Pleistocene climatic oscillations in Neotropical open areas: Refuge isolation in the rodent *Oxymycterus nasutus* endemic to grasslands

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**Abstract**

Pleistocene climatic oscillations favoured the expansion of grassland ecosystems and open vegetation landscapes throughout the Neotropics, and influenced the evolutionary history of species adapted to such environments. In this study, we sampled populations of the rodent *Oxymycterus nasutus* endemic to open areas in the Pampas and Atlantic Forest biomes to assess the tempo and mode of population divergence using an integrative approach, including coalescence theory, ecological niche models, and morphometry. Our results indicated that these *O. nasutus* populations exhibited high levels of genetic structure. Six major mtDNA clades were found, structuring these biomes into distinct groups. Estimates of their divergence times was indicated to be 0.571 myr. The high degree of genetic structure is reflected in the analyses of geometric morphometric; skull differences between lineages in the two ecoregions were detected. During the last glacial maximum, there was a strong increase in suitable abiotic conditions for *O. nasutus*. Distinct molecular markers revealed a population expansion over time, with a possible demographic retraction during the post-glacial period. Considering that all clades coalesce with the last interglacial maximum, our results indicated that reduction in suitable conditions during this period may have resulted in a possible vicariance associated with refuge isolation.

**Introduction**

The glaciations occurred in the Pleistocene (2.58–0.01 million years ago [myr]), a period of climatic history that is known with near accuracy [1], which led to the migrations and reductions in the population sizes of several species, followed by recolonization and population expansion as glaciers retreated [2–4]. Therefore, the divergence promoted by glaciers have probably affected populations distributed in allopatri, which could be detected in genealogies, as observed in typical glacial refuges [5].
In the recent years, some regions in the Neotropics have been systematically studied [6], as the high biological diversity provides an opportunity to test refuge hypotheses associated with climatic oscillations in the Quaternary Epoch, especially in complex ecoregions such as the Atlantic Forest [7,8]. A remarkable landscape in South America are the open areas, such as the Pampas biome, the largest temperate grasslands in the world [9], which are excellent environments to evaluate the influence of historical climatic fluctuations on the origin and evolutionary dynamics of species. The cool and dry weather during the glaciations allowed grassland ecosystems and open vegetation landscapes to expand over much of South America [10], which created new ‘suitable’ areas. Thus, it is expected that a species endemic to open areas respond to Pleistocene climatic oscillations in a different way than a species adapted to forest environments, [11–15], particularly, those that occupy heterogeneous grasslands ecoregions such as the Akodontine rodent *Oxymycterus nasutus* (Waterhouse, 1837).

*O. nasutus* is an abundant species adapted to wet areas, thriving at sea level in the southernmost Uruguay and Rio Grande do Sul State, Brazil, to higher elevations (up to 900 m) on the Santa Catarina and Parana States, Brazil, where it is restricted by the coastal mountains [16–17] (Fig 1). It inhabits coastal sandbanks, wet grasslands, steppes, and other phytosocieties of the Pampas, a biome that dominate Uruguay and the plains of southernmost Brazil. Moreover, it is highly dispersed throughout the southern Brazilian plateau in high-elevation field domains, and is probably the most abundant small mammal in the high-elevation grasslands in the states of Rio Grande do Sul and Santa Catarina (Atlantic Forest domain) [17–19].

The relationship between the grasslands and the forests during the late Quaternary is well documented in the studies on pollen records in the South and Southeast Brazil [20, 10]. Overall, general patterns of grasslands distributions up to the last glacial period in the Pleistocene (42,000–10,000 years BP) is linked with the rise of vegetation formations in the late Holocene [21]. In Southern Brazil, the expansion of grasslands was promoted by cold weather conditions and dry climate owing to the glaciation periods, with a mean decrease of temperature between 5–7°C in high-altitude (Meridional Plateau) areas [10, 21]. In addition, during the Last Glacial Maximum ([LGM] ~21,000 years BP), grasslands were also present abundantly in the coastal lowlands and on the exposed continental shelf, where forests were almost absent [22–23,10].

Since *O. nasutus* is intrinsically related to the grasslands and is abundantly found in these habitats [17–19], cyclic events of the dynamic expansion and retraction of open areas and forests that occurred during glacial and interglacial periods have probably influenced the evolutionary history of this species. Accordingly, to understand the tempo and mode of population divergence and to contribute insights into the aspects of the biogeographic history of the South America, we analysed the phylogeography, population genetics, and skull morphometry across the entire distributional range. We characterized the geographical patterns of genetic and morphological variation and used these data to investigate whether glacial and interglacial periods influenced the extant distribution of *O. nasutus* in distinct biomes, hypothesizing the existence of significant divergence between Pampas and Atlantic Forest lineages. Additionally, we estimated the current and historical potential distribution area of *O. nasutus* based on paleoclimatic models to allow a detailed evaluation of the demographic history and phylogeographical patterns.

**Materials and methods**

**Ethics statement**

Skull and tissue samples of *O. nasutus* surveyed in the study were obtained from the specimens deposited in the scientific collections (Museo Nacional de Historia Natural [MNHN], Museu de História Natural Capão da Imbuia [MHNCI], Museu de História Natural of the Universidade Maestri received fellowship from FAPERGS (16/2551-0000485-4) and CNPq (150391/2017-0), respectively. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Luterana do Brasil [MCNU], Fundação Zoobotânica-Museu de Ciências Naturais [FZB-MCN], Universidade Federal do Paraná [UFPR] and Universidade Regional de Blumenau [FURB]), who kindly lent the specimens for use in this study. Specimens deposited in the Universidade Federal do Rio Grande do Sul [UFRGS] were collected (previously) by our research group with the license issued by the Brazil Government authorities.

Sample collection

A total of 135 specimens of *O. nasutus* from 45 localities covering the entire known distribution of this species (Fig 1; Table 1) were surveyed. Field collection (permits granted by the Ministério do Meio Ambiente, Brazil—SISBIO 30204–1, 52650–1 and 29358–1) followed the guidelines of the American Society of Mammalogists for the use of wild mammals in research (https://www.mammalsociety.org/committees/animal-care-and-use). Specimens...
were euthanized via overdoses of isofluorane and cervical dislocation, procedures authorized by The Animal Care and Use Committee of the Institute of Biological Sciences at UFRGS, Brazil, for this study. Specimens trapped were deposited both in the mammal collections of UFRGS and MCNU.

**Phylogenetic relationships**

DNA was isolated from 107 specimens using the PureLink Genomic DNA extraction kit (Invitrogen, Life Technologies), following the manufacturer’s instructions. Polymerase chain reaction (PCR) was performed to amplify the mitochondrial DNA (mtDNA) cytochrome b (Cytb) gene (801 bp) and the nuclear locus beta-fibrinogen, intron 7 (Fgb-I7) (408 bp) according to the method described by Smith and Patton [24] and Matocq et al. [25]. The nuclear marker was chosen as it represents a single-copy locus, and is informative and evolving at a different rate compared to the mtDNA [26]. PCR products were stained with GelRed (Biotium) and checked on 1% agarose gel, purified with enzymatic method (Exonuclease and Alkaline Phosphatase; Amersham Biosciences, Piscataway, NJ) and sequenced with Sanger method using both primers (forward and reverse). All sequences obtained were deposited in GenBank under the accession numbers: Cyt b, MF766110 to MF766188 and Fgb-I7, MF766189 to MF766256.

Alignment and editing of the mtDNA Cytb sequences was performed in the Clustal W algorithm using the MEGA 7 software. For the nuclear Fgb-I7, sequences were aligned using MAFFT v.7.245 [26] with the auto setting. Variable sites of this nuclear marker were assessed in the original chromatograms to ensure correct identification of the heterozygotes. Heterozygous sites were identified when two different nucleotides were indicated at the same position in the chromatograms, with the weakest peak reaching at least 25% of the strongest signal. The IUPAC symbols were applied for coding the double peaks. The gametic phase of each haplotype was identified computationally using the program PHASE v2.1 [27, 28] as implemented in DnaSP v5.10 [29] to reconstruct putative alleles of the nuclear marker for use in downstream haplotype-based analyses. The program was run with 500 burn-in steps and 500 iterations, including the allowed intragenic recombination for the given data set. We also used a 0.6 output probability threshold for haplotypes and genotypes, as this was shown to reduce the number of unidentified genotypes with or without slight increase in the number of false positives [30].

The most appropriate substitution model for mtDNA phylogeny from the haplotypic data was the HKY+G that was determined based on the Akaike Information Criterion in the jModelTest v2.1.10 software [31]. The phylogeny was reconstructed using Bayesian Inference (BI) in the software BEAST 1.8.4 [32]. Two independent Markov Chain Monte Carlo (MCMC) runs, each with four streams per 25 million steps of the MCMC, sampled every 1000 generations, and discarding 2.5 million burn-in (about 10% trees discarded), starting the initial trees with randomness, without restriction were performed. Speciation tree prior was modelled on Birth-Death Process using the BEAST software. Parameter convergence was checked in Tracer v1.5 [33] as to whether effective sample sizes (ESS) reached 200. The remaining trees were used to calculate the posterior probabilities for each node.

