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Original Article

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Phylogeography and ecological niche modelling of the South American cricetid rodent *Graomys griseoflavus*, with insights into its chromosomal evolution

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ABSTRACT

The rodent *Graomys griseoflavus* has a wide geographical distribution in the Arid Diagonal of South America, showing variation in the diploid number ($2n = 33–38$) caused by three different Robertsonian (Rb) translocations. Two contrasting hypotheses have been proposed to explain the origin and evolution of this polymorphism: (i) unique and sequential events; or (ii) multiple and independent origins of Rb translocations. Here, we combined phylogeography and ecological niche modelling to elucidate the evolutionary history of *G. griseoflavus* and help to contrast these hypotheses. The results indicated a demographic increase that would have begun ~150 kya from the High Monte ecoregion. The spatial distributions of these populations were coincident with the areas of highest habitat suitability. The palaeoclimatic projections suggested the presence of two main glacial refugia coincident with the main genetic clusters. *Graomys griseoflavus* underwent two pulses of southward expansion at ~220 and ~210 kya. More recently (~20 kya), there was a secondary contact between the southern populations (Low Monte ecoregion) expanding northwards and the northern populations (High Monte ecoregion) expanding into marginal areas of the Chaco ecoregion. Combined with the cytogenetic evidence, our results support the multiple and independent origins of Rb translocations.

Keywords: Argentina; chromosome evolution; demographic history; Phyllotini; mitochondrial DNA; Robertsonian polymorphism; South American Arid Diagonal

INTRODUCTION

Differences in the number of chromosomes are frequently associated with taxonomic distinction at the species level. This has been described particularly among species of the order Rodentia, in which karyotypic divergence plays a causal role as part of species differentiation in addition to being an adaptive component in phylogenetic evolution (Patton and Sherwood 1983). However, chromosomal rearrangements can persist as a polymorphism if their effect on fitness is neutral or mildly under-dominant and if the meiotic cost (errors during meiosis leading to chromosomal abnormalities) is compensated for by a selective advantage (Rieseberg 2001, Dumas and Britton-Davidian 2002, Dobigny *et al.* 2017).

Among South American cricetid rodents, there are several examples of species that exhibit karyomorphic polymorphism (Nachman and Myers 1989, Nachman 1992a, b, Theiler and Gardenal 1994, Zambelli *et al.* 2004, Lanzone *et al.* 2007, 2011, 2014, Swier *et al.* 2009, Ventura *et al.* 2009, Armella *et al.* 2017). For instance, *Graomys griseoflavus* (Waterhouse, 1837) is a medium-sized phyllotine rodent widely distributed in Argentina

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(from about the Tropic of Capricorn to 50°S; Braun and Patton 2015), largely found in semi-arid environments, with a remarkable Robertsonian (Rb) autosomal polymorphism (Theiler and Gardenal 1994, Zambelli *et al.* 2004, Ferro and Martínez 2009, Martínez and Di Cola 2011, Lanzone *et al.* 2014). These chromosomal rearrangements imply centric chromosomal fusions resulting in a reduction in the diploid number (Robertson 1916). *Graomys griseoflavus* consists of cytotypes with $2n = 33$, 34, 35, 36, 37, and 38. Zambelli *et al.* (1994) proposed that the different cytotypes were generated by Rb rearrangements of an ancestral cytotype $2n = 42$ (now belonging to a different species, *Graomys chacoensis* Allen, 1901), from which the remaining cytotypes were derived sequentially. These different karyomorphs were considered by Catanesi *et al.* (2002) as an indicator of different species. However, several studies on cytogenetics, reproductive behaviour, and gonadal histology derived from inter-cytotype crosses demonstrated reproductive isolation between specimens with $2n = 42$ and the remaining kariomorphs, $2n = 36$–38, but not within this latter group of cytotypes (Theiler and Blanco 1996, Theiler *et al.* 1999). Later, additional analysis, including information on skull morphometrics and genetic divergences, supported the specific status for populations $2n = 42$ and $2n = 36$–38, respectively. The taxonomic arrangement accepted nowadays is that $2n = 42$ represents *G. chacoensis*, distributed mainly in the chaco ecoregion, whereas the remaining cytotypes belong to *G. griseoflavus*, distributed mainly in the Monte Desert ecoregion (Tiranti 1998, Ferro and Martínez 2009, Martínez *et al.* 2010, 2022, Martínez and Di Cola 2011, Braun and Patton 2015, Martínez and Gardenal 2016).

Regarding the chromosomal evolution, Zambelli *et al.* (2004) proposed a unique and sequential origin for the Rb cytotypes present in *G. griseoflavus*, derived from the occurrence of a founder effect (i.e. genetic drift) during chromosomal differentiation. However, this hypothesis was refuted, in part, based on additional cytogenetic evidence (Lanzone *et al.* 2014, De Cena *et al.* 2023). Also, several authors have highlighted that the high levels of isozyme heterozygosity, the high nucleotide diversity, and similar haplotype diversity compared with *G. chacoensis* reveal the absence of significant bottlenecks in the speciation of *G. griseoflavus* (Theiler and Gardenal 1994, Theiler *et al.* 1999, Martínez *et al.* 2010, 2022, Martínez and Gardenal 2016). Moreover, Lanzone *et al.* (2014) found that the generation and/or maintenance of a new chromosomal fusion is not necessarily preceded by fixation of the others, and they proposed the hypothesis that chromosomal evolution in *G. griseoflavus* occurred through multiple and independent centric fusions.

Here, we evaluate the above-mentioned hypotheses in light of a combined analysis of phylogeography and ecological niche modelling. Phylogeography allows us to make inferences about selection, population genetic structure, and demographic changes based on neutrality tests and coalescence. This approach helps to clarify the evolutionary and geographical determinants of genetic variation, in addition to the relationships between genetic structure and cytogenetic differentiation (Avise 2009, Poplavskaya *et al.* 2019). Furthermore, ecological niche modelling allows us to project potential present and past geographical distributions based on the relationship between a set of environmental variables, confirmed current occurrence data, and palaeoclimatic scenarios (Phillips *et al.* 2006, Pearson 2010, Peterson *et al.* 2011, Zurell *et al.* 2020). Thus, we combine these approaches to evaluate the demographic and spatial consequences of the origin and maintenance of Rb polymorphism in *G. griseoflavus*, providing insights into past range dynamics and historical demography associated with diversification of mitochondrial DNA variants.

MATERIALS AND METHODS

Studied samples

We analysed 99 sequences of the mitochondrial gene cytochrome *b* (*Cytb*), covering most of the geographical range of *G. griseoflavus* (Fig. 1). Of these, 79 were downloaded from GenBank, and 20 were newly produced sequences covering previously unrepresented areas (see Supporting Information, Table S1). Our database included sequences from 44 localities extending for 20° of latitude (from 25 to 45°S), covering ~2300 km, the five ecoregions where the species is present, and an elevational variation from sea level to 2650 m a.s.l. Importantly, our dataset includes specimens that can be considered topotypes of two nominal forms. These are *Mus* (*Phyllotis*) *griseo-flavus* Waterhouse, 1837, with type locality restricted to the mouth of the Río Negro (see Hershkovitz 1962: 453), with the type specimen being an animal collected in El Espigón, 29 km S Balneario El Cóndor, and *Phyllotis cachinus* Allen, 1901, with type locality at 'Upper Cachi River, Salta Province, Argentina' (Allen 1901: 409), which is represented in our study by a specimen collected from Escuela Nevado de Cachi, 30 km northeast of Cachi. More detailed information about sampling localities is provided in the Supporting Information (Table S1; see also Fig. 1).

