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Multiple refugia and glacial expansions in the Tucumane–Bolivian Yungas: The phylogeography and potential distribution modeling of *Calomys fecundus* (Thomas, 1926) (Rodentia: Cricetidae)

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Abstract

The Yungas, a subtropical mountain rainforest of South America, has been little studied in relation to the evolutionary history of the large-bodied species of the genus *Calomys*. Particularly, two species have been synonymized: *C. boliviae* and *C. fecundus;* the first is only known from its type locality in the northern Bolivian Yungas, whereas the second is known along the Tucumane–Bolivian Yungas shared by Bolivia and Argentina. In this study, we combined a phylogeographic approach with ecological niche modeling, with samples covering most of the geographic range of *C. fecundus*. One mitochondrial and two nuclear genes were used for population genetic analyses. Current and paleoclimatic models were obtained. Nuclear genes resulted uninformative by retention of ancestral polymorphism with other species of *Calomys*. The mitochondrial marker revealed a complex network showing signals of several population expansions. Three genetic clusters in a latitudinal sense were detected, which are coincident with the three stable climatic zones estimated by current and paleoclimatic models. We determined a pattern of expansion during glacial cycles and ancestral refugia during interglacial cycles. None of the potential distribution models predicted the presence of *C. fecundus* in the type locality of *C. boliviae*. Therefore, we recommend making integrative taxonomic studies in the Bolivian Yungas, to determine whether or not *C. fecundus* and *C. boliviae* correspond to the same species.

KEYWORDS

Calomys, glacial cycles, niche modeling, phylogeography, Pleistocenic refugia, Yungas ecoregion

1 | INTRODUCTION

Genetic variation within and among populations from different geographic regions throughout a species range results from historical, ecological, and evolutionary processes. Research of the evolutionary history of a species can provide an explanation of how the species reached its current patterns of diversity. Frequently, genetic footprints can be associated with past climatic events which molded the current diversity and distribution of a given species (Hewitt, 2004; Thanou, Paragamian, & Lymberakis, 2020). Phylogeography provides a robust analytical framework to study the processes that govern the geographical distribution of intraspecific lineages (Avise, 2009).

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However, understanding species distribution also requires consideration of environmental components of the geographic space that they occupy, given their ecological requirements for survival (Pearson, 2010). Ecological niche models (ENMs) can predict a species' potential distribution given a set of environmental variables and known presences, and those models can be projected in different paleoclimatic scenarios, informing about its potential past distributions (Peterson et al., 2011). Studies combining ecological and phylogeographic analyses have provided a considerable amount of information regarding the evolutionary history of species and have contributed to significant advances in biogeography (Alvarado-Serrano & Knowles, 2014). In South America, several phylogeographic studies have been performed in rodents, for example, Martínez et al. (2010) in *Graomys chacoensis* (J. A. Allen, 1901) / *G. griseoflavus* (Waterhouse, 1837), González-Ittig and Gardenal (2004) in *Calomys musculinus* (Thomas, 1913),or González-Ittig et al. (2010) and Palma and Rodríguez-Serrano (2018) in *Oligoryzomys longicaudatus* (Bennett, 1832). However, in the Chaco and Yungas ecoregions, there are no studies in rodents combining phylogeography with ecological niche modeling (Turchetto-Zolet, Pinheiro, Salgueiro, & Palma-Silva, 2013).

South American Chaco is a large continuous neotropical dry forest (800,000 km^2), formed by the vegetation of the plains of northern Argentina, western Paraguay, and south-eastern Bolivia (Prado, 1993). Its climate is characterized by a strong seasonality, with a dry season in winter and spring and a rainy one in summer. Rainfalls decline from over 1,000 mm/year in the east to less than 500 mm/year in the west, determining two sub-ecoregions: the Humid and the Dry Chaco (Figure 1) (Pennington, Prado, & Pendry, 2000). The western limit of the Chaco is determined by the Andean and Subandean mountains. Heavy precipitations fall in the western slopes of these mountains in summer, giving origin to the cloud rain forest of the Yungas ecoregion (Quiroga & Premoli, 2007). In north-western Argentina and Southern Bolivia, the Yungas occur in a narrow strip of 100 km, between 350 and 3,500 m.a.s.l. (Figure 1). Several woody plants occurring in the piedmont areas of the Yungas are found in drier south-eastern areas of the Chaco, which makes it difficult to define the precise boundaries of the two neighboring areas (del Río, Morrone, & Lanteri, 2015).

Among the species inhabiting, the Chaco and the Yungas transitions are the rodents of the *Calomys callosus* complex. According to the different taxonomic revisions performed, the genus *Calomys* is composed of several small species and one large-bodied group (see the review of Salazar-Bravo, 2015). Molecular phylogenetic studies showed that this group include the species *C. cerqueirai* Bonvicino, Oliveira, and Gentile, 2010, *C. expulsus* (Lund, 1840), and *C. callosus* species complex [*C. fecundus* (Thomas, 1926) or *C. boliviae* (Thomas 1901), *C. venustus* (Thomas, 1894), *C. tocantinsi* Bonvicino, Lima, and Almeida, 2003, *C. callosus* sensu stricto *stricto* (s.s.) (Rengger, 1830), and *C. callidus* (Thomas, 1916)] (Bonvicino et al., 2010). However, the evolutionary history of the species within this clade is still a matter of discussion (Salazar-Bravo, 2015). The taxonomic status of the species from Argentina and Bolivia has changed repeatedly

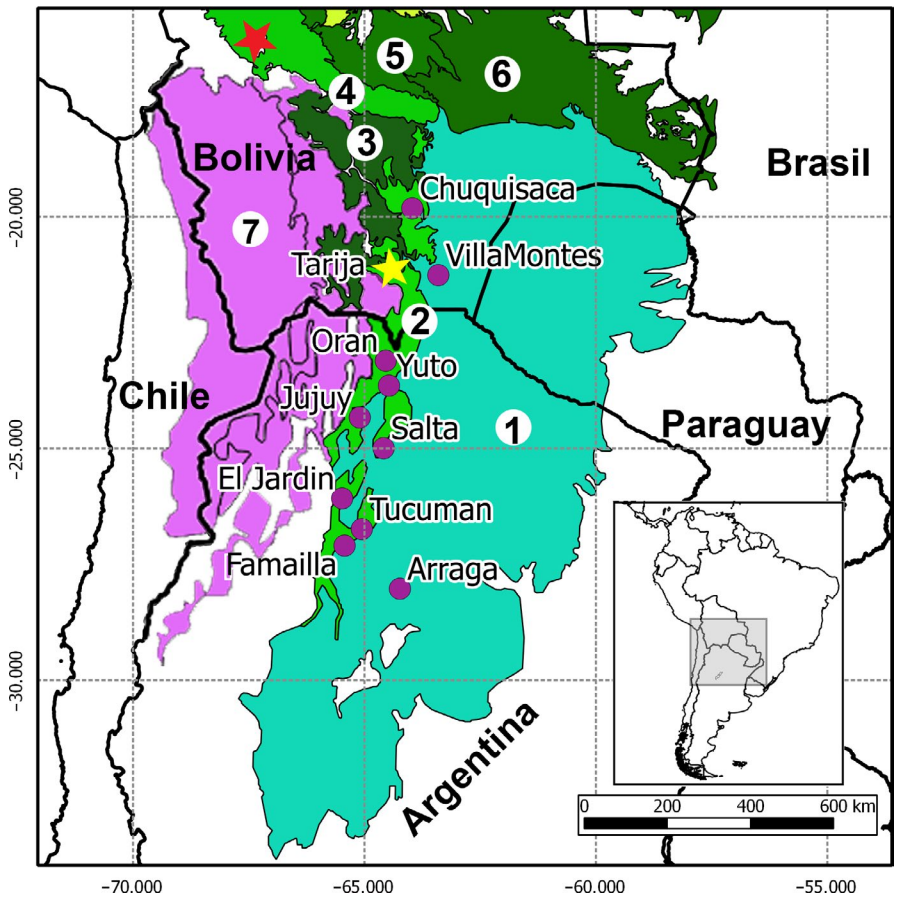

FIGURE 1 Collecting sites of individuals of *Calomys fecundus* and ecoregions relevant for this study: 1—Dry Chaco, 2—Tucumane–Bolivian Yungas, 3—Bolivian Montane Dry Forest, 4—Bolivian Yungas, 5—Amazon Moist Forest, 6—Chiquitano Dry Forest, 7—Puna. Red star: Type locality of *C. boliviae*, Yellow star: Type locality of *C. fecundus*

from synonyms or subspecies to valid species. For example, *C. boliviae* (type locality: Río Solocame, La Paz, Bolivia; Figure 1) was considered to be a valid species, including *C. fecundus* (type locality: Tablada, Tarija, Bolivia; Figure 1) as a synonym, by Musser and Carleton (1993), while both species were considered synonyms of *C. venustus* by Olds (1988) and Anderson (1997). In his original descriptions, Thomas reported larger morphological measures for *C. fecundus* than for *C. boliviae*. In addition, Olds (1988) noticed that adult females of *C. boliviae* presented five pairs of mammae, whereas those of *C. fecundus* presented seven pairs. Based on molecular characters and chromosomal data, Salazar-Bravo et al. (2002) and Dragoo, Salazar-Bravo, Layne, and Yates (2003) considered *C. fecundus* to be a valid species.

