Biological Journal of the Linnean Society, 2021, **XX**, 1–19. With 5 figures.

Phenotypic and genomic differences between biomes of the South America marsh rat, *Holochilus brasiliensis*

JOYCE R. PRADO[1,*], ALEXANDRE R. PERCEQUILLO[1], RENATA M. PIRANI[2] and ANDREA T. THOMAZ[3]

[1]*Departamento de Ciências Biológicas, Escola Superior de Agricultura 'Luiz de Queiroz', Universidade de São Paulo, Piracicaba, SP, Brazil*
[2]*Biology Department, University of Nevada-Reno, Reno, NV, USA*
[3]*Facultad de Ciencias Naturales, Universidad del Rosario, Bogotá, DC, Colombia*

Received 10 June 2021; revised 25 August 2021; accepted for publication 26 August 2021

Abiotic factors can influence genetic and phenotypic divergence in several ways, and identifying the mechanisms responsible for generating this variation is challenging. However, when evaluated in combination, ecological characteristics and genetic and phenotypic information can help us to understand how habitat preferences can influence morphological and genetic patterns exhibited by taxa distributed between distinct biomes, such as the Atlantic Forest and Pampas biomes in South America. By combining distributional, environmental, phenotypic and genomic information from a habitat-specialist semi-aquatic rodent (*Holochilus brasiliensis*), we quantified the relationship between ecological niche differences and the phenotypic and genetic variation. The results demonstrate notable segregation among the ecological niches of *H. brasiliensis* within each biome, although we could not refute the hypothesis of niche similarity or equivalency. Such differences are consistent with a solid morphometric variation associated with the size of these rodents. However, the ecological and morphometric differentiation is not accompanied by the same pattern of genetic variation. Despite differences in the connectivity patterns in both biomes, the genetic differences corroborate a consistent level of migration history between biomes. Additionally, the association tests show that the environment explains a small and non-significant part of the genetic variation but a significant portion of the morphometric variation.

ADDITIONAL KEYWORDS: Atlantic Forest – Ecological Niche – *Holochilus brasiliensis* – Morphometrics – Neotropics – Pampas – RADseq – Rodentia – Wetlands.

INTRODUCTION

Abiotic factors can influence genetic and phenotypic divergence in several ways. The genetic differences of neutral genes can inform us about demographic processes (e.g. drift, expansion, changes in effective population size; Avise, 2000; Knowles, 2009), whereas phenotypic differences can affect the performance of species in distinct environments (e.g. affecting survival, reproductive success, dispersal, colonization and persistence; Zamudio *et al.*, 2016), potentially attributable to mechanisms that determine whether a species can persist *in situ*, such as adaptation and plasticity (Chevin *et al.*, 2010). Identifying which mechanisms are responsible for generating this trait variation is challenging, given the necessity of high-resolution genomic data and experimental approaches (Hendry *et al.*, 2008). However, when evaluated in combination, neutral genetic information and phenotypic variation allow an understanding of the evolutionary dynamics that operate currently and historically and might help to explain observed patterns (Zamudio *et al.*, 2016), such as how a species is capable of responding to environmental differences over its distribution.

Species with relatively large distributions over different biomes offer us a unique opportunity to study how species deal with abiotic differences over their range. Over larger areas, the capability of responding (or not) to an environmental gradient needs to be evaluated to understand the evolutionary processes that might have happened during the history of the species, such as responses to climatic changes that impact

*Corresponding author. E-mail: joycepra@gmail.com

connectivity and population structures (Massatti & Knowles, 2014, 2016). The position of a species in the environmental gradient, here defined broadly by ecological niche, can influence the partitioning of resources in biological communities (Hixon & Beets, 1993; Cavender-Bares *et al.*, 2004; Moroti *et al.*, 2020), leading to phenotypic differentiation dictated by local environmental variables (Schluter & Grant, 1984; Rosenblum, 2006; Feldhamer *et al.*, 2014) and affecting the phylogeographical structure of species (Massatti & Knowles, 2014, 2016). Changes in the ecological niche between populations of the same species along an environmental gradient are a little evaluated but fruitful area for research, given that the variance in the ecological niche within species might favour larger distributional ranges that could encompass different biomes. However, species with strong specialization for a specific habitat might be constrained to respond to ecological niche variation over its distribution.

South America houses some of the most diverse environments globally, which is reflected in its vast classification of biomes (Olson *et al.*, 2001). Among them, two neighbouring biomes with distinct characteristics are found in the coastal region of the continent, namely the Pampas and the Atlantic Forest. The Pampas biome is a non-forest ecosystem dominated by grasslands and subtropical climate (Overbeck *et al.*, 2015), with isolated patches of bushlands and forest associated with rivers in the plains of Uruguay, Argentina, Paraguay and southern Brazil (Olson *et al.*, 2001). A great part of this biome is occupied by the Humid Pampas ecoregion (Olson *et al.*, 2001), dominated by shallow lagoons that can be permanent or temporary, exhibiting complex ecological patterns driven by local conditions (Cabrera, 1976; Josens *et al.*, 2012). Despite its high endemism of terrestrial species of vertebrates (de Freitas *et al.*, 2012; Turchetto *et al.*, 2014; Felappi *et al.*, 2015), it has been little studied in South America (Lawler *et al.*, 2006; Beheregaray, 2008; Turchetto-Zolet *et al.*, 2013), with a knowledge gap regarding the processes that led to the biological diversification in this biome (Ramos-Fregonezi *et al.*, 2017).

To the north of the Pampas is the Atlantic Forest biome, which has been the focus of several phylogeographical studies (e.g. Carnaval & Moritz, 2008; Carnaval *et al.*, 2009; Thomé *et al.*, 2010, 2014; Sabbag *et al.*, 2018; Thomaz & Knowles, 2020). This biome is covered by a tropical forest that extends from southern to north-eastern Brazil and a portion of Paraguay and Argentina (Olson *et al.*, 2001). Apart from the dominant forests, the biome is a mosaic of vegetation types, which include formations that are typically forestry, but also shrublands and grasslands, in addition to the aquatic ecosystems (wetlands) and the ecotonal areas in all their extension (Marques *et al.*, 2021). Additionally, it harbours some of the most threatened Brazilian wetlands, for which little information exists (Junk, 2013). These wetlands are restricted to riparian forests along streams and interfluvial lowland and montane fens, bogs and hygrophile forests (Junk, 2013). The Atlantic Forest biome is also a biodiversity hotspot, and it exhibits a complex arrangement of biogeographical units (e.g. Martins, 2011), which might be explained by its topographic complexity, large latitudinal and elevational range and strong seasonality (Ab'Saber, 1977), reflecting complex evolutionary divergence patterns (e.g. Cabanne *et al.*, 2008; Prado *et al.*, 2021).

In this study, we focus on the evolutionary responses associated with the environmental differences between biomes in a South American marsh rat species, *Holochilus brasiliensis* (Desmarest, 1819) (Rodentia: Cricetidae), distributed throughout the coastal region of the Pampas and Atlantic Forest biomes (Fig. 1; Prado *et al.*, 2021). Species of *Holochilus* are characterized by a large body size, several morphological specializations for a semi-aquatic lifestyle and a herbivorous diet (feeding mainly on herbaceous plants); they are considered to be important crop pests in agricultural fields (Hershkovitz, 1955; Gonçalves *et al.*, 2015). Although the genus is widely distributed over almost the entire continent, inhabiting several distinct biomes, its distribution is restricted to wetlands and open areas (riparian or marshy habitats with deep herbaceous ground cover; Gonçalves *et al.*, 2015). Even when inhabiting forested biomes, such as the Amazon and the Atlantic Forest, these rodents are associated with inundated grass patches along the river banks and lakes or in agricultural fields in proximity to rivers (Emmons & Feer, 1997; Patton *et al.*, 2000; Gonçalves *et al.*, 2015). These rodents also demonstrate high mobility, with males moving large distances (almost 1 km in one night) in inundated areas, with a possible polygynic mating system (Eiris & Barreto, 2009).

Previous results did not reveal strong genomic structure among *H. brasiliensis* populations, with models recovering a large area of stable distribution through time within the Pampas biome (Prado *et al.*, 2019). The study also suggested that genetic diversity within this marsh rat species is explained primarily by natural history traits and secondarily by differences between biomes, such as spatiotemporal environmental variation and historical stability. Therefore, in this contribution, we focus on assessing whether the ecological niche of *H. brasiliensis* changes across the different biomes and how this has influenced the evolution of the morphological and genetic patterns exhibited by this species. Many species of sigmodontine rodents appear to be restricted to specific habitat types (D'Elía & Pardiñas, 2015), and *Holochilus* seems to be one of those cases (Gonçalves *et al.*, 2015).

