimately related to the landscape resulting from the evolution of the Paraná
351 River, from its beginning as a broad river corridor, up to its current position, movement that resulted
352 in the formation of the Ibera Wetland as a large mosaic of lentic bodies. Historically, the Parana River
353 flowed into the Paranaense Sea (14-5 Mya, Orfeo 2005), which covered a large portion of the
354 Argentine territory including the Mesopotamia region (Corrientes, Entre Rios and Misiones
355 Provinces). After the marine regressions in the Late Miocene (7-3 Mya, Orfeo 2005), the Parana
356 River changed its course and began to pour its waters in north-eastern Argentina, initially at the
357 boundary between Misiones and Corrientes towards the Uruguay River. Afterwards, it changed its
358 course again flowing to the west and up to the north, resulting in its current geographic location
359 (Castellanos 1959, Popolizio 2004). This shift occurred approximately between 0.78-0.13 Mya
360 (Iriondo 1979, Popolizio 2004). According to the species tree, the appearance of the *Corrientes*
361 group of *Ctenomys* occurred within this time range, some 0.119-0.079 Mya. Therefore, the *Corrientes*
362 group seems to have colonised this region from East to West, which also explains its phylogenetic
363 inclusion in the torquatus group of species also present in Brazil and Uruguay.

364 Projection of the SDM's into the past suggests that the suitable habitat for *Ctenomys* since
365 the Last Inter Glacial (140,000 – 120,000 yrs BP) has undergone expansions and contractions as
366 expected with the dry and wet periods that have been observed in the Paleoclimatological record
367 since the Late Pleistocene (Stevaux 2000), changing the suitability of the landscape for the species.

368 Our model projection into the climatic environment of the LGM, some 21,000 yrs BP suggests a
369 decrease in the proportion of suitable habitat at this time, with a slight range shift northward.
370 According to palaeoclimatic analysis, following the LGM the climate in Argentina remained relatively
371 cold and arid until the Holocene, with a particular cold snap during the Younger Dryas 10,000 yrs BP
372 (Iriondo & Garcia 1993). The final climate switch to the warmer and moister conditions of today, some
373 8,000 yrs BP coincides with the broader range observed in the current SDM (Iriondo & Garcia 1993).

374 Projecting the distribution model for this region into the year 2060, based on the IPCC4 CIAT
375 climate model, suggests a decline in habitat suitability with a potential range shift towards the south
376 as temperature increases. *Ctenomys* are predicted to experience maximum temperatures above
377 those experienced today, and potentially those estimated after the LGM. In the heterogeneous
378 landscape of the Iberá wetlands this is likely to lead to further population fragmentation, population
379 isolation and the associated loss of diversity and local extinction.

380 Interestingly, a comparison of the SDM's models from LIG to 2060 reveals a region between
381 the Santa Lucía River and the Iberá marsh that appears to remain climatically stable and suitable for
382 *Ctenomys* through time (Fig. 3). The results of the GESTE analysis showed that genetic
383 differentiation is negatively correlated with current probability of *Ctenomys* presence but not with
384 LGM presence. Furthermore, there is a strong correlation with niche stability from the LGM to the
385 present. These results are in agreement with our expectations that climatically stable areas tend to
386 favour diversification and sustain genetically more diverse populations, in comparison with regions
387 that have suffered greater climatic changes.

388 The more stable, diverse, and genetically structured area comprised between the Santa
389 Lucía River and the Iberá marsh is currently occupied mainly by one (DEFG) of the six main lineages

390 described in Fernández *et al.* (2012). This evolutionary lineage is highly variable, with the highest
391 number of microsatellites alleles and mitochondrial haplotypes, as it would be expected if this lineage
392 originated from a Late Quaternary refugia (Carnaval *et al.* 2009). Furthermore, migration assessment
393 (see Fernández *et al.* 2012) suggests that there was a fluid but low movement between *Ctenomys*
394 lineages in the past, but more recently migration seem to have ceased. The current metapopulation
395 dynamics seems to be driven by changes in habitat suitability throughout this region, with asymmetric
396 migration occurring across environmentally stable habitats. Collectively, these results allow to
397 characterize this region as a hotspot of species distribution in Corrientes, which acted as a potential
398 refuge for the species during climate extremes. Unfortunately, this area is currently under strong
399 commercial forestation, which makes efforts for conservation even more urgent, as this region faces
400 future climate and anthropogenic changes.