DNA extraction and sequencing

We extracted total genomic DNA using the standard salt extraction method described for ethanol-preserved tissues (Bruford *et al.* 1992). The DNA was then precipitated in absolute ethanol, dried, and stored in sterile water. We amplified the mitochondrial *Cytb* sequences using the primers Mus 14095 (5′-GACATGA AAAATCATCGTTGTAATTC-3′), Mus 15398 (5′-GAATATC AGCTTTGGGTGTTGRTG-3′) (Anderson and Yates 2000), and GoTaq Colorless Master Mix (Promega), following the cycling protocol outlined by Ferro and Martínez (2009). We purified and sequenced the PCR products at Macrogen Korea (http://dna.macrogen.com). Then, we performed a multiple alignment of the sequence matrix using the program MUSCLE (Edgard 2004) with default parameters, available online at the European Bioinformatics Institute (https://www.ebi.ac.uk). Then, we checked the alignment manually and trimmed it to a common length. The newly generated sequences were deposited in GenBank with the accession numbers OP83947–OP83966 (Supporting Information, Table S1). The alignment is available as Supporting Information (File S1).

Phylogeography: spatial diffusion analyses

We assessed the spatial dynamics of populations through time using a lognormal relaxed random walk diffusion model implemented in BEAST v.1.10.4 (Drummond and Rambaut 2007). To perform this analysis, we included all sequences ($N = 99$) and their geographical coordinates. We used a normally distributed diffusion rate, a coalescent Bayesian skyride model (BSR),

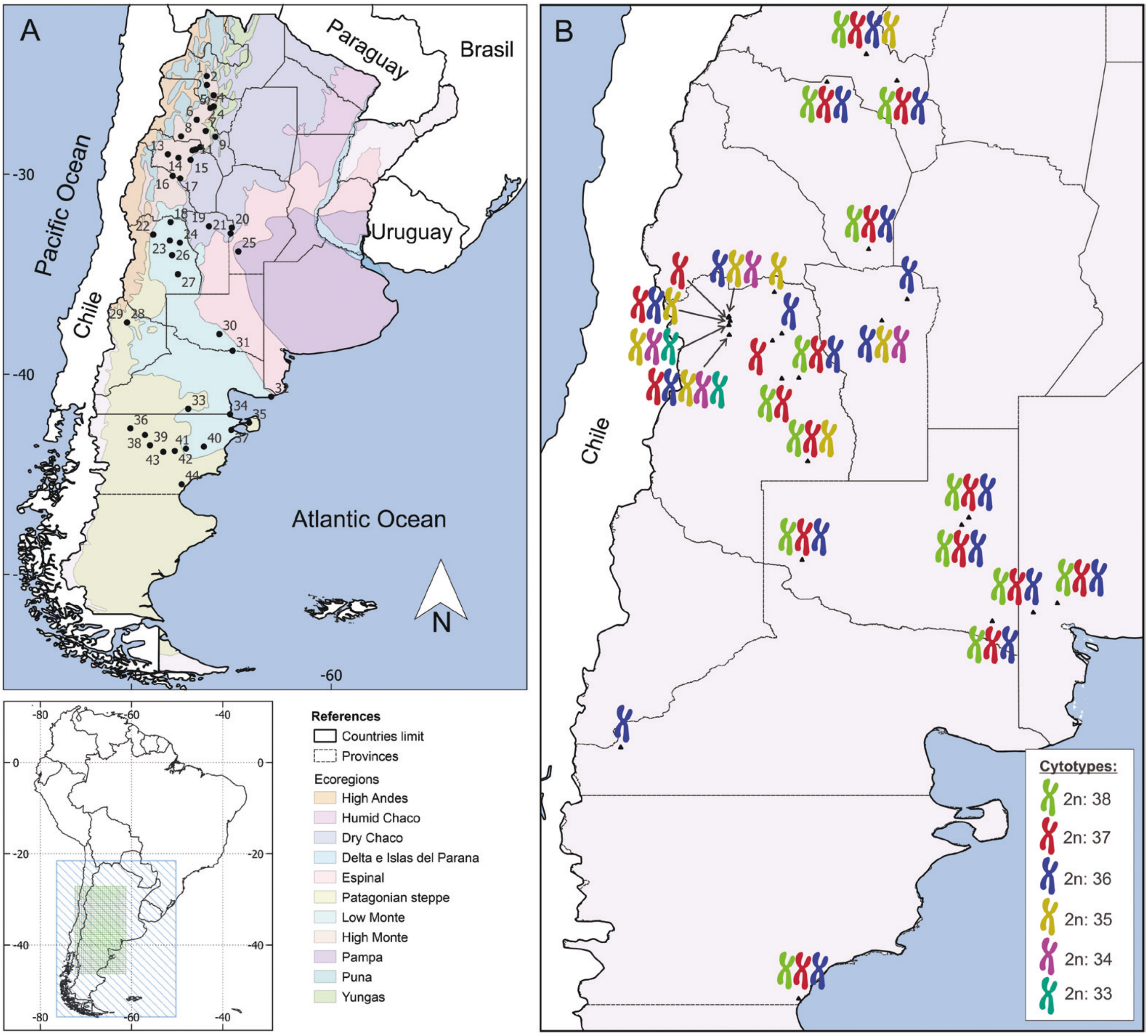

Figure 1. A, map of the study area and sampling location of *Graomys griseoflavus* across its entire geographical range. B, the distribution of the karyotypes ($2n = 33–38$) described in previous studies. The current ecoregions of Argentina are indicated. Additional information on each sampled locality can be found in the Supporting Information, Appendix 1.

and the HKY+I+G substitution model, according to the model selection analysis following the Akaike information criterion in jModeltest v.2.1.3 (Darriba *et al.* 2012). We included the parameters of this model in BEAST v.1.10.4 and used a substitution rate of 2.38%/Myr, which was selected according to the rate of divergence obtained for the tribe Phyllotini (Smith and Patton 1999, Martínez and Gardenal 2016). We set the jitter option to .01 to add variation to sequences with the same coordinates (Dellicour *et al.* 2021). A total of 800 million generations were run to reach convergence and sampled every 80 000 generations. We inspected the stationarity parameters with Tracer v.1.7. To summarize the posterior distribution of ancestral ranges using the relaxed random walk model, we annotated nodes in a maximum clade credibility tree using the program TreeAnnotator v.1.7.5. Finally, we used this tree as input for the software SpreaD3 v.0.9.7 (Bielejec *et al.* 2016) to reconstruct and visualize the pattern of spatial diffusion through the geographical range of the species.

Haplotype network and molecular diversity patterns

To estimate the genetic structure of *G. griseoflavus* populations across its geographical range, we grouped populations as north, central, and south (N, C, and S, according to the first three migrations identified in spatial diffusion analyses; see below), and by ecoregions (Martínez and Gardenal 2016), and calculated nucleotide and haplotype diversity indices using DnaSP v.5 (Librado and Rozas 2009). Using the minimum spanning network algorithm of PopART v.1.7 (Bandelt *et al.* 1999), we constructed the haplotype network according to the three groups (N, C, and S) and by ecoregions. Additionally, we conducted a hierarchical analysis of molecular variance (AMOVA) (Excoffier *et al.* 1992) to estimate the genetic differentiation between the three groups and by ecoregions. The assessment of genetic divergence within and between groups could be a useful approach to understand chromosomal variation in this species. To estimate genetic distances, we used the Kimura two-parameter model (Kimura 1980), implemented in MEGA X (Kumar *et al.* 2018), taking into account both latitudinal groups and ecoregions. Standard errors were calculated using the bootstrap method with 1000 replicates.

Demographic history analysis

To test for demographic changes, we conducted Tajima's *D* and Fu's F_S (Tajima 1989, Fu 1997) neutrality tests. Both tests assume that populations have been in mutation–drift balance for long periods of time. If this assumption is false (e.g. owing to sudden expansion), these indices retrieve negative values. We also carried out mismatch distribution analyses of nucleotide pairwise differences among individuals. We performed the *D* and F_S neutrality tests and analysed the mismatch distribution using the software DnaSP v.5 (Fu 1997, Librado and Rozas 2009). To complement these analyses, we conducted a Bayesian skyline plot analysis in BEAST v.1.10.4 (Drummond and Rambaut 2007) to infer past population demographics and estimate the timing of demographic events. We ran the analysis for 30 million generations using the substitution model HKY+G+I, with a substitution rate of 2.38%/Myr (Smith and Patton 1999, Martínez and Gardenal 2016). Finally, we assessed chain convergence, effective sample size, and confidence intervals for each parameter, and we reconstructed the Bayesian skyline plot using Tracer v.1.7 (Rambaut and Drummond 2003).