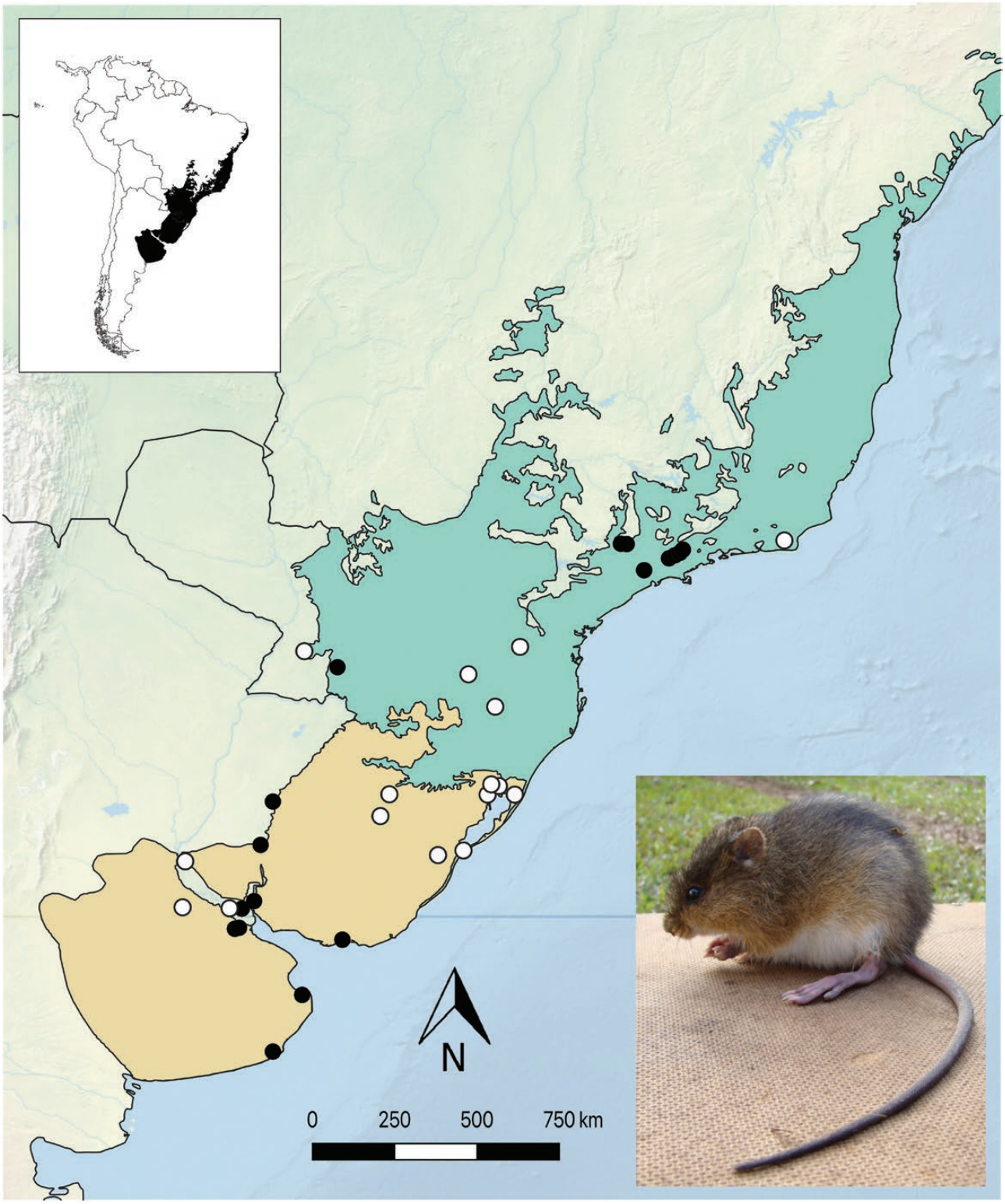

Figure 1. Map showing the distribution of *Holochilus brasiliensis* samples in the Pampas (yellow) and the Atlantic Forest (green) biomes. Black dots indicate phenotypic samples and white dots genetic samples. Photograph: P. R. O. Roth.

Given the high abiotic specificity demonstrated by *H. brasiliensis* to wetland environments (see Prado *et al.*, 2019), it can be hypothesized that there will be no significant differences in the ecological niche occupied by this species when comparing the environmental space of each biome. As a result, no morphological difference directly associated with the two biomes should be observed, and genetic diversity should be higher in the biome where wetlands are patchier (i.e. Atlantic Forest), as a consequence of less movement of individuals, owing to restricted environmental connectivity. However, if genetic differences are small, the high dispersal capability can overcome environmental patchiness, homogenizing populations by gene flow. Alternatively, if differences in the ecological niche play an important role between the biomes, we expect to observe differences in the environmental space occupied by the populations in each biome, accompanied by morphological differences. If these differences restrain the movement of individuals between the two biomes, genetic structure should be observed between the Pampas and the Atlantic Forest. As a means to test these biological hypotheses, our goals are as follows: (1) to characterize the ecological niche similarity between populations in both biomes; (2) to test for genetic and morphometric differentiation along the species range; (3) to evaluate the level of individual movement between biomes; and (4) to search for an association between environmental variables and genomic and morphometric variation. Finally, we discuss how these findings can help us to understand the role of environmental variation in shaping ecological niche characteristics and genetic and morphometric structure within a species.

MATERIAL AND METHODS

Sampling

Species occurrence data were gathered by searching for the georeferenced occurrence data representative of the entire range of *H. brasiliensis*, which were obtained by direct examination of specimens (Supporting Information, Tables S1.1 and S1.2) and from specific bibliographic sources (Hershkovitz, 1955; Pardiñas & Teta, 2011; D'Elía *et al.*, 2015; Gonçalves *et al.*, 2015).

Genomic data were generated for 20 individuals of *H. brasiliensis* distributed throughout the species distribution, encompassing both biomes (thirteen individuals from the Pampas and seven from the Atlantic Forest; Fig. 1; Supporting Information, Table S1.1). Additionally, genomic DNA was generated for the species *Holochilus nanus* and used as an outgroup for the phylogenetic analyses. The genomic data applied in this study are a subsampling of four double-digest restriction-site associated DNA (ddRAD) libraries generated with samples from the entire genus. For a complete description of the ddRAD library preparation and sequencing protocol, see Prado *et al.* (2019, 2021).

The morphometric data were generated for 61 adult individuals (*sensu* Voss, 1991), both males and females (following Abreu Junior *et al.*, 2012), distributed throughout most of the species distribution. From this total, 25 individuals were sampled from the Pampas biome and 36 from the Atlantic Forest biome (Fig. 1; Supporting Information, Table S1.2).

Ecological niche model

An ecological niche model (ENM) was generated to characterize the environmental space occupied by *H. brasiliensis* per biome. Thirty-one bioclimatic and topographic variables from the present were gathered from WorldClim (Hijmans *et al.*, 2005) and ENVIREM (Title & Bemmels, 2018) databases with a resolution of 30 arc-s (Supporting Information, Table S2.1). The geographical extent applied for all variables in the ENM corresponded to the area inhabited by the species at each biome separately, with a 50 km buffer. From the entire set of masked variables, a principal components analysis (PCA) was calculated with the *prcomp* function in R (R Core Team, 2020) to identify the subset of environmental variables responsible for explaining 90% of the observed environmental variation. A correlation analysis was also performed to select only the variables with a correlation of < 0.7, with the *layerStats* function from the R package raster (Hijmans, 2020). When correlated variables were identified, the variable that presented the highest number of correlations was discarded.

After these steps, eight variables were selected in the Atlantic Forest biome (mean diurnal range, bio2; mean temperature of the wettest quarter, bio8; precipitation of the wettest month, bio13; relief, ETOPO; relative wetness and aridity, climaticMoistureIndex; monthly mean of the potential evapotranspiration of the driest quarter, PETDriestQuarter; monthly mean of the potential evapotranspiration of the warmest quarter, PETWarmestQuarter; and the topographic wetness index, topoWet) and seven variables in the Pampas biome (annual potential evapotranspiration, annualPET; monthly mean of the potential evapotranspiration of the driest quarter, PETDriestQuarter; terrain roughness index, tri; relief, ETOPO; isothermality, bio3; mean temperature of the wettest quarter, bio8; and precipitation seasonality, bio15). We used the combination of both sets of variables to perform the ENM analysis at each biome, totalling 12 environmental variables. The occurrence data were thinned, applying a 10 km buffer to reduce

the spatial autocorrelation of the points attributable to the sample bias.

