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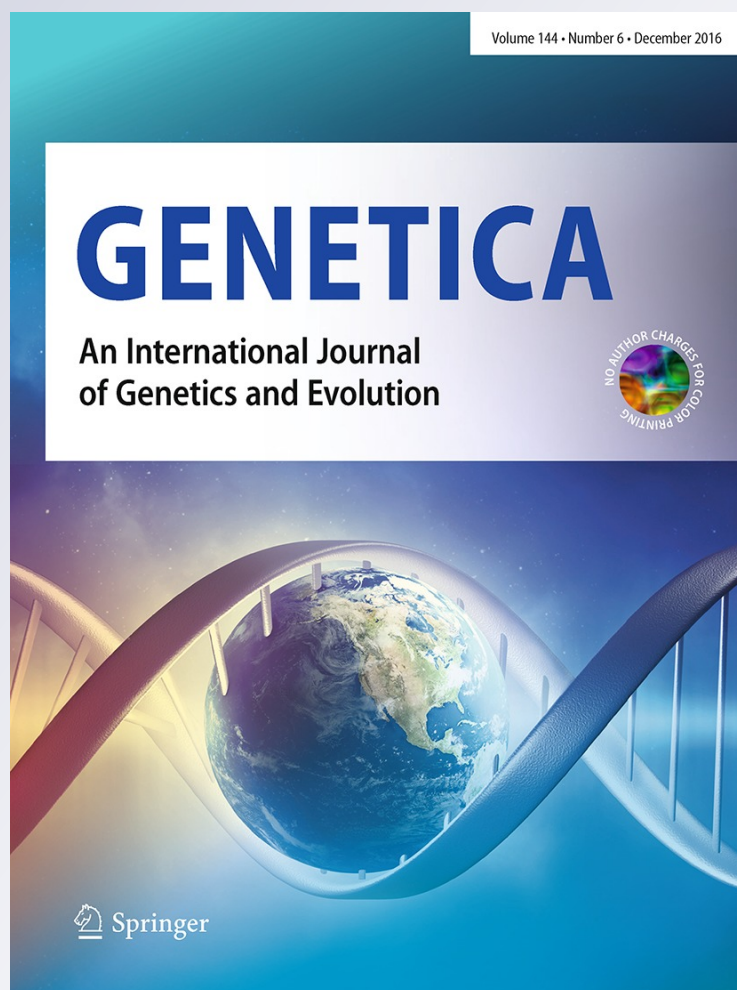
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Influence of environmental heterogeneity on the distribution and persistence of a subterranean rodent in a highly unstable landscape

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Abstract In this study we combine information from landscape characteristics, demographic inference and species distribution modelling to identify environmental factors that shape the genetic distribution of the fossorial rodent *Ctenomys*. We sequenced the mtDNA control region and amplified 12 microsatellites from 27 populations distributed across the Iberá wetland ecosystem. Hierarchical Bayesian modelling was used to construct phylogenies and estimate divergence times. We developed species distribution models to determine what climatic variables and soil parameters predicted species presence by comparing the current to the historic and predicted future distribution of the species. Finally, we explore the impact of environmental variables on the genetic structure of *Ctenomys* based on current and past species distributions. The variables that consistently correlated with the predicted distribution of the species and explained the observed genetic differentiation among populations included the distribution

of well-drained sandy soils and temperature seasonality. A core region of stable suitable habitat was identified from the Last Interglacial, which is projected to remain stable into the future. This region is also the most genetically diverse and is currently under strong anthropogenic pressure. Results reveal complex demographic dynamics, which have been in constant change in both time and space, and are likely linked to the evolution of the Paraná River. We suggest that any alteration of soil properties (climatic or anthropic) may significantly impact the availability of suitable habitat and consequently the ability of individuals to disperse. The protection of this core stable habitat is of prime importance given the increasing levels of human disturbance across this wetland system and the threat of climate change.

Keywords *Ctenomys* · Habitat fragmentation · Iberá Wetland · Metapopulation · Population genetics · Species distribution modelling

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Introduction

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

A landscape consists of a mosaic of patches of suitable habitat characterized by different environmental features within a matrix of unsuitable habitat (Fahrig and Merriam 1985). The ability of an animal to use resources

within a landscape patch is determined not only by the distance between patches but also by the nature of the routes connecting them (Taylor et al. 1993). Consequently, the degree of isolation between subpopulations depends on the actual capacity of movement of the species in relation to the landscape heterogeneity (Bowne and Bowers 2004; Anderson et al. 2015).

