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Habitat Stability Does Not Influence Size Variation in Morphological Features of Lizards

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Abstract

Global climatic fluctuation has significantly impacted biodiversity by shaping adaptations across numerous species. Pleistocene climate changes notably affected species' geographic distributions and population sizes, especially fostering post-glacial expansions in temperate regions. Evolutionary theory suggests spatial sorting of morphological traits associated with dispersal in recently expanded species. However, evidence of predicted intraspecific trait variation is scant. We investigated intraspecific trait variation in five lizard species along a forest-savanna gradient affected by Pleistocene climate. Lizards serve as an ideal group to test these ideas due to climate's known influence on their morphological traits linked to essential functions like feeding and locomotion. We assessed two hypotheses: (i) niche variation and (ii) spatial sorting. For the niche variation hypothesis, we predicted increased intraspecific variability in head dimensions with distance from stable areas. For spatial sorting, we anticipated larger hind limb sizes with increased distance from stable areas. We gathered data on five quantitative traits from 663 samples across species. There was no evidence supporting either hypothesis across the five species. Limited sample sizes, challenges in habitat modeling, or other factors might explain this lack of support. Nonetheless, our study illuminates complexities in exploring trait variation within species. The data collected here, although inconclusive, represent a crucial test for evolutionary theory.

Historical global climate oscillations over the past millions of years have deeply affected biodiversity by changing species ranges and promoting evolutionary change. Pleistocene climate fluctuations (2 million–11 thousand years ago) are some of the most well-documented historical events (Cheng et al., 2013; Deininger et al., 2019). During this epoch, the globe experienced cyclical periods of cooling and ice sheet advance (i.e., glacial periods) followed by warming and ice sheet retreat (i.e., interglacial periods). During glacial periods, lower temperatures and reduced precipitation likely caused demographic declines and reduced genetic diversity, forcing many species to migrate to new areas or survive in isolated refuges with stable climates (Burbrink et al., 2016; Gehara et al., 2017; Hewitt, 2000, 2004). In areas of climatic stability, species tend to maintain larger, more viable populations over time, with these populations acting as sources of individuals dispersing to newly suitable habitats as temperatures rise in post-glacial periods (Bennett & Provan, 2008; Costa, Mesquita, et al., 2008; Graham et al., 2006). The stable regions, with high species diversity, likely increased interspecific competition and source-sink dynamics (Bennett & Provan, 2008; Costa, Mesquita, et al., 2008; Graham et al., 2006). Despite numerous molecular studies highlighting spatial and demographic changes due to Pleistocene climate oscillations (Burbrink et al., 2016; Camargo et al., 2013; Fonseca et al., 2023), less is known about the impact of those climatic changes on functional traits like morphology, which can reflect adaptations to ecological niches and vary across climatic stability gradients. Variation in morphology, such as head dimensions (e.g., depth, length, and width), may reflect adaptations to specific ecological niches. Morphological variation in traits indicates different feeding strategies and environmental interactions, emphasizing the link between physical adaptations and ecological specialization.

The niche variation hypothesis suggests that morphological variability within a species can be influenced by spatial factors and climatic dynamics (Van Valen, 1965). According to the niche variation hypothesis, populations in narrow ecological niches tend to show less morphological variation compared to those in broader niches due to limiting effects of interspecific competition (Van Valen, 1965). Consequently, populations in optimal habitats (usually central to their range) are expected to exhibit less morphological variability than those in less favorable habitats at range edges. Expanding on the concept, climatic stability further impacts these dynamics by fostering intense interspecific competition in areas with higher species diversity and endemism (Graham et al., 2006). Consequently, we expect less morphological variability in populations from climatically stable regions, as opposed to those from unstable areas, where the presence of fewer species and unbalanced population dynamics (Storch et al., 2022) likely increases morphological diversity. Therefore, evolutionary patterns

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of morphological variability across different environmental gradients apparently are driving complex interactions among ecological, spatial, and climatic factors.