Calomys boliviae is only known in its type locality in north-western Bolivia in the ecoregion named Bolivian Yungas (Figure 1), while that of *C. fecundus* is in southern Bolivia in the Tucumane–Bolivian Yungas ecoregion. It is worth noting that these ecoregions are climatically and biogeographically very distinct; the Bolivian Yungas are more humid and less seasonal because they benefit from the humidity brought by the trade winds, while the Tucumane–Bolivian Yungas suffer more directly from southerly cold fronts (Ibisch & Mérida, 2004). Besides, both ecoregions are separated by the Bolivian Montane Dry Forest, which might be a barrier for gene flow between the northern and southern forms of *Calomys*, and thus, it is not unreasonable to think that they might represent two different species. Taxonomic clarification is especially important when the species of concern are implicated in zoonotic diseases (Mills & Childs, 1998). In this sense, several species of *Calomys* have been associated with different genotypes of the genus *Orthohantavirus*. The genotype Laguna Negra (etiologic agent of the Hantavirus Pulmonary Syndrome) has been associated with three species of the *C. callosus* complex: *C. fecundus* (Levis et al., 2004), *C. callosus* (Carroll et al., 2005), and *C. callidus* (Travassos da Rosa et al., 2012), but, hitherto, not with *C. boliviae*.

Based on the biogeographical and taxonomic implications explained above, the aim of this study was to contribute to the knowledge of the evolutionary history of *C. fecundus* of the Tucumane–Bolivian Yungas using a phylogeographic approach combined with present and past ecological niche modeling. In this way, we could understand how the past climatic events determined the current distribution and genetic diversity of the species. Using this methodology, we helped to clarify the taxonomic problems between *C. fecundus* and *C. boliviae*.

2 | MATERIALS AND METHODS

2.1 | Specimens and localities

A total of 81 live-trapped animals were collected in 17 localities from Argentina. Rodents were sedated by isofluorane inhalation, photographed, weighed, and measured, and a small portion of the tail was stored in ethanol. Specimens were released in the site of collection and georeferenced. Animals were handled following the guidelines of the American Society of Mammalogists (Sikes, 2016) and the current laws of Argentina. For comparison purposes, we included mitochondrial *cytochrome b* (*Cyt-b*) sequences of *C. fecundus* published by Salazar-Bravo et al. (2002) and available in GenBank: AF385592 and AY033173 from Tucumán, Argentina; AY033158 and AY033164 to AY033167 from Tarija, Bolivia; AY033159 to AY033163 from Chuquisaca, Bolivia; and AY033169 to AY033172 from Villa Montes, Bolivia. The addition of these sequences leads to a total of 97 individuals distributed in 24 localities, covering the full range extent of the species. Due to low sample sizes in some localities or the proximity between them, specimens were grouped in 11 units for the a priori defined population statistical analyses (Figure 1). For the a posteriori analyses, individuals were ungrouped, and the original geographic localities were used. The original coordinates and the names of the grouped localities are detailed in Table S1.

2.2 | DNA extraction and sequencing

DNA was extracted from alcohol-preserved tissues following the method described in Gemmell and Akiyama (1996). A biosafety level 1 vertical laminar flow cabinet was used to manipulate tissues potentially infected with viruses. For the 81 specimens collected in the field, the complete *Cyt-b* gene was amplified with primers Mus 14095 (5′-GACATGAAAAATCATCGTTGTAATTC-3′) and Mus 15398 (5′-GAATATCAGCTTTGGGTGTTGRTG- 3) (Anderson & Yates, 2000) following the conditions described in González-Ittig, Salazar-Bravo, Barquez, and Gardenal (2010).

To evaluate whether nuclear genes could be useful for the intraspecific analysis of *C. fecundus*, we sequenced the first exon of the retinol binding protein 3 (*Rbp3*) of 18 individuals with primers A1 5′-ATGCGGAAGGTCCTCTTGGATAAC-3′ and B2 5′-ATGAGGTGTTCCGTGTCCTG-3′, following the conditions described in Jansa and Voss (2000) and Weksler (2003) and the intron 7 of the β Fibrinogen gene (*i7Fgb*) of 20 individuals with primers β17-mammL 5′-ACCCCAGTAGTATCTGCCGTTTGGATT-3′ and βfib- mammU 5′-CACAACGGCATGTTCTTCAGCAC-3′ with the conditions described in Matocq, Shurtliff, and Feldman (2007). For comparison purposes, we amplified these genes in the species *C. venustus*, *C. callosus* s.s., and *C. callidus* of the *Calomys callosus* complex. Identification codes and GenBank accession number are detailed in Table S1. PCR products were sequenced at Macrogen Korea (http://www.dna.macrogen.com) using the primers mentioned above. The alignment was obtained with the software Mega x (Kumar, Stecher, Li, Knyaz, & Tamura, 2018) using the Muscle algorithm. Then, the alignment was inspected by eye and manually edited when needed. Mitochondrial and nuclear DNA sequences were deposited in GenBank with accession numbers MN105616 to MN105732. Alignments of the three genes are available as File S1 for the *Cyt-b* gene, File S2 for the *i7Fgb* gene and File S3 for the *Rbp3* gene.

2.3 | Genetic diversity, haplotype/allele networks, and population genetic structure

Mitochondrial haplotypes and nuclear alleles were inferred with DnaSP v.5 (Librado & Rozas, 2009). For the nDNA sequences, individual alleles were resolved with the Bayesian coalescent-based method implemented in Phase v2.1 (Stephens, Smith, & Donnelly, 2001). For the a priori analyses, haplotype/allele diversity (h/S), nucleotide diversity (π), and mean number of pairwise differences (p) were calculated for each of the 11 populations with DnaSP v.5.

Genetic networks were inferred using the median-joining algorithm implemented in PopART v1.7 (Leigh & Bryant, 2015). Mitochondrial haplotypes were colored according to the 11 populations, while nuclear alleles were colored according to the species within the *C. callosus* complex. The genetic structure was estimated with Geneland v3.2.2 (Guillot, Mortier, & Estoup, 2005). Sequences' variable sites and individuals' geographic coordinates were used as the input data. Ten independent runs with 10,000,000 iterations and a thinning of 10,000 were performed for K (number of genetic clusters) varying from 1 to 14. The K value with the highest posterior probability was selected, and three independent runs with K fixed and a chain length of 20,000,000 were performed. We used the correlated frequency model, with uncertainty of coordinates set in 0.05km and a matrix of variables sites only.