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

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

Subterranean rodents of the genus *Ctenomys* have specific habitat requirements and restricted dispersal capacity, promoting the establishment of a strong genetic structure, which can result in spatial patterns where geographically close populations become genetically distinct

(Reig and Kiblicky 1969; Reig et al. 1990; Wlasiuk et al. 2003, Kittlein and Gaggiotti 2008; Fernández et al. 2012). Populations of *Ctenomys* living around the Iberá Wetlands belong to the *torquatus* species group, which has been extensively studied during the past few years (Giménez et al. 2002; Mirol et al. 2010; Fernández et al. 2012). Although three nominal species (*C. roigi*, *C. perrensis* and *C. dorbinyi*) had been previously described inhabiting this area, a recent study based on microsatellite data has found that all the populations are part of a larger metapopulation of six distinct evolutionary lineages with different degrees of genetic isolation (Fig. 1; Fernández et al. 2012). Although these lineages are currently described as discrete genetic clusters, their future structure will depend on the temporal dynamics of their unstable habitat; i.e., these lineages may become distinct species or continue to evolve as a single metapopulation.

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

Materials and methods

Sampling and genetic data

The sampling of *Ctenomys* populations covered 27 localities throughout Corrientes Province, and denotes a representative sample of sites with known favourable habitat characteristics for this group in the area. Genetic data used in this study consisted of 354 individuals genotyped for 12 microsatellite loci (Lacey et al. 1999; Lacey 2001), of which 195 were also sequenced for a DNA fragment of 374 bp of the mitochondrial (mtDNA) control region. These data were generated by our lab and previously