The burn-in was determined in Tracer v1.6 based on the trajectory parameters, and 10% of the initial trees were removed and summarized in TreeAnnotator. The consensus tree generated was visualized and edited in Figtree v1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/). There were no fossils for *Oxymycterus* that could be used for a specific estimate of the substitution rate. Hence, we used an evolutionary rate already published [34] of 2.37% per million years (Myr⁻¹) for the genus. Accordingly, the divergence times were estimated in BEAST assuming an uncorrelated relaxed clock model and a normal distribution for the substitution
rate, with a mean of 2.37% Myr-1, and a standard deviation of 0.25% Myr-1, to allow some uncertainty in the evolutionary rate. One representative sequence for each haplotype was used. Sequences of *Oxymycterus delator* (U03525), *Oxymycterus dasytrichus* (AF454768), *Oxymycterus amazonicus* (AF454765), and sister lineages of *O. nasutus* [35], were included in the analysis. The time of the most recent common ancestor (TMRCA) for relevant nodes and major mitochondrial clades was reported as the mean value of node height with 95% highest posterior density interval (HPD). The nodes were supported with the posterior probability (PP) of ≥ 90% [36]. We also reconstructed the phylogenetic tree among nuclear DNA (nDNA) haplotypes (Fgb-I7) data set with phased alleles and single-copy sequences using BI in BEAST. However, due to the low variability and lack of support, we performed downstream analysis based on the haplogroups inferred by mtDNA. The topology obtained from Fgb-I7 evolutionary tree is shown in S1 Fig.

The evolutionary relationships between the haplotypes for each data set (i.e. mtDNA and nDNA) were estimated using the median-joining method implemented in the Network v5.0 [37] (http://www.fluxus-engineering.com). For assessing the evolutionary relationships of the Fgb-I7 gene, haplotypes were identified by coalescent-based Bayesian method implemented in PHASE v2.1. Missing/gaps sites were verified and invariant sites were removed from the data set. Indels were treated as single mutational events.

### Intraspecific variation and historical demography

Statistical parameters of diversity, such as the number of variable sites (S), number of haplotypes (h), haplotype diversity (Hd), nucleotide diversity (π), and average number of nucleotide differences (k) were measured using DNAsP v5.10 [29]). We calculated the genetic distance between the recognized haplogroups of *Cytb* (average distances) using p-distance model with 1000 bootstrap replications using MEGA v7 [38]. The defined clades were assumed based on the phylogeny obtained through Bayesian analysis.

Historical demographic changes such as signatures of demographic expansion, and equilibrium or decline were examined for the species and for each haplogroup using neutrality tests, such as Tajima’s D [39] and Fu’s Fs [40] statistics, employing the software DNAsP v5.10. Pairwise mismatch distribution was performed in DNAsP v5.10 to infer the historical demography of *O. nasutus*, calculated with the expected frequency based on a population growth-decline model. The sum of squared deviations (SSDs) between the observed and expected mismatch distribution and the raggedness index (r) were calculated to test the null hypothesis of spatial expansion using ARLEQUIN v3.5 [41]. In addition, we performed a Bayesian skyline plot (BSP) analysis, which does not assume a priori any growth model and infers the effective population size through time based on coalescent theory [42]. The BSP was used to estimate the dynamics of alterations in the overall population size over time for the *O. nasutus* dataset. BEAST v1.8.4 [32] was used for estimating the BSP of mtDNA as described above, with minor alterations in the number of parameters sampled at every 10,000 steps. For nDNA, a posterior distribution model of effective population size through time was generated using a MCMC sampling scheme. Two independent analyses were ran for 40 x 10^7 generations (sampling at every 10,000 and 10% burn in) under a HKY+G substitution model (obtained through the jModelTest 2 software), assuming a relaxed molecular clock model. However, we used the substitution rates and dates estimated from previous studies to calibrate a relaxed molecular clock and approximate divergence times for the main phylogroups retrieved in our study. We implemented a lognormal prior distribution based on the substitution rate for the cricetid genus *Peromyscus* (0.006 ± 0.003 substitution/site/million years) for the Fgb-I7 data set [43]. Skyline reconstruction was performed in Tracer v1.5, and the median and 95% credibility interval were plotted as a function of time.
Ancestral area reconstruction

Aiming to reconstruct the ancestral range of *O. nasutus*, we performed a Statistical dispersal–vicariance analysis (S–DIVA) on the maximum clade credibility tree generated from the BEAST analysis using the software RASP 3.02 [44–46]. The DIVA method [44] develops an approach based on parsimony events and reconstructs ancestral distributions based on a simple biogeographic model and a three-dimensional cost matrix. Penalties are not assigned when speciation is the result of vicariance; however, dispersal and extinction events have a penalty of one per unit area added to or deleted from a distribution. S-DIVA method rectified the problems in DIVA analysis and suggested possible ancestral ranges at each node and also calculated the probabilities for each ancestral range at the nodes. Due to the current area of occurrence and based on historical aspects of the region (expansion and retreat of forest formations), we reconstructed the ancestral areas only within the Pampas (A) and Atlantic Forest/grassland mosaic (B) regions. According to the results obtained by BI and the presence of restricted haplogroups in a single ecoregion, the clades recognized in *O. nasutus* were categorized in these areas. We used the output files generated by BEAST (total and condensed trees) for the analysis.

Morphological analyses

Digital photographs were taken from the dorsal, lateral, and ventral views of the skull of 89 specimens of *O. nasutus* (Table 1). Only adult specimens were photographed to minimize the ontogenetic effects (the complete eruption of the third molar was the criterion to separate juveniles from adults). Two-dimensional digital images were taken using a Canon PowerShot G10 camera with 14.7 megapixels resolution (4416 x 3312) in the macro function of the automatic mode and without flash or zoom. The pictures were taken from a standard distance of 127 mm for all the specimens. A total of 17 landmarks were digitized in the dorsal view, 16 landmarks in the lateral view, and 32 landmarks in the ventral view (S2 Fig), using the TpsDig2 software [47]. The same person (WTP) conducted the digitization. The choice of landmarks was based on previous studies with sigmodontines [48,49]. In the dorsal and ventral view, all individuals were marked on both sides of skull. The description of all the landmarks employed is given in S1 Appendix. After digitization, a Generalized Procrustes Analysis (GPA) was conducted on the matrix of landmark coordinates to remove the effects of scale, position, and orientation [50]. The centroid size, derived by the square root of the sum of squared distances of each landmark from the centroid of the configuration [51], was used as a measure of size. We appended the natural log-transformed centroid size into the matrix of shape coordinates to work in the form of space for further analyses.

Our goal was to investigate whether morphology is divergent among the haplogroups found in the genetic analyses (Northwest, Central, Eastern, Steppes Plain, Southern, and Taim Wetland), and also between physiognomies or “environmental groups” (Pampas versus Atlantic Forest). First, we conducted a principal component analysis (PCA) in the form matrix for each view. The number of PCs necessary to achieve 100% variation in each view (16 PCs for dorsal view, 31 PCs for ventral view, and 29 PCs for lateral view) were used as response variables for downstream analyses. We performed multivariate analyses of variance (MANOVA) and discriminant analyses with Jackknife cross validation (DA) to investigate how morphology was structured among the six genetic haplogroups (first predictor) and the two environmental groups (second predictor). MANOVAs and DAs were performed independently for each predictor and for each skull view. All procedures were carried out in the software R [52], with the packages geomorph [53] and Morpho [54]. Visualization of shape changes was made by comparing the shapes among groups using discriminant functions implemented in MorphoJ [55].
Table 1. Sampling localities of *Oxymycterus nasutus* in Southern Brazil and Uruguay.

| #  | Sample sitea | Lat. (S), Long. (W) | N | Voucherb | Haplotyp e | Cytb | Fgb-I7 | Haplogroup | Skull |
|----|--------------|---------------------|---|----------|------------|------|-------|------------|-------|
| 1  | BR: PR, Quatro Barras | -25.3658, -49.0769 | 4 | MHNCI 4605, 4607, 4608, 4595 | H1 | H3, H15, H24 | Eastern | + |
| 2  | BR: PR, Piraquara | -25.4419, -49.0627 | 8 | UFPR-P42, 53, 54, 56, 59, 60, 62, 65 | H1-H5 | H3, H17 | Eastern | - |
| 3  | BR: PR, Curitiba | -25.4789, -49.3307 | 1 | UFPR-P86 | H1 | - | Eastern | - |
| 4  | BR: PR, Curitiba, Parque Regional do Iguacu | -25.5229, -49.2229 | 1 | MHNCI 3433 | H1 | H1 | Eastern | + |
| 5  | BR: SC, Sã o José dos Pinha s | -25.5799, -49.1753 | 11 | UFPR-P969, 995*, 1008*, 1018*, 1019*, 1039*, 1049*, 1051*, 1053*, 1055, 1061 | H1 | H3, H18, H19 | Eastern | + |
| 6  | BR: SC, Castro | -24.7908, -50.0120 | 4 | MHNCI 816–818, 0821 | - | - | Central | + |
| 7  | BR: SC, Ponta Grossa | -25.2440, -50.0227 | 7 | UFPR-P76, MHNCI 642, 657, 709, 723, 838, 839 | H6 | - | Central | + |
| 8  | BR: SC, Sã o Mateus do Sul | -25.8738, -50.3827 | 1 | MHNCI 3192 | H7 | - | Northwest | + |
| 9  | BR: SC, Cândido | -25.5708, -52.0527 | 1 | CZFURB 18228 | H8 | H1; H2 | Northwest | + |
| 10 | BR: SC, Sã o Domingos | -26.6163, -52.5388 | 2 | CZFURB 18119, 18153 | H11 | H8, H11, H12 | Central | + |
| 11 | BR: SC, Agua Doce | -26.9977, -51.5558 | 2 | CZFURB 9365, 9856 | H12, H13 | H1, H3-5 | Central | + |
| 12 | BR: SC, Ponta Alta do Norte | -27.1153, -50.4577 | 2 | MHNCI 4951, 4596 | H9, H10 | H1, H10 | Northwest | + |
| 13 | BR: SC, Indaiatuba | -27.0830, -49.1166 | 1 | CZFURB 9825 | H10 | H15; H15 | Central | + |
| 14 | BR: SC, Abdon Batista | -27.6108, -51.0227 | 1 | CZFURB 20520 | H2 | H13; H14 | Central | + |
| 15 | BR: RS, Erechim | -27.6338, -52.2738 | 2 | CMLCE-UFRGS HFE 2, 4 | H2 | H1; H6, H7 | Central | - |
| 16 | BR: RS, Campo Belo do Sul | -27.9625, -50.8231 | 4 | CZFURB 15106*, 15109, 15140, 15154* | H2 | H8, H9 | Central | + |
| 17 | BR: RS, Vacaria | -28.511944, -50.933889 | 1 | MCNU 2498 | H2 | - | Central | - |
| 18 | BR: RS, Cambarã do Sul | -29.191667, -50.0975 | 2 | CMLCE-UFRGS AS5, 17 | H14 | H1, H14, H20 | Eastern | - |
| 19 | BR: RS, Sã o Francisco de Paula | -29.428322, -50.259444 | 10 | MCNU 3043, 3210, 3658, 3656*, 3657*, CMLCE-UFRGS PM 100, 104, 74, 79, 86 | H14, H15 | H1, H8, H16-17, H21-24 | Eastern | + |
| 20 | BR: RS, Montenegro | -29.682555, -51.466450 | 1 | FZB-MCN 547 | - | - | Steppes | + |