The *ENMevaluate* function from the R package ENMeval (Muscarella *et al.*, 2014) was applied to select the best combination of parameters to achieve a balance between goodness of fit and model complexity. Models were tested over combinations of regularization parameters from 0.5 to 3 in intervals of 0.5, under different combinations of the feature parameters linear (L), quadratic (Q), product (P), threshold (T) and hinge (H) (L, Q, H, L + Q, L + Q + H, L + Q + H + P, and L + Q + H + P + T), following MAXENT recommendations. MAXENT v.3.4 (Phillips *et al.*, 2006) was used to perform the ecological niche modelling analysis, with 100 bootstrap runs and 70/30 partition percentage for the training/testing datasets. From the selected model, the suitability threshold was inferred using the Akaike information criterion (AIC; Warren & Seifert, 2011) and the area under the receiver–operator curve (AUC; Swets, 1988). The threshold was applied to convert the model into a binary prediction for each biome.

CHARACTERIZATION OF THE ECOLOGICAL NICHE

To characterize the ecological niche similarity between both biomes, we applied two different approaches: (1) the multivariate environmental niche overlap, quantified with the 'PCA-env' (Broennimann *et al.*, 2012); and (2) the estimation of *n*-dimensional environmental hypervolume (Hutchinson, 1957; Blonder *et al.*, 2014). The approach proposed by Broennimann *et al.* (2012) is a robust two-dimensional statistical framework that describes and compares niches in a gridded environmental space and tests hypotheses regarding niche conservatism. It is appropriate for studying niche differences between species or populations that differ in their geographical distributions. The approach proposed by Blonder *et al.* (2014) has the advantage of using a multidimensional set of variables to quantify the geometrical shape of the fundamental niche.

First, we performed a PCA with the 12 abiotic variables selected to build the ENMs for each biome using the rasterPCA function from the R package RStoolbox (Leutner *et al.*, 2019). This function allows a PCA calculation to be performed directly on raster files. From the PCA of the biome, specific environmental values for the species occurrence records were extracted. Additionally, from the binary suitability map generated for each biome, a set of 1000 random points were selected, and from them the PCA environmental values of the biome were also extracted. Both datasets (environmental values for the species occurrence records and from the 1000 random points) were used to perform the 'PCA-env' and generate the hypervolume of the species in each biome.

The 'PCA-env' was performed with the *dudi.pca* function from the R package ade4 (Dray & Dufour, 2007). Based on the first two principal components (PCs) from the 'PCA-env' space, niche overlap between biomes was quantified using Schoener's (1970) *D* index, niche equivalency and similarity, all of which were assessed with the R package Ecospat (Broennimann *et al.*, 2021). Specifically, the environmental PCs were used to create a grid with occurrence densities along the environmental gradient for each biome separated using the *ecospat.grid.clim.dyn* function, and the two occurrence density grids were compared using the *ecospat.niche.overlap* function. To assess whether the niches were more or less similar than expected by chance (niche conservatism vs. niche divergence), we performed a niche similarity test with the *ecospat.niche.similarity.test* function. The niche similarity test assesses whether the niche occupied in one range is more similar to the one occupied in the other range than would be expected by chance (Broennimann *et al.*, 2012). A niche equivalency test was performed with the *ecospat.niche.equivalency.test* function to determine whether niches of two populations with distinct geographical ranges were constant when randomly reallocating the occurrences of both lineages among the ranges. Both tests were performed with 2000 replicates. Also, we estimated the overlap between the hypervolumes from both biomes using the *hypervolume_gaussian* function from the R package Hypervolume (Blonder, 2018). The values of each hypervolume and their similarity (the Sørensen similarity index) were estimated with the *get_volume* and the *hypervolume_overlap_statistics* functions, both from the R package Hypervolume (Blonder, 2018).

GENOMIC DATA AND BIOINFORMATICS

Raw sequence reads were processed with the STACKS v.2.54 pipeline (Catchen *et al.*, 2013). Specifically, the reads were demultiplexed and filtered using *process radtags*. One mismatch in the adapter sequence (--adapter_mm) and a barcode distance of two (--barcode_dist) was allowed, and individuals with < 500 000 reads were excluded. The USTACKS module was used to create a *de novo* assembly of reads with a minimum coverage depth (m = 5) and a maximum distance in nucleotides (M = 2), enabling the *removal algorithm* (-r), the *deleveraging algorithm* (-d) and the model type equal bounded (--model_type) settings, in addition to an error bound rate (ε) of 0.1 (--bound_high). A catalogue of consensus loci among individuals was constructed with the CSTACKS module, allowing for two mismatches between sample tags when building the catalogue (-n 2); loci were identified using SSTACKS under default options. After SSTACKS, both TSV2BAM and GSTACKS programs were used

to transpose data to be oriented and build a contig by locus, align reads per sample, and call variant sites and genotypes for each individual. Files were then loaded into the POPULATIONS module, requiring a locus to be present in at least two populations to be considered (-p 2). This single nucleotide polymorphism (SNP) dataset was exported in Variant Call Format (vcf). We performed a whitelist in R, in which the SNPs positioned at the 18 last base pairs at the end of all loci were removed because of an artificially increased number of SNPs observed at these last positions. Also, loci with high theta values (> 95th percentile) were removed, given that these are suggestive of sequencing and assembly errors (script available at: https://github.com/joycepra; Thomaz *et al.*, 2017). With this whitelist, we re-ran POPULATIONS to generate a vcf file without these dubious variants and only a putative random SNP per locus. Finally, we created a dataset with only 20% of missing data (total of 24 264 SNPs; Supporting Information, Table S2.2) and used it in all analyses. The filtering step for missing data was performed with the toolset PLINK v.1.90 (Purcell *et al.*, 2007), and all bioinformatics processing with STACKS was performed in the high performance computing cluster at the Universidade de São Paulo.

GENOMIC DIVERSITY AND STRUCTURE

Summary statistics were calculated with POPULATIONS in STACKS and used to estimate genetic diversity and differentiation between the Pampas and the Atlantic Forest populations, including nucleotide diversity (π), observed heterozygosity (H_{obs}), expected heterozygosity (H_{exp}), Wright's inbreeding coefficient (F_{IS}) and fixation index (F_{ST}). Additionally, Student's unpaired *t*-test was used to check for significant differences in the genetic diversity summary statistics (R package BSDA; Arnholt, 2017).

To evaluate genomic structure and measure whether the assignment of individuals into genetic clusters followed the environmental distinction between biomes, we used the software STRUCTURE v.2.3.4 (Pritchard *et al.*, 2000), with individuals not conditioned to a population a priori. The dataset was analysed for a *K*-value (number of populations) of two to check whether there was genetic structure between the biomes. For this analysis, 15 independent runs were performed, with 300 000 Markov chain Monte Carlo iterations each and the first 100 000 discarded as burn-in. The software CLUMPAK (Kopelman *et al.*, 2015) was used to assign individuals graphically to their ancestral history.

In order to visualize the major axes of population genetic variation, a PCA was performed with the *dudi.pca* function from the R package ade4 (Dray & Dufour, 2007). After the PCA, a Tracy–Widom test (Tracy & Widom, 1994) was performed with the *tw* function from the R package AssocTests (Wang *et al.*, 2020) to select the significant number of PCs that could be interpreted as genetic clusters. To test the correlation between genetic and geographical distances among individuals, we used the results from the PCA to generate a genetic distance matrix between individuals that was used in the Mantel test (*mantel.rtest* function) with 10 000 permutations with the R package ade4 (Dray & Dufour, 2007), considering each biome separately and combined. For that, the number of PCs from the genetic data that corresponded to 70% of the total variation were the input for the *distance* function (R package ecodist; Goslee & Urban, 2007) to calculate the genetic distance matrix with the Mahalanobis method. The geographical distance matrix among individuals was generated with the *earth.dist* function from the R package *fossil* (Vavrek, 2011).

To test whether the relatedness history among individuals corresponded to each biome, we estimated relationships among individuals using the program SVDQUARTETS (Chifman & Kubatko, 2014). We evaluated all possible quartets, selecting trees using the Quartet Fiduccia-Mattheyses quartet assembly, and we also performed bootstrapping with 1000 replicates to calculate branch support.

To uncover the colonization history and calculate the number of migrants between the two biomes, demographic parameters were estimated using a composite-likelihood simulation-based approach implemented in FASTSIMCOAL2 (Excoffier & Foll, 2011; Excoffier *et al.*, 2013) based on the site frequency spectrum (SFS). We tested three different demographic models: (1) strict divergence between the two biomes (three parameters to be estimated in the model); (2) divergence with symmetrical migration rate between biomes (four parameters to be estimated); and (3) divergence with asymmetrical migration rate between biomes (five parameters to be estimated). A python script was used to remove all missing data from the POPULATIONS output file and to calculate the joint SFS between biomes [modified from He & Knowles (2016) and available on GitHub at: https://github.com/joycepra]. This script selects the five individuals with smaller levels of missing data from each population at each locus to calculate the SFS.