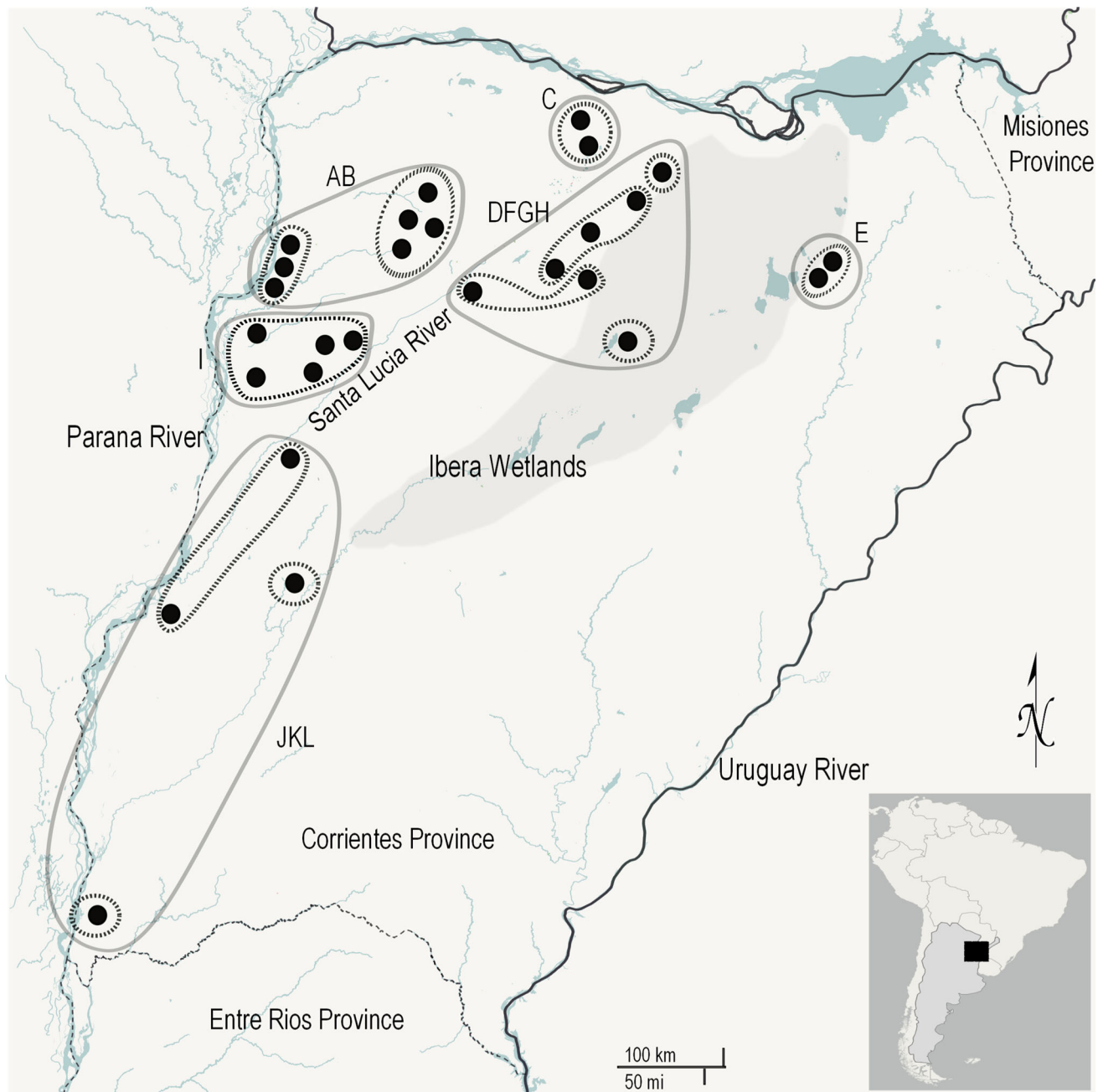


Fig. 1 Map of Corrientes Province showing the sampled localities of *Ctenomys*. The 12 genetic clusters defined for the *Ctenomys* group are shown by *black broken lines* and the 6 lineages by *grey solid lines*. Adapted from Fernandez et al. (2012)

reported in Fernández et al. (2012) (Fig. 1; Table 1, JQ686014–JQ686050) and Mirol et al. (2010) (Fig. 1; Table 1), except for three new sequences from sampling site Ea. Tacuaritas, which did not render any new haplotype. We also obtained 41 additional control region sequences and 67 cytochrome b sequences (426 bp) of related species from Genbank (species and Genbank accession numbers are reported as Supplementary Information, Table S1 and in

Giménez et al. (2002) Genbank: AF500038–AF500070). This species group of *Ctenomys*, identified as the Corrientes's group, has been the subject of previous studies (Giménez et al. 2002; Mirol et al. 2010; Fernández et al. 2012) where historical and recent gene flow has been found between genetically independent groups. Therefore, the Corrientes group can be considered a metapopulation of multiple groups interacting through gene flow.

Species tree

We used the hierarchical Bayesian model implemented in *BEAST v. 1.7.1 (Heled and Drummond 2010) to test species trees for the six lineages delimited in Fernández et al. (2012) belonging to the Corrientes group, along with the other five species of the torquatus group, using both mtDNA fragments (236 control region and 67 cytochrome b sequences). BEAST estimates a multispecies coalescent based on multiple gene trees embedded in a shared species tree, along with the effective population size of both extant and ancestral species (Heled and Drummond 2010). For gene tree inferences, we used JMODELTEST (Posada 2008) to select the nucleotide substitution model that best fitted the data and a Yule speciation process. With the species tree we also estimated the dates of the appearance of *Ctenomys* in the Province of Corrientes and its separation from the rest of the torquatus group. We used two mutation rates previously estimated for the *Ctenomys* mtDNA control region: 4.42% (Mora et al. 2013) and 2.96% (Roratto et al. 2015) per million years. The analysis was run twice, with 2.5×10^8 MCMC iterations, a sample size of 25×10^3 and with the first 20% excluded as burn-in. Both runs were combined into a single chain with the program LOG COMBINER 1.7.1 (Drummond and

Rambaut 2007). Convergence was evaluated using the program TRACER (Rambaut and Drummond 2007) and the cladogram was created from the posterior distribution of species trees with Fig Tree v 1.4.0 (Rambaut 2012).