2.4 | Demographic and spatial diffusion analyses

To assess the past demographic history of the species, neutrality tests D (Tajima, 1989) and F_S (Fu, 1997) were calculated with DnaSP v.5. The mismatch distribution of nucleotide pairwise differences among individuals was calculated with Arlequin v3.5.1.3 (Excoffier, Laval, & Schneider, 2005). We conducted a Bayesian skyline plot (BSP) analysis implemented in Beast v1.10 (Drummond & Rambaut, 2007) to infer past population demographics. Previous to this analysis, the HKY + G+I model was selected using jModeltest v2.1.3 (Darriba, Taboada, Doallo, & Posada, 2012) following the Akaike information criterion. Parameters of this model were included in Beast 1.10, and then, the analysis was run for 400 million generations using a substitution rate of 2.3% per million years estimated by Smith and Patton (1993) for the *Cyt-b* gene for South American rodents. Chain convergence, estimations of effective population size, parameters' confidence intervals, and BSP reconstructions were checked using Tracer v1.7 (Rambaut, Drummond, Xie, Baele, & Suchard, 2018). The species spatial dynamics through time was assessed using a lognormal relaxed random walk (RRW) diffusion model implemented in Beast v1.10. The analysis was performed including all sequences (n = 97) and geographic coordinates. A normally distributed diffusion rate, a coalescent Bayesian Skyride model, and the HKY + I+G substitution model were used. The remaining priors were the same than those used in BSP analysis. The jitter option was set to 0.01 to add variation to sequences with the same coordinates. Several runs were made to reach convergence; the final run had 800 million generations sampled every 80,000 of them. To check for stationarity, we inspected parameters with Tracer v1.7. To summarize the posterior distribution of ancestral ranges using the RRW model, we obtained the maximum clade credibility tree using TreeAnnotator v1.7.5. This tree was then used as input for Spread v1.0.7 (Bielejec et al., 2016) to reconstruct the pattern of spatial diffusion.

2.5 | Current and paleodistribution modeling

Species distribution models under different past and present climatic scenarios were analyzed. The presence data used for modeling were obtained from our captures in different field trips, from the Museo Argentino de Ciencias Naturales collection, and from the global biodiversity information facility (www.gbif.org). Due to the taxonomic problems in the genus, the presences identified online as *C. callosus*, *C. venustus*, *C. boliviae*, and *C. fecundus* were downloaded. A convex hull with all the individuals identified genetically as *C. fecundus* with a 70 km buffer was built in QGIS 2.18.15 (http://www.qgis.org/), and the database was filtered, keeping only the presences which geographic coordinates intersected the polygon created. The presence records were then filtered temporally to match the period of the bioclimatic layers used (from 1968 until present). After these processes, 97 records remained as presence points for *Calomys fecundus*. ENMs were developed using Maxent 3.3.3k (Phillips, Aneja, Kang, & Arya, 2006). As climatic data, we used the 19 bioclimatic layers from CHELSA (www.chelsa-climate.org). Following the suggestions of Barve et al. (2011), these layers were clipped off according to the species accessible historical area. The list of presence records and the accessible historical area of the species are included in Table S2 and Figure S1. Covariation between variables was tested by the Pearson r coefficient. Those pairs of variables with an absolute r-value of 0.80 or higher were considered as significantly correlated; models with different combinations of uncorrelated variables were run. The maximum background points were set to 50,000, while 25% of records were set aside for testing, and ten replicates were run for each model. Variables were evaluated by the shape of their marginal and individual response curves, percentages of contribution and permutation, jackknife tests, and biological significance. The partial area under the receiver operating curve (pROC) was calculated to evaluate the performance of the models. The ten percentile minimum value of training presence was used as presence threshold (Peterson et al., 2011).

To estimate how species distributions may have changed through time, this model was projected on the paleoclimatic scenarios available online (www.worldclim.org): the last interglacial period (LIG; 130–114 Kya), the last glacial maximum (LGM; 21 Kya), and the mid-Holocene (6 Kya). For the last two periods, we used the climatic layers from the Community Climate System Model (CCSM4)

and those of the Model for Interdisciplinary Research on Climate (MIROC). The predicted presence probabilities obtained with both models were averaged to obtain a combined result. Analyses using separated models are available as Figure S2.

Local differences in the climatic variables influence were examined using the limiting factors analysis (Elith, Kearney, & Phillips, 2010). In any grid cell, the climatic variable which value influences most the model prediction is identified as the limiting factor for that grid cell. The same analysis was performed for current and paleoclimatic layers. To map the haplotype and nucleotide diversity indices on the *C. fecundus* geographic range, we interpolated the values obtained at each of the 11-defined populations into the modeled distribution of the species using the inverse distance weighting spatial interpolation method (Watson, 1992) in QGIS 2.18.

3 | RESULTS

3.1 | Nuclear genes

A total of 20 individuals were analyzed for the *i7Fgb* gene, obtaining sequences of 659 bp. There were 19 polymorphic sites (16 parsimony-informative), and 26 alleles were inferred. To ensure the reliability of polymorphisms, just clear mutations were considered and chromatogram double peaks were resolved with the software Phase v2.1. The species *C. fecundus*, *C. callidus*, and *C. callosus* s.s. have high values of mean number of pairwise differences and allele and nucleotide diversities, while *C. venustus* has much lower values in those indexes. A total of 29 alleles were inferred from the 18 individuals analyzed for the *Rbp3* gene. Sequences have a length of 1,201 bp, with 26 polymorphic sites

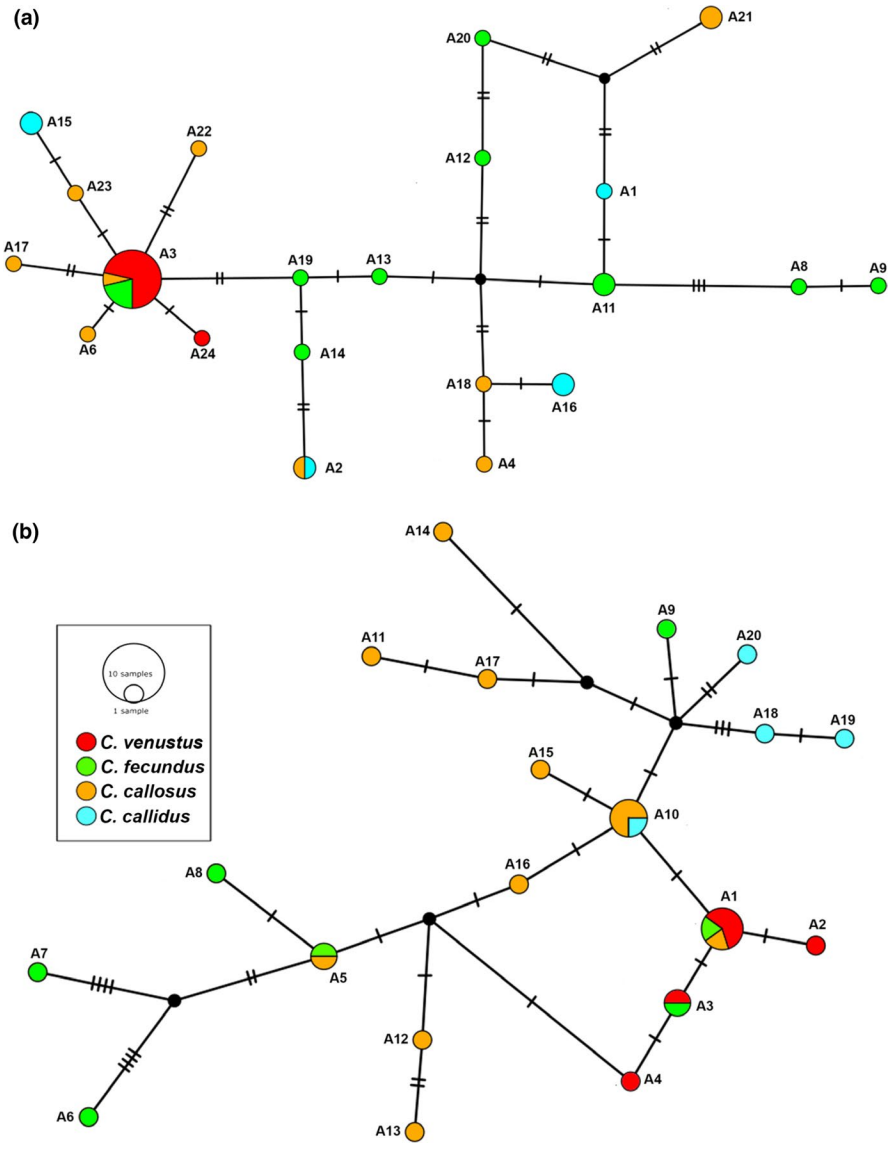

FIGURE 2 Haplotype network of the nuclear genes *i7Fgb* (a) and *Rbp3* (b) of the lowland clade species of *Calomys* present in Argentina. Colors indicate the species identification, as indicated in the figure. Circle sizes are proportional to allele frequencies. Segments between dashes indicate mutational steps; black dots represent missing intermediate alleles not observed in the analyzed individuals

(10 parsimony-informative). Again, *C. venustus* presented the lowest values. This information is summarized in Table S3.