To improve the performance of the models, one population parameter (the effective population size of the Pampas biome, *N1*) was fixed and calculated directly from the empirical data based on the nucleotide diversity (π) of variant and invariant sites and on a genomic mutation rate, μ, of 3.67×10^{-8} per site per generation (estimated for another rodent species, *Peromyscus maniculatus*; Pfeifer *et al.*, 2018). Other parameters, such as *N2* (the effective population size of the Atlantic Forest biome), ancestral

population size (N_{ANC}), divergence time (T_{DIV}) and migration rates (M for asymmetric; *M12* and *M21* for the asymmetric scenarios), were estimated from the SFS using uniform priors (Supporting Information, Table S2.3) and a generation time of 2 years. A total of 40 FASTSIMCOAL2 runs were conducted for each model. Each run was performed with 250 000 simulations per likelihood estimation, 40 expectation-conditional maximization (ECM) cycles, and the flag --removeZeroSFS (which allows the omission of monomorphic sites). To select the best-fitting model to the observed data, we used the AIC for the single run with the highest composite likelihood. A parametric bootstrap was used to estimate 95% confidence intervals on the parameter estimates for the more probable model selected based on 100 simulated SFSs and that were used to re-estimate the parameters each time (Excoffier *et al.*, 2013).

Morphometric structure

Skull and dental measurements were taken only from adult specimens under a stereomicroscope and with digital callipers (accurate to 0.01 mm). These measurements included 21 variables: length of upper molar series (LM), breadth of first upper molar (BM1), length of incisive foramen (LIF), breadth of incisive foramen (BIF), breadth of the incisor tips (BIT), breadth of palate (BP), length of nasal (LN), breadth of nasal (BN), least interorbital breadth (LIB), breadth of braincase (BB), breadth of zygomatic plate (BZP), depth of incisor (DI), breadth of the occipital condyles (BOC), length of palatal bridge (LPB), breadth of orbital fossa (BOF), breadth of rostrum (BR), length of interparietal (LI), breadth of interparietal (BI), breadth of bulla (BBU), lambdoidal breadth (LBB) and length of condyle–zygomatic (CZL). Additionally, external body measurements were taken from the scientific collection labels of specimens, such as length of body (LB), length of tail (LT), length of ears (LE) and length of hindfoot (LH). See the Supporting Information (Table S2.4) for a complete description of all the measurements.

Skull and dental measurements were analysed via a multivariate approach. Initially, to check whether the variables follow a multivariate normal distribution, we applied Mardia's test with the *mult.test* function from the R package QuantPsyc (Fletcher, 2012). Then, a PCA and a discriminant function analysis were performed with $\log_{10}$-transformed data. The PCA was used to obtain an exploratory view of the data and was applied to unveil how the morphometric variation was distributed in the multivariate space. The discriminant function analysis was used to test the morphometric differences between individuals in both biomes statistically. The discriminant function analysis was performed with the *lda* function from the R package MASS (Venables & Ripley, 2002), and the PCA was performed with the *prcomp* function from the R package stats. Additionally, to measure the proportion of total variance in the variables that was accounted for by the grouping of specimens in the two biomes, we performed a multivariate analysis of variance (MANOVA) with the *manova* function from the R package stats.

Given the high frequency of missing data in the external body measurements, significant differences between biomes for these variables were assessed only with a univariate approach. Shapiro–Wilk univariate normality tests were performed with the *shapiro.test* function from the R package stats. For variables that followed a normal distribution, we applied unpaired Student's *t*-test, using the *t.test* function from the R package stats. Otherwise, we applied a Mann–Whitney *U*-test with the *wilcox.test* function from the R package stats. Boxplots displaying the mean and the 95% confidence interval of each of the external variables are also shown. Additionally, descriptive statistics for all variables are presented, including the sample size, mean, standard error, minimum and maximum value for each variable (Supporting Information, Table S2.5).

Association between environment, genome and morphometrics

We tested for an association between environmental variables and genomic and morphometric distance by performing a distance-based redundancy analysis (dbRDA; Legendre & Anderson, 1999). This technique summarizes linear relationships between components of response variables explained by a set of explanatory variables. Specifically, in this study, we tested the linear relationship between pairwise genomic and morphometric distances explained by environmental variables with and without the effect of the geographical distance. The function *capscale* from the R package vegan (Oksanen *et al.*, 2020) was used for the dbRDA.

We used the results from the PCAs to extract the genomic and morphometric distance matrixes between individuals with the function *distance* from the R package Ecodist (Goslee & Urban, 2007) using the Mahalanobis method. For that, the number of PCs from each data type that corresponded to 70% of the total variation (11 for the genomic data and four for the morphometric data) were extracted (note that for the morphometric distance, only the skull/dental measurements were used). Data from the corresponding environmental variables were obtained based on the PC1 performed with the 12 layers used in the ENMs. The geographical distance matrix among individuals was generated with the

earth.dist function from the R package *fossil* (Vavrek, 2011). Given that dbRDA relates a response matrix to rectangular predictors, we used the function *pcnm* from the R package vegan (Oksanen *et al.*, 2020) to transform the geographical distance matrix into continuous rectangular vectors via principal coordinates analyses.

RESULTS

Ecological niche characteristics

The set of environmental characteristics that represent the niche of *H. brasiliensis* (i.e. niche hypervolume) in the Atlantic Forest and Pampas biomes is very different (Sørensen similarity index = 0.0373), as is Schoener's *D* overlap metric (*D* = 0.4485), suggesting distinct segregation in the ecological niches that this taxon occupies within each biome (Fig. 2; Table 1). This segregation is conspicuous when the environmental PCs of both biomes are compared (Fig. 2A), with most of the centroids for each hypervolume not overlapping. Additionally, the niche volume of the Atlantic Forest biome is much larger than the niche volume of the Pampas (Table 1). The observed differentiation among the occupied ecological niches at each biome was not enough to reject either the niche equivalency hypothesis (*P*-value = 0.376) or the niche similarity hypothesis (*P*-value Pampas → Atlantic Forest = 0.072; *P*-value Atlantic Forest → Pampas = 0.079; Supporting Information, Fig. S2.1).

The current environmental characteristics of the biomes inhabited by *H. brasiliensis* (Pampas and Atlantic Forest) differ clearly (i.e. segregation of the environmental scores in Fig. 2B, and the differences in colour in the environmental map in Fig. 2C), as does the proportion of the area of potential distribution of the species in each biome (Fig. 2D), which is predicted to be more suitable in the Pampas. Additionally, environmental PC1 and PC2 account for most of the environmental variation in both biomes (69.16% in the Pampas biome and 57.03% in the Atlantic Forest biome). Also, the variables that contributed the most to these axes within each biome do not overlap between them. Within the Atlantic Forest, the variables that most contributed to the variation in each of PC1 and PC2 were the annual potential evapotranspiration (annualPET) and the relief (ETOPO). In the Pampas, the most important variables were the relative wetness and aridity (climaticMoistureIndex) and isothermality (bio3).

Structuring of genetic variation

The comparison between biomes performed on the genetic summary statistics recovered nearly identical and non-significant results (see Supporting Information, Table S2.6). Nevertheless, the Atlantic Forest population presented lower levels of genetic diversity with a higher inbreeding coefficient in comparison to the Pampas.

The genetic structure results accessed with PCA (Fig. 3A, B) and STRUCTURE (Fig. 3C) recovered no population structure directly associated with biomes. Also, STRUCTURE analysis supported two probable patterns of population structure for $K = 2$ (Fig. 3C), indicating a mixed signal at the genome. Additionally, the Tracy–Widom test corroborated the existence of a single cluster. These results agree with the low genetic differentiation between biomes exhibited by F_{ST} (0.099).

The individual relationships evaluated with SVDQuartets also did not recover reciprocal monophyly for each biome. Indeed, individuals of the Atlantic Forest from Paraguay clustered with individuals from Argentina in the Pampas biome (see Supporting Information, Fig. S2.2).

Furthermore, the isolation-by-distance pattern for the entire distribution was not significant, indicating that genetic distances between individuals were not associated with geographical distances (Mantel test = 0.21, *P* = 0.12). However, when the biomes were analysed separately, a significant association was found for each of them (Atlantic Forest, Mantel test = 0.31, *P* = 0.02; Pampas, Mantel test = 0.39, *P* = 0.003).