401 In conclusion, in this study we found that environmental variables that consistently correlated
402 with genetic differentiation and predicted species distribution of *Ctenomys* in this region, were those
403 linked to specific habitat requirements associated with the lifestyle of these underground rodents.
404 Results presented in this study describe the demographic dynamics of a complex metapopulation
405 system, which has been in constant change both in time and space. Our results suggests that the six
406 lineages previously described for the *Corrientes* group of *Ctenomys* have not yet fully diverged, and
407 that their location and connectivity coincide with the distribution of sandy hills that cross the province
408 between the main rivers originated by the evolution of the Parana River (Popolizio 2004). Future
409 alteration of soil properties (e.g., due to climatic or anthropic changes) may alter the availability of
410 suitable habitat and consequently the ability of *Ctenomys* individuals to disperse. Finally, we were
411 able to identify a stable, genetically rich and structured area at the core of the species distribution in
412 the wetland marshes of the Corrientes Province. Protecting this area is of prime importance because

413 of the increasing human disturbance across this wetland ecosystem, and the current trends in global
414 climate change.

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420

421 Data Archiving

422 For Mitochondrial DNA sequences and microsatellite data were see Fernández *et al.* 2012.

423

424 Conflict of interest.

425 The authors declare no conflict of interest.

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428 This article does not contain any studies with human participants performed by any of the
429 authors.

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Table 1. Environmental factors used in the GESTE analysis. a- calculated with the software IDRISI, b- from the Project Soils of Argentina (Cruzate *et al.* 2006-2009, SAGyP - INTA), c- obtained from the National Weather Service, the Meteorological Information Center and the Hydrological Integrated Data Base of the Ministry of Water Resources

Factor	Description
Habitat proportion (HTPR) _a	Percentage of area covered by habitat, as a measure of the structure of the landscape.
Normalized vegetation index (Ndvi) _a	Estimator of the quality of the landscape and represents the average value of the normalized vegetation index, which is a measure of vegetation cover. Has a range between 1 to -1. In the case of tuco-tucos, values are expected to be negatively correlated with habitat quality, because they mostly occur in poorly vegetated areas (Vleck 1979, 1981; Mapelli and Kittlein 2009)
E _a	Average elevation above the sea, tuco-tuco occupies the highest areas of the province of Corrientes
Relative Bulk density (RBd) _b	Approximate value of soil density through the textural class and the percentage of sand present at each site. It is generally defined by the mass of soil per unit volume (solids + pore space)
Drainage class (Dc) _b	Related to water movement through the soil and frequency and duration of wet periods. We use 5 classes: somewhat excessively drained (1), well drained (2), moderately well drained (3), somewhat poorly drained (4) and poorly drained (5).
Precipitation (Pp) _c	Annual average precipitation
Distance (D)	Average distance between the focal population with respect to all other populations of the system
Fragmentation (PC1-PC2)	<p>We quantified habitat fragmentation using 5 class-level metrics: 1-Mean Patch Area, 2-Total Core Area, 3-Total Edge, 4-Aggregation Index, 5- Patch Cohesion Index.</p> <p>To incorporate these metrics we transformed each metric into a fragmentation distance (the average value between the focal population with respect to all other populations in the system) similarly to what was done for the distance.</p> <p>We perform a Principal Component analysis and used as variables for the environmental survey the projected values of the first two components (PC1 and PC2).</p>

a.

Table 2. Sum of posterior probabilities of models that include a given factor. GESTE analyses included all 9 factors. Bold value indicates the factor with highest score.

Factor	Sum of posterior probabilities	
	Cluster level	
	Locality	Genetic cluster
E	0.387	0.259
RBd	0.997	0.199
Dc	0.997	0.823
Ndvi	0.081	0.430
HTPR	0.093	0.294
Pp	0.068	0.128
D	0.993	0.852
PC1	0.482	0.339
PC2	0.063	0.277

Table 3. Posterior probability of the most probable model (a) and posterior estimates of regression parameters for such model (b) when the five variables with the highest explanatory power are considered.