The *i7Fgb* gene network (Figure 2a) does not show any evident group for *C. venustus*, *C. callosus* s.s., *C. callidus*, or *C. fecundus*; individuals of each species are all mixed up within the network. Interestingly, *C. venustus* has a different pattern, having nine identical alleles and just one differing in one mutational step (A3 and A24, respectively). Allele A3 is also shared with individuals of *C. callosus* s.s. and *C. fecundus*. Similarly, the *Rbp3* gene network (Figure 2b) lacks an evident structure; there are three alleles (A1, A5, and A10) that are shared by two or three species of the complex.

3.2 | *Cytochrome b*

A total of 97 individuals were analyzed for the *Cyt-b* gene, obtaining sequences of 1,143 bp. There were 59 polymorphic sites (34 parsimony-informative). The nucleotide diversity is 0.00453, and the mean number of differences is 5.179. From the 97 sequences, 54 haplotypes were recovered. It is worth noting the great haplotype diversity (0.9792) and the high number of endemic haplotypes (47 out of 54). All analyzed populations have great haplotype diversity, ranging from 1 to 0.7273, with the lowest values belonging to Arraga, Chuquisaca, and Jujuy (Table 1). These localities also have low values of nucleotide diversity and mean number of pairwise differences, whereas Villa Montes presents the highest values (Table 1).

The haplotype network (Figure 3) shows no genetic structure and an intricate pattern, being haplotypes differentiated by just one or few mutational steps. A noticeable star-like pattern around haplotype H3 (with the highest frequency) and around H30 (with a low frequency) can be observed. There are four other frequent haplotypes H21, H24, H26, and H31. All localities have more than one haplotype, which are scattered within the network. There are a few divergent haplotypes (diverging by four or more mutational steps): H7, H49, H50, H51, and H53. In summary, the haplotype network shows a complex pattern with no evidence of geographic structure.

Geneland v3.2.2 detected that $K = 3$ showed the highest probability; Figure 4 shows a map with an estimation of the geographic position of the three genetic clusters recovered. The northern cluster is composed of individuals from the locality of Chuquisaca. The central cluster includes individuals from Tarija and Villa Montes in Bolivia and Orán and Yuto in Argentina. Finally, the Southern cluster is composed of individuals of the rest of the localities (Arraga, Tucumán, Famaillá, El Jardín, Salta, and Jujuy).

Neutrality tests were negative and significant ($D = -1.84158$, $p < .05$; $F_S = -32{,}953$, $p < .001$). The mismatch distribution analysis shows a unimodal curve with its maximum in four pairwise nucleotide differences (Figure 5a). The sum of squared deviation (SSD) and Harpending's raggedness index were both non-significant (SSD = 0.0004, $p = .735$; Rag = 0.0111, $p = .506$), supporting the null hypothesis of population growth. The BSP analysis indicates that the demographic expansion started around 100,000 years ago (Figure 5b).

The spatial diffusion analysis shows that the origin of the species would have been close to the current locality of Yuto (Figure 6a), coinciding with the zone of the highest posterior probability of the central cluster in Geneland analyses. Around 200,000 years ago, the species migrated southward to Salta and Tucumán Argentinian provinces (Figure 6b), and around 170,000, it arrived to southern Bolivia (Figure 6c). Then, it remained with no range expansion for approximately 70,000 years and without contact between these zones. One hundred thousand years ago, the species started both a northward and southward range expansion which ended with the arrival to the localities of Chuquisaca and Arraga 60,000 years ago (Figure 6d,e). This expansion is coincident with the effective population size growth inferred in the BSP. After that, there were multiple mixing events among Salta, Tucumán, and Jujuy provinces (Figure 6f), which could have caused the complexity and the lack of structure observed in the median-joining network (Figure 3).

TABLE 1 Diversity indexes of the *Cyt-b* gene of *Calomys fecundus* for the 11 localities analysed. Number of individuals (*N* ind.), Number of haplotypes (*N* hap), Haplotidic diversity (*H*), Nucleotide diversity (π) and mean number of pairwise differences (*p*) are shown

Gene	Locality	*N* ind.	*N* hap	*H* (±*SD*)	π (±*SD*)	*p* (±*SD*)
Cyt-b	Chuquisaca	5	3	0.70 (0.2)	0.0019 (0.0015)	2.2 (1.4)
	Tarija	5	3	0.80 (0.2)	0.0033 (0.0023)	3.8 (2.3)
	Oran	2	2	1.00 (0.5)	0.0035 (0.0039)	4.0 (3.2)
	Yuto	24	16	0.93 (0.1)	0.0043 (0.0024)	5.0 (2.5)
	Jujuy	5	3	0.70 (0.2)	0.0028 (0.0020)	3.0 (2.0)
	Salta	4	4	1.00 (0.2)	0.0031 (0.0023)	3.5 (2.2)
	El Jardin	2	2	1.00 (0.5)	0.0017 (0.0021)	2.0 (1.7)
	Tucuman	9	7	0.94 (0.1)	0.0042 (0.0026)	4.9 (2.6)
	Famailla	25	15	0.96 (0.1)	0.0040 (0.0022)	4.5 (2.2)
	Arraga	12	5	0.73 (0.1)	0.0015 (0.0010)	1.7 (1.1)
	VillaMontes	4	4	1.00 (0.2)	0.0082 (0.0057)	9.3 (5.4)

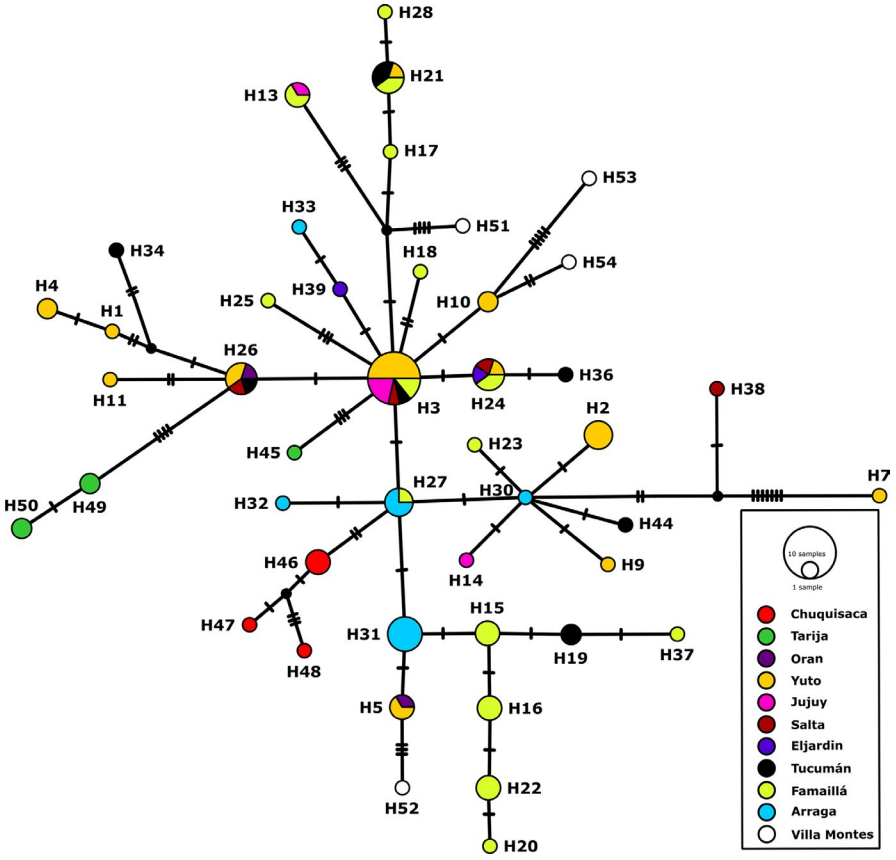

FIGURE 3 Haplotype network of the *Cyt-b* gene of *Calomys fecundus*. Colors indicate the localities where each haplotype was found. Circle sizes are proportional to haplotype frequencies. Segments between dashes indicate mutational steps; black dots represent missing intermediate haplotypes not observed in the analyzed individuals