Current and palaeodistribution modelling

We built ecological niche models (ENMs) to predict the potential distribution of the species under different past and present climatic scenarios, given a set of environmental variables and localities of known presence. We obtained 104 reliable presence localities for *G. griseoflavus* from our own captures during different field trips and from specimens housed at Colección Nacional de Mastozoología, Museo Argentino de Ciencias Naturales 'Bernardino Rivadavia', Ciudad Autónoma de Buenos Aires, Argentina, and Colección Mamíferos Lillo at Facultad de Ciencias Naturales Universidad Nacional de Tucumán, in Tucumán, Argentina. We also included some additional records from the literature and from the global biodiversity information facility, GBIF (www.gbif.org; Supporting Information, Table S2).

We built a convex hull polygon with all verified presence records for the species in QGIS v.3.22.6 (http://www.qgis.org/). Then we filtered the GBIF dataset, keeping only those occurrences that did not exceed the range limits of the created polygon. Finally, we filtered this record temporally according to the time period covered by the bioclimatic layers. To reduce potential spatial autocorrelation, we eliminate all occurrence locations within a distance of 10 km from each other with the '*spThin*' R package (Aiello-Lammens *et al.* 2015) . We performed ENMs using the software MaxEnt 3.3.3k (Phillips *et al.* 2006) through the '*dismo*' R package (Hijmans *et al.* 2017) in R v.4.0 software (R Core Team). The background points were set to 50 000, with 20% of the occurrence points for testing and 80% for replicate training.

As predictors, we used topographic and bioclimatic variables (Supporting Information, Table S3 and Table S4). The topographic variables were the elevation and slope obtained from the WorldClim digital elevation model (Hijmans *et al.* 2005). We built the slope variable using the GDAL module in the software QGIS v.3.22.4 (QGIS Core Team). We used the 19 bioclimatic variables for the present, Last Glacial Maximum (LGM; 25 kya), and Last Interglacial (LIG; 130–114 kya) periods downloaded from WorldClim v.2.1 with a resolution of 2.5 arc minutes (5 km^2) (https://www.worldclim.org/data/worldclim21.html) (Hijmans *et al.* 2005). Following Barve *et al.* (2011), we cropped all bioclimatic layers to span the distribution of the three species of *Graomys* with widest known distribution [i.e. *Graomys domorum* (Thomas, 1902), *G. griseoflavus* and *G. chacoensis*] and the respective ecoregions in which each of them is probably present [High Monte (HM), Low Monte (LM), Patagonian Steppe, Pampa, Dry Chaco, and Yungas forest]. To evaluate possible multicollinearity among the topographic and bioclimatic variables we performed a variance inflation factor (VIF) analysis (Zuur *et al.* 2010) and progressively eliminated variables until the VIF value was <10 (Pearson's *r* <.5). We performed this analysis with the function vif.cca of the 'vegan' R package (Oksanen *et al.* 2019).

To assess the performance of the model, we analysed the area under the receiver operating characteristic curve and the true skill statistic. The area under the curve was calculated in MaxEnt, based on the presence points and pseudo-absence generated by this algorithm. To calculate the true skill statistic, we converted the continuous values of the distribution model to a binary variable that represents the presence or absence of a suitable environment, then we evaluated how well presence and absence were predicted by the model (Allouche *et al.* 2006). Indices analysed in the external validation were based on the 10% threshold, following Dalapicolla and Leite (2018). The model was projected for each scenario (present, LGM, and LIG) to evaluate whether there was variation in the suitability of the area over time.

Spatial distribution of the chromosomal polymorphism

To test the two hypotheses about the origin of the Rb variants among populations of *G. griseoflavus*, we conducted an exhaustive bibliographical search of published evidence on the location of every karyotype polymorphism. We then compared our findings on the demographic and spatial history of this species with the results of these studies, which were carried out in various locations across the species distribution. These studies, including those by Rodriguez and Theiler (2007) and De Cena *et al.* (2023), provided valuable information on the distribution of these karyotypes, which is essential for our study. By contrasting this information with the demographic patterns resulting from our phylogeographical analysis, we were able to gain further insights into the origin of the Rb variants.

RESULTS

Phylogeography

After trimming the *Cytb* aligned sequence matrix to the same length, we obtained a dataset of 753 bp, with 93 polymorphic sites, and a nucleotide diversity of .00688; the mean number of differences was 5.157. For the 99 individual sequences, we found 66 haplotypes with a high haplotype diversity (.986). The molecular diversity indices for each group and ecoregion are detailed in Table 1.

The spatial diffusion analysis revealed that *G. griseoflavus* most probably originated from what is now southern La Rioja province, as shown in Figure 2, ~290 kya. The species then expanded both northwards (275 kya) and southwards, eventually reaching La Pampa province at ~260 kya (Fig. 2A). Additionally, it is possible that the species also reached most of the central western part of the Buenos Aires province during the same time period. The southward expansion of *G. griseoflavus* continued, reaching the Patagonian steppe of Chubut and Santa Cruz provinces at ~200 kya (Fig. 2B). Subsequently, ~160 kya, the population in La Rioja Province expanded again, both southwards and northwards, reaching Mendoza and Catamarca provinces (Fig. 2C). Shortly after 80 kya, populations in La Pampa province also expanded to reach Mendoza province. From that time, a generalized drastic demographic expansion occurred, and the species spread sequentially to all provinces where it is recorded nowadays, including the marginal areas of the southern Chaco and the Espinal ecoregions: Tucumán and Salta provinces (140 kya), San Luis province (136 kya), Neuquén province (23 kya), and finally, Córdoba (10 kya) and Buenos Aires provinces. Finally, within the last 25 kya, mixing events occurred among the north-central populations (in Mendoza, La Rioja, Catamarca, Salta, and Tucumán provinces) and the southern populations in Chubut and Santa Cruz provinces (Fig. 2D), indicating a secondary contact event between HM and LM ecoregion populations.

In summary, the history of geographical distribution for *G. griseoflavus* can be clustered into three main groups: the northern populations (including the possible ancestral distribution area) corresponding to the HM ecoregion; the central populations, including the provinces of San Luis and Mendoza in the HM ecoregion and surrounding areas such as the Chaco and Espinal ecoregions; and the southern populations, which include the Argentine Patagonia populations and correspond to the LM and surrounding ecoregions, such as the Patagonian Steppe.

Genetic structure and molecular diversity patterns

The haplotype network analysis revealed a star-like pattern centred mainly around haplotypes Hap 51 and Hap 63 distributed across both the northern and southern areas (Fig. 3). The haplotypes of the southern group were distributed into two sections of the network and connected to Hap 51 and Hap 63 of the northern group (Fig. 3). The network exhibited a dispersal pattern from the northern group towards the south and central groups. Considering the distribution of haplotypes in the ecoregions, both central haplotypes of the star-like network, Hap 51 and Hap 63, were present in the HM ecoregion and in the LM and Patagonian Steppe, respectively. The Patagonian Steppe haplotypes were distally located in the network and connected to haplotypes in the HM and LM ecoregions by a few mutational steps, except for the haplotypes Hap 45 and Hap 1, which differed by seven mutational steps between them (Supporting Information, Fig. S1). All ecoregions had more than one haplotype dispersed in the network; this network also shows a dispersal pattern from the HM ecoregion towards the LM and then to the peripheral ecoregions (Supporting Information, Fig. S1). The nucleotide diversity was higher in the northern group and in the Monte ecoregion (.00715 and .00712, respectively) than in the southern group or in the southern ecoregions (.00584, .00605, and .00620 for the South, Patagonian Steppe, and Espinal, respectively).

The AMOVA performed for localities grouped into three geographical regions (southern, central, and northern) showed significant apportionment of genetic variance among regional groups (the genetic differentiation among groups, as measured by Φ_{ST}, was found to be .33). The among-group component of variance was 6.49%. The variation within populations was 26.56%, and it was 66.95% among populations within groups (Table 2). The AMOVA performed for ecoregions also showed significant variation among ecoregions, with 4.79% of variation

Table 1. Genetic diversity indices and neutrality tests for demographic analyses in the *Graomys griseoflavus Cytb* dataset according to dispersal groups (spatial diffusion analysis) and ecoregions. Abbreviations: *h*, haplotype diversity; *k*, number of haplotypes; *N*, number of sequences; NA, not applicable; π, nucleotide diversity; *p*, mean number of pairwise differences; *S*, polymorphic sites.