Migration history

Results from FASTSIMCOAL2 supported divergence with asymmetrical migration as the best-fitting model (Supporting Information, Table S2.7), but migration estimates were generally low. The lowest migration estimate per generation was from the Pampas biome to the Atlantic Forest (7×10^{-7} vs. 1.2×10^{-5} in the opposite direction), indicating less than one individual per generation migrating between biomes (i.e. 0.19 and 0.7 individuals migrating per generation, respectively).

Structuring of morphometric variation

Morphometric differences between biomes were supported by both external and cranial/dental datasets. In general, adult specimens from the Pampas had overall larger body sizes than the Atlantic Forest specimens (see Figs 4, 5; Supporting Information, Tables S2.8 and S2.9). A multivariate normality test on the cranial/dental measurements showed that the data follow a normal distribution. Comparing cranial/dental measurements, the PCA displays a conspicuous separation along PC1 (corresponding to 46.99% of the total variation), but a small differentiation along PC2 (explaining 10.76% of the total variation; Fig. 4A). The

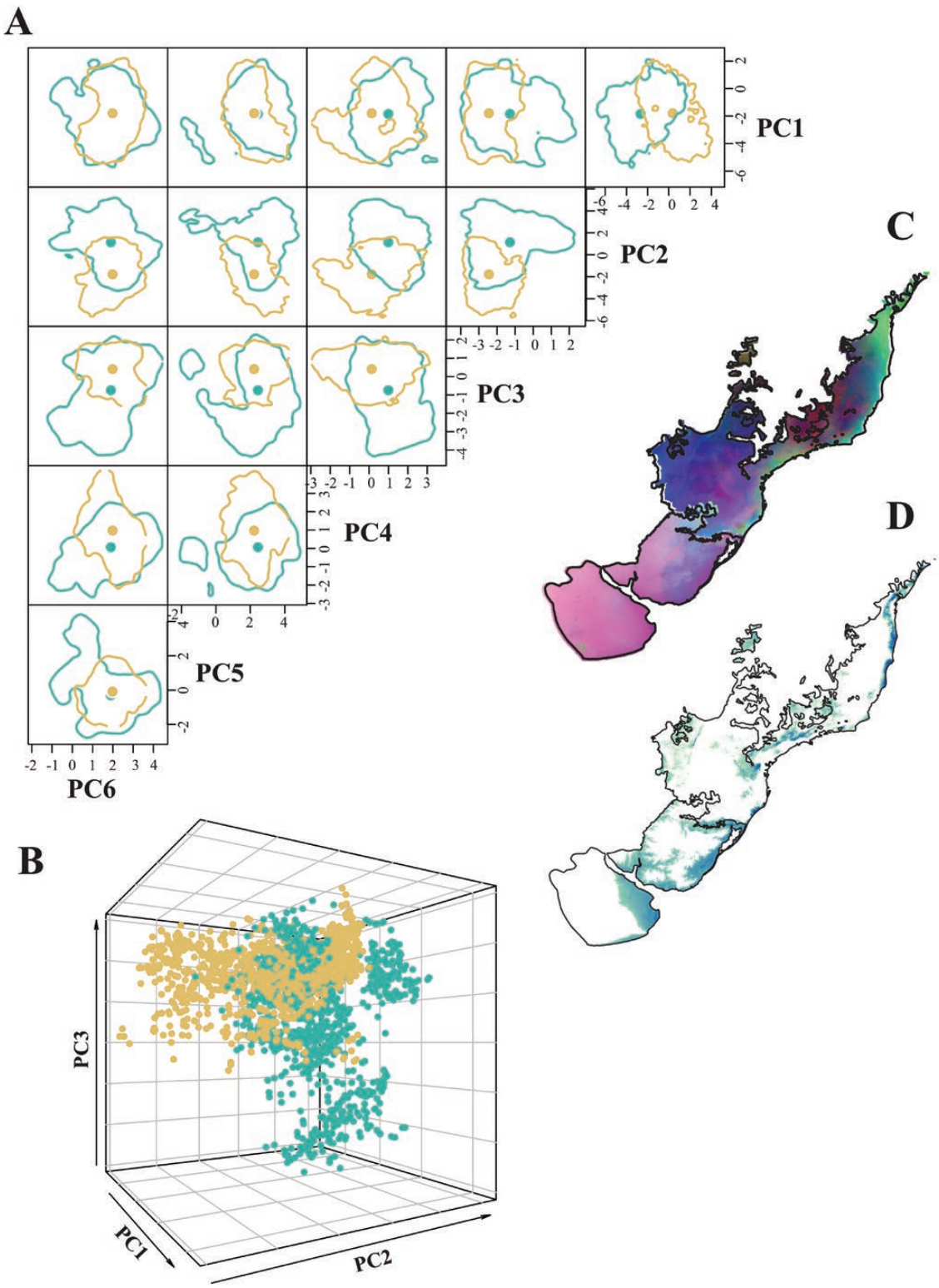

Figure 2. Environmental differentiation across biomes. A, ecological niche partitioning for the two biomes, Pampas (yellow) and Atlantic Forest (green). Hypervolumes are shown as two-dimensional projections for all combinations of principal component (PC) axes. B, three-dimensional projections of the first three environmental PCs. C, map of the environmental variation across the region where the species are distributed. Differences in colour represent the geographical regions that

Table 1. Pairwise niche overlap values in terms of *D* (Schoener's overlap metric), equivalency and niche similarity *P*-values, hypervolumes per biome, total hypervolume, intersection and Sørensen overlap of *Holochilus brasiliensis*

Niche overlap	Equivalency	Similarity Pa → AF	Similarity AF → Pa	Volume of Pampas	Volume of Atlantic Forest	Volume of union	Volume of intersection	Sørensen
0.4485742	0.376	0.0729	0.0799	142.6294	645.6976	773.621333	14.705616	0.03730

Sørensen indices range from zero (no overlap) to one (identical). Abbreviations: AF, Atlantic Forest; Pa, Pampas.

most important variables explaining the morphometric differences between biomes along PC1 are LPB, LBB and CZL; in PC2, BIF, LN and LIB were the most relevant variables (Fig. 4B; Supporting Information, Table S2.8). There is also some geographical variation in size throughout the geography (Supporting Information, Fig. S2.3): the mean values of PC1 of pooled samples from south-eastern Brazil, Paraguay (both from the Atlantic Forest), southern Brazil plus Uruguay, and Argentina (both from the Pampas) indicate an increase in size in a clinal variation from north to south, with a discontinuity in this trend coincident with the Rio da Plata estuary, with Argentinean samples exhibiting scores similar to those from Paraguay.

The discriminant function analysis also displayed significant discrimination between both biomes (Fig. 4C); however, unlike the PCAs, the variables that contributed most to the individual discrimination between biomes in this analysis were BM1 and BBU (Supporting Information, Table S2.9). The MANOVA results also confirm the significant difference in the cranial and dental measurements between Atlantic Forest and Pampas biomes ($F = 12.88$, $P = 0.000$).

The univariate normality test performed on the external variables recovered a normal distribution pattern for the variables LB, LT and LH. The LE did not follow a normal distribution. Statistical tests performed on all variables showed significant differences ($P < 0.05$) between biomes, except for the LB comparison, which was marginally significant ($P = 0.06$). The morphometric differences between biomes are also conspicuous when displayed graphically (Fig. 5).

Association between environment, genome and morphometry

The association tests (dbRDA) between the environmental variables, geographical distance and genomic and morphometric distances corroborated previous results. We did not find statistical significance in any of the comparisons including the genomic data. Specifically, the associations between geographical distance and genomic distance and between genomic distance and environmental variables were not significant (even controlling for the effects of geography), indicating that factors other than the environment influence genomic differences more significantly within *H. brasiliensis*. Only 4.01% of the genomic variation can be explained by the environmental variables. This amount is even lower when the association between genomic distance and environmental variables is conditioned on the geographical distances (2.96%; Table 2).

The lack of significance of the dbRDAs for the genomic data contrasts with the significant associations with the morphometric data. That is, the role of the environment in explaining morphometric differences is suggested by the significant results of the association tests between the environmental variables and the morphometric distance. The results show that the environment can explain 11.34% of the morphometric variation. This proportion is smaller than the percentage of the morphometric variance accounted for by the geographical distance (59.56%) but is still statically significant ($P = 0.001$). However, when the association between morphometric distance and environmental variables is controlled for the effects of geography (conditional test), the results are not significant (1.23%, $P = 0.281$; Table 2), being even smaller than the conditional tests performed for the genomic data.