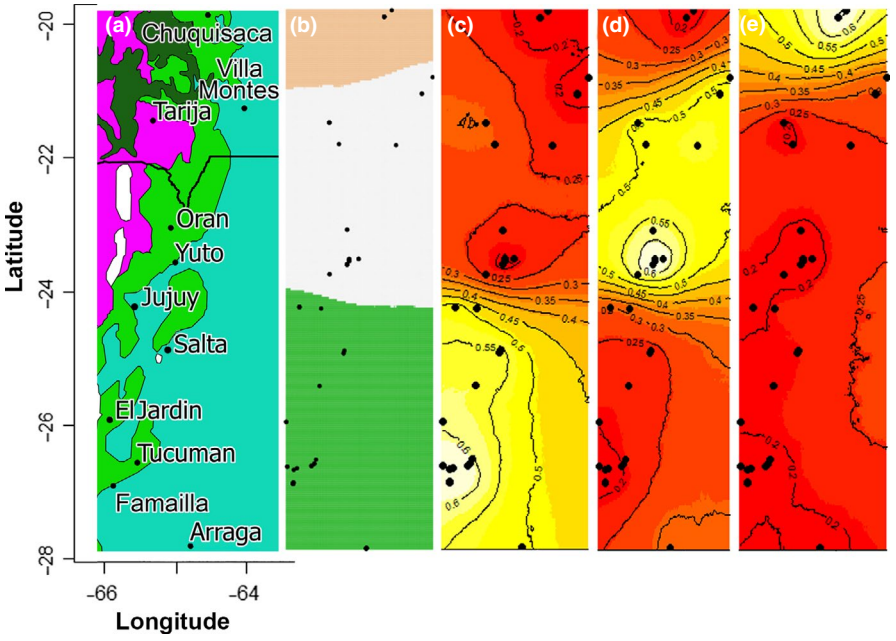

FIGURE 4 Maps of posterior probabilities of cluster membership based on Geneland analyses. Panel (a) shows the localities and ecoregions of Figure 1. Panel (b) shows a synthetic map of the mode of the posterior probability distribution for each pixel belonging to each inferred population. Panels (c–e) show maps of the studied area with the relative posterior probability of belonging to each of the three inferred population clusters: Black dots represent the geographical position of sampled locations, and lighter color reflects a higher posterior probability of membership to a cluster

3.3 | Current and paleoclimatic modeling

The current model (Figure 7a) shows a great association with the Tucumane–Bolivian Yungas and is well demarcated in the west by the Puna ecoregion and northwards by the Bolivian Montane Dry Forest (Figure 1). The eastern distribution is highly limited by the Chaco ecoregion, except for two suitable zones, one in the Argentinian province of Santiago del Estero, and the other in the Bolivian department of Chuquisaca.

Paleoclimatic models indicate that the species retracted its southern limit near the locality of Tucumán during the mid-Holocene

(6,000 years ago). The distribution is still well-delineated westwards, but the potential distribution expanded to the east. At this time, the distribution was less continuous than in the current model, showing three zones of high suitability: (a) a southern one in Tucumán, (b) a central one in Jujuy and the northern portion of Salta in Argentina, and (c) a northern one in Chuquisaca and Santa Cruz in Bolivia (Figure 7b). A similar pattern with three highly suitable zones is observed for the model of the LGM (21,000 years ago). The main difference between these periods is the expansion to the east (Figure 7c). During the last interglacial (130,000 years ago), the distribution models also showed the same three zones with high suitability, but the southern nucleus is broader than in the LGM. The eastern expansion is similar to the one of the LGM, and the southern limit expanded southwards, reaching similar latitudes than the current model (Figure 7d). The three suitable zones determined by the models are in concordance with the Geneland and the spatial diffusion results (Figures 4 and 6).

The most limiting factor analysis (Figure 8) shows that the temperature of the warmest month (Bio 5) limits the distribution of *C. fecundus* in the Chaco ecoregion at present; thus, a temperature of 39°C would be limiting the rodents' distribution. On the other hand, the minimum temperature of the coldest month (Bio 6) limits the range of the species in the west; scilicet, −3°C seems to be the lowest tolerable temperature. In the south, the species is limited by precipitations of the wettest month (Bio 13) and in the north by precipitations of the driest month (Bio 14) (Figure 8a). In the paleoclimatic models, temperature variables are less important in terms of distribution constraints, while precipitations of the wettest month (Bio 13) seem to gain relevance, being determinant in almost all the range limits of the species, excluding the northern limit which remains similar to the current model (Figure 8b–f).

The interpolation of the genetic diversity over the potential distribution shows that localities where the species have arrived last (Chuquisaca and Arraga) have low values of haplotype and nucleotide diversity indexes (Figure 9). Furthermore, the regions with the highest values of genetic diversity are coincident with the zones of high and stable suitability detected in all paleodistribution models.

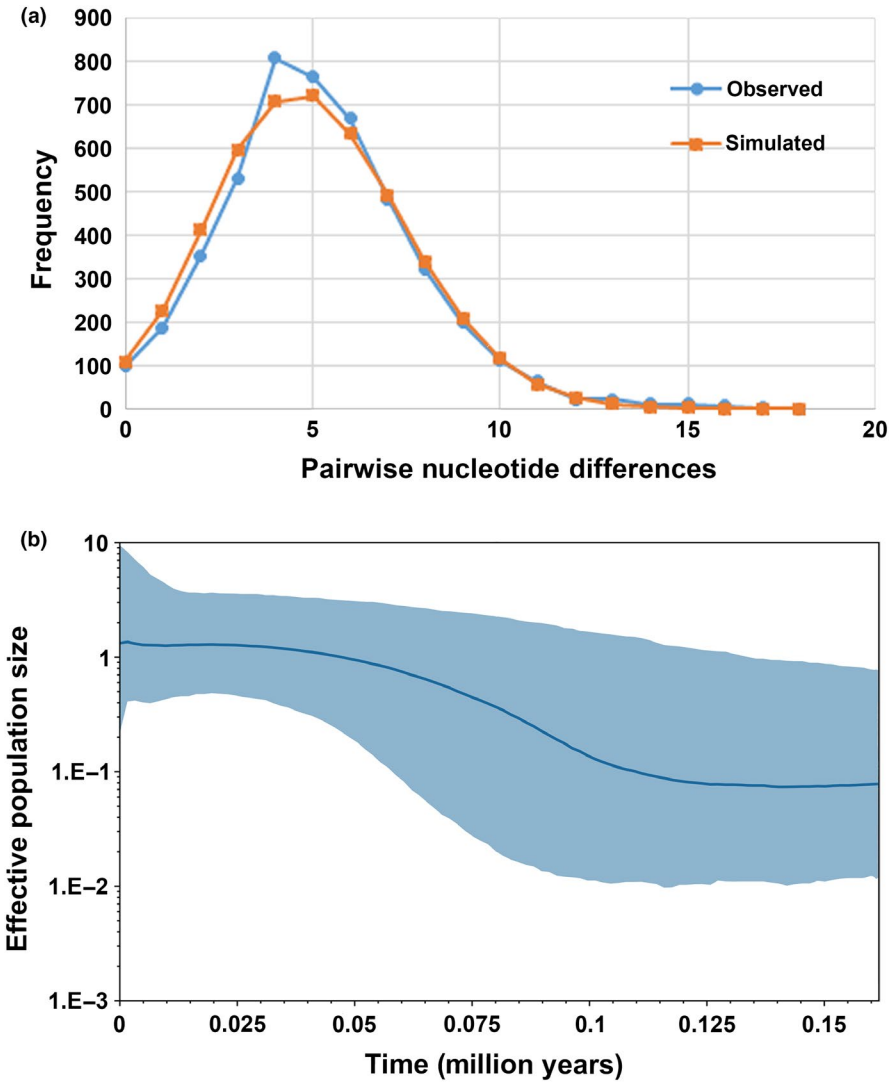

FIGURE 5 Inferred demographic history of *Calomys fecundus* using the *Cyt-b* gene sequence data: (a) Mismatch distribution analysis in which the blue line delineates the observed values and the orange line is the expected pattern under a model of population growth. (b) Bayesian skyline plot in which the *y*-axis represents the effective population size and the *x*-axis represents time in million years. The bold line represents the median value, while the light blue area shows the 95% highest posterior density limits

4 | DISCUSSION

4.1 | Genetic implications

In the present study, we characterized the genetic diversity of *C. fecundus* using two nuclear and one mitochondrial markers. Nuclear *i7Fgb* and *Rbp3* genes resulted uninformative because allele networks showed very low intraspecific polymorphism and alleles were shared among the species analyzed within the *C. callosus* complex. These two genes showed lower values of diversity indexes than the *Cyt-b* gene. This lack of resolution could be explained by the much lower substitution rates and by a fourfold larger effective population size of nuclear genes in comparison with mitochondrial DNA. These factors increase nuclear genes coalescence times (Avise, 2009). Considering the relatively short time elapsed since the divergence of these closely related species (Almeida, Bonvicino, & Cordeiro-Estrela, 2007), the nuclear allele networks obtained may be revealing incomplete lineage sorting and retention of ancestral polymorphisms. Probably, these genes could be useful for studies implicating larger scale interspecific phylogenetic relationships, but they are uninformative for intraspecific level studies.