Groups	***n***	***S***	**π**	***p***	***k***	***h***	**Tajima's *D***	**Fu's F_S**
Species dataset								
	99	93	.00688	5.157	66	.986	−2.41685**	−4.93947**
Geographical distribution								
North	48	54	.00715	5.386	31	.971	−2.02960*	−3.06019*
Central	20	26	.00598	4.505	17	.979	−1.49538	−1.95120
South	31	39	.00584	4.378	22	.953	−2.01689*	3.44524*
Ecoregion								
High Monte	46	54	.00712	5.359	31	.973	−2.06278*	−3.02114*
Low Monte	32	36	.00607	4.558	25	.972	−1.77351	−3.10350*
Patagonian Steppe	13	21	.00605	4.538	10	.923	−1.41376	−2.15412
Espinal	3	7	.00620	4.667	2	.667	NA	
Dry Chaco	5	8	.00505	3.800	4	.900	−0.07339	−0.07686

$^{*}P < .05$, $^{**}P < .01$.

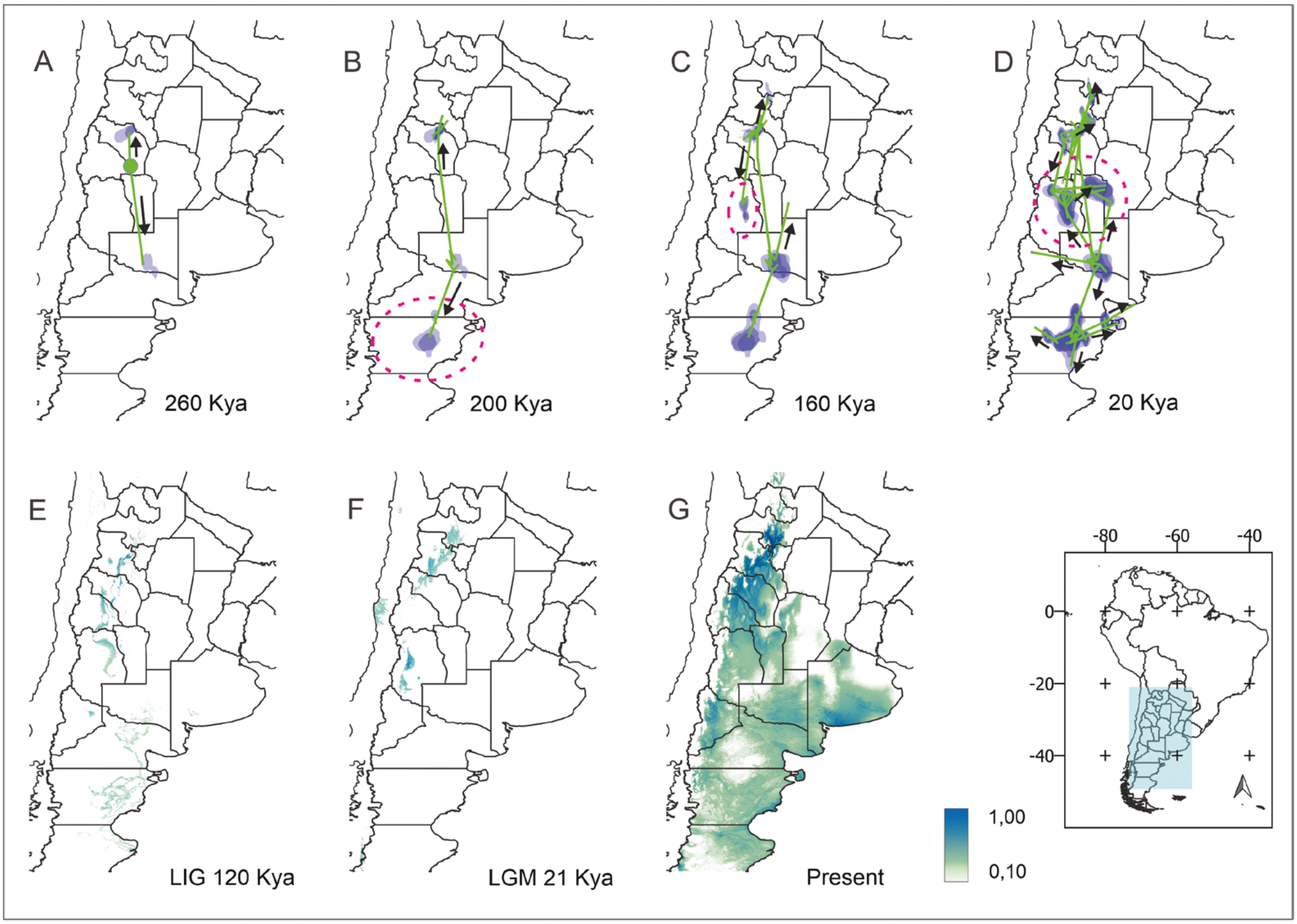

Figure 2. Bayesian spatiotemporal diffusion analysis of *Graomys griseoflavus* and ecological niche modelling projections. A–D, the spatial projections of the diffusion pattern over time in four time slices: 260 (A), 200 (B), 160 (C), and 20 kya (D) before present. The pattern is based on the maximum clade credibility tree derived from a Bayesian phylogeographical analysis conducted in the program BEAST. The green lines represent the branches of the maximum clade credibility tree, and the blue areas indicate the 80% highest posterior density uncertainty in the location of ancestral branches. The light-to-dark gradient reflects the progression of diffusion events from older to most recent, with the arrows indicating the trajectory of the events. E–G, the probability areas indicating the species occurrence under three climatic scenarios: Last Interglacial (LIG; 120 kya; E), Last Glacial Maximum (LGM; 21 kya; F), and current conditions (G). The blue gradient represents habitat suitability, with darker blue indicating higher suitability.

between ecoregional groups, and the remaining percentage of variation was partitioned within populations (27.65%) and among populations within groups (67.56%). These results suggest a low signal of population structure and that populations are moderately differentiated (Table 2). The genetic distances within each latitudinal group exhibit a gradient from north to south, with the northern group displaying the highest value (.0072, .006, and .0059 for north, central, and south, respectively). Regarding ecoregions, the highest value corresponds to HM, followed by the Espinal (.0072 and .0063, respectively) (Supporting Information, Table S5). In contrast, the distances between latitudinal groups reveal similar levels of divergence than within groups. The comparison between N and C has the highest values, followed by N and S (.0072 and .007, respectively). For ecoregions, HM and Espinal exhibit the highest value (.00749) (Supporting Information, Table S5).

Demographic changes

Tajima's and Fu's neutrality tests were negative for all geographical groups, but significant only for the north and south groups, consistent with the HM and LM ecoregions (Table 1). The mismatch distribution of pairwise differences for the ecoregions revealed unimodal curves for both larger populations, the HM and the LM, although LM showed a small second peak (Fig. 4B). The unimodal shape for both indicates a demographic and geographical expansion for these populations. In contrast, the bimodal pattern in the pairwise difference distribution for the Patagonian Steppe (Fig. 4C) is attributable to recent haplotypes with different origins (see the spatial diffusion analysis). Finally, Dry Chaco and Espinal showed a multimodal pattern (Fig. 4D, E).

The Bayesian skyline plot analysis indicated that the increase in the population effective size would have begun ~150 kya, with a 10-fold increase in the mean effective population size (Fig. 5).