Impact of environmental factors on genetic structure and genetic diversity

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

Using environmental data for each sampling site, we performed the analysis according to two spatial scales: sample locality (SLoc) on one hand and the 12 genetic clusters (GC) inferred using TESS in Fernández et al. (2012) (see Fig. 1) on the other. The idea behind this two-

Table 1 Environmental factors used in the GESTE analysis

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 and -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)	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 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 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)

^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

level analysis was to evaluate what environmental variables influence the genetic structure of the *Ctenomys* group at different spatial scales. In the GC case, environmental values corresponding to each cluster were the average value across the sample localities included in each group. To verify the robustness of the estimated parameters we performed three independent runs. We used 10 pilot runs of 10^3 iterations, followed by an additional burn-in period of 5×10^6 iterations. The thinning interval was 50, and the sample size used for posterior distribution estimates was 6×10^4 .

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

Species distribution modelling

To determine which environmental factors best predicted the probability of presence of *Ctenomys*, SDMs were developed using maximum entropy in MaxENT v3.3.3 (Phillips et al. 2006) based on the current distribution of this species in Corrientes and Entre Ríos ($N = 54$). Details of the climatic variables and methods used to model the current, past and future distribution of *Ctenomys*, are found in Supplementary Data. Multivariate Environmental Similarity Surfaces (MESS) analysis was undertaken to determine whether climatic conditions in the predicted range of the past and future differed significantly from those in the current species distribution (Elith et al. 2011).

Niche stability

In order to assess whether the current genetic structure is better explained by past or current climatic conditions, we performed a General Linear Model within GESTE using three explanatory variables: (1) the probability of *Ctenomys* presence during the Last Glacial Maximum (LGM) approximately 21,000 years ago; (2) the probability of current presence (Current) and (3) the niche stability (N_S) since the Last Inter-Glacial (LIG). N_S was estimated following Ortego et al. (2012) calculations as: (1) $1 - |\ln_{\text{CURRENT}} - N_{\text{LGM}}|$, where N_S is the niche stability between LGM-Current and (2) $1 - |\ln_{\text{LGM}} - N_{\text{LIG}}|$ where N_S is the niche stability between LIG-LGM. Since all three

variables are highly correlated, we performed a separate run for each of them and used the variance left unexplained by each model as the criterion to choose the best model (i.e., the lower the variance left unexplained, the better the fit).

Results

Estimation of species tree

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

Impact of environmental factors on the genetic structure

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

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

In order to infer the importance of the effect of each variable, we used the estimates of the regression coefficients of the best model (Table 3b). Their sign indicates whether the factor increases or decreases with the genetic differentiation, and its absolute value shows the magnitude of the effect (Foll and Gaggiotti 2006). For both clustering levels, drainage (Dc) shows the highest absolute value and

Table 2 Sum of posterior probabilities of models that include a given factor. GESTE analyses included all 9 factors

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

Bold value indicates the factor with highest score

a positive relationship with genetic differentiation measured through local F_{ST} . This means that the most poorly drained localities revealed the highest differentiation with the rest of the metapopulation. For clustering level SLoc, the second and third most influential variables were RBd ($\alpha_2 = -0.91$) and D ($\alpha_4 = 0.57$), with a negative and positive relationship with genetic differentiation respectively. For clustering level GC two other factors included in the best model increased genetic differentiation, D ($\alpha_5 = 0.94$) and Ndvi ($\alpha_3 = 0.85$).

Species distribution modelling

All models had high predictive probability for *Ctenomys* presence and did not overfit the presence data. The SDM for the current distribution containing all variables at a resolution of 30 arc seconds (Fig. 2) had a model average

AUC > 0.90. Distribution models with variables used for past and future projections with current presence data all had model averages AUC > 0.85. The variables that contributed the most and positively, to the current SDM were soil characteristics, followed by a negative correlation with temperature seasonality and precipitation in the wettest month. Soil characteristics which positively predicted the probability of presence in the model, included soils with low flood risk, good drainage, and ‘Sandy’ and ‘Sand-Silt’ soil types. On the other hand, ‘Silt-Sand’ had a negative correlation with probability of presence. Lastly, soil types susceptible to erosion and those with low fertility in the upper layer were also important in the model. When the current distribution was modelled at the lower resolution, the SDM demonstrated lower probability of *Ctenomys* presence in the region, with the same three variables contributing most to the model (Fig. 2).