DISCUSSION

The response of a species to environmental change depends on its habitat preferences (Massatti & Knowles, 2016) and/or natural history traits (Prado *et al.*, 2019). By considering the differences in the

differ most from each other. Specifically, the red–green–blue colour composite was calculated and plotted on the map with the PC1 set as the red scale, PC2 as the green scale and PC3 as the blue scale. D, MAXENT ecological niche model map of habitat suitability for *Holochilus brasiliensis*. Lighter colours indicate low habitat suitability; darker colours indicate high habitat suitability.

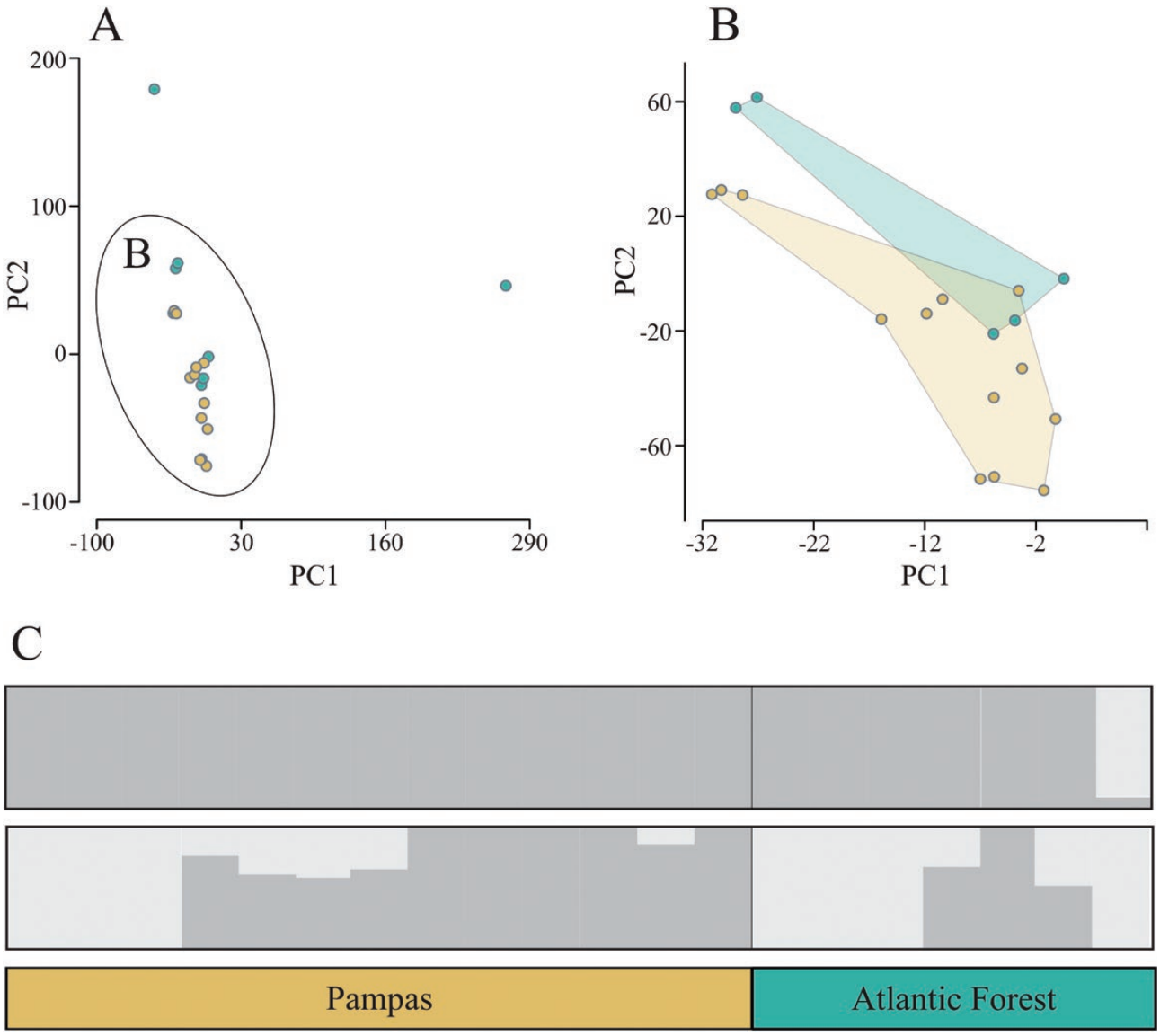

Figure 3. Genetic variation across Pampas (yellow) and Atlantic Forest (green) biomes. A, distribution of individuals along principal component 1 (PC1) and principal component 2 (PC2) of genetic variation based on the analysis of polymorphic single nucleotide polymorphisms. B, detail of the plot highlighted in A. C, results from STRUCTURE analyses depicting the genetic structure with $K = 2$ groups and the ancestry of the two biomes represented by different colours.

local environmental space, genetic differentiation of neutral alleles and morphological variation between populations that inhabit different biomes, our study provides important insights into how intrinsic factors of a highly specialized taxon respond to abiotic factors, and their impact in the species dispersal history. We found that the niche differences between the studied biomes are conspicuous but do not necessarily represent a function of habitat selection. Nonetheless, such differences are consistent with a strong morphological variation associated with the size of these rodents between the two biomes. However, the ecological and phenotypic differentiation is not accompanied by the same pattern of genetic variation. Below, we discuss how these findings can help us to understand the role of environment changes (at a biome scale) in shaping: (1) ecological niche characteristics; and (2) genetic and morphological variation.

Ecological niche differences in specialized taxa

Although natural populations might present intraspecific niche differentiation (Ashman *et al.*, 2018), the magnitude

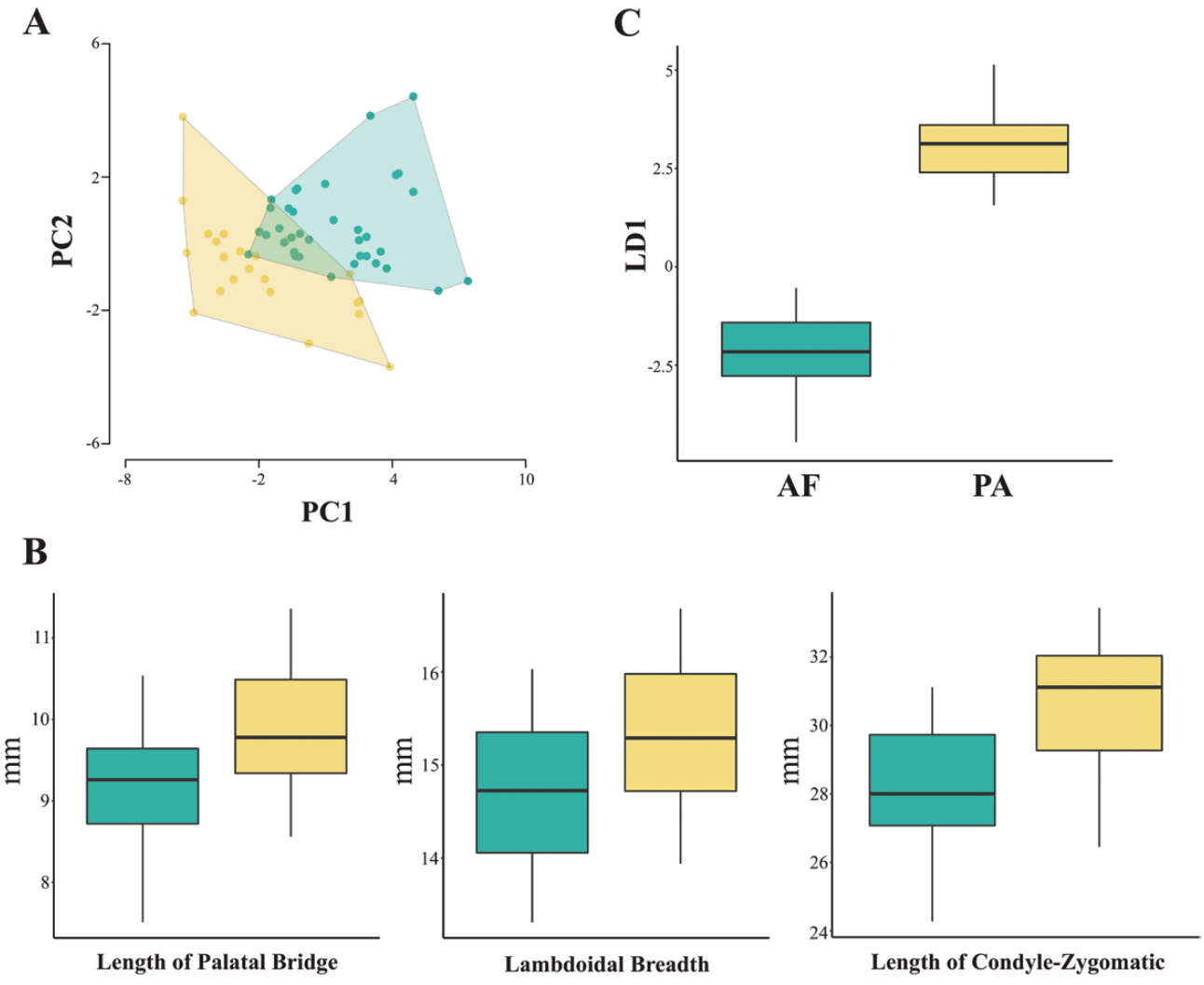

Figure 4. Morphometric variation across Pampas (yellow) and Atlantic Forest (green) biomes. A, distribution of individuals along principal component 1 (PC1) and principal component 2 (PC2) of morphometric variation. B, boxplot showing the descriptive statistics for the most important variables explaining the morphometric differences between biomes along PC1, which explain 46.99% of the total variation [length of palatal bridge (LPB), the lambdoidal breadth (LBB) and the length of condyle–zygomatic (CZL)]. C, boxplot showing the score of individuals along the first discriminant function (LD1) of morphometric variation.