(Continued)
Table 1.  

| #   | Sample sitea                      | Lat. (S), Long. (W) | N  | Voucherb                  | Haplotype | Haplogroup | Skull |
|-----|-----------------------------------|--------------------|----|---------------------------|-----------|------------|-------|
| 21  | BR: RS, Eldorado do Sul           | -29.997139, -51.307861 | 1  | FZB-MCN 675              | H16       | H1, H3, H24, H26, H33-34 | Steppes Plain + |
| 22  | BR: RS, Guaíba                    | -30.113889, -51.325  | 11 | MCNU 3211, 3652, 3228, 3119, 3009, 3141, 3146*, 3142, 3149, 3212, 3653 | H16, H17, H18 | H1, H33-35 | Steppes Plain + |
| 23  | BR: RS, Barra do Ribeiro          | -30.290833, -51.300833 | 6  | MCNU 3144, 3654*, 3147, 3230, 3039, 3135 | H16, H17, H18 | H1, H33-35 | Steppes Plain + |
| 24  | BR: RS, Sentinel do Sul           | -30.610833, -51.578889 | 1  | MCNU 314               | H19       | H1; H29      | Steppes Plain + |
| 25  | BR: RS, Tapes                     | -30.669849, -51.429707 | 1  | MCNU 3132             | H19       | -           | Steppes Plain + |
| 26  | BR: RS, Camaquã                    | -30.850833, -51.811944 | 10 | CMLCE-UFRGS FQ 47; MCNU 3011, 3012, 3110, 3116, 3122, 3124, 3133, 3214, 3227; CZFURB 6249*, 6250 | H20-H24 | - | Steppes Plain + |
| 27  | BR: RS, Cristal                    | -31.002778, -52.050  | 3  | MCNU 4331; CMLCE-UFRGS FQ 63, 72 | H22 | H1; H30 | + |
| 28  | BR: RS, São Lourenço do Sul       | -31.365, -51.977778 | 5  | MCNU 3225, 3109, 3123, 3115, 3010 | H19, H26 | H1, H26; H31 | Steppes Plain + |
| 29  | BR: RS, Rosário do Sul            | -30.247938, -54.924036 | 1  | FZB-MCN 648           | -         | -           | Steppes Plain + |
| 30  | BR: RS, Dom Pedro                  | -30.975882, -54.666567 | 2  | FZB-MCN 710, 1011      | -         | -           | Steppes Plain + |
| 31  | BR: RS, Bagé                       | -31.330833, -54.106944 | 2  | CMLCE-UFRGS ALL 12, 13 | H25       | H1, H25-27 | Steppes Plain - |
| 32  | BR: RS, Pelotas                    | -31.771944, -52.342778 | 4  | CMLCE-UFRGS PL 300; MCNU 3223, 3041,3042 | H19, H27 | H1, H28-30 | Steppes Plain + |
| 33  | BR: RS, Rio Grande                 | -32.035, -52.098889 | 2  | CMLCE-UFRGS MEV 01; MCNU 3014 | H31       | H31 | Taim Wetland - |
| 34  | BR: RS, Rio Grande, ESEC Taim     | -32.7425, -52.574444 | 3  | MCNU 3661*, 3131, 3660  | H32, H33      | H31 | Taim Wetland + |
| 35  | BR: RS, Rio Grande, APA Lagoa Verde| -32.139934, -52.181064 | 1  | MCNU 3660               | H33       | -           | Taim Wetland + |
| 36  | BR: RS, Pedro Osório               | -31.863889, -52.822778 | 4  | CMLCE-UFRGS POS 18, 20, 25, 27 | H28, H29 | H26, H36 | Southern - |
| 37  | BR: RS, Herval                     | -32.023889, -53.395833 | 1  | CMLCE-UFRGS HL 01      | H30       | H31; H37   | Southern - |
| 38  | UY: Rocha, Parque Santa Teresa     | -34.008180, -53.552735 | 1  | MNHN SN EMG1809        | -         | H3; H3 | Southern - |

(Continued)
Geographic distribution maps and modelling

Ecological niche modelling (ENM) was carried out in MAXENT v 3.3.3e [56] to predict suitable present and past potential distribution areas of *O. nasutus* based on climatic variables. Such modelling has been performed favourably compared to other analytical alternatives for presence-only data [56–59]. Information on the geographic distribution of the species was based on 51 occurrence records obtained from literature and collections (S2 Appendix). We verified the accuracy of the geo-referenced data in ArcGIS v 10.0. To avoid data clustering [60], we limited our database to a single record per km$^2$. For modelling settings, the function ‘Auto features’ was selected, and the distributions were modelled through the ‘cross-validate’ parameter, applying a maximum number of iterations at 500. We verified model performance using the area under the ‘Receiver Operating Characteristic (ROC) Curve’ (AUC) calculated by MAXENT. Values between 0.7 and 0.9 indicated good discrimination [61].

Projections for the past climatic conditions were developed for three periods: LGM c. 21 ka; Last InterGlacial (LIG) c. 120–140 ka; and mid-Holocene c. 6 ka. Current and past distributions were modelled using 19 bioclimatic data layers available from the WorldClim database (http://www.worldclim.org) at 30 arc-sec resolution (~1 km$^2$), with the exception of the layers

### Table 1. (Continued)

| Sample site* | Lat. (S), Long. (W) | N | Voucher* | Haplotype | Cytb | Fgb-I7 | Haplogroup | Skull |
|--------------|---------------------|---|----------|-----------|-------|--------|------------|-------|
| 39 UY: Rocha, Laguna de Castillos | 34.35, -53.866667 | 2 | MNHN SN SCV 108, 110 | H34 | H38, H29; H30 | Southern | - |
| 40 UY: Rocha, Route 9 km 304.800 | 34.257743, -54.064845 | 1 | MNHN SN GD 577*** | H35 | - | Southern | - |
| 41 UY: Rocha, La Paloma, La Palma | 34.655896, -54.181969 | 2 | MNHN SN CA 614, 617** | H34 | H24; H24 | Southern | + |
| 42 UY: Maldonado, San Carlos | 34.915632, -54.865456 | 2 | MNHN SN GD 723; MVZ 182701 (CA458)** | H37, H38 | H3, H31 | Southern | - |
| 43 UY: Maldonado, Pan de Azucar | 34.779218, -55.232399 | 1 | MNHN SN CA 680* | - | - | Southern | + |
| 44 UY: Maldonado, Solís Grande | 34.783273, -55.334011 | 1 | MNHN SN CA 695** | H37 | - | Southern | + |
| 45 UY: Canelones, La Floresta | 34.770278, -55.588333 | 1 | MNHN 5615 (EMG1567) | H36 | H26; H31 | Southern | - |

Localities (#) are mapped in Fig 1. Vouchers, Cytb/Fgb-I7 haplotypes and presence (+)/absence (-) of skull samples per site are presented.

*Abcervations: BR: Brazil, UY: Uruguay; PR, Paraná; SC, Santa Catarina; RS, Rio Grande do Sul states.*

*Acronyms of collections: UFPR-P, Scientific Collection of the Cytogenetic and Conservation Laboratory at the Universidade Federal do Paraná; MHNCI, Museu de História Natural Capão da Imbuia; CZFURB, Zoological Collection of the Universidade Regional de Blumenau; CMLCE-UFGRGS, Mastozoological Collection of the Cytogenetic Laboratory and Evolution at the Universidade Federal do Rio Grande do Sul; MCNU, Museu de História Natural of the Universidade Luterana do Brasil; MNHN, Museo Nacional de Historia Natural (Uruguay); FZB-MCN, Fundação Zoobotânica-Museu de Ciências Naturais; MVZ, Museum of Vertebrate Zoology at the University of California/Berkeley.