Cluster level	Model Probability	Factors included		
a) Locality	0.41	D+Dc+RBd		
Genetic cluster	0.38	D+NDVI+Dc		
	Factor	Regression coefficient	Mode	95% HPDI
b) Locality	constant	α_0	-0.86	[-1.22 ; -0.491]
	D	α_4	0.57	[0.198 ; 0.955]
	Dc	α_3	1.03	[0.549 ; 1.65]
	RBd	α_2	-0.914	[-1.49 ; -0.412]
		σ_2	0.693	[0.363 ; 1.43]
Genetic cluster	constant	α_0	-1.19	[-1.69 ; -0.633]
	D	α_5	0.936	[0.334 ; 1.60]
	Ndvi	α_3	0.845	[0.212 ; 1.45]
	Dc	α_2	1.4	[0.662 ; 2.13]
		σ_2	0.527	[0.206 ; 1.69]

Titles and legends to figures

Fig. 1.

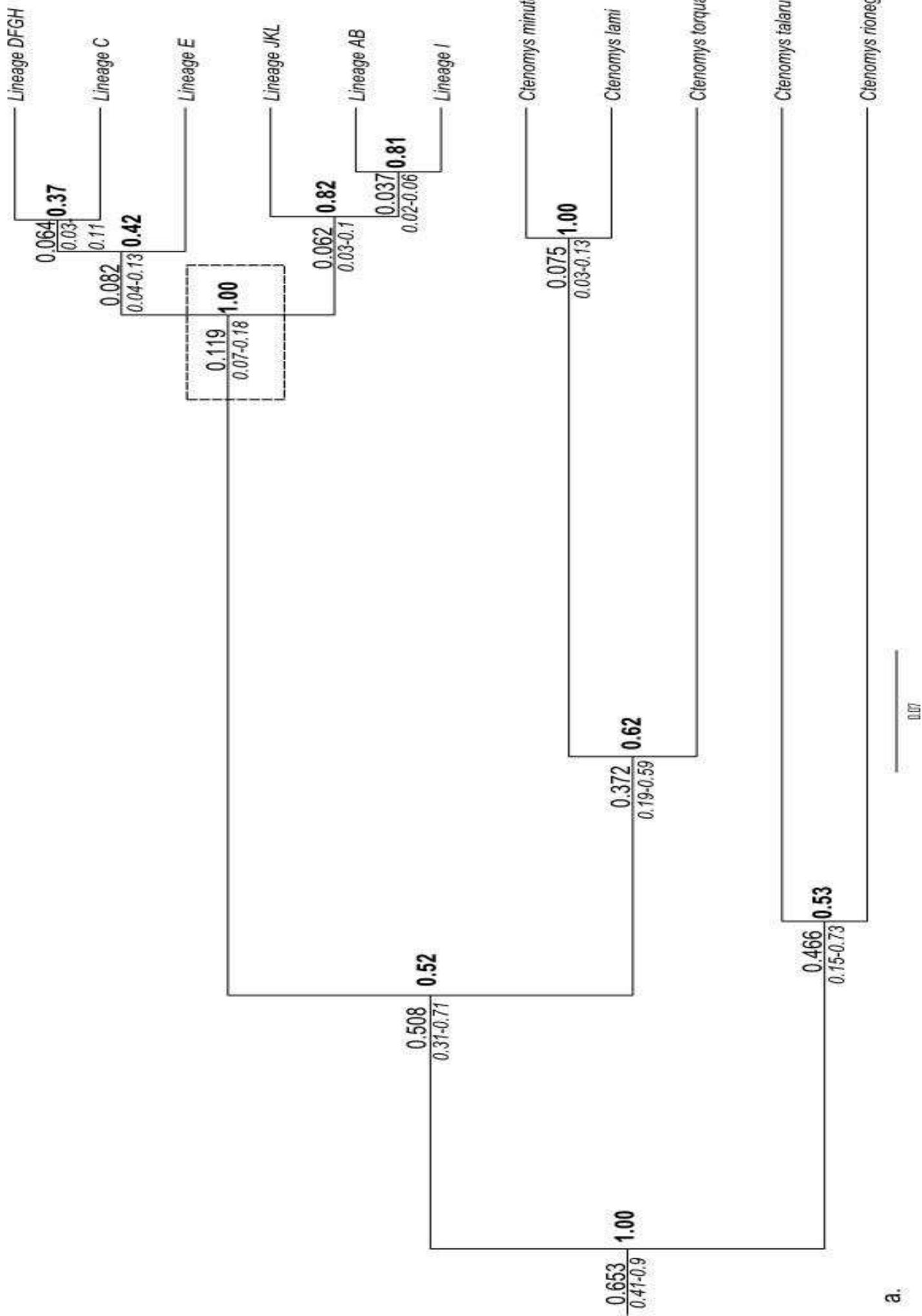
Map of Corrientes Province showing the sampled localities of *Ctenomys*. The 12 genetic clusters defined for the *Ctenomys* group are shown by black broken lines and the 6 lineages by grey solid lines. Adapted from Fernandez *et al.* 2012