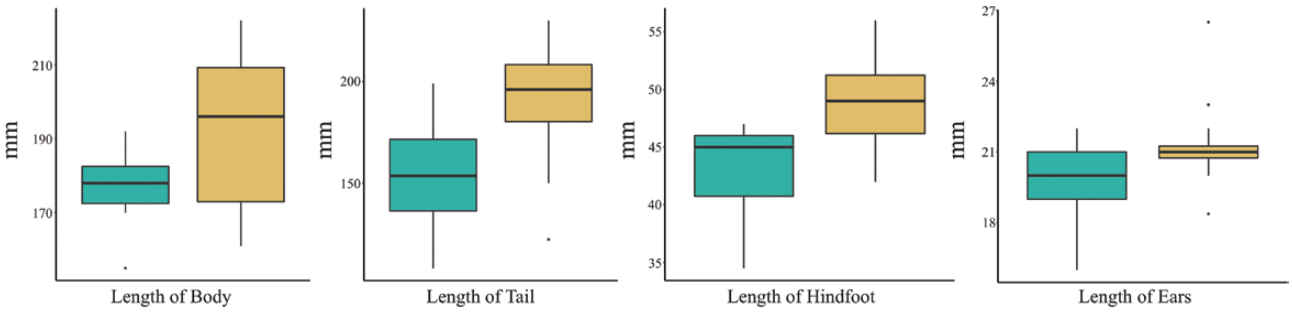

Figure 5. Boxplot showing the descriptive statistics for the external variables explaining the morphometric differences between Pampas (yellow) and Atlantic Forest (green) biomes.

Table 2. Tests of association between genetic and morphometric distances with environmental differences or geographical distance between individuals using distance-based redundancy analysis

		Marginal tests			Conditional tests		
	Variable	F	*P*-value	Percentage of variance	*F*	*P*-value	Percentage of variance
Genomic	Environmental	0.7526	0.473	4.01	0.5154	0.757	2.96
	Geographical	0.8728	0.667	33.74	–	–	–
Morphometric	Environmental	7.547	**0.001**	11.34	1.2631	0.281	1.23
	Geographical	3.178	**0.001**	59.56	–	–	–

Results are given for each geographical and environmental variable separately (marginal tests) and conditioned on the effects of geographical distance (conditional tests). The *F*-statistics, *P*-values and the percentage of variance explained by each variable are presented, and values in bold are significant *P*-values.

of niche variation within populations is context dependent (Costa-Pereira *et al.*, 2018). For instance, environmental gradients across the landscape can shape variation in niche preferences that is more continuous than with an abrupt shift in the environment (Borzée *et al.*, 2016). Our findings demonstrate differences in the ecological niche between biomes that are dictated by the physical characteristics of each biome.

Environmental variables related to temperature, precipitation, evapotranspiration and relief were the most important ones in shaping these differences. In the non-forested biome (Pampas), variables related to precipitation (relative wetness and aridity) and temperature (isothermality) had significant weight in the environmental PCAs. In the Atlantic Rainforest, variables related to evapotranspiration (annualPET) and relief (ETOPO) were the most important. As already mentioned, the Pampas biome is dominated by extensive grassland plains with a subtropical climate (Overbeck *et al.*, 2015), with extensive lagoons that can be permanent or temporary (Cabrera, 1976; Josens *et al.*, 2012). In these circumstances, variables related to precipitation and temperature could determine the species habitat occupation. In contrast, the Atlantic Forest exhibits a much more complex topographic arrangement and a climatic regime frequently associated with species occurrence and endemism (e.g. Vale *et al.*, 2018; Brown *et al.*, 2020). For an organism with a preference for flooded plains or *várzea* areas, a rugged relief such as that of the Atlantic Forest, predominantly covered by dense forest, can limit the spaces occupied by these rodents in this biome to plains, intermontane basins and coastal lowlands. Physical characteristics of the biomes can also explain the large differences in their volumes. The Pampas is a biome much more homogeneous than the Atlantic Forest, meaning that the environmental variables that influence the ecological niche of *H. brasiliensis* within this biome are less changeable.

Niche similarity results confirm that ecological niches located in both biomes do not tend to be more or less similar than expected at random (Supporting Information, Fig. S2.1), suggesting a lack of niche conservatism. However, even with the conspicuous difference in ecological niche between biomes (Fig. 2), we could not to refute the hypothesis of niche equivalency (Supporting Information, Fig. S2.1), suggesting that the ecological niches of *H. brasiliensis* are interchangeable (i.e. it is still precise to imply niche characteristics for one biome based on the niche of the other biome). These findings might indicate that the characteristics of the species history traits (preferences for specific habitats, such as open wetlands) might constrain the degree of ecological differentiation observed in this species between these two biomes (reducing the niche divergence among individuals), even when the environmental variation between biomes would be strong enough to reduce the level of niche conservatism among individuals. The differences between hypervolume and 'PCA-env' approaches (i.e. conspicuous differences in the hypervolume analysis but the lack of significance of niche equivalency or similarity tests) can be explained by the different ways in which these methodologies handle the environmental data. The 'PCA-env' approach considers niche overlap in only two dimensions, whereas the hypervolume approach compares five different dimensions.

The interpretation of the differences in the ecological niche between these two biomes must also consider the potential shortfalls associated with ENM. Ecological niche modelling is a powerful tool to examine niche overlap (e.g. Culumber & Tobler, 2016; Scriven *et al.*, 2016), and current methods that allow the use of PCA provide a reliable way to test hypotheses regarding niche divergence and conservatism (Broennimann *et al.*, 2012). However, niche overlap analyses using ENMs could be problematic regarding unquantified statistical artefacts related to model fitting and the distribution of environmental gradients in the study area. Hence, the environmental space shifts much more abruptly in the Atlantic Forest owing to its complex geography and its heterogeneous phytophysiognomy

than in the Pampas biome, which could be problematic for capturing microhabitat in the former.

Genetic and phenotypic responses to different biomes

Despite differences in the patterns of connectivity between wetland patches in both biomes (e.g. patches of open wetlands in the Atlantic Forest vs. large contiguous open wetlands in the Pampas), genetic diversity and differentiation (Fig. 3; Supporting Information, Table S2.6) are similar within each biome, corroborating a previous study (Prado *et al.*, 2019).

The pattern of regional structure (i.e. environmental differences between biomes) does not seem to be the main factor structuring genetic variation within this species (see Fig. 3C; Supporting Information, Fig. S2.1). The lack of genetic structure is in agreement with a migration history indicating that biome differences do not restrain gene flow between them. The evidence of gene flow between biomes and the absence of an independent ancestral history in each biome separately (Fig. 3), together with the inferred homogeneous distribution of the species since the Last Glacial Maximum (Prado *et al.*, 2019), reduces the impact of differences in biomes in the genetic diversity within this species. Alternatively, the origin of *H. brasiliensis* is recent, dating from ~0.87 Ma (Prado *et al.*, 2021). This recent origin estimate is consistent with the large amount of shared ancestry between biomes, suggesting that there might not have been enough time to develop a genetic structure related to biomes.