We included soil characteristics in the SDM’s projected during the LGM, the LIG and for the year 2060 (Fig. 3), in order to account for their potential influence on species distribution. A negative correlation with temperature seasonality, followed by precipitation in the wettest month, were the most important projected variables. MESS analysis showed that climatic conditions during the LGM had parallels with today’s conditions, with few areas in the core of the species current range predicted to have had conditions very different from the present day. However, the most suitable habitat for *Ctenomys* based on these climatic features showed a significant shift northward. MESS analyses indicate that temperature seasonality during the LIG, among other climatic variables, might have extended outside their present range of values. Predictions of habitat suitability for the LIG should thus be treated with caution as the model trained with present conditions included a

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	
Cluster level	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)
Genetic cluster		σ_2	0.693	(0.363; 1.43)
	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)

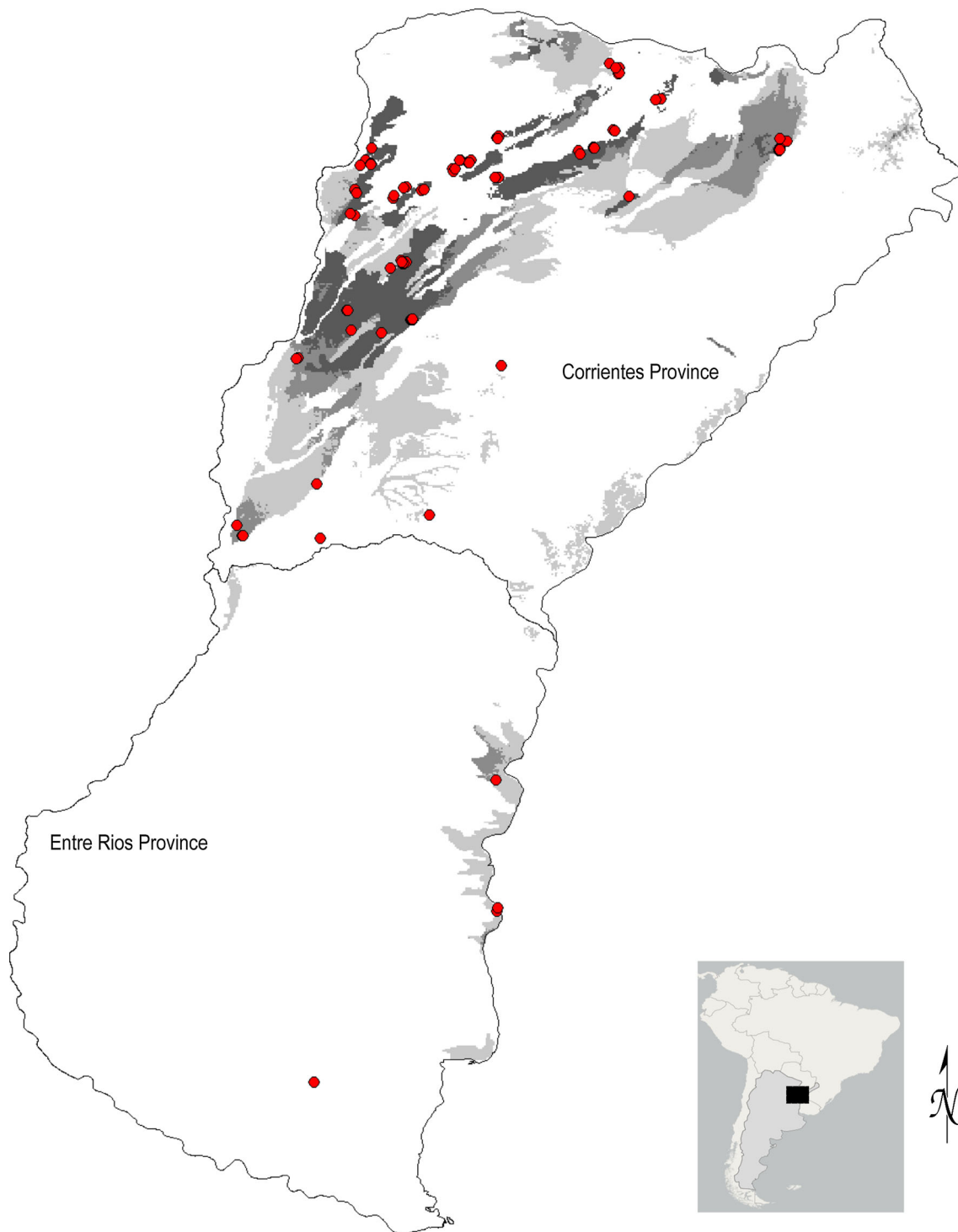


Fig. 2 Maps showing the probability of *Ctenomys* presence in Corrientes and Entre Ríos Provinces, Argentina, based on the location of the 54 presence records (red circles). Shading indicates logistic probability of presence and range from 0 to 1, with increasingly