Therefore, we continued with the mitochondrial *Cyt-b* gene data because it separate all the species of the *C. callosus* complex (Almeida et al., 2007) and the utility of this gene has been shown for phylogeographical studies of neotropical rodents (Da Silva et al., 2018; Machado et al., 2019). In the present study, the network revealed that only seven haplotypes are shared by two or more populations and that most genetic variants of each locality are intermingled in the genealogical reconstruction; these phenomena could be the evidence of secondary contact between the haplotypes. The low number of mutations between haplotypes could be caused by the short time elapsed since the formation of the species. All neutrality tests allow us to reject the neutral mutation hypothesis, being the negative sign of the indexes compatible with a recent range expansion of the species (Fu, 1997). This is also supported by the mismatch distribution and the Bayesian skyline plot results (Figure 5).

Geneland analyses recovered three clusters divided in a latitudinal sense. The spatial diffusion analysis also shows a pattern of diversification in a latitudinal sense, suggesting that the origin of *C. fecundus* occurred nearby the locality of Yuto around 220,000 years ago. After several expansion stages, the species would have reached the localities of Chuquisaca and Arraga around 60,000 years ago. These expansions are coincident with BSP analysis and would have caused the population mixing detected in the network.

4.2 | Niche modeling scenarios

The current potential distribution of *C. fecundus* shows a great association with the Tucumane–Bolivian Yungas, but also has two zones of high suitability in the Chaco ecoregion (Figure 7d).

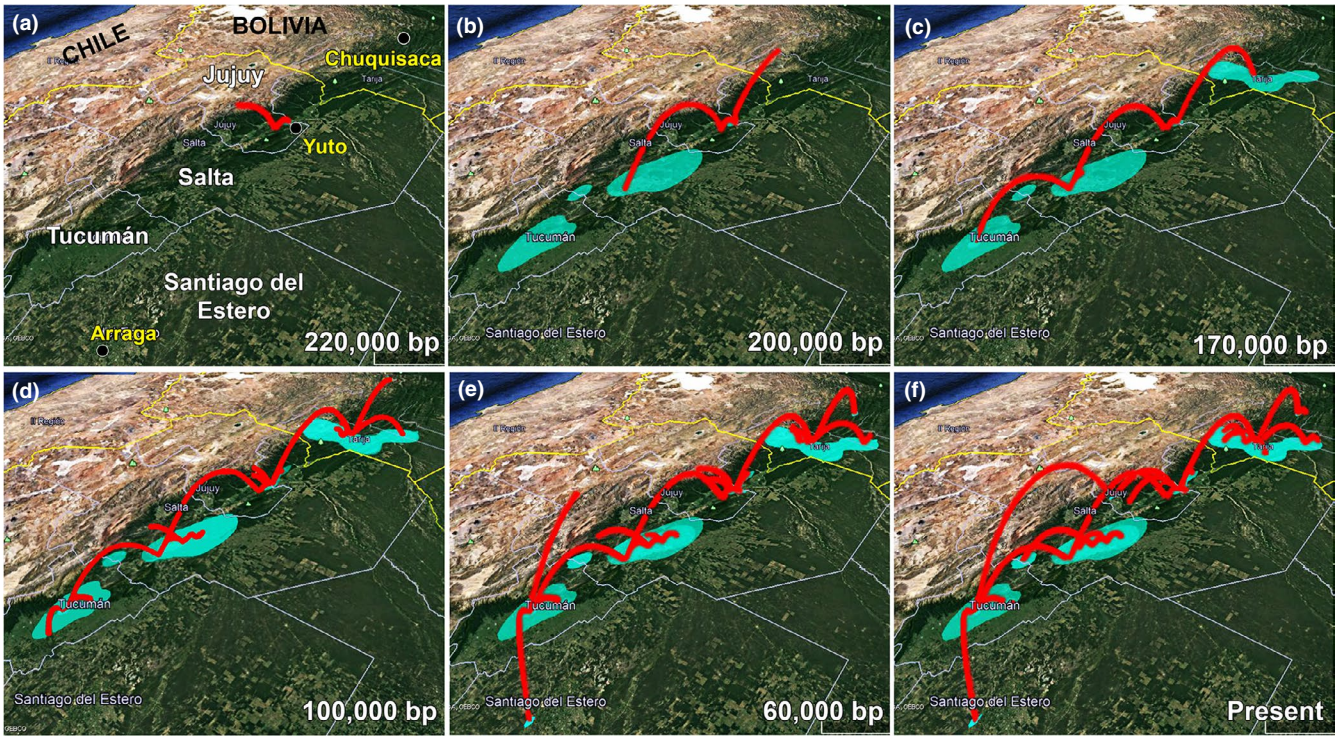

FIGURE 6 Bayesian spatiotemporal diffusion analysis of lineages of *Calomys fecundus* at different time points based on the maximum clade credibility tree. Panels (a - f) represent the time slices indicated in each image. Lines represent branches of the MCC tree; shaded areas: 80%-HPD uncertainty in the location of ancestral branches. In (a) fonts for Chile and Bolivia are in black, Argentinean provinces names in white and localities mentioned in the text in yellow