Fig. 2

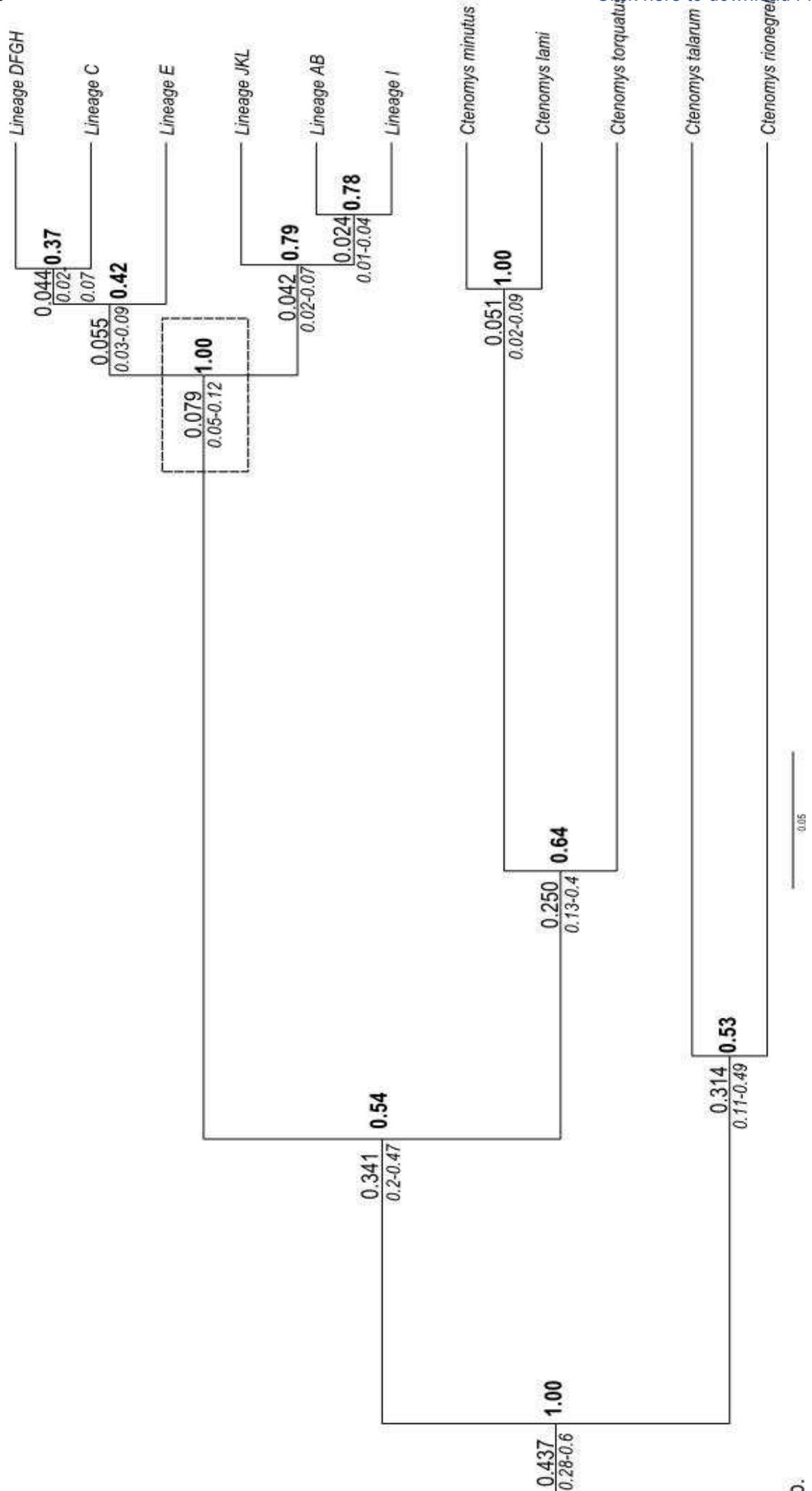
Maps showing the probability of *Ctenomys* presence in Corrientes and Entre Ríos Provinces, Argentina, based on the location of the 54 presence records (red circles). Shading indicates logistic probability of presence and range from 0 to 1, with increasingly darker shades of grey indicating increasing habitat suitability. Models based on 7 Bioclim variables, altitude and several measures of soil type at a scale of 30 arc seconds.