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

narrower range of values (Elith et al. 2011). Similarly, MESS analysis of predicted habitat suitability for the year 2060 suggested that several key habitats for *Ctenomys* will experience a very different climate from that known today.

In particular areas to the north and west are predicted to experience maximum temperatures above those recorded today, and potentially higher than those estimated since the LGM.

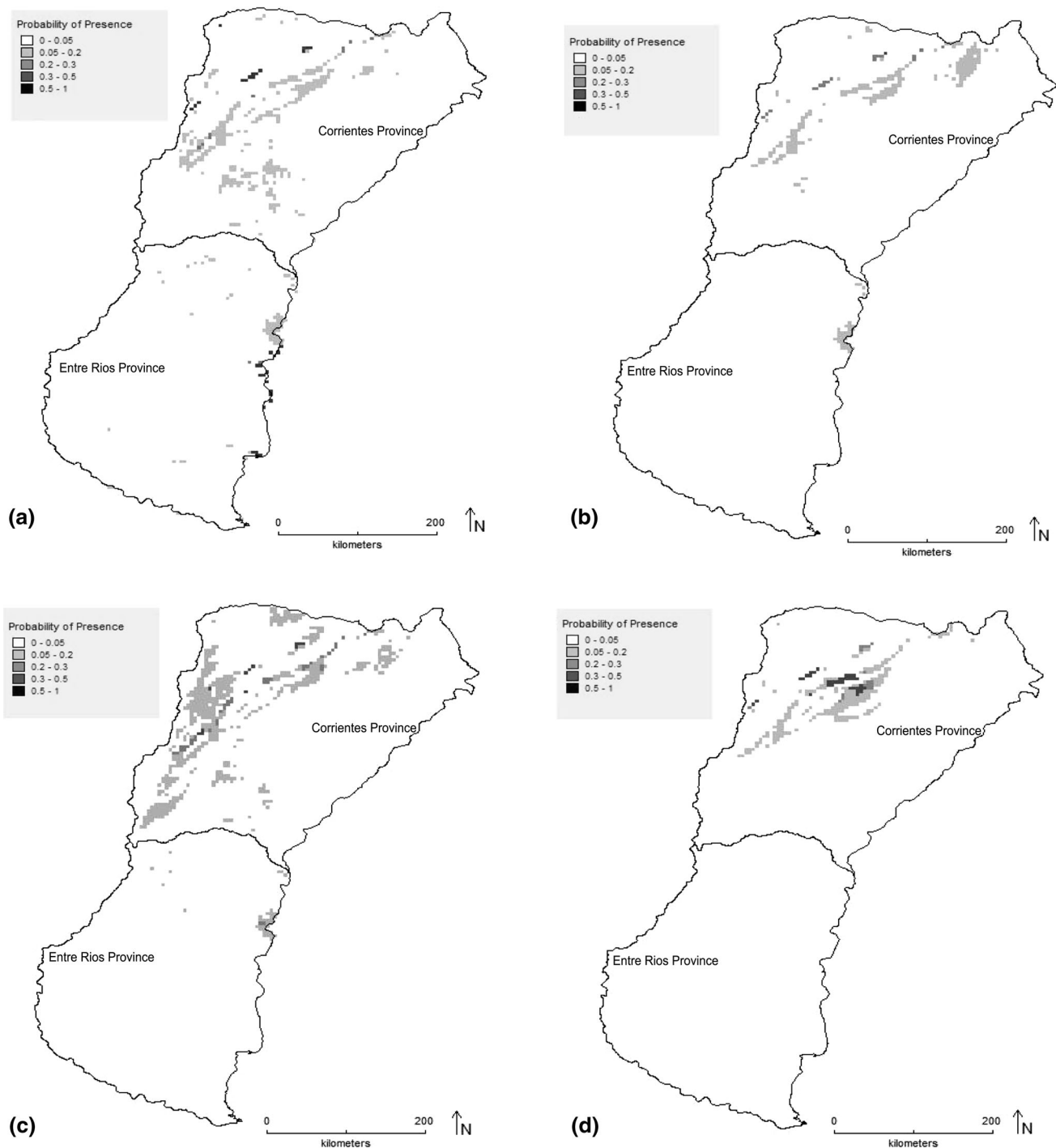


Fig. 3 Predicted and projected distribution of *Ctenomys* in Corrientes and Entre Ríos Provinces, Argentina, based on 7 Bioclim variables, altitude, various measures of soil type, and 54 presence records. **a** Projected distribution during the Last Inter-Glacial period (ca. 120,000–140,000 years BP); **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

Niche stability

Regarding the relative importance of recent and historical events on the genetic structure we found that for genetic

clusters (GC), the genetic structure is best explained by *Ctenomys* recent demographic events. First, the best model that includes the SDM probabilities of presence for the Current distribution results in $p = 1.00$ (regression

coefficients $\alpha_1 = -0.472$ 95%CI [-1.270; 0.299]; $\sigma_2 = 1.280$, 95%CI [0.540; 3.73]), whereas in the run with SDM probability of presence for the LGM distribution the model with the highest probability was the null model that did not include any variable. Second, when studying the possible influence of climatic stability we found that the model that best explains the genetic differentiation includes the niche stability obtained between the LGM and current conditions ($p = 0.674$; regression coefficients $\alpha_1 = 0.69$, 95%CI [0.145; 1.26]; $\sigma_2 = 1.06$, 95%CI [0.465; 2.73]), while for the niche stability between LGM-LIG the model with the highest probability was the null one. Comparing the regression parameters of both analyses (probabilities for Current distribution and niche stability LGM-Current), the model with niche stability had the lowest residual variance, and therefore, was the one that best explains genetic structure of the *Ctenomys* metapopulation (i.e., the higher the niche stability, the weaker the effect of genetic drift).

Discussion

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