**Vouchers presenting only skulls.

**Data obtained from NIH genetic sequence database (Genbank; https://www.ncbi.nlm.nih.gov/genbank/)

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from the LGM period, which were available in 2.5 arc-min resolution (~5 km²). Climatic variables for the present study represented the average climate changes from 1950 to 2000. Projections for the LGM and mid-Holocene were derived from the CCSM4 [62] atmosphere-ocean general circulation models (AOGCM). All the analyses were performed in QuantumGIS 2.18 software.

**Results**

**Intraspecific genetic variation and evolutionary patterns**

We identified 73 variable sites in the mtDNA dataset resulting in 38 haplotypes and 32 variable sites in the Fgb-I7 locus resulting in 40 haplotypes (with one 2-bp indel) (Tables 1 and 2). Standard diversity indices (haplotype diversity, nucleotide diversity, mean number of pairwise differences) for both the markers are presented in Table 2.

Bayesian consensus tree based on mtDNA dataset depicted six highly supported (BPP > 0.90) haplogroups corresponding to two distinct biomes: Northwest, Central, and Eastern clades are distributed along the Atlantic Forest biome, and Steppes Plain, Taim Wetland, and Southern clades assigned to the Pampas biome. However, the relationship between these groups was not entirely clear, considering that they did not cluster in major clades exclusive to each of the two biomes (Fig 2). The haplotype network based on the mtDNA also supported such tree structure. Nonetheless, despite the several mutational steps between haplogroups, the haplotype network showed several median vectors indicating non-sampled or extinct ancestral sequences (Fig 3). The two main widespread haplogroups were Central and Southern clades. The Central clade occurred throughout the domain of Atlantic Forest, mainly dispersed over the Araucaria forests and the mosaic of forest/highland grasslands (Fig 2). This clade was

Table 2. Genetic variability of *O. nasutus* using mitochondrial (Cytb) and nuclear (Fgb-I7) markers.

| Group          | S  | N_H | Hd ± SD     | π ± SD     | k     | Fu-Fs (P-value)       | Tajima’s D (P-value)       |
|----------------|----|-----|-------------|------------|-------|-----------------------|---------------------------|
| **Cytb:**      |    |     |             |            |       |                       |                           |
| Northwest      | 4  | 12  | 4.0000 ± 0.1768 | 0.00885 ± 0.00256 | 6.83  | -0.12436 (0.2570)    | 0.44358 (0.7550)          |
| Central        | 13 | 10  | 0.6282 ± 0.1431 | 0.00229 ± 0.00093 | 1.76  | -0.36009 (0.3760)    | -1.80161 (0.0160)*        |
| Eastern        | 18 | 8   | 0.7451 ± 0.0790 | 0.00280 ± 0.00037 | 2.16  | -0.24573 (0.4710)    | -0.24280 (0.4240)         |
| Steppes Plain  | 30 | 18  | 0.8874 ± 0.0329 | 0.00379 ± 0.00047 | 2.92  | -3.34189 (0.0670)    | -1.22763 (0.1100)         |
| Southern       | 13 | 11  | 0.9103 ± 0.0559 | 0.00422 ± 0.00065 | 3.25  | -1.96098 (0.1040)    | -0.32877 (0.4000)         |
| Taim Wetland   | 4  | 6   | 0.8333 ± 0.2224 | 0.00410 ± 0.00172 | 3.16  | 0.81143 (0.5720)     | -0.31446 (0.5510)         |
| All            | 82 | 73  | 0.9624 ± 0.0083 | 0.01190 ± 0.00066 | 9.18  | -11.24103 (0.0070)** | -1.23865 (0.0720)         |
| **Fgb-I7:**    |    |     |             |            |       |                       |                           |
| Northwest      | 3  | 8   | 0.9333 ± 0.1217 | 0.00640 ± 0.00123 | 3.66  | -0.90493 (0.2050)    | -0.60642 (0.4550)         |
| Central        | 14 | 14  | 0.9500 ± 0.0364 | 0.00606 ± 0.00062 | 3.45  | -4.51905 (0.0060)**  | -1.22325 (0.1120)         |
| Eastern        | 16 | 13  | 0.8407 ± 0.0557 | 0.00481 ± 0.00053 | 2.92  | -5.23937 (0.0140)*   | -0.90384 (0.1990)         |
| Steppes Plain  | 29 | 11  | 0.7629 ± 0.0538 | 0.00293 ± 0.00052 | 2.00  | -3.31470 (0.0160)*   | -1.05889 (0.1440)         |
| Southern       | 9  | 13  | 0.9216 ± 0.0417 | 0.00639 ± 0.00098 | 3.53  | -3.67515 (0.0280)    | -0.68862 (0.2940)         |
| Taim Wetland   | 3  | 0   | -             | -           | -     | -                     | -                         |
| All            | 68 | 32  | 0.8778 ± 0.0225 | 0.00497 ± 0.00034 | 2.98  | -26.44071 (0.00)***  | -1.93728 (0.0050)**       |

Groups are based on Cytb phylogenetic inferences (see Fig 2). Neutrality tests are indicated by Fu’s FS and Tajima’s D.

N ind: Number of individuals sequenced, S: Number of segregating sites, N_H: number of haplotypes; Hd: Haplotype diversity, π: Nucleotide diversity, SD: Standard deviation; k = Mean number of pairwise differences.

*P<0.02 or **P<0.01 for Fu’s FS or Tajima’s D, respectively.

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distributed across the entire extension of the Araucaria Forest coverage. The Southern clade was dispersed along the Pampas biome in Uruguay and the southernmost of Brazil, a region dominated by steppes or grassland. The other haplogroups were isolated and covered smaller areas when compared to Central or Southern haplogroups, except for Eastern clade with two isolated zones of occurrence. The most diversified haplogroup was the Steppes Plain, including 12 distinct haplotypes dispersed throughout the state of Rio Grande do Sul, Brazil (Table 1).

Only Piraquara locality in Parana state, Brazil, comprised haplotypes from two distinct mtDNA clades (Fig 1). nDNA network revealed 36 low-frequency haplotypic variants, with a reticulate evolutionary relationship.

The majority of haplotypes differed by one substitution site. None of the six major clades in the mitochondrial tree was recovered for the Fgb-I7 sequences. However, nuclear haplotype H1 was present in almost all mtDNA clades (except Taim Wetland). All mtDNA clades showed the presence of unique alleles, but Taim Wetland group shared the haplotype 31 with Steppes Plain and Southern clades.

The genetic distances between the Bayesian clades ranged from 1% to 2.5% for mtDNA, whereas it was close to 0 for Fgb-I7 (S1 Table).

The most common ancestor for all clades of *O. nasutus* was estimated to the middle Pleistocene (0.5715 myr; 95% HPDs = 0.3657–0.8471 mya) (Fig 2, Table 3). The mtDNA haplotypes (n = 38) clustered into six lineages; individual clades diverged between 265.8 and 147.3 myr (HPDs = 0.438.8–0.046.7 myr). The ancestral haplotype could not be inferred for this marker due to the presence of several median vectors in the network. However, the BEAST-derived tree indicated H7 (Northwest clade) as the oldest clade. Tajima’s D and Fu’s Fs tests were negative and non-significant for both mtDNA and nDNA markers, indicating that *O. nasutus* might have experienced a recent population expansion (Table 2).
Only Fu’s Fs test showed significant P values for mtDNA ($P < 0.01$). Considering the mtDNA, only the Central haplogroup depicted significant P values in Tajima’s D test (-1.80161, $P = 0.0160$), indicating an ongoing demographic expansion or mutational selection (too many

![Evolutionary relationship of O. nasutus haplotypes.](https://doi.org/10.1371/journal.pone.0187329.g003)

**Fig 3. Evolutionary relationship of** *O. nasutus* **haplotypes.** Median joining network based on the mitochondrial Cytb fragment and the nuclear Fgb-I7 locus. Color coding denotes the major mtDNA clades obtained in the Bayesian dated phylogeny (see Fig 2).

Table 3. Estimates of the time to the most recent common ancestor (TMRCA) for the nodes addressed in this study using unique haplotypes for each of the mitochondrial and overall clades for *O. nasutus.*

| Clade          | TMRCA (Ma) | 95% HPD       |
|---------------|------------|---------------|
| Northwest     | 0.2658     | 0.1275–0.4388 |
| Central       | 0.1529     | 0.0691–0.2675 |
| Eastern       | 0.1658     | 0.0689–0.2894 |
| Steppes Plain | 0.1911     | 0.1057–0.3017 |
| Southern      | 0.1822     | 0.0905–0.3087 |
| Taim Wetland  | 0.1473     | 0.0467–0.2902 |
| All           | 0.5715     | 0.3657–0.8471 |

[https://doi.org/10.1371/journal.pone.0187329.t003](https://doi.org/10.1371/journal.pone.0187329.t003)
segregating sites/too few pairwise differences). On the other hand, Northwest haplogroup showed positive values for Tajima’s D test (0.44358, P = 0.7550), which could be an indicator for a contraction (too few segregating sites/too many pairwise differences). Central, Eastern, and Steppes Plain clades showed significant P values for Fu’s Fs tests performed on nDNA marker, as expected from a recent population expansion. This indicated recent demographic expansion or departure from the null hypothesis of selective neutrality and population equilibrium. Nonetheless, a unique haplotype for nDNA was found in Taim Wetland mtDNA clade, which did not allow neutrality tests to be performed. This clade also showed positive values for the Fu’s Fs test for mtDNA, thereby suggesting recent population bottleneck for this lineage (Table 2).