Current and palaeodistribution modelling

For ecological niche modelling, 9 of the 19 bioclimatic and topographic variables were selected according to the VIF (Supporting Information, Table S4). The three most important climatic variables depicting the species environmental suitability were as follows: minimum temperature of coldest month (Bio

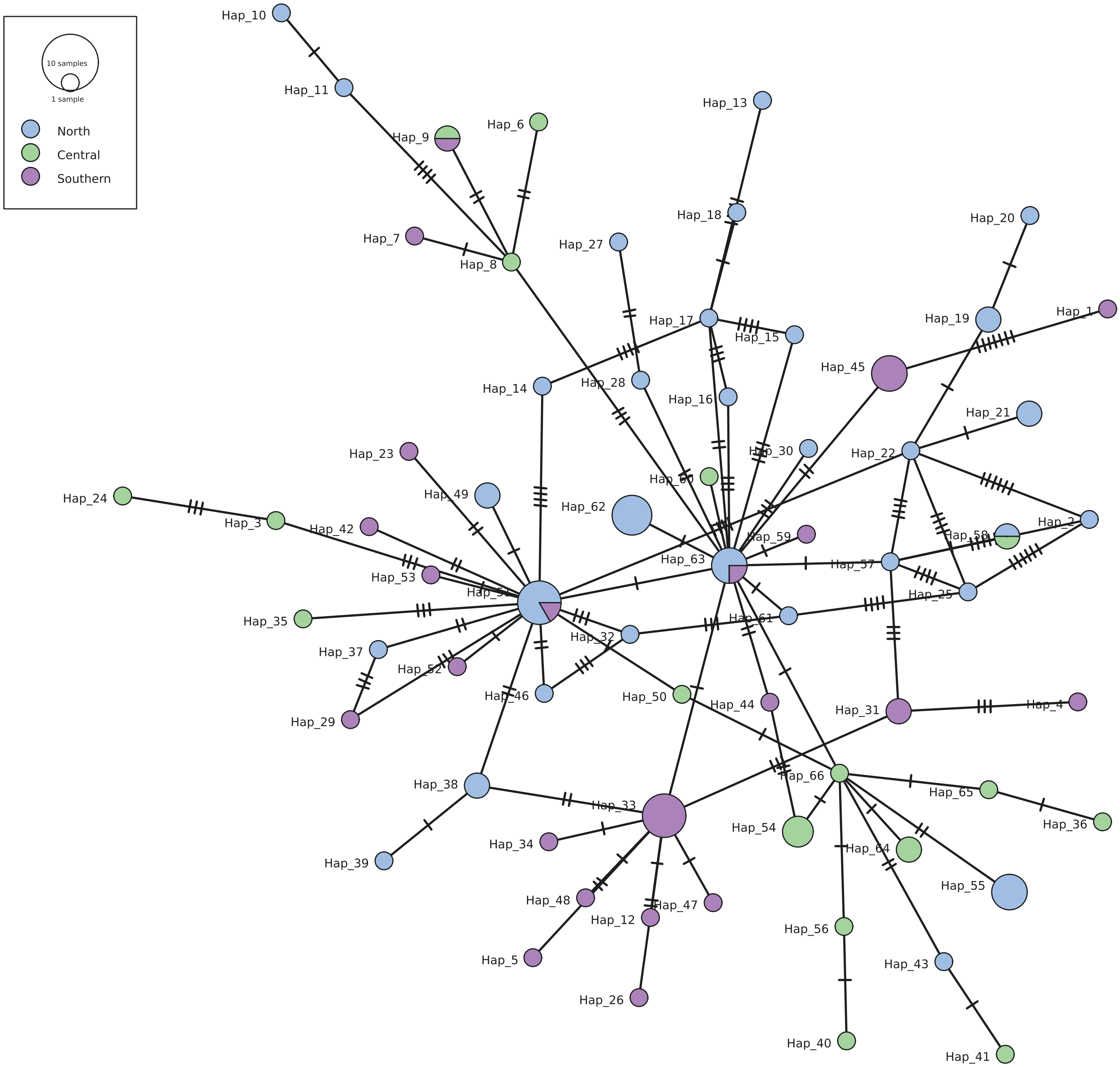

Figure 3. Haplotype network based on *Cytb* mitochondrial DNA sequence data from the 99 individuals of *Graomys griseoflavus* used in this study. The haplotypes are classified according to their geographical distributions: northern, central, and southern. The network was estimated using a minimum spanning network algorithm (for details, see the main text). Circle sizes within the network are proportional to haplotype frequencies, with larger circles representing more common haplotypes. The segments between dashes on the network represent mutational steps.

6; 26.2% of contribution); precipitation seasonality (Coefficient of Variation) (Bio 15; 22.9%); and precipitation of the driest month (Bio 14; 21.3%; see Supporting Information, Table S4). The best model for the current environmental conditions was obtained with an area under the curve of .82 ($P < .0001$); sensitivity = .86; specificity = .40; and true skill statistic of .44. These values indicated a good performance of the MAXENT algorithm, allowing us to project it to past scenarios. The areas with high probability of occurrence were coincident with the South American Arid Diagonal and roughly with the ecoregions of HM and LM (Fig. 2E–G). The model of palaeodistribution during the LIG (120 kya) showed a slight coincidence related to the current distribution of the species (Fig. 2E). During the LGM, the model showed a considerable retraction of suitability, keeping a low probability (30–40%) of occurrence only in central western Argentina, in the provinces of Mendoza, La Rioja, and Catamarca, where the HM ecoregion exists currently (Fig. 2F). The distribution model for the present environmental conditions showed a wide geographical area of high occurrence probability across the five ecoregions occupied by the species: the HM, LM, Espinal, Dry Chaco, and Patagonian Steppe (Fig. 2G). The model for the present climatic conditions indicates a

Table 2. Hierarchical AMOVA in *Graomys griseoflavus Cytb* for north (N), central (C), and south (S) groups (according to dispersal groups identified in the spatial diffusion analysis), and for the five ecoregions (CH, Dry Chaco; E, Espinal; HM, High Monte; LM, Low Monte; PE, Patagonian Steppe). The significance of the variance component (*P*) was tested by 1000 permutations according to Excoffier *et al.* (1992).

Source of variation	Percentage of variation	Fixation indices (Φ-statistics)
Among groups (N, C, S)	6.49	.33049***
Among populations within groups	26.56	
Within populations	66.95	
Among ecoregional groups (HM, LM, PE, E, CH)	4.79	.32438***
Among populations within ecoregional groups	27.65	
Within populations	67.56	

***$P < .01$.

notable increment of suitable areas in the southeast ecoregions after the LGM. The area corresponding to the centre–south of Salta, centre of La Rioja, and the north of Catamarca maintained suitable conditions in all projections carried out.

Spatial distribution of the chromosomal polymorphism

Following the literature review, we have identified 26 localities where at least one karyotype for *G. griseoflavus* has been detected (Fig. 1B). The central area of the species distribution, particularly in Mendoza province, exhibits the highest karyotype diversity for both the number of chromosomes and the types of Rb fusions, as indicated by the latest report on the distribution of chromosome variation for the genus (De Cena *et al.* 2023). The most widely distributed karyotype was 38–36 chromosomes, which is present in populations across the northern, central, and southern regions. Although there are a few localities where only a single karyotype has been recorded (for example 35, 36, or 37), these occurrences appear to be distributed randomly, without a discernible distribution pattern. For instance, there is a record of karyotype $2n = 36$ in the southern populations of the Patagonian Steppe ecoregion in the Rio Negro province, and another one in the Chaco ecoregion of San Luis province. The karyotypes with the lowest chromosome count (34–33) are all found in Mendoza province, near the transition between the HM and LM regions (Fig. 1B).

DISCUSSION

In this study, we investigated the demographic and spatial evolutionary history of the cricetid rodent *G. griseoflavus* across its entire range. We used original data and multiple complementary analytical tools. This study significantly enhances our knowledge of the evolutionary history of this rodent species by increasing the available *Cytb* sequences by ~20% and covering most of its geographical range. We provide a comprehensive description of the demographic and distributional changes of *G. griseoflavus*, shedding light on the overall phylogeographical history and the karyological evolution of the species.