We first explored what variables best explained the observed pattern of genetic differentiation among subpopulations at the local level and across the metapopulation as a whole. Of all the variables studied, two of them, distance between habitat patches and soil drainage ('Distance' and 'Drainage class') were most strongly associated with genetic structure at both spatial scales (locality and genetic cluster). Given the low mobility of *Ctenomys*, the strong positive association of distance with genetic structure is not surprising. In a previous study, Fernández et al. (2012) reported that the genetic structure was consistent with a model of isolation by distance only across short distances. This result is in agreement with a study on the Brazilian species *C. flamarioni* (Stolz 2006), which reported

movements of approximately 0.25 km per year in continuous habitats. In the case of the *Corrientes* group, where the landscape presents natural as well as rapidly increasing artificial discontinuities and thus less connectivity throughout the system as a whole, the movement of the individuals could be even more restricted. In our study, the shortest average distance between localities was approximately 78.02 km, which is approximately ~ 300 times longer than their natural annual dispersal distance based on estimates reported by (Stolz 2006).

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

When examining the impact of environmental variables on the metapopulation as a whole (i.e., on the 12 genetic clusters inferred in Fernández et al. 2012), in addition to 'Distance' and 'Drainage class', 'Vegetation index' (Ndvi) becomes important, showing a positive relationship with genetic structure. In a study on the landscape ecology of *Ctenomys porteousi*, Mapelli and Kittlein (2009) found four habitat variables explaining patch occupancy, which included habitat quality in the matrix surrounding the patch, average vegetation cover (Ndvi) in the patch, minimum vegetation cover in the matrix surrounding the patch,

and area of the nearest neighbor patch. Regarding N_{dvi}, Mapelli and Kittlein (2009) found that low values in the habitat patch as well as in the area surrounding habitat patches, corresponding to poorly vegetated soils, were positively correlated with occupancy. Our data suggests that N_{dvi}, besides its influence on occupancy, is also an important factor determining genetic structure as higher values of vegetation cover correlate with higher values of F_{ST} . Both results are expected given that vegetation cover favors soil development, and enlargement of the root system increases soil compactness, where burrowing becomes energetically more expensive (Mapelli and Kittlein 2009). The fact that we found N_{dvi} as a factor in the model explaining genetic structure only at the metapopulation level, probably indicates its influence not only on habitat patches but also on their surrounding areas.