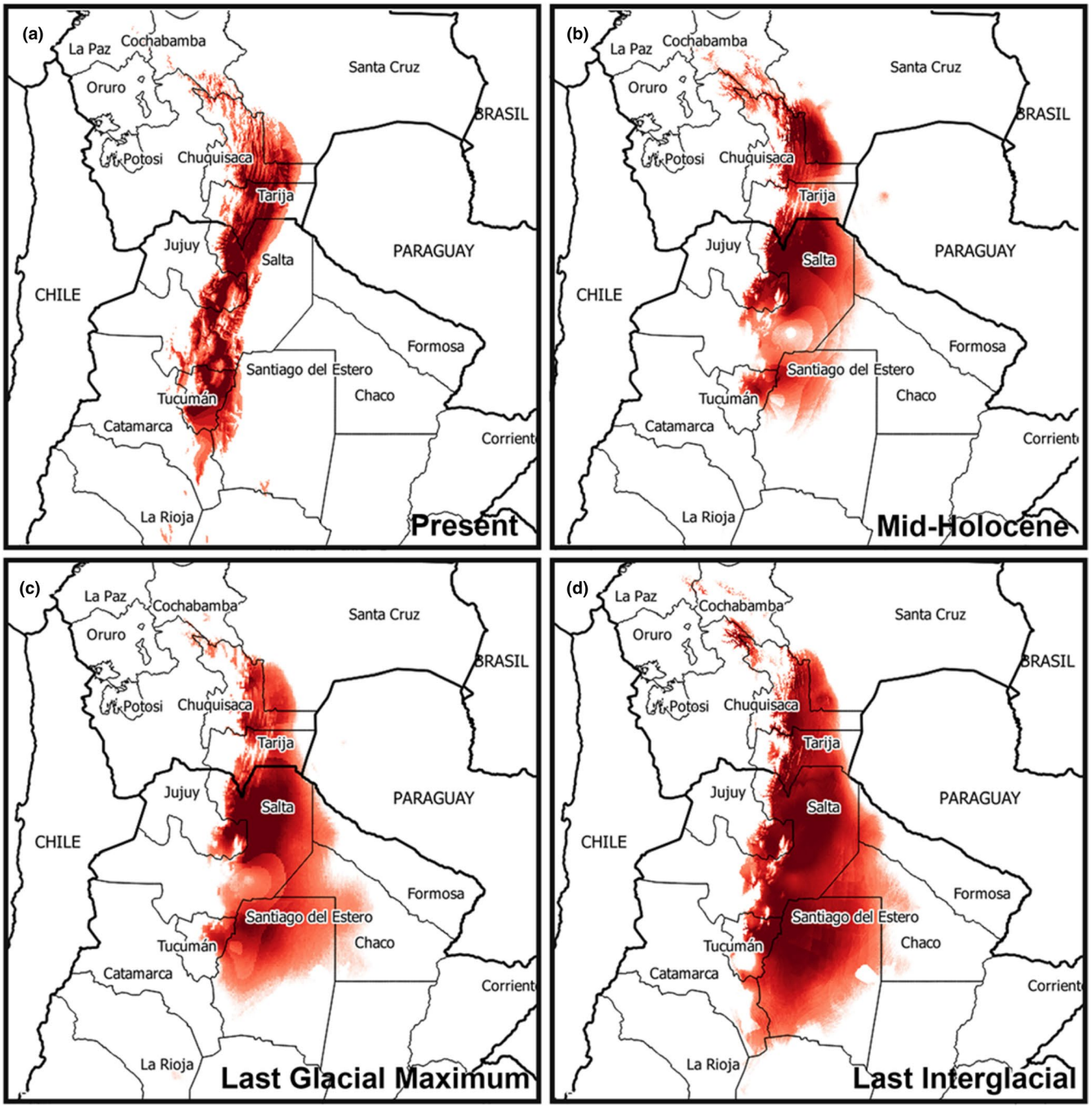

FIGURE 7 Spatial projections of *Calomys fecundus*' climatic niche across several climatic scenarios. (a) Current conditions, (b) Mid-Holocene (6 Kya), (c) Last Glacial Maximum (LGM; 21 Kya), and (d) last interglacial (LIG; 130 Kya). The red gradient indicates the habitat suitability for the species, with a darker red indicating higher suitability. The pROC value obtained after 500 iterations was 1.89 ± 0.02

Notably, according to the spatial diffusion analysis, these zones are the ones the species arrived last (Figure 6e,f). It is also remarkable that the model did not predict areas of presence in the Bolivian Yungas where the type locality of *C. boliviae* is found (Figure 1), reinforcing the idea that *C. fecundus* and *C. boliviae* may be separate entities. In all the paleoclimatic projections, the highest suitability values are coincident with the north, central, and south clusters recovered by Geneland analyses (Figure 7). This would indicate that these three zones had suitable and stable climatic conditions for *C. fecundus*.

The most limiting factors of the current distribution seem to be temperature variables, denoting a cold westward barrier and a hot eastward barrier. In the same way, the species is limited both northward and southward by precipitation variables (Figure 8). Noticeably, the precipitation variable of the wettest month (Bio 13) is the only important one in the paleoclimatic projections. This is plausible for

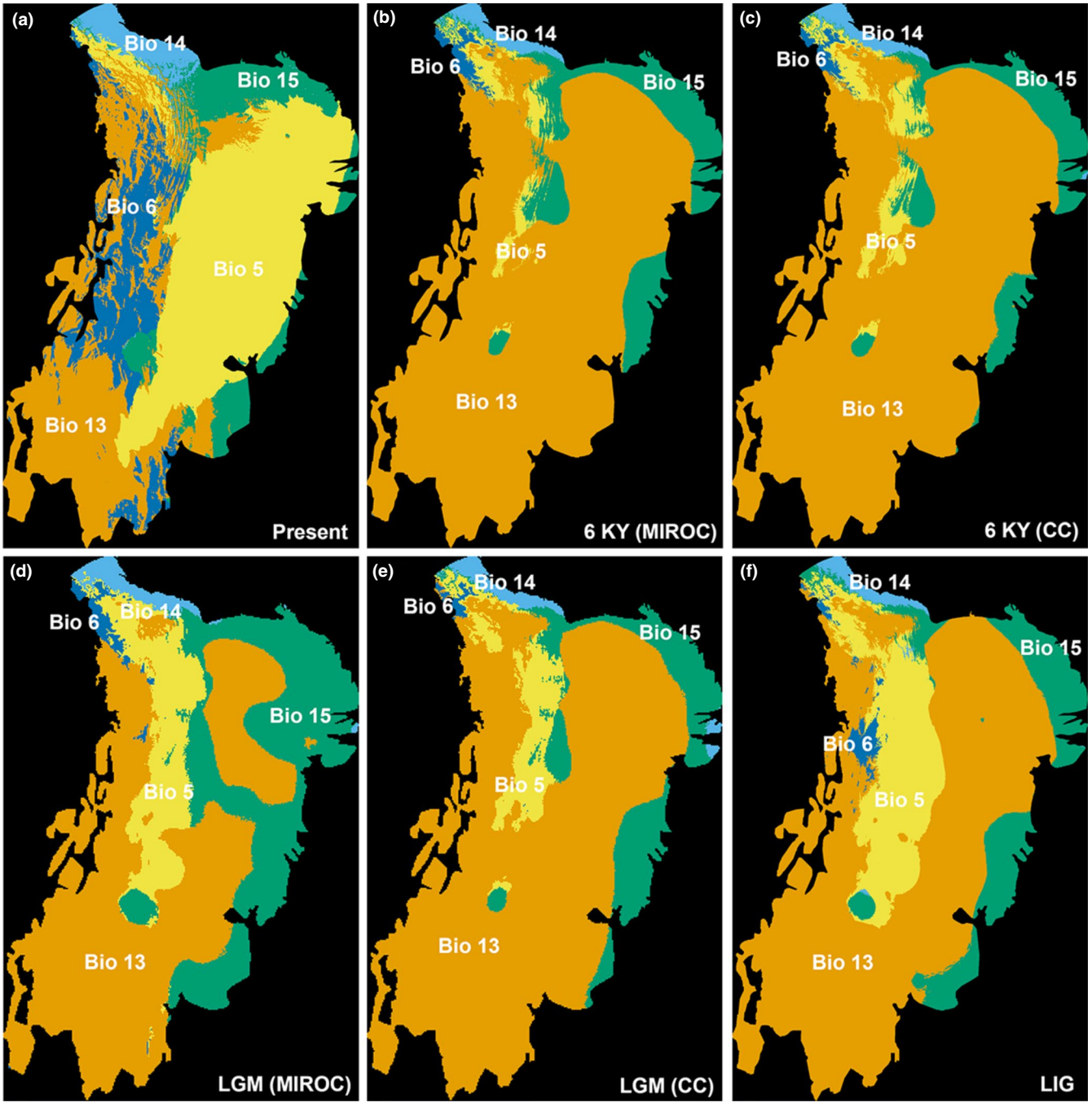

FIGURE 8 Most limiting factor analysis for *Calomys fecundus* across its historical accessible area applied to the following: (a) current climate, (b) Mid-Holocene MIROC (−6 Kya), (c) Mid-Holocene CCSM4 (−6 Kya), (d) Last Glacial Maximum MIROC (LGM, −21 Kya), (e) Last Glacial Maximum CCSM4 (LGM, −21 Kya), and (f) last interglacial (LIG, −130 Kya). Most relevant variables recognized as limiting: BIO5 = maximum temperature of warmest month, BIO6 = minimum temperature of coldest month, BIO13 = precipitation of wettest month, BIO14 = precipitation of driest month, and BIO15 = precipitation seasonality

the LGM, an epoch characterized by low temperatures and high aridity (Pennington et al., 2000). In the last interglacial and the mid-Holocene, maximum temperatures observed for Bio 5 are much lower than the maximum observed in the present (32.4°C and 35.3°C for the LIG and mid-Holocene periods, respectively, against 40.6°C for the present). This could be the cause of the broader areas suitable for the species in what is now recognized as Dry Chaco sub-ecoregion and the lack of limiting effect of temperature in the paleoclimatic projections.