Latitudinal genetic variation along the Arid Diagonal

The genetic variation across the overall geographical range of *G. griseoflavus* revealed a high haplotype diversity (.986) but a low nucleotide diversity (.00688) in comparison to the congeneric and also widespread species, *G. chacoensis* (Martínez and Gardenal 2016). This genetic footprint might be indicative of a low effective population size followed by a rapid population expansion (Grant and Bowen 1998). However, the process generating a reduced effective population size and the consequent drop in nucleotide variability might be either a colonization of an isolated area by a population subset, known as the founder effect, or a consequence of a drastic reduction in species abundance, termed a population bottleneck (Grant and Bowen 1998, Charlesworth 2009). Considering the spatial diffusion analysis, the process that shaped *G. griseoflavus* populations might be depicted as geographical blocks, with two main populations: the northern (HM ecoregion) and the southern (LM and Patagonian Steppe) populations, with marginal populations restricted to Dry Chaco and Espinal ecoregions in Argentina (Fig. 2C, D). The decreasing nucleotide diversity from the north (HM) to south (LM and Patagonian Steppe) throughout the Argentinean portion of the South American Arid Diagonal (Abraham *et al.* 2020), might suggest a colonization pattern from HM to the south by means of the founder effect. Furthermore, the haplotypes of HM are scattered through the haplotype network, indicating that this ecoregion conserves the ancestral haplotypes (particularly, the haplotypes Hap 51 and Hap 63) and the highest genetic diversity. Conversely, most haplotypes of LM are related to those of HM by a few mutational steps (Supporting Information, Fig. S1), supporting the hypothesis that these represent a population subset derived from the northern HM. Besides, the spatial patterns revealed in the spatial diffusion analysis showed a secondary contact between haplotypes of HM and LM. The haplotypes occurring in the Dry Chaco, Patagonian Steppe, and the Espinal ecoregions were not grouped, but scattered across the haplotype network. This pattern might suggest either multiple colonization events or an ancestral disruption from the HM core. Additionally, haplotypes from these ecoregions showed similar genetic diversity values between them. In contrast, the haplotypes Hap 45 and Hap 1, belonging to the Patagonian Steppe, which are located close to the Andes, like a wedge between the northern (HM) and southern (LM) populations, have both low nucleotide diversity and low haplotype diversity. The restricted location of these haplotypes in the network, linked to a single haplotype of both populations (HM and LM ecoregion populations) suggests recent punctual colonization and isolation of the southernmost populations, possibly using the Neuquén River as a route. In fact, the dispersal of *G. griseoflavus* via this type of corridor was highlighted for the several fluvial systems that dissect

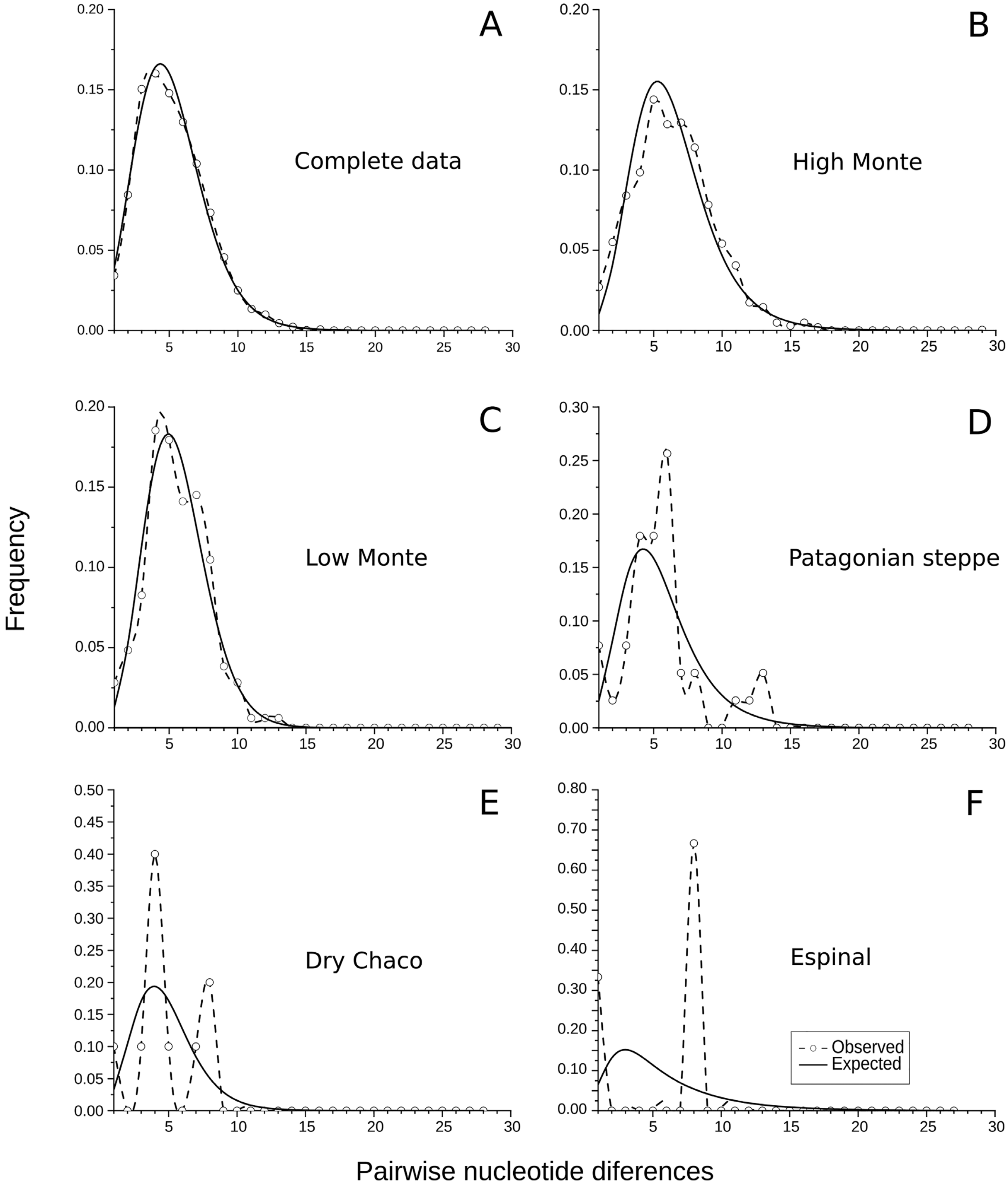

Figure 4. Mismatch distribution of *Cytb* mitochondrial DNA sequence data from *Graomys griseoflavus* according to ecoregions: complete data (A), High Monte (B), Low Monte (C), Patagonian Steppe (D), Dry Chaco (E), and Espinal (F). The dashed line delineates the observed values, and the continuous line is the expected pattern under a model of population growth.

the Patagonian region west–east (Udrizar Sauthier *et al.* 2011). Our results are concordant with the hypothesis of an ancestral range in the HM and subsequent expansion into the southern ecoregions across the South American Arid Diagonal, reaching marginally the eastern Chaco and Espinal ecoregions (Lessa *et al.* 2010, Martínez *et al.* 2010, Martínez and Gardenal 2016).