Fig. 3.

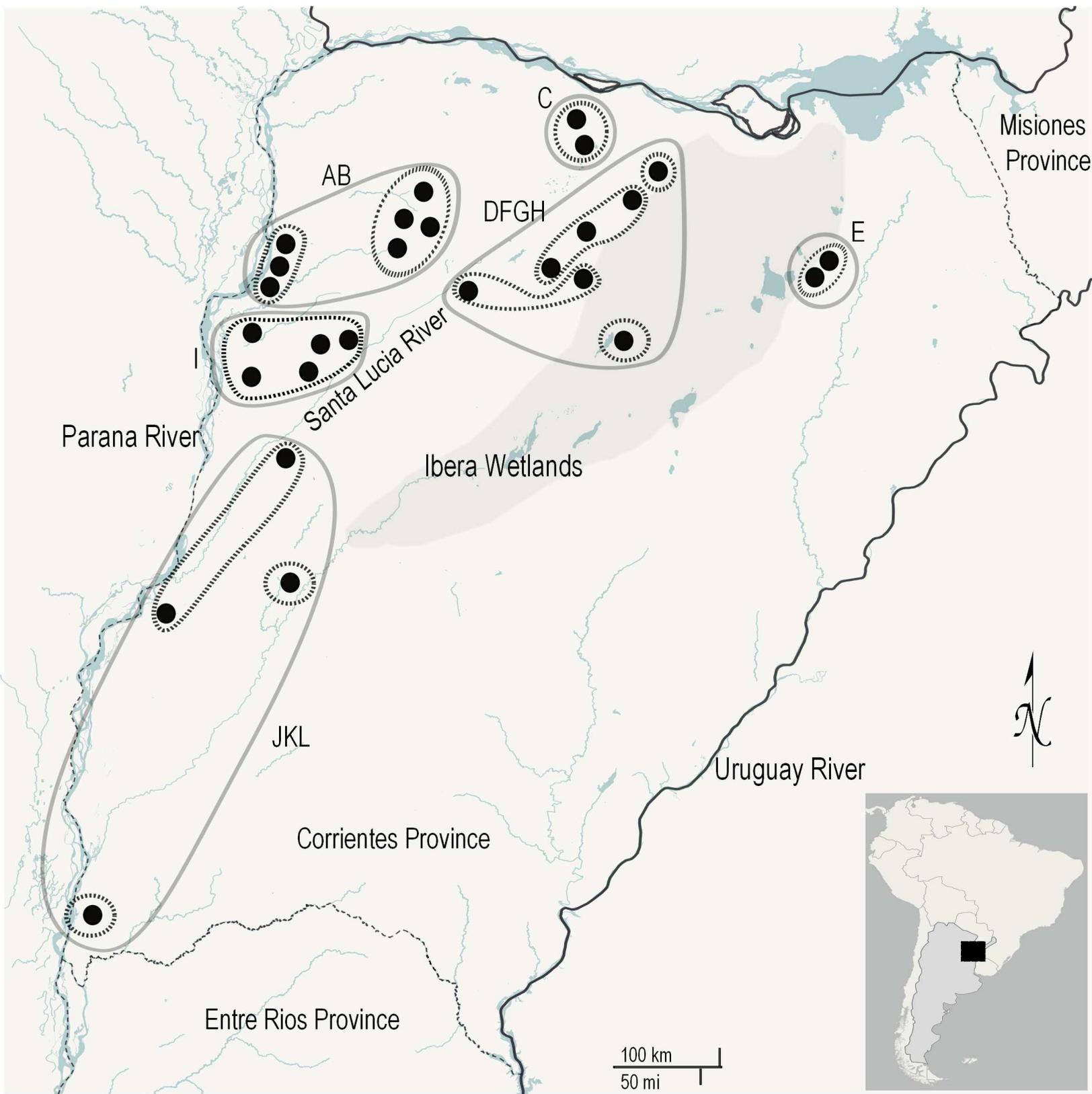
Predicted and projected distribution of *Ctenomys* in Corrientes and Entre Ríos Provinces, Argentina, based on 7 Bioclim variables, altitude, various measures of soil type, and 54 presence records. a) Projected distribution during the Last Inter-Glacial period (ca. 120,000 – 140,000 years BP); b) Projected distribution during the Last Glacial Maxima (ca. 21,000 years BP); c) Current predicted distribution of *Ctenomys* for the year 2013.d) Predicted distribution for the year 2060. Shading indicates logistic probability of presence and range from 0 to 1, with increasingly darker shades of grey indicating increasing habitat suitability.