The interpolation of nucleotide and haplotype diversity across the potential distribution of *C. fecundus* showed high values in the central and southern zones with climatic stability determined by the paleoclimatic projections (Figures 7 and 9). The northern stable zone has low values of genetic diversity, but this is congruent with the

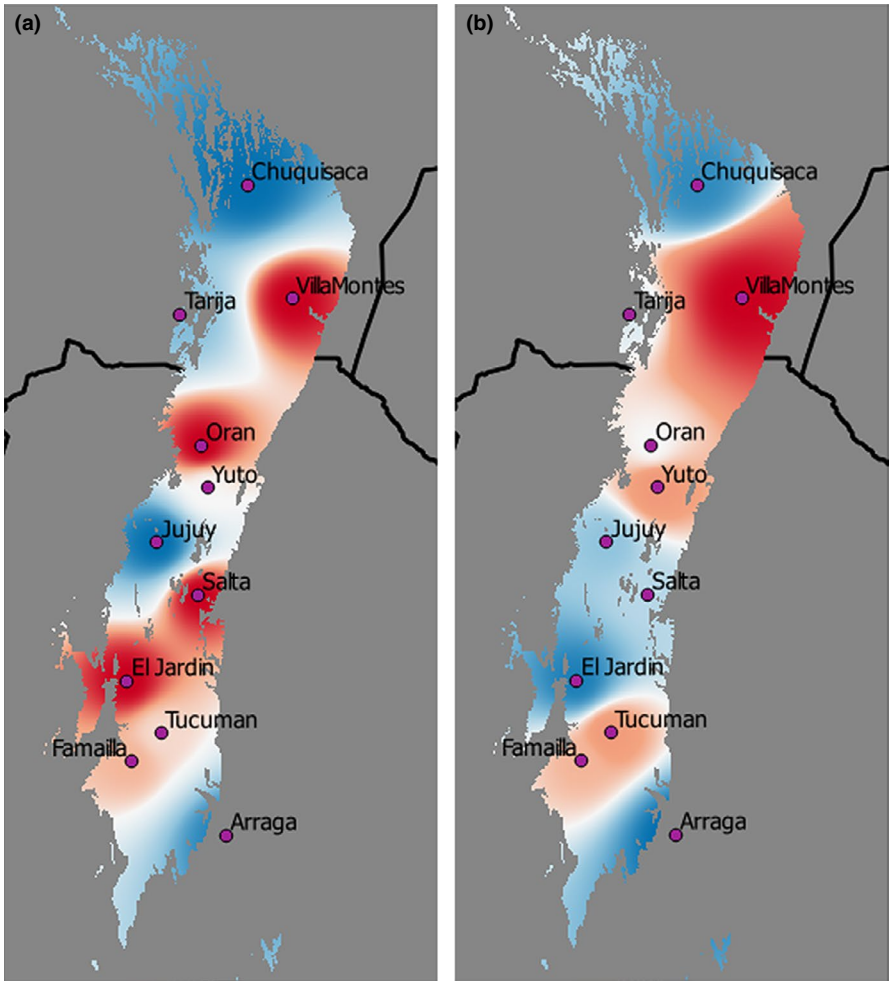

FIGURE 9 Inverse distance weighting spatial interpolation of haplotype (a) and nucleotide (b) diversity across the potential distribution of *Calomys fecundus*. Red colors indicate the highest and blue colors the lowest molecular diversity

spatial diffusion analysis, which detected that the species arrived later to this zone.

4.3 | Calomys fecundus evolutionary history

Considering the genetic and niche modeling results, we propose that the species exhibited a complex evolutionary history, suffering range expansions during glacial times and remaining stable in refugia during interglacial periods. The species would have originated 220,000 years ago in the actual upper basin of the Bermejo river, and later, it would have suffered a range expansion southwards and northwards during the RISS glaciation (around 200,000–170,000 years ago). Based on the Geneland analysis, the paleoclimatic projections, and the interpolation of the genetic diversity indices in the current potential distribution of *C. fecundus* (Figures 4, 7, and 9), we consider that the species presented two refugia between 170,000 and 100,000 years ago (during the last interglacial): one southern refugium, in the current area of the Tucumán province; and a central one, in Salta and Jujuy provinces. Coinciding with our results, a genetic study of *Cedrela lilloi* C. DC., a tree with logging importance, proposes the upper basin of the Bermejo river and the eastern slopes of Sierra de Aconquija in Tucumán as Pleistocenic refugia for this species (Inza, Zelener, Fornes, & Gallo, 2012). A study in the harvestman *Discocyrtus dilatatus* Sørensen, 1884, which also combined phylogeography and niche modeling tools, recovered the same putative refugia (Vergara, Acosta, González-Ittig, Vaschetto, & Gardenal, 2017).

During the WURM glaciation (approximately 100,000 years ago, according to the BSP and spatial diffusion analyses), the species suffered a second range expansion, reaching the actual limits of its distribution. From that moment, a third northern refugium appears in the department of Chuquisaca (Figure 7b,c). Apparently, after the expansion to Chuquisaca, this population remained isolated, as is shown by the spatial diffusion analyses. Besides, this population is the only geographic group in the haplotype network (H46, H47 and H48 in Figure 3). In some point between the present time and 6,000 years ago, the species suffered a secondary contact between the south and the central refugia (Figure 6f). This contact could have happened due to the onset of favorable pluvial conditions in the Pleistocene/Holocene boundary (Pennington et al., 2000).

This pattern of expansion during glacial cycles has also been reported for *Bulnesia sarmientoi* Lorentz ex Griseb., an emblematic tree species of the Dry Chaco (Camps, Martínez-Meyer, Verga, Sérsic, & Cosacov, 2018) and for the mentioned harvestman *D. dilatatus* (Vergara et al., 2017). During glaciations, semi-arid forests of the Chaco would have occupied a larger area and montane species

tended to occur in lowland areas (Colinvaux, De Oliveira, & Bush, 2000). As *C. fecundus* inhabits the Yungas and the ecotone Chaco–Yungas, these conditions could have promoted the species geographic range enlargement during the glacial periods.

4.4 | The Calomys fecundus-boliviae problem

We analyzed the phylogeography and paleodistribution of the lowland *Calomys* species which inhabits the Tucumane–Bolivian Yungas in the western slopes of the Subandean mountains. Our results indicate that the species has no genetic structuring and that it has never had a distribution beyond the Bolivian Montane Dry Forest (Figures 1 and 7). Therefore, we consider that *C. fecundus* is a valid taxon as was defined by Thomas 1926, with its type locality in Tablada, department of Tarija (−21.55 S, −64.78 W, 2,000 m.a.s.l). The species lives in the ecotone between the Argentinian Yungas and the Dry Chaco and is limited northwards by the Bolivian Montane Dry Forest and southwards by a climatic hot barrier (Figures 1 and 7d).

The holotype of *C. boliviae* Thomas, 1901 is located in the central Bolivian Yungas, with its type locality in Rio Solocame, La Paz, Bolivia (−16.35 S, −67.53 W, 1,200 m.a.s.l). Unfortunately, there are no genetic studies involving *Calomys* individuals from this zone. The evidence obtained in the potential distribution suggests that the Bolivian Montane Dry Forest could have acted as a barrier for the differentiation between *C. boliviae* and *C. fecundus*. Besides, adult females from the Bolivian Yungas have five pairs of mammae, while those from southern Bolivia and Argentina have seven pairs and are larger in body size than northern samples (Olds, 1988). This leads us to think that it would be necessary to perform integrative studies focused on the species inhabiting the Bolivian Yungas (putatively *C. boliviae*) to determine its taxonomic place within the genus *Calomys*.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Historical accessible area determined for *Calomys fecundus*.

Figure S2. Paleoclimatic projections of the models CCSM4 and MIROC.

Table S1. Specimens of *Calomys* included for genetic studies. The grouped localities are in bold, while the original localities are listed below with their corresponding geographic coordinates. For each individual, GenBank Accession Numbers for the markers used are also indicated. Sequences downloaded from GenBank are marked with †.

Table S2. Final presences used for modelling the potential distribution of *Calomys fecundus*.

Table S3. Diversity indexes of nuclear genes for the large-bodied species of *Calomys* present in Argentina. Number of individuals (*N* ind), number of alleles inferred (*N* alleles), allele diversity (*S*), nucleotide diversity (π) and mean number of pairwise differences (p) are shown.

File S1. Fasta file containing all sequences of the *Cytochrome b* gene (*Cyt b*) used in this study (available online).

File S2. Fasta file containing all alleles inferred for the intron 7 of the β Fibrinogen gene (*i7FGB*) used in this study (available online).

File S3. Nexus file containing all alleles inferred for retinol binding protein 3 (*Rbp3*) used in this study (available online).

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