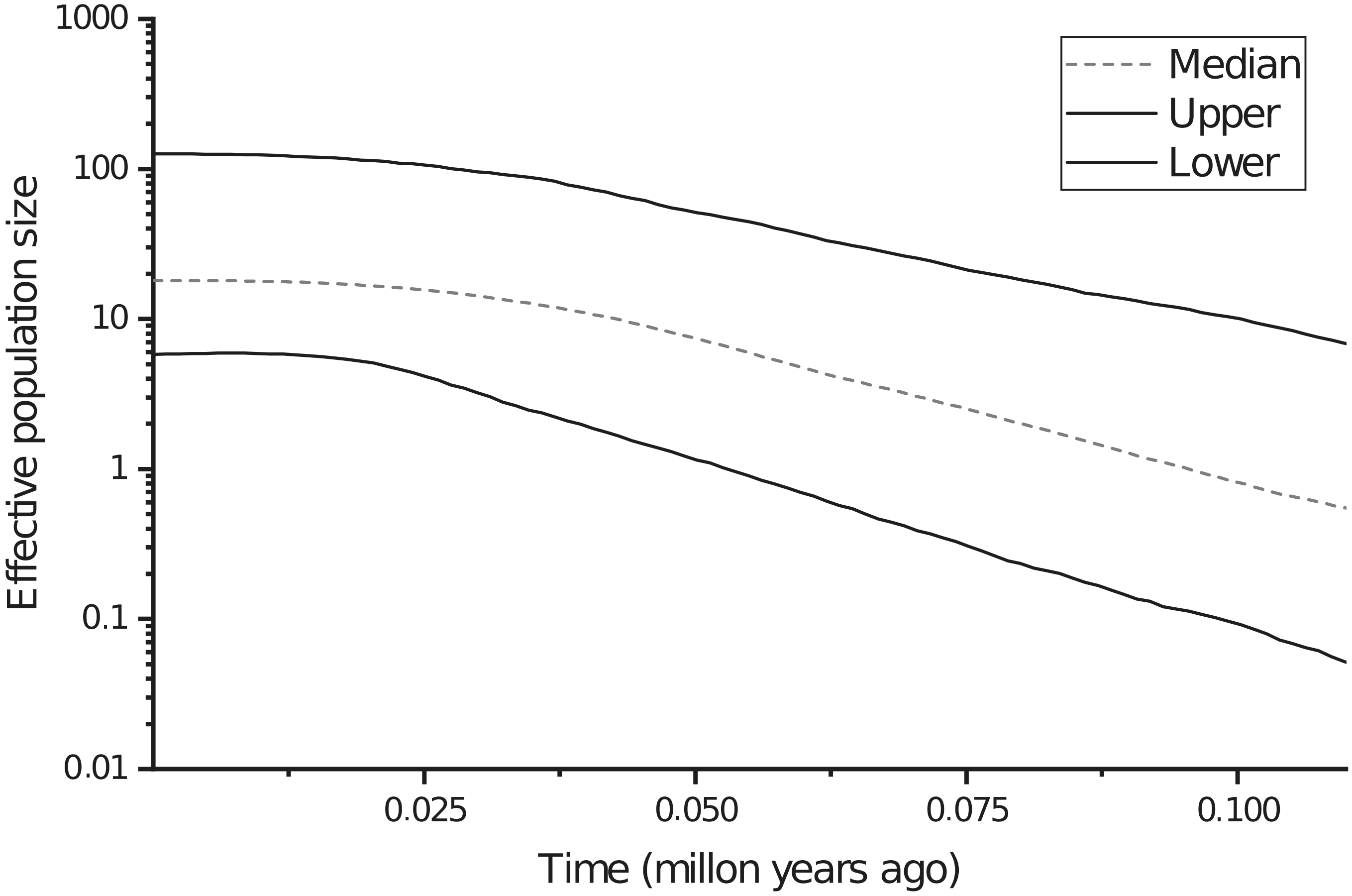

Figure 5. Bayesian skyline plot for *Graomys griseoflavus*. On the *x*-axis, the time scale is in millions of years before the present. The effective population size multiplied by generational time is shown on the *y*-axis and expressed on a logarithmic scale. Dashed lines give the median of effective population size through time, and continuous lines represent the 95% highest posterior densities over the median estimates along the coalescent history of the lineages.

Furthermore, similar patterns of genetic diversity have already been reported for other rodent species with distributions across the South American Arid Diagonal, such as *Phyllotis xanthopygus* (Albright 2004), *Tympanoctomys barrerae* (Ojeda 2010, Gallardo *et al.* 2013), and *Abrothrix olivacea* (Quiroga-Carmona *et al.* 2022), suggesting a shared phylogeographical history, probably as a result of similar responses to environmental changes. It is worth mentioning that a phylogeographical study on *Monttea aphylla*, an endemic plant of the Monte Desert, displays a genetic structure that is predominantly latitudinal as a result of glacial events in southern South America (Baranzelli *et al.* 2017). The spatial distribution and latitudinal phylogeographical structure shown by this aphyllus shrub are strikingly similar to our findings for *G. griseoflavus*, suggesting that geological and climatic events might affect a whole biota in a similar way. These genetic patterns seem to be the result of population persistence in periglacial refuges during glacial–interglacial cycles. Although the response to these events is largely species specific (Lessa *et al.* 2010), the patterns of genetic structure of the species studied in this region are similar, and thus likely to be produced by the environmental variations during glacial events.

In the specific case of *T. barrerae*, the phylogenetic structure and demographic history apparently triggered the emergence of a new species (*Tympanoctomys kirchnerorum*), owing to expansion, colonization, and subsequent isolation in a cyclic glacial scenario (Teta *et al.* 2014). Likewise, based on morphological and molecular analysis, Ojeda *et al.* (2021) suggested that *P. xanthopygus* represents a species complex, and thus elevated to species rank those taxa previously recognized as subspecies (e.g. *Phyllotis vaccarum*; see Hershkovitz 1962). For *G. griseoflavus*, however, the haplotypes in the network were connected by a few mutational steps. This connectivity hinders our ability to interpret differences at the specific level. This is not trivial because, in the light of genomic studies, the recent boom of revalidation and novel species based on single genetic markers or a few genetic markers might have inflated taxonomic nominal forms artificially (e.g. Dufresnes and Jablonski 2022).

Of the five nominal forms classically allied to *G. griseoflavus*, the five subspecies recognized by Cabrera (1961), three are names now regarded as synonyms of *G. chacoensis* (i.e. *Graomys centralis*, *G. chacoensis*, and *Graomys medius*). For those two that persisted associated with *griseoflavus*, *cachinus* would be an available name for northern populations, with the nominotypical form applicable to southern ones (Braun and Patton 2015). *Phyllotis cachinus* Allen, 1901, with a type locality in Salta Province, Argentina, was erected as different in the first revision of the entire genus to be conducted (Allen 1901). Allen (1901: 408) also recognized in the same work *Phyllotis chacoensis*, from animals collected in Paraguayan Chaco (Pardiñas *et al.* 2018). Reading comparatively the diagnoses made by this author, the observed differences in ventral coloration guided his taxonomic judgment. Given that he had the opportunity to examine this trait directly in the holotype of *P. griseoflavus*, he stated that in *P. chacoensis* '… the fur on the ventral surface being wholly white to the base, instead of basally pale plumbeous', whereas *P. cachinus* has '… the pelage of the ventral surface pale grayish at extreme base' (Allen 1901: 409). Colour differences are important features to characterize species, but are often influenced strongly by regional environmental conditions (e.g. Hofreiter and Schöneberg 2010).

The processes generating the genetic variation discussed above can be evaluated further through additional evidence on historical demography and variations in environmental suitability for the species over time. As previously described by Martínez *et al.* (2010) and Martínez and Gardenal (2016), the populations of *G. griseoflavus* initiated a significant demographic growth and expansion ~150 kya. In this study, we analysed population growth parameters, but divided the entire range of *G. griseoflavus* into geographical divisions represented by the ecoregions. We found a remarkable expansion of the populations belonging to HM and LM ecoregions as revealed by negative Tajima's and Fu's neutrality tests and mismatch distribution analysis. However, both neutrality tests were significant only for populations in the northern HM ecoregion. Likewise, the mismatch distribution of pairwise differences was clearly unimodal for HM populations, whereas it presented a small secondary peak for populations in the southern LM ecoregion. This would indicate that these demographic changes occurred at different times, uncoupled from each other. Likewise, the neutrality tests showed a pattern of demographic expansion in all ecoregions, but both tests were significant for the northern HM ecoregion populations, and only Fu's was significant for the southern populations in the LM ecoregion. The mismatch distributions of pairwise differences for the Patagonian Steppe, Dry Chaco, and Espinal ecoregions were bimodal, indicating no expansion but multiple immigration for these populations of *G. griseoflavus*, as also suggested by the haplotype network. However, we should interpret these results cautiously because populations inhabiting Patagonian Steppe, Dry Chaco, and Espinal ecoregions are represented by very few samples from which to draw robust inferences.