Spatial sorting is another process that may account for morphological variability across a species' range, particularly influenced by demographic expansions. The spatial sorting hypothesis posits that faster-dispersing individuals, often found at the forefront of expanding populations, are more likely to mate assortatively and produce offspring with traits advantageous for dispersal, such as longer hind limbs (Lindström et al., 2013; Pelletier & Carstens, 2016; Phillips et al., 2006; Shine et al., 2011; Simmons & Thomas, 2004). Traits that enhance dispersal capability are hypothesized to increase in frequency toward edges of species' distributions. Additionally, climatically stable areas, which serve as refugia during glacial periods and sources during interglacial periods, are thought to influence spatial distribution of these traits, suggesting that dispersal ability correlates positively with distance from these stable zones. Interestingly, the patterns of morphological variability influenced by spatial sorting, interspecific competition, and niche variation might contrast with those of genetic diversity, which tends to be higher in more stable climatic regions (Knowles et al., 2007).

Squamate reptiles are an excellent study system for exploring the niche variation and spatial sorting hypotheses due to their ectothermic nature, low thermal tolerance, and strong link between ecophysiology and morphology (Losos, 2009). The narrow physiological tolerance of squamate reptiles raises questions about the significant impact of historical climate on their ecological and evolutionary patterns (Camargo et al., 2010). Frequently used to study rapid ecomorphological shifts, lizards serve as models for understanding how historical climates shape spatial variations in crucial morphological traits for feeding, locomotion, and competition. This research tests predictions that two spatial processes—niche variation and spatial sorting—drive intraspecific morphological diversity. We hypothesized that as the distance from climatically stable areas increases, there will be more variability in lizard head dimensions (depth, length, and width) and larger hind limb sizes (HLS). To evaluate the hypotheses about geographic variation in morphology, we collected morphometric data and occurrence records for five lizard species along a forest-savanna gradient. Using environmental niche modeling (ENM) for three Pleistocene climatic periods (present, mid-Holocene, and last glacial maximum – LGM), we identified climatically stable areas and correlated these with morphological measurements to assess the influence of niche variation and spatial sorting.

MATERIALS AND METHODS

Morphometric Measurements.—We collected morphometric measurements for five species of lizards across five different families: *Iguana iguana* (Iguanidae), *Micrablepharus maximiliani* (Gymnophthalmidae), *Notomabuya frenata* (Scincidae), *Tropidurus oreadicus* (Tropiduridae), and *Tupinambis teguixin* (Teiidae). We selected these focal species for our study because they were readily accessible and represented a diverse cross-section of taxonomic families in the study area. Individual morphometric measurements for each species were collected through field expeditions led by GRC or FPW over the last 30 years in many locations throughout Brazil. Lizards were collected using traps, active search, or during occasional encounters. Collected specimens were deposited in two biological collections: Herpetological Collection of Brasília University (CHUNB) and the Collection of Amphibians and Reptiles from the National Institute of Amazonian Research (INPA-H) (see Appendix 1 for specimen vouchers). We collected morphometric information for each species from seven or more localities.

We analyzed five unique morphometric measurements: snout-vent length (SVL), hind limb size (HLS), and head size (depth, HD; length, HL; width, HW). We only selected morphometric measurements with known ecological implications. For example, HLS is a proxy of dispersal ability, so that longer limbs have been correlated with more effective dispersal (Phillips et al., 2006). Variation in head size (depth, length, and width) is positively correlated to SVL. In turn, SVL is often used to infer niche breadth (Costa, Vitt, et al., 2008). Therefore, higher variation in body size (SVL) corresponds to more variation in head size, and higher niche breadth. In exploratory analyses, we found individuals with unusual proportions (e.g., higher SVL with smaller HLS or head measurements) likely due to incorrect measurements in the field. We identified apparent outliers using the interquartile range (IQR) method of outlier detection (Hadi, 2020), and removed individuals with morphometric proportions (i.e., morphometric measurement divided by SVL) lower and/or higher than the lower (Q1 − 1.5 * IQR) and upper (Q3 + 1.5 * IQR) boundaries, respectively (Table S1). In addition, it is well-known that individual measurements are influenced by SVL, so that larger lizards will have larger hind limbs and head sizes than smaller lizards. In fact, we found that larger lizards had greater hind limb and head measurements (linear regression; all $P < 0.001$). Because of that, we used residuals from linear regression between SVL and HLS as our response variable in downstream analyses. Conversely, the response variable for traits associated with the head was to divide each trait, for example head depth (HD), by SVL to calculate the proportion of the head measurement according to body size. Following standardization for body size, we calculated the standard deviation of the resultant measurement for each locality, to estimate the variance.