The results of the mismatch distribution for mtDNA and nDNA analysis was approximately unimodal (Fig 4). Non-significant SSD statistic (SSD = 0.00112542, P = 0.944) and raggedness index value (r: 0.0032, P = 0.9770) under the spatial expansion models failed to reject the spatial expansion model. Similar patterns suggested demographic expansion for nDNA (SSD = 0.00322295, P = 0.570; r: 0.01631205, P = 0.780). Strong evidence of demographic expansion came from the BSP. The results showed different patterns between the mitochondrial and the nuclear datasets. For the mtDNA, a constant population size was noted over the last 0.375–1 mya after a long phase of demographic stability; the population then appeared to have experienced an accelerated demographic expansion phase approximately 0.030–0.300 myr, followed by a decrease in population size after 0.030 myr. For the nDNA marker, results showed a slow growth in the effective population size across time (0.400–1.2 mya), demographic stability for 30–400 myr, with smooth decrease posteriorly (Fig 4).

Fig 4. Demographic history of *O. nasutus* with signatures for population expansion. A, mismatch distributions of pairwise differences of Cytb and Fgb-I7 haplotypes obtained under a model allowing expansion. B, Bayesian Skyline Plot for Cytb and Fgb-I7 datasets. Bold lines indicate the median of effective population size through time and the coloured lines represent the 95% highest posterior densities over the median estimates along the coalescent history of the species.

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Skull morphometric variation

The occupation of different physiognomies, or environmental groups (Atlantic Forest or Pampas) explained 17.3% of the variation in the dorsal view (Wilk’s $\Lambda = 0.271$, $p < 0.001$), 17.9% in the ventral view (Wilk’s $\Lambda = 0.23$, $p < 0.001$) (Fig 5), and 15.4% in the lateral view (Wilk’s $\Lambda = 0.31$, $p < 0.001$) of the *O. nasutus* skull (S3 Fig). Genetic haplogroups indicated 24.7% of the variation in the dorsal view (Wilk’s $\Lambda = 0.084$, $p < 0.001$), 26.3% in the ventral view (Wilk’s $\Lambda = 0.014$, $p < 0.001$) (Fig 5), and 23.5% in the lateral view (Wilk’s $\Lambda = 0.021$, $p < 0.001$) of the *O. nasutus* skull (S3 Fig). The percentage of correct classification among the haplogroups were: 62.92% for the dorsal view, 65.16% for the ventral view, and 55.05% for the lateral view. Among the environmental groups, the percentages of correct classification were: 93.25% for the dorsal view, 82.02% for the ventral view, and 80.89% for the lateral view. The percentages of correct classification were similar between the genetic and the environmental haplogroups, considering the total number of groups in each one (i.e. six haplogroups would result in ~16% of the correct classification by chance, while with two groups this percentage will rise to 50%; deviations from the random classification are similar between the predictors). Detailed results of discriminant analyses can be found in the S2 and S3 Tables.

Ecological niche modelling and ancestral area reconstruction

Relatively high AUC values showed an excellent predictive power of the ENMs (current, AUC: 0.952; MID-HOL, mean AUC: 0.952; LGM, mean AUC: 0.952; LIG, AUC value: 0.958). The
ENM results (Fig 6) and the potential LGM distributions were more widespread. On the other hand, the potential distribution of MID-HOL was reduced, which showed more similarities with the current conditions. The potential distributions of the LIG were the most restricted of all the ENMs. Suitable habitats in the LGM showed a great expansion in relation to the LIG. However, in all the models tested, areas of low suitability present between the Pampas and...
Atlantic Forest domains were identified, as well as in the western part of the territory of Uruguay. In addition, the coastal areas did not have suitable conditions during the LGM.

According to S-DIVA analysis (Fig 7), Pampas ecoregion (A/AB, node 1) was the most likely region for the origin of the dispersal of *O. nasutus*. Dispersal events probably progressed from Pampas domain (A) to areas that are currently translocated into the Atlantic Forest domains (B) and also ranging to other regions of the Pampas. Vicariance signals, detected on node 2 (AB), indicated that vicariance events occurred between the Northwest clade and the other haplogroups that had dispersed between the Pampas and the Atlantic Forest. A new dispersion event was detected on node 3 (A/AB), scattering the haplogroups (Southern, Steppes Plain, Central, and Eastern) along the Pampas and the current Atlantic Forest areas. Finally, on node 4, a new vicariance was verified between the haplogroups that were present on the Atlantic Forest areas (Central and Eastern) and the Pampas (Steppes Plain).

### Discussion

**Genetic and morphological variation in the Pampas and Atlantic Forest**

The absence of Cytb haplotypes that was common to Atlantic Forest and Pampas groups was intriguing. However, the scenario was intricate, as a lack of further structuring between the two biomes in the Bayesian and median-joining network analysis was evident. This pattern indicated a possible isolation following the historical scenario of gene flow, as depicted by the Fgb-17 network. Patterns of skull form were also roughly in agreement with the molecular findings. The differentiation between the physiognomies of *O. nasutus* populations were
evident in the classification after the cross-validation. Subtle differences in the skull shape of *O. nasutus* populations were also depicted among the environments. Such skull differences might be related to genetic grouping or with environmental factors, exerting some form of local selection and/or guiding phenotypic plasticity in each physognomy [8]. Similarly, studies on haplogroups of sympatric akodonts (e.g., *Deltamys kempi* [63, 64] and *Scapteromys* spp. [65, 66]). were also divergent in the geometric or linear morphometric analysis of the skull.

A conspicuous geomorphological feature of the connection among the southern Atlantic Forest and Pampas landscapes is the escarpment of Serra Geral (Meridional Plateau), which might have acted as a long-term barrier to gene flow between the Pampas and the current Atlantic Forest open areas at the eastern point of contact of these two biomes. Thus, the altitudinal gradient formed might have influenced certain level of differentiation, thereby rendering a group of lineages dispersing across the highlands of the Meridional Plateau, and others through the sedimentary lowlands of the Paraná basin, and later spreading along the coastal plain. Mountain ranges and ridges have been considered as topographic elements associated with speciation processes in akodontine rodents [67–71]. Since the differentiation in *O. nasutus* populations is recent (time-tree revealed that the diversification probably began in the Middle Pleistocene) and the divergence process seems incomplete, it can be considered at the population-level and not at species-level yet.

The population structure of *O. nasutus* was quite complex; therefore, it is possible that the recent expansion of forest formations of the South Atlantic Forest, such as the Araucaria Forest and the seasonally dry Forest lato sensu [21], along with the escarpment of Serra Geral have created a new geographical barrier that separated the Atlantic Forest and Pampas lineages. Hence, divergence in the mtDNA clades suggested that the lineages have persisted in isolation across time, characterizing an ‘grassland refuge’ pattern. Accordingly, the species adapted to dry and/or cold environments and restricted to small areas surrounded by unsuitable habitats can persist through interglacial microrefugium [72].

**Phylogeographic patterns**

The phylogenetic tree revealed a more complex evolutionary history in the open areas of Pampas and Atlantic Forest instead of simple reciprocally monophyletic groups. Six major mtDNA clades were observed, which fall within the range of intraspecific divergence described for the Akodontini tribe [63, 64, 71, 73]. The mtDNA haplotype network analysis in this study was congruent with such tree structure, representing the six haplogroups separated by several mutational steps, and inserted in specific ecoregions. However, median joining vectors present in the network did not rule out the hypothesis of non-sampled or possibly extinct haplotypes. Although differentiation was less evident, patterns found in nDNA haplotypes provided indication of a demographic expansion event. Similarly, results of mtDNA analysis also suggested demographic growth. In addition, intraspecific *Fgb*-17 variability was conspicuously lower than *Cytb* variability, as reported in other studies [43, 63, 74]. Molecular markers such as mtDNA and nDNA generally show different phylogeographic patterns for the same biogeographical history owing to differentiation in effective population sizes, recombination, and mutation rates [75, 76].

The estimates of genetic distances for the mtDNA clades were moderately high (up to 2%). This might be the result of an ongoing process of isolation, and because mtDNA accumulates substitutions 5–10 times faster than the single-copy nuclear DNA [75]. The highest distances were observed among Taim Wetland versus other clades and between Southern and Northwest clades; however, none of these genetic distances were recovered for the nuclear locus (divergence close to zero). The highest genetic distance observed for these clades (Taim
Wetland, Northwest, and South) might relate to the ancestral condition of these groups, as depicted by BI and mtDNA network, along with the fact that they are geographically distant from each other. According to the S-DIVA analysis, tandem dispersion events followed by vicariance might have probably influenced the phylogenetic pattern of mtDNA observed in the study.