We also assessed environmental factors that predicted the distribution of populations of the *Corrientes* group. The SDMs models demonstrated that the probability of *Ctenomys* presence was higher in areas with a small range in seasonal temperature and low levels of precipitation in the wettest month. However, in all SDMs, soil characteristics and particularly sandy soils with good drainage, clearly played a major role in accurately predicting species presence. Given the underground life style of these rodents, it is clear that subterranean burrows play an important role in their interactions with the environment. Burrowing requires 360–3400 times as much energy as moving the same distance across the surface (Jarvis and Bennet 1991), so the distribution of subterranean life will be strongly limited to habitats where tunnel excavation is energetically feasible (Busch et al. 2000).

Furthermore, the genetic differentiation among groups of *Ctenomys* found in Corrientes Province seems to be intimately related to the landscape resulting from the evolution of the Paraná River, from its beginning as a broad river corridor, up to its current position, movement that resulted in the formation of the Iberá Wetland as a large mosaic of lentic bodies. Historically, the Parana River flowed into the Paranaense Sea (14–5 Mya, Orfeo 2005), which covered a large portion of the Argentine territory including the Mesopotamia region (Corrientes, Entre Rios and Misiones Provinces). After the marine regressions in the Late Miocene (7–3 Mya, Orfeo 2005), the Parana River changed its course and began to pour its waters in north-eastern Argentina, initially at the boundary between Misiones and Corrientes towards the Uruguay River. Afterwards, it changed its course again flowing to the west and up to the north, resulting in its current geographic location (Castellanos 1959; Popolizio 2004). This shift occurred approximately between 0.78 and 0.13 Mya (Iriondo 1979; Popolizio 2004). According to the species tree, the appearance of the *Corrientes* group of *Ctenomys* occurred

within this time range, some 0.119–0.079 Mya. Therefore, the *Corrientes* group seems to have colonised this region from East to West, which also explains its phylogenetic inclusion in the torquatus group of species also present in Brazil and Uruguay.

Projection of the SDM's into the past suggests that the suitable habitat for *Ctenomys* since the Last Inter Glacial (140,000–120,000 years BP) has undergone expansions and contractions as expected with the dry and wet periods that have been observed in the Paleoclimatological record since the Late Pleistocene (Stevaux 2000), changing the suitability of the landscape for the species. Our model projection into the climatic environment of the LGM, some 21,000 years BP suggests a decrease in the proportion of suitable habitat at this time, with a slight range shift northward. According to palaeoclimatic analysis, following the LGM the climate in Argentina remained relatively cold and arid until the Holocene, with a particular cold snap during the Younger Dryas 10,000 years BP (Iriondo and García 1993). The final climate switch to the warmer and moister conditions of today, some 8000 years BP coincides with the broader range observed in the current SDM (Iriondo and Garcia 1993).

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

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

The more stable, diverse, and genetically structured area comprised between the Santa Lucía River and the Iberá marsh is currently occupied mainly by one (DEFG) of the six main lineages described in Fernández et al. (2012). This evolutionary lineage is highly variable, with the highest number of microsatellites alleles and mitochondrial haplotypes, as it would be expected if this lineage originated

from a Late Quaternary refugia (Carnaval et al. 2009). Furthermore, migration assessment (see Fernández et al. 2012) suggests that there was a fluid but low movement between *Ctenomys* lineages in the past, but more recently migration seem to have ceased. The current metapopulation dynamics seems to be driven by changes in habitat suitability throughout this region, with asymmetric migration occurring across environmentally stable habitats. Collectively, these results allow to characterize this region as a hotspot of species distribution in Corrientes, which acted as a potential refuge for the species during climate extremes. Unfortunately, this area is currently under strong commercial forestation, which makes efforts for conservation even more urgent, as this region faces future climate and anthropogenic changes.

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

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Data archiving For Mitochondrial DNA sequences and microsatellite data were see Fernández et al. (2012).

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