Distribution Data and Environmental Predictors.—We used environmental niche models (ENMs) to gain insight into potential distribution of our focal species in three different time slices: present, mid-Holocene (8.3–4.2 thousand years ago, kya), and LGM (ca. 21 kya). For all species, we obtained occurrence data through the Sistema de Avaliação do Risco de Extinção da Biodiversidade database (SALVE; ICMBio, 2024). All occurrence points were carefully checked to avoid including errors (e.g., points outside the known distribution of the species and/or points in the marine regions; Graham et al., 2008). We also removed duplicated points to increase effectiveness of the ENMs and avoid excessive weight of overrepresented localities in the final model.

We obtained current climatic data to build ENMs from the WorldClim database (19 bioclimatic variables; Hijmans et al., 2005; available at https://www.worldclim.org) at a spatial resolution of 2.5 arc minutes (4.5 x 4.5 km at the equator). We used the function *getData* from the “raster” package (Hijmans, 2020) to download climatic data. In addition, we manually downloaded elevation data from the WorldClim website. All 19 bioclimatic variables are derived from temperature and precipitation records, representing means and extreme seasonal values (Hijmans et al., 2005).

We further processed environmental layers and kept only noncorrelated layers. Correlated predictors, if not controlled, can generate erroneous interpretations of statistical models by inflating parameter variance in regression models, increasing uncertainty and decreasing model efficiency (De Marco & Nóbrega, 2018; Dormann et al., 2013; Rissler & Apodaca, 2007). We removed highly correlated variables using the variance inflation factor (VIF) through the R package “uncertainty analysis for species distribution models” (usdm; Naimi et al., 2014). We only kept variables with VIF values lower than two. The variables retained in the model were: Mean Diurnal Range (Mean of monthly (max temp − min temp)) − BIO2; Isothermality ((BIO2/BIO7) (*100)) − BIO3; Mean Temperature of Wettest Quarter − BIO8; Precipitation Seasonality (Coefficient of Variation) − BIO15; Precipitation of Warmest Quarter − BIO18, and Precipitation of Coldest Quarter − BIO19. We also obtained the same bioclimatic variables from the WorldClim website for the mid-Holocene and LGM. To reduce possible database sampling bias, we applied an environmental filter using the *envSample* function from Varela et al. (2014) and *sample.envR* from Castellanos et al. (2019).

Environmental Niche Modeling.—We conducted environmental niche models through the R package “biomod2” (Thuiller et al., 2020). We used a total of eight different modeling algorithms. Three are machine learning algorithms (Random Forest − RF, Generalized Boosting Model − GBM, and Classification Tree Analysis − CTA), and the remaining are regression methods (Generalized Linear Model − GLM; Generalized Additive Model − GAM; Artificial Neural Network − ANN; Surface Range Envelop − SER, and Flexible Discriminant Analysis − FDA). We adjusted the machine learning models using pseudo-absence data, such that the number of occurrences in each class were equivalent, and conducted regression models with 10,000 pseudo-absence points and 10 replicates of pseudo-absences in all models. We used the True Skill Statistic (TSS) metric to measure accuracy of the model and, to decrease uncertainty, we constructed a consensus model considering all models with TSS values above 0.70. Next, we used the best-fit ENM model for each species to project habitat suitability to the mid-Holocene and LGM.

We generated a total of three unique suitability maps: (i) present, (ii) mid-Holocene, and (iii) LGM. Climatically stable area was estimated for each species by averaging these three unique suitability maps (Fig. 1A) such that areas with higher values of suitability through time were quantified as more stable than areas with correspondingly lower values. Next, we identified climatically stable regions across the distribution of each species. In the climatically stable regions, individuals presumably survived and thrived during climatic fluctuations of the