The Northwest clade was the northernmost lineage; so, the effects of the dynamics of forest formations/open areas might have reached earlier in this group, making it more suitable to environmental pressure. Positive values for the Tajima’s D test (0.44358, P = 0.7550) for this clade (from mtDNA) and other negative but non-significant values (for both markers), indicated a possible contraction effect on this lineage. The divergence between the Steppes Plain and Central and Eastern clades dates back to 0.350 myr (0.220–0.505 myr). Moreover, some forest formation similar to Seasonal Forest, which at present is adjacent to the escarpment of the Serra Geral might have acted concomitantly. This hypothesis was supported by the results of the ENM that indicated an area of very low suitability between the zone of contact of these two ecoregions. This is consistent with the escarpment of Serra Geral, which might have corroborated mainly due to the vicariance between the Steppes Plain and Central and Eastern clades, as detected by S-DIVA analyses. Similarly, a strong phylogenetic break in this region was evident for other akodonts recently described, such as *Scapteromys meridionalis* [71] and *Deltamys araucaria* [64]. An evident pattern is the genetic divergence within the Southern clade in the Pampas ecoregion (Uruguay). In this study, ENM supported up to a limited dispersion, indicating a possible historical barrier acting in this region. In this case, the Rio Negro could be possibly acting as a barrier for Southern clade, wherein areas of low suitability were identified in all the prediction models. Southern clade was one of the most spread throughout the Pampas ecoregion. Although neutrality test indicated expansion, Southern clade was the only clade where suitable areas were identified mainly in the coastal regions and continental shelf, according to the LGM niche models.

A remarkable phylogeographic aspect was the genetic divergence of the Taim Wetland clade. The mtDNA dataset revealed a statistically supported phylogenetic break between the Taim Wetland clade and other clades, indicating a possible historical barrier acting in this region. This lineage originated about 0.147 myr (95% HPD = 0.047–0.290 myr) and was the first to have dispersed in the S-DIVA analysis. This clade was inserted on the plains of southernmost Brazil (RS), where climatic oscillations occurred during the middle Pleistocene and Holocene, resulting in marine transgressions and regressions of relative sea level that shaped the South Atlantic Coastal Plain (SACP). The formation of the SACP is related to the sedimentary processes associated with the sea transgressive events known as ‘Barriers’, which occurred in the middle Pleistocene with the formation of Barrier I (~ 0.400 myr), Barrier II (~ 0.325 myr), Barrier III (~ 0.120 myr), and Holocene, when Barrier IV (~0.005 myr) was established [77]. Possibly, this clade accessed this region after the formation of barriers II and III, between 0.325 and 0.120 myr, respectively. The geological evolution of the “Barriers” also formed the Patos-Mirim complex, the largest lagoon-barrier system in South America [77]. In addition, three paleochannels related to the Jacuí, Camaquã, and Jaguarão rivers were identified in the middle and southern coastal zone of RS [78]. These paleochannels were associated with the large persistent hydrographic elements such as Mirim Lagoon, São Gonçalo Channel, and the Patos Lagoon estuarine channel, which might have possibly contributed to the isolation of this lineage in the southernmost domains of the SACP. Similarly, recent studies show a strong phylogenetic break in the same region for *Scapteromys tumidus* [65] and *Deltamys kempi* [64]. Thus, major elements of Patos-Mirim lagunar complex might constitute a geographical barrier for the historical gene flow.
Climate change and landscape

Overall, substantial demographic changes associated with Pleistocene climatic oscillations were observed in more than half of the biota investigated in South America. Considering only the taxa to be associated with open vegetation, a total of 68% of the studied species experienced population expansion during the glacial periods [6]. The historical biogeographic approach presented herein appeared to corroborate this pattern detected for the open areas-dwelling species.

All divergence times within *O. nasutus* populations estimated by BEAST occurred during the Middle Pleistocene, wherein a minimum of eight glaciations occurred in the Middle–Late Pliocene in the southern Andes of South America [79]. However, the greatest glaciations occurred during the early Pleistocene, such as the Great Patagonian Glaciations, between 1.16 and 1.01 mya, when the glaciers advanced up to 200 km east of the Andes mountains, and stretching along the Pacific and south of Atlantic coast [79,80]. After the Great Patagonian Glaciations, 13 minor glacial and interglacial periods were recorded for this region in the Early-Middle Pleistocene that reached a maximum around 25,000 and 16,000 years BP (see [79]).

Although the emergence of *O. nasutus* occurred in the earlier periods (ca. 500 kyr), the patterns showed by the present and past niche models depicted expansion of high probability areas during the LGM (~21,000 years BP), favouring its distribution. Probably, a pattern similar to LGM might have occurred during the Great Patagonian Glaciations, where the effects of glaciations might have increased the open areas and retracted the forest formations. The areas of Southern Atlantic Forest were dominated by grasslands between 42,000–10,000 years BP; the period comprising the LGM. Forest elements were restricted to sites in deep river valleys and in coastal lowlands, indicating a cold and dry climate [21]. In general, grasslands adapted to cold conditions prevailed in the southern and southeastern Brazil until around 11,500 years BP [81]. Since *O. nasutus* is intrinsically related to open areas and is abundantly found in these habitats [17–19], cyclic events of the dynamic expansion and retraction of open areas and forests that occurred during glacial and interglacial periods have probably caused species dispersal and historical distribution. Such expansions of open areas promoted by glaciations made the environment more suitable for establishment of new areas, which was detected by S-DIVA analysis. It was interesting to reveal that no suitable areas in the coastal zone were increased during LGM according to the niche model. Palynological studies on the coasts of the states of Santa Catarina and Paraná indicated that fields were abundant in this region and also on the exposed continental shelf during the LGM period, whereas tropical tree species were practically absent [23]. The continental shelf during LGM presented a dry climate, hampering the colonization by any species adapted to humid/wet habitats. Areas of moderate suitability were found in the coastal region of southern Brazil and Uruguay during the LIG, Middle Holocene, and current models, with a reduction of suitability in the inland areas. Thus, the coastal regions in the Pampas ecoregion might have served as a refuge for *O. nasutus* during the interglacial periods.

*O. nasutus* is known to inhabit the subtropical grassland in wet or flooded seasons [17]. Accordingly, the species is adapted to humid environments, being found in bunch grass and in tall grass near the streams and rivers [82], sandbanks near wet lands across coastal semi-fossorial habit [19], and coastal vegetation in the South Atlantic Coastal Plain [83]. Thus, in addition to grassland, *O. nasutus* might have followed river routes in search of humid environments. Such habitat specialization might reflect the dispersion ability to more extensive regions during the glacial periods.

In this context, we suggest that the two major rivers in Rio Grande do Sul state (the Jacuí and Ibicuí) might have acted (and still be acting) as river barrier for the Steppes Plain clade. The haplotypes of this clade are distributed across the western edge of the Patos Lagoon and the
interior of the plains of southern Brazil. Therefore, ENM suggested that these areas might be unsuitable for the restriction caused mainly by the river Jacuí. Others rivers could have affected the limits of dispersion in \textit{O. nasutus}. Rivers Uruguay and Paraná also might have acted in limiting the dispersion during the glacial periods. The paleoecological niche modelling suggested that at LGM, the River Uruguay limited the dispersion for Steppes Plain and Southern clades in Uruguay and Southern Brazil to reach new areas to the west. Similarly, the River Paraná limited the dispersion for Northwest and Central Clade in highlands. Finally, Paraná, and Paranapanema rivers might have acted as physical barriers in the dispersion to the north, mainly for the Northwest clade, due to their amplitude and water volume. These inferences were based on the ENM results, wherein areas of low suitability were verified in all the prediction models.

**Supporting information**

**S1 Fig.** Bayesian consensus time-tree based on the \textit{Fgb-I7} sequences. Values above nodes correspond to posterior probabilities $> 0.90$.

(TIF)

**S2 Fig.** Position of the landmarks (circles) digitized on the dorsal, ventral, and lateral views of the \textit{O. nasutus} skull. A description of each landmark is presented in S1 Appendix.

(TIF)

**S3 Fig.** Scatter plot of the first Canonical Variate axis for the skull of \textit{O. nasutus} in the lateral view, with groups following the genetic haplogroups (A, B) and the physiognomies (C). Changes in the shape for each axis are given. Solid lines indicate positive scores and dashed lines indicate negative ones.

(TIF)

**S1 Appendix.** Definition of the landmarks positioned at the three skull views of \textit{O. nasutus} analysed specimens (see. S2 Fig).

(DOCX)

**S2 Appendix.** Additional localities retrieved from the literature for the spatial distribution modelling analysis.

(DOCX)

**S1 Table.** Genetic divergence (using \textit{p}-distance) between pairs of \textit{Cytb} haplotypes recovered from different clades of \textit{O. nasutus} determined by the Bayesian phylogeny.

(DOCX)

**S2 Table.** Percentage of correct classification by discriminant analysis, using Jackknife cross-validation, for the dorsal, ventral, and lateral views of the \textit{O. nasutus} skull for the mtDNA clades.

(DOCX)

**S3 Table.** Percentage of correct classification by discriminant analysis, using Jackknife Cross-validation, for the dorsal, ventral, and lateral views of the \textit{O. nasutus} skull for the two ecoregions.