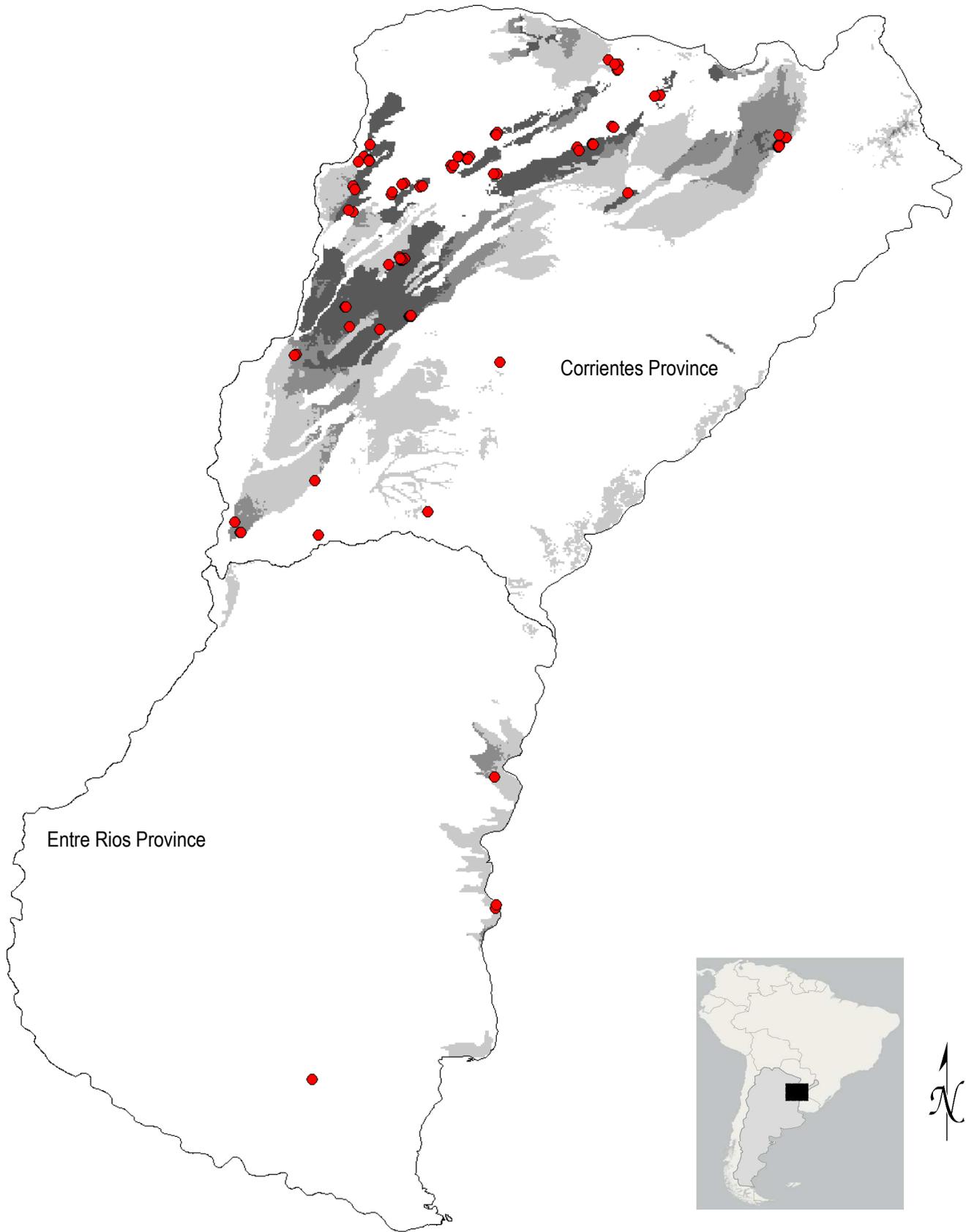


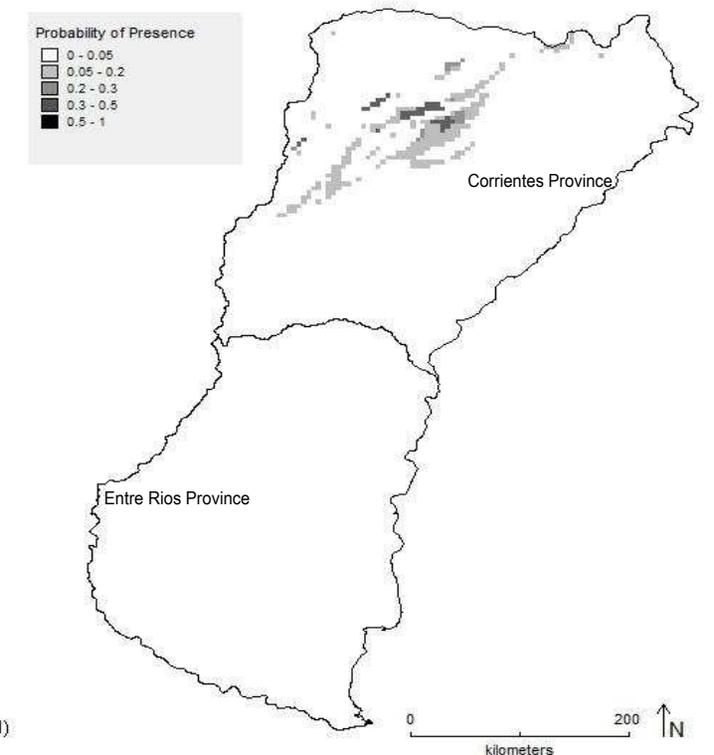
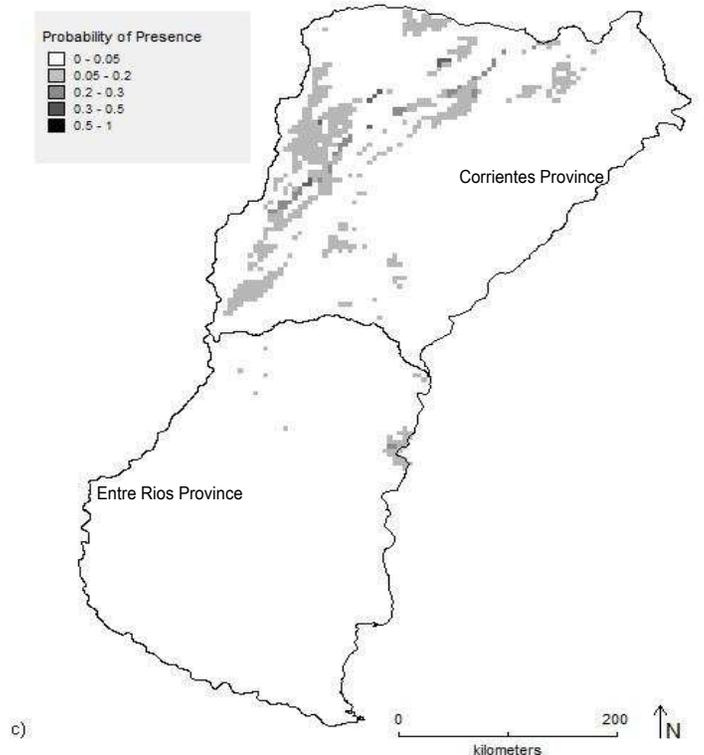