Rather than being associated purely with the two biomes that the taxon inhabits, the genetic structure seems to respond to different drivers, such as geographical distance within each biome and phytophysiognomic heterogeneity. Our genetic samples from the Pampas biome were all collected in grasslands, savannas and scrubland ecoregions. In contrast, our genetic samples from the Atlantic Forest were collected in three very different phytophysiognomies. The sample from Rio de Janeiro state in Brazil on the east is from the Serra do Mar coastal forest ecoregion, a tropical moist broadleaf forest. Samples from the Brazilian Paraná and Santa Catarina states in the south are from a region characterized by *Araucaria* moist forest, a coniferous forest ecoregion that includes open areas. Paraguayan samples on the west were collected in a border region between an open area surrounded by grasslands, savannas, scrublands (the Humid Chaco ecoregion) and a humid broadleaf forest (the Parana interior forest) on the banks of the Tebicuary River. Paraguayan samples share a phytophysiognomy much more similar to the samples from the Pampas biome than to other locations in the Atlantic Forest, which could explain the pattern of historical relatedness found in the phylogenetic tree (Supporting Information, Fig. S2.2). Also, according to the PCAs (Fig. 3; Supporting Information, Fig. S2.4), the samples from the southern part of Brazil, from both the Pampas and Atlantic Forest biomes, are more genetically close to each other than to other samples from the same biome, suggesting that geographical distance explains the pattern better than differences between biomes.

In contrast to the genetic data, phenotypic data recovered significant differences between biomes related to the overall size of the adult individuals (see Figs 4, 5). In all cases, specimens from the Pampas were larger than specimens from the Atlantic Forest. Allometric traits (i.e. overall size) are often recognized with the potential to change dramatically over short evolutionary time scales (e.g. Barnosky *et al.*, 2003; Millien *et al.*, 2006) as a rapid response to variation in the natural history of the species (Arendt, 2007), such as diet (Price & Hopkins, 2015; Pineda-Munoz *et al.*, 2017; Grossnickle, 2020) and locomotion (Biewener, 1990; Lovegrove & Haines, 2004; Kilbourne & Hoffman, 2013, 2015). Empirical and theoretical studies also show that variation in size is frequently correlated with changes in environmental variables, such as temperature, precipitation, moisture and food resources (for a review, see Meiri & Dayan, 2003).

A classic hypothesis to explain this pattern would be Bergmann's rule. The Pampas is located south of the Atlantic Forest, exhibiting colder environments and, as a consequence, larger body sizes than the samples from the Atlantic Forest. However, this should be interpreted cautiously and investigated further, given that rodents might not adhere to this generalization (Meiri & Dayan, 2003).

Another potential explanation is the 'resource rule' (McNab, 2010), which predicts that larger body size is a consequence of higher resource availability (e.g. food sources and water). Contradicting macroevolutionary patterns found for other rodents (Alhajeri *et al.*, 2020), this rule might fit well for *H. brasiliensis*. Individuals of the genus *Holochilus* are most associated with riparian or marshy open habitats with deep herbaceous ground cover, being specialized small grazers, feeding mainly on grasses and herbaceous plants but also on cultivated plants in agricultural fields (such as wet rice plantations); these characteristics are more commonly observed in the Pampas than in the Atlantic Forest. However, we did not evaluate the availability of resources in both biomes; hence, we cannot adhere to this generalization, and further investigations are needed to confirm this hypothesis. Although we do not have body mass data to compare, our results indicate that

sites with larger specimens (and, in consequence, possibly heavier ones) occurred at higher latitudes, in the Pampas biome. This pattern is the opposite of the one proposed in macroevolutionary analyses for sigmodontine rodents (Maestri *et al.*, 2016). Contradictions to these macroevolutionary studies (Maestri *et al.*, 2016; Alhajeri *et al.*, 2020) point to the importance of considering the specific biological attributes of each species and its variation across the species distribution; knowledge that we believe to be helping to build with the present study.

Although our statistical framework points to a clear morphometric differentiation between the two biomes, with a significant proportion of the variance being explained by the environment, the effects of the environment become insignificant after controlling for geographical distance. This suggests that geography, which also explains latitudinal environmental change, might be the primary driver of the patterns of morphometric variation. We do not have samples from localities close to the border between both biomes to disentangle this effect. These samples would be essential to test for the association and relative importance of the environment and geography in the morphometric variance of these rodents. Even so, we believe that our study raises new and important data to advance knowledge about how ecology, genetics and morphometrics interact to generate patterns of diversity.

Conclusion

The biomes that *H. brasiliensis* inhabits are very distinct from each other, and the ecological niches in the biomes are also somewhat distinct. The differences in niche favour morphometric dissimilarities related to the size of the individuals, but even so there is still movement and gene flow between individuals inhabiting these two biomes, precluding the establishment of genetic structure. Overall, the observed differences between biomes in terms of the general size and ecological factors seem to reflect a complex combination of factors that include geographical distance, environmental differences and evolutionary history (Cooper & Purvis, 2010). Additional samples from both biomes are necessary to provide a more colourful and detailed painting of the evolutionary processes that shaped the variation in this unique species.

ACKNOWLEDGEMENTS

This study was funded by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP; Proc: 2014/22444-0, 2012/24099-3, 2009/16009-1 and 2016/20055-2) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES; 88887.336032/2019-00). We are grateful to the curators and staff of the scientific collections studied in this contribution: Museum of Vertebrate Zoology (MVZ, USA), Museum of Southwestern Biology (MSB, USA), University of Michigan Museum of Zoology (UMMZ, USA), Universidade Federal de Santa Catarina (UFSC, Brazil), Museu Nacional (MN, Brazil), Museu de Ciências Naturais da Ulbra (MCNU, Brazil), Universidade Federal de Minas Gerais (UFMG, Brazil), Natural History Museum (BMNH, UK), Museo Argentino de Ciencias Naturales 'Bernardino Rivadavia' (MACN, Argentina), Muséum National d'Histoire Naturelle (MNHN, France) and the Knowles laboratory for the technical support during the genomic library construction and bioinformatics. We are also indebted to the anonymous reviewers who contributed in the review of this manuscript.

DATA AVAILABILITY

Dataset collected, codes, and all the Supporting Information are available in the figshare repository (https://figshare.com/articles/dataset/Biome_effect_on_phenotypic_and_genomic_differences_in_the_South_America_marsh_rats/16589744).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. Summaries of geographical information and genomic data.
Table S1.1. Sampled specimens used in next-generation sequencing, with species, voucher number, locality of origin and biome (AF, Atlantic Forest; PA, Pampas).
Table S1.2. Sampled specimens used in phenotypic analysis, with species, voucher number, locality of origin and biome (AF, Atlantic Forest; PA, Pampas).
Appendix S2. Summaries of methodological settings and results.
Table S2.1. For each biome, the number of distribution points and variables used in niche modelling are shown.
Table S2.2. Sampling and genomic sequences pre- and postprocessing in STACKS for each individual, with biome (AF, Atlantic Forest; PA, Pampas), voucher number, number of raw reads, final number of loci and mean coverage.
Table S2.3. Priors used in the FASTSIMCOAL2 analysis.
Table S2.4. Descriptions of the morphometric variables used in the present study.
Table S2.5. Descriptive statistics of each biome. Sample size, mean ± SE, minimum and maximum value for each variable.
Table S2.6. Summaries of genetic diversity [average observed heterozygosity (H_{obs}), expected heterozygosity (H_{exp}), average nucleotide diversity (π) and Wright's inbreeding coefficient (F_{IS})] per sampled biome, in addition to sample sizes and percentage of loci that were polymorphic. Significance values (*P*-values) of unpaired Student's *t*-test are also shown.
Table S2.7. Results of divergence time estimation with FASTSIMCOAL2.
Table S2.8. Results of principal components analysis based on 21 craniometric variables for the two biomes, Pampas and Atlantic Forest. The coefficient of the first eight principal components (which account for almost 85% of the variation) and the proportion of variance (as a percentage) are provided.
Table S2.9. Results of discriminant function analysis based on 21 craniometric variables for the two biomes, Pampas and Atlantic Forest. Coefficients of the single discriminant function are provided.
Figure S2.1. Pairwise comparisons of niches in climatic space (PCA-env), illustrating the niche occupied by *Holochilus brasiliensis* in each biome (the density of occurrences per cell is shaded in grey, and the continuous and dashed lines represent 100% and 50% of the available environmental space, respectively), the contribution of the principal component-derived variables on the two axes of the principal components analysis and the explanatory power of the two main axes, in addition to the position of the observed niche overlap in the niche equivalency and similarity tests.
Figure S2.2. Individual tree estimated with SVDQUARTETS, using a matrix of 24 264 single nucleotide polymorphisms, with 1000 bootstrap replicates.
Figure S2.3. Dice-Leraas diagrams showing the geometric mean of the individual scores of the first principal component of the geographical groups sampled in each biome.
Figure S2.4. Distribution of individuals along principal component (PC) 1 and PC2 of genetic variation based on the analysis of polymorphic single nucleotide polymorphisms, with information about the geographical location of the samples.