(DOCX)

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**References**

1. Lisiecki LE, Raymo ME. Plio-Pleistocene climate evolution: Trends and transitions in glacial cycle dynamics. Quaternary Sci Rev. 2007; 26, 56–69. [https://doi.org/10.1016/j.quascirev.2006.09.005](https://doi.org/10.1016/j.quascirev.2006.09.005)
2. Hewitt GM. Some genetic consequences of ice ages, and their role in divergence and speciation. Biol. J. Linn. Soc. 1996; 58: 247–276.
3. Hewitt G. The genetic legacy of the Quaternary ice ages. Nature. 2000; 405: 907–913. [https://doi.org/10.1038/35016000](https://doi.org/10.1038/35016000). PMID: 10879524
4. Hewitt G. Genetic consequences of climatic oscillations in the Quaternary. Philos. Trans. R. Soc. Lond. B: Biol. Sci. 2004; 359: 183–195. [https://doi.org/10.1098/rstb.2003.1386](https://doi.org/10.1098/rstb.2003.1386)
5. Avise JC. Phylogeography: the history and formation of species. Cambridge, Harvard University Press; 2000.
6. Turchetto-Zolet AC, Pinheiro F, Salgueiro F, Palma-Silva C. Phylogeographical patterns shed light on evolutionary process in South America. Mol Ecol. 2013; 22: 1193–1213. https://doi.org/10.1111/mec.2013.12164 PMID: 23279129

7. Carnaval AC, Moritz C. Historical climate modelling predicts patterns of current biodiversity in the Brazilian Atlantic forest. J Biogeogr. 2008; 35: 1187–1201. https://doi.org/10.1111/j.1365-2699.2007.01870.x

8. Maestri R, Fornel R, Gonçalves GL, Geise L, Freitas TRO, Carnaval AC. Predictors of intraspecific morphological variability in a tropical hotspot: comparing the influence of random and non-random factors. J Biogeogr. 2016; 43: 2160–2172. https://doi.org/10.1111/jbi.12815

9. Bilenca DN, Maestri R, Fornel R, Geise L, Freitas TRO, Carnaval AC. Predic tors of intraspecific morphological variability in a tropical hotspot: comparing the influence of random and non-random factors. J Biogeogr. 2016; 43: 2160–2172. https://doi.org/10.1111/jbi.12815

10. Behling H, Pillar VD. Late Quaternary vegetation, biodiversity and fire dynamics on the southern Brazil oian highland and their implication for conservation and management of modern Araucaria forest and grassland ecosystems. Philos T R Soc B. 2007; 362: 243–251. https://doi.org/10.1098/rstb.2006.1984

11. Behling H, Negrellre RRB. Tropical Rain Forest and Climate Dynamics of the Atlantic Lowland, Southern Brazil, during the Late Quaternary. Quaternary Res. 2001; 56:3, 383–389. https://doi.org/10.1006/qres.2001.2264

12. Smith MF, Patton JL. The diversification of South American murid rodents: evidence from mitochondrial DNA sequence data for the akodontine tribe. Biol J Linn Soc. 1993; 50: 149–177. https://doi.org/10.1016/0006-bijl.1993.1052

13. Maestri R, Fornel R, Gonçalves GL, Geise L, Freitas TRO, Carnaval AC. Predic tors of intraspecific morphological variability in a tropical hotspot: comparing the influence of random and non-random factors. J Biogeogr. 2016; 43: 2160–2172. https://doi.org/10.1111/jbi.12815

14. Cristiano MP, Cardoso DC, Fernandes-Salomão AN. Biogeographical history and diversification of the subterranean rodent Ctenomys talarum: integrating demographic and habitat histories. J Mammal. 2013; 94: 459–476. https://doi.org/10.1644/1406-bijl.2013.0053.2014

15. Fregonezi JN, Turchetto C, Bonatto SL, Freitas LB. Biogeographical history and diversification of the subterranean rodent Ctenomys talarum: integrating demographic and habitat histories. J Mammal. 2013; 94: 459–476. https://doi.org/10.1644/1406-bijl.2013.0053.2014

16. Cristiano MP, Cardoso DC, Fernandes-Salomão AN. Integrating Paleo distribution Models and Phylogeography in the Grass-Cutting Ant D1 (Hymenoptera: Formicidae) in Southern Lowlands of South America. PLoS One. 2015; 10(2): e0118162. https://doi.org/10.1371/journal.pone.0118162 PMID: 25692471

17. Oliveira JA, Gonçalves AP, Lessa EP, Vassallo AI, D'Anatro A, Mapelli FJ. Phylogeography and population genetic structure of the Talas tuco-tuco (Ctenomys talarum): integrating demographic and habitat histories. J Mammal. 2013; 94: 459–476. https://doi.org/10.1644/1406-bijl.2013.0053.2014

18. Paíse G, Vieira EM. Daily activity of a Neotropical Rodent (Oxymycterus nasutus) and influence of Environmental factors. J Mammal. 2006; 87 (4): 733–739. https://doi.org/10.1644/05-MAMM-A-158R.51

19. González EM. Guía de campo de los mamíferos de Uruguay. Introducción al estudio de los mamíferos. Montevideo: Vida Silvestre 339p, 2001.

20. Behling H, Bauer mann SG, Neves PCP. Holocene environmental changes in the Sào Francisco de Paula region, southern Brazil. J Am Earth Sci. 2001; 14: 631–639. https://doi.org/10.1016/S0895-9811(01)00040-2

21. Overbeck GE, Muller SC, Fidelis A, Pfadenhauer J, Pillar VD, Blanco CC, et al. Brazil’s neglected biome: The South Brazilian Campos. Perspect Plant Ecol. 2007; 9: 101–116. https://doi.org/10.1016/j.ppees.2007.07.005

22. Behling H, Pillar VD. Late Quaternary vegetation, biodiversity and fire dynamics on the southern Brazilian highland and their implication for conservation and management of modern Araucaria forest and grassland ecosystems. Philos T R Soc B. 2007; 362: 243–251. https://doi.org/10.1098/rstb.2006.1984

23. Behling H, Negrellre RRB. Tropical Rain Forest and Climate Dynamics of the Atlantic Lowland, Southern Brazil, during the Late Quaternary. Quaternary Res. 2001; 56:3, 383–389. https://doi.org/10.1006/qres.2001.2264

24. Smith MF, Patton JL. The diversification of South American murid rodents: evidence from mitochondrial DNA sequence data for the akodontine tribe. Biol J Linn Soc. 1993; 50: 149–177. https://doi.org/10.1016/0006-bijl.1993.1052

25. Matocq MD, Shurtliff QR, Feldman CR. Phylogenetics of the woodrat genus Neotoma (Rodentia: Muridae): implications for the evolution of phenotypic variation in male external genitalia. Mol Phylogenet Evol. 2007; 42:637–652. https://doi.org/10.1016/j.ympev.2006.08.011 PMID: 17208019
26. Katoh K, Standley DM. MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. Mol Biol Evol. 2013; 30: 772–780. https://doi.org/10.1093/molbev/msm010 PMID: 23329690

27. Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. Am. J. Hum. Genet. 2001; 68: 978–989. https://doi.org/10.1086/319501 PMID: 11254454

28. Stephens M, Donnelly P. A comparison of Bayesian methods for haplotype reconstruction from population genotype data. Am J Hum Genet. 2003; 73:1162–1169. https://doi.org/10.1093/molbev/ms379378 PMID: 14574645

29. Librado P, Rozas J. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics. 2009; 25: 1451–1452. https://doi.org/10.1093/bioinformatics/btp187 PMID: 19346325

30. Garrick RC, Sunnucks P, Dyer RJ. Nuclear gene phylogeography using PHASE: dealing with unresolved genotypes, lost alleles, and systematic bias in parameter estimation. BMC Evol. Biol. 2010; 10: 118. https://doi.org/10.1186/1471-2148-10-118 PMID: 20429950

31. Darriba D, Taboada GL, Doallo R, Posada D. jModelTest 2: more models, new heuristics and parallel computing. Nat Methods. 2012; 30: 772. https://doi.org/10.1038/nmeth.2109

32. Drummond AJ, Suchard MA, Xie D, Rambaut A. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol Biol Evol. 2012; 29:1969–73. https://doi.org/10.1093/molbev/mss075 PMID: 22367748

33. Parada A, Elia G, Palma R. The influence of ecological and geographical context in the radiation of Neotropical sigmodontine rodents. BMC Evol. Biol. 2015; 15:172. https://doi.org/10.1186/s12862-015-0440-z PMID: 26307442

34. Hoffmann FG, Leasa EP, Smith MF. Systematics of Oxymycterus with description of a new species from Uruguay. J Mammal. 2002; 53: 408–420.