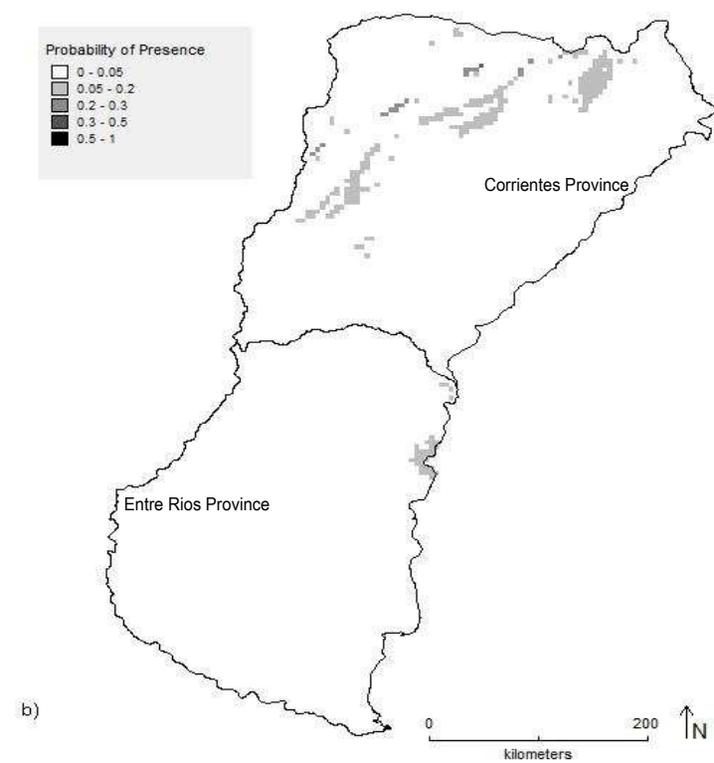
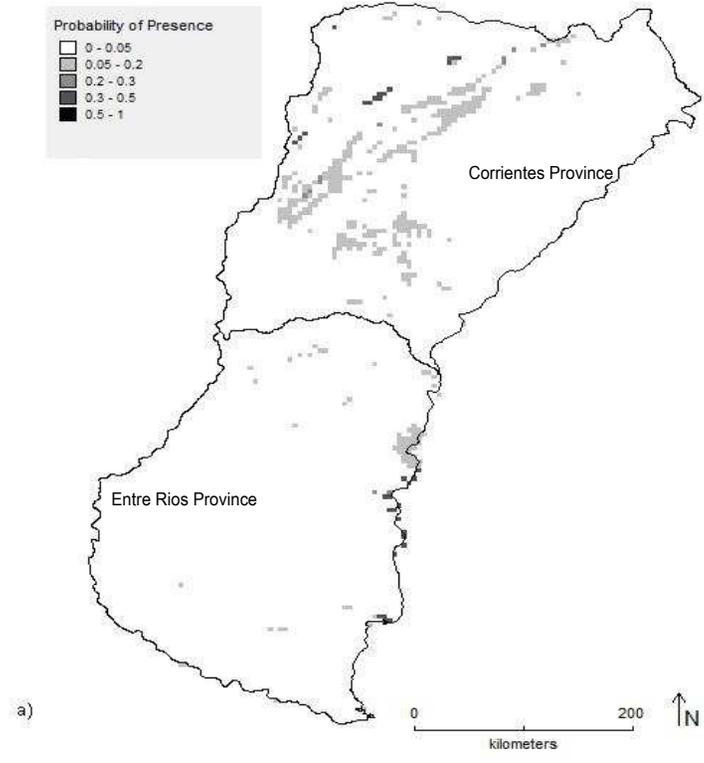
a.

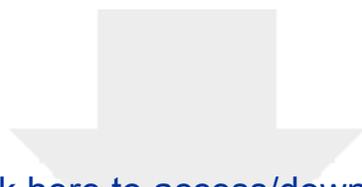


b.









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Supplemental Materials

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