The Bayesian skyline results support the finding described above, indicating a drastic demographic growth that began 100 kya, characterized by an increase of >10 times the population mean. Our results are in agreement with those reported previously by Martínez and Gardenal (2016). However, the presence of wedge ice casts in northeastern Patagonia suggests that much of the non-glaciated territory also experienced harsh environmental conditions (Liaudat 2008). Moreover, the spatial diffusion analysis indicates that the species expanded its geographical range from the boundary between the LM and HM ecoregions, in southern La Rioja province, 290 kya, and it reached the northern Patagonian Steppe 200 kya, establishing the southern populations of the LM ecoregion (Fig. 2B). This indicates a range expansion of the species from central-western Argentina northwards and southwards across the arid environments of the South American Arid Diagonal. Then, 160 kya, the species reached the marginal areas of the Chaco and the Espinal ecoregions in San Luis and Córdoba provinces from the southern populations of La Pampa province, and reached the centre–east of Mendoza province from the north of La Rioja province. This analysis reinforces the hypothesis suggested by the haplotype network topology of a secondary contact between HM and LM populations, multiple subsequent colonization of localities in Mendoza province from the LM ecoregion, and a single intrusion into Neuquén province (Patagonian Steppe), also from the LM populations.

Although the fossil record attributed to *G. griseoflavus* is far from complete, several localities indicate that during the Pleistocene the species greatly surpassed its current geographical range in central–eastern Argentina (Pardiñas 1999). The oldest occurrence for *G. griseoflavus* is from northeastern Buenos Aires Province, based on materials recovered in deposits representing approximately the Brunhes–Matuyama limit (~780 kya; Tonni *et al.* 1988, Voglino and Pardiñas 2005). Late Pleistocene evidence for the species also indicates extralimital localities, including records in northeastern (Luján; Pardiñas 1995a), central (Tandil; see Pardiñas 1995b), southeastern (Centinela del Mar; Pardiñas 1999), and south (Cascada del Paleolama; Pardiñas *et al.* 1996) Buenos Aires province.

Overall, this scenario can be contrasted with projections of the ENM for the species into the past and allows us to identify ancestral suitable areas for occurrence of the species during the LIG, ~120 kya. Climate simulations of the LGM indicate that the greatest cooling occurred at mid- to high latitudes in South America (Labraga and Villalba 2009). Using Gramineae and herbaceous pollen dispersal patterns, a northward shift of the Patagonian Steppe in response to cooler climates has been inferred (Labraga and Villalba 2009, Baranzelli *et al.* 2017). These characteristics might have caused the populations to retreat to less hostile environments in the north of the species distribution, while the LM populations persisted in a refuge that allowed them to withstand these unfavourable conditions until after the LGM (Fig. 2F). The time when conditions were hostile for the southern LM populations not only disrupted gene flow with northern HM populations but could also have had demographic implications by slowing the population growth. One aspect to highlight from our past ENM results is the fact that the current area corresponding to the HM (from Salta to La Rioja provinces) maintained suitable conditions for occurrence of the species in all projected scenarios. This could be explained by the climatic implications triggered by the increment of continentality during the LGM. It is a fact that the sea level decreased by ≥105 m, exposing huge portions of the current continental shelf along the Atlantic coast of Argentina and, therefore, increasing continental conditions (e.g. Violante *et al.* 2014), which can be interpreted as a general decrease in temperature and a direct impact on evapotranspiration, resulting in more arid and cold conditions in most parts of central Argentina (Vuilleumier 1971, Hulton *et al.* 2002, Ponce *et al.* 2011). In agreement with this, the minimum temperature of the coldest month was among the main influential variables for our ENM, in addition to precipitation-related variables (precipitation seasonality and precipitation of the driest month). Given that the species is currently associated with arid environments, it was probably the low-temperature conditions that influenced the evolutionary history of this species the most (Cavides-Vidal *et al.* 1987).

The origin of chromosomal polymorphism

Chromosomal rearrangements often play a prominent role in the speciation process between parapatric or sympatric populations due to recombination suppression, promoting the survival of incipient species that have encountered their progenitor (Noor *et al.* 2001, Rieseberg 2001, Zhang *et al.* 2021). *Graomys griseoflavus* has a remarkable chromosomal polymorphism that varies from $2n = 33$ to $2n = 38$, but reproduction between individuals with these different cytotypes appears not to be affected (Theiler and Gardenal 1994). Two hypotheses have been proposed to explain the origin of this polymorphism, postulating either a non-random sequential origin of Rb events leading to the current karyotypic divergence (Zambelli *et al.* 2004) or, alternatively, multiple and independent centric fusions (Lanzone *et al.* 2014).

The former hypothesis postulates $2n = 42$ as the ancestral karyotype (currently restricted to *G. chacoensis*). However, despite the absence of geographical barriers to gene flow, reproductive isolation between the $2n = 42$ cytotype (*G. chacoensis*) and the $2n = 33$–38 cytotypes (*G. griseoflavus*) has been proved experimentally, owing to pre- and post-reproductive barriers and non-viable offspring of sterile hybrids (Theiler and Gardenal 1994, Theiler and Blanco 1996).

The sequential model would require a substantial reduction in population size, via genetic drift, to fix the different Rb variants that might have emerged in the population. Thus, according to the model proposed by Zambelli *et al.* (1994) and Catanesi *et al.* (2002), the different variants should have originated from the $2n = 38$ karyomorph, and by means of founder effects and centric fusions, differentiated sequentially to the diploid number reduction, with the karyotype $2n = 33$ being the most recent one.

Our analysis refutes the hypothesis of a single, sequential origin for the Rb variants in *G. griseoflavus*. If this hypothesis were correct, we would expect the different Rb variants to be correlated with the pattern of population expansion described by our spatial diffusion analysis. In other words, the hypothesized intermediate states of Rb variants should have been recorded in intermediate areas of the species range expansions across the South American Arid Diagonal. However, the record of $2n = 38$–36 cytotypes for the southernmost limit of the species (Rodriguez and Theiler 2007) could be interpreted as ancestral populations that reached these southern latitudes and constituted the LM populations when they became separated from the rest of the populations. Nonetheless, to support the hypothesis of a sequential origin of Rb rearrangement, the most recent karyotype variants ($2n = 34$–33) should be found in the most recently established populations at the southernmost distribution in the South American Arid Diagonal. On the contrary, these cytotypes ($2n = 34$–33) are found in the northern populations of the LM ecoregion, close to the ancestral range area of the species, where the population size has increased continuously because climatic conditions were optimal, thus neglecting the influence of population bottlenecks (Figs 1B, 2B, D, F), according to our findings and those of previous studies for northern populations of *G. griseoflavus* (Martínez *et al.* 2010, Martínez and Gardenal 2016). Furthermore, Lanzone *et al.* (2014) studied the cytogenetics of populations with polymorphic Rb rearrangements from Mendoza and Catamarca provinces and described a new fusion restricted to a single population, indicating that it is a recent, independently acquired chromosomal mutation. Furthermore, Rb translocations to achieve the $2n = 36$ cytotype do not necessarily involve the exclusive intervention of the same set of chromosomes (see De Cena *et al.* 2023). These results indicate that the generation and/or maintenance of new fusion is not necessarily conditioned by previous fixation of another one. Thus, our results support the hypothesis of chromosomal evolution in *G. griseoflavus* by multiple and independent centric fusions.

CONCLUSION

Phylogeography provides insights into the association between population genetic structure and geography, also yielding information on the demographic processes and the environmental dynamics involved in genetic differentiation. The results of the phylogeographical approach benefit from ENM analysis, because this method allows estimation of current and past geographical distribution models of the species and, furthermore, provides insights regarding the environmental suitability to provide a better representation of its evolutionary history. In this research, we combined the evolutionary history of *G. griseoflavus* based on reconstructions of historical demographic processes and the environmental dynamics involved in genetic differentiation across the entire geographical range of the species (~2300 km). Our results suggest that this cricetid rodent experienced an early range expansion from central western Argentina, followed by a faint population isolation during the glacial climatic oscillations, and secondary contacts among populations. This evidence contrasts with the distribution of the diverse chromosomal variation in *G. griseoflavus* and supports multiple and independent origins of Rb translocations.

SUPPLEMENTARY DATA

Supplementary data is available at *Biological Journal of the Linnean Society* online.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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DATA AVAILABILITY

The data underlying this article are available in the Supporting Information.

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