35. Altaro ME, Zoller S, Lutzoni F. Statistical tests of neutrality against population growth, hitchhiking and background selection. Genetics. 1997; 147: 915–925. PMID: 9335623

36. Excoffier L, Lischer HEL. Arlequin suite version 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour. 2010; 10 (3): 564–567. https://doi.org/10.1111/j.1755-0998.2010.02847.x PMID: 21565059

37. Drummond AJ, Rambaut A, Shapiro B, Pybus OG. Bayesian Coalescent Inference of Past Population Dynamics from Molecular Sequences. Mol Biol Evol. 2005; 22: 1185–1192. https://doi.org/10.1093/molbev/msi103 PMID: 15703244

38. Platt RN, Amman BR, Keith MS, Thompson CW, Bradley RD. What Is Peromyscus? Evidence from nuclear and mitochondrial DNA sequences suggests the need for a new classification. J Mammal. 2015; 96: 708–719. https://doi.org/10.1093/jmammal/gvy067 PMID: 26937047

39. Ronquist F. Dispersal-Vicariance analysis: a new approach to the quantification of historical biogeography. Syst Biol. 1997; 46: 195–203. https://doi.org/10.1093/sysbio/46.1.195

40. Yu Y, Harris AJ, He. S-DIVA (Statistical Dispersal-Vicariance Analysis): a tool for inferring biogeographical histories. Mol Phylogenet Evol. X 2010; 56: 848–850. https://doi.org/10.1016/j.ympev.2010.04.011 PMID: 20399277

41. Yu Y, Harris AJ, Blair C, He XJ. RASP (Reconstruct Ancestral State in Phylogenies): a tool for historical biogeography. Mol Phylogenet Evol. 2015; 87: 46–49. https://doi.org/10.1016/j.ympev.2015.03.008 PMID: 25819445

42. Rohlf FJ. The tps series of software. Hystrix. 2015; 26:9–12. https://doi.org/10.4404/hystrix-26.1–11264

43. Martinez JJ, Di Cola V. Geographic distribution and phenetic skull variation in two close species of Gramomys (Rodentia, Cricetidae, Sigmodontinae). Zool Anz, 2011; 250: 175–194. https://doi.org/10.1016/j.jcz.2011.03.001
49. Maestri R, Fornel R, Galiano D, de Freitas TRO. Niche Suitability Affects Development: Skull Asymmetry Increases in Less Suitable Areas. PLoS One. 2015; 10(4): e0122412. https://doi.org/10.1371/journal.pone.0122412 PMID: 25874364

50. Rohlf FJ, Slice D. Extensions of the Procrustes method for the optimal superimposition of landmarks. Syst. Zool. 1990; 39: 40–59.

51. Bookstein FL. Morphometric tools for landmark data: geometry and biology. Cambridge, Cambridge University Press, UK; 1991.

52. R Core Team. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria; (2014)

53. Adams DC, Otárola-Castillo E. geomorph: and R package for the collection and analysis of geometric morphometric shape data.—Methods Ecol. Evol. 2013; 4: 393–399. https://doi.org/10.1111/2041-210X.12035

54. Schlager S. “Morpho and Rvcg—Shape Analysis in R.” In: Zheng G, Li S and Szekely G (editors), Statistical Shape and Deformation Analysis, pp. 217–256. Academic Press; 2017.

55. Klingenberg CP. MorphoJ: an integrated software package for geometric morphometrics. Mol Ecol Res. 2011; 11: 353–357. https://doi.org/10.1111/j.1755-0998.2010.02924.x

56. Phillips SJ, Anderson RP, Schapire RE. Maximum entropy modeling of species geographic distributions. Ecol Model. 2006; 190, 231–259. https://doi.org/10.1016/j.ecolmodel.2005.03.028

57. Elith J, Graham CH, Anderson RP, Dudík M, Ferrier S, Guisan S. et al. Novel methods improve prediction of species’ distributions from occurrence data. Ecography. 2006; 29: 129–151. https://doi.org/10.1111/j.2006.0900-7590.04996.x

58. Peterson AT, Papes M, Eaton M. Transferibility and model evaluation in ecological niche modeling: a comparison of GARP and Maxent. Ecography. 2007; 30: 550–560. https://doi.org/10.1016/j.ecolmodel.2007.11.008

59. Peterson AT, Papes M, Soberón J. Rethinking receiver operating characteristic analysis applications in ecological niche modeling. Ecol. Model. 2008; 213: 63–72.

60. Hernandez PA, Graham CH, Master LL, Albert DL. The effect of sample size and species characteristics on performance of different species distribution modeling methods. Ecography. 2006; 29: 773–785. https://doi.org/10.1111/j.0906-7590.2006.04700.x

61. Swets JA. Measuring the accuracy of diagnostic systems. Science. 1988; 240: 1285–1293 PMID: 3287615

62. Gent PR, Danabasoglu G, Donner LJ, Holland MM, Hunke EC, Jayne SR et al. The Community Climate System Model version 4. J. Climate, 2011; 24: 4973–4991. https://doi.org/10.1175/2011JCLI4083.1

63. Montes MA, Oliveira LFB, Bonatto G, Donner LJ, Holland MM, Hunke EC, Jayne SR et al. The Community Climate System Model version 4. J. Climate, 2011; 24: 4973–4991. https://doi.org/10.1175/2011JCLI4083.1

64. Elith J, Graham CH, Anderson RP, Dudík M, Ferrier S, Guisan S. et al. Novel methods improve prediction of species’ distributions from occurrence data. Ecography. 2006; 29: 129–151. https://doi.org/10.1111/j.2006.0900-7590.04996.x

65. Phillips SJ, Anderson RP, Schapire RE. Maximum entropy modeling of species geographic distributions. Ecol Model. 2006; 190, 231–259. https://doi.org/10.1016/j.ecolmodel.2005.03.028

66. Elith J, Graham CH, Anderson RP, Dudík M, Ferrier S, Guisan S. et al. Novel methods improve prediction of species’ distributions from occurrence data. Ecography. 2006; 29: 129–151. https://doi.org/10.1111/j.2006.0900-7590.04996.x

67. Elith J, Graham CH, Anderson RP, Dudík M, Ferrier S, Guisan S. et al. Novel methods improve prediction of species’ distributions from occurrence data. Ecography. 2006; 29: 129–151. https://doi.org/10.1111/j.2006.0900-7590.04996.x

68. Elith J, Graham CH, Anderson RP, Dudík M, Ferrier S, Guisan S. et al. Novel methods improve prediction of species’ distributions from occurrence data. Ecography. 2006; 29: 129–151. https://doi.org/10.1111/j.2006.0900-7590.04996.x

69. Elith J, Graham CH, Anderson RP, Dudík M, Ferrier S, Guisan S. et al. Novel methods improve prediction of species’ distributions from occurrence data. Ecography. 2006; 29: 129–151. https://doi.org/10.1111/j.2006.0900-7590.04996.x

70. Elith J, Graham CH, Anderson RP, Dudík M, Ferrier S, Guisan S. et al. Novel methods improve prediction of species’ distributions from occurrence data. Ecography. 2006; 29: 129–151. https://doi.org/10.1111/j.2006.0900-7590.04996.x
71. Quintela FM, Gonçalves GL, Althoff SL, Sbalqueiro IJ, Oliveira LFB, Freitas TRO. A new species of swamp rat of the genus *Scapteromys* Waterhouse, 1837 (Rodentia: Sigmodontinae) endemic to *Araucaria angustifolia* Forest in Southern Brazil. Zootaxa. 2014; 3811:207–225. https://doi.org/10.11646/zootaxa.3811.2.3

72. Rull V. Microrefugia. J Biogeogr. 2009; 36: 481–484. https://doi.org/10.1111/j.1365-2699.2008.02023.x

73. D'Elía G, Pardiñas UFJ. Systematics of Argentinian, Paraguayan, and Uruguayan swamp rats of the genus *Scapteromys* (Rodentia, Cricetidae, Sigmodontinae). J Mammal. 2004; 85: 897–910. https://doi.org/10.1644/BRB-201

74. D'Elía G, Hanson JD, Mauldin MR, Teta P, Pardiñas UFJ. Molecular systematics of South American marsh rats of the genus *Holochilus* (Muroidea, Cricetidae, Sigmodontinae). J Mammal. 2015; 96: 1081–1094. https://doi.org/10.1093/jmammal/gyv115

75. Avise JC. Phylogeography: retrospect and prospect. J Biogeogr. 2009; 36: 3–15. https://doi.org/10.1111/j.1365-2699.2008.02032.x

76. Toews DPL, Brelsford A. The biogeography of mitochondrial and nuclear discordance in animals. Mol Ecol. 2012; 21:3907–3930. https://doi.org/10.1111/j.1365-294X.2012.05664.x PMID: 22738314

77. Tomazelli LJ, Villwock JA. Mapeamento geológico de planícies costeiras: o exemplo da costa do Rio Grande do Sul. Gravel. 2005; 3:109–115.

78. Weschenfelder J, Correa ICS, Aliotta S, Baitelli R. Paleochannels related to late Quaternary sea-level changes in Southern Brazil. Braz J Oceanogr. 2010; 58:35–44. https://doi.org/10.1590/S1679-87592010000600005

79. Rabassa J, Coronato A, Salamme M. Chronology of the Late Cenozoic Patagonian glaciations and their correlation with biostratigraphic units of the Pampean region (Argentina). J S Am Earth Sci. 2005; 20: 81–103. https://doi.org/10.1016/j.jsames.2005.07.004

80. Rabassa J, Clapperton C (1990) Quaternary glaciations of the southern Andes. Quaternary Sci Rev. 1990; 9:153–174. https://doi.org/10.1016/0277-3791(90)90016-4

81. Behling H, Jeske-Pierschka V, Schüler L, Pillar VP, Campos Sulinos, conservação e uso sustentável da biodiversidade. In: Pillar VP, Müller SC, Castilhos ZMS, Jacques AVA, editors. Ministério do Meio Ambiente, Brasília, Brazil, 2009.

82. Barlow JC. Observations on the biology of rodents in Uruguay. Life sci. contrib., R. Ont. Mus. 1969; 75:1–59.

83. Quintela FM, Santos MB, Christoff AU, Gava A. Pequenos mamíferos não-voadores (Didelphimorphia, Rodentia) em dois fragmentos de mata de restinga de Rio Grande, Planicie Costeira do Rio Grande do Sul. Biota Neotrop. 2012; 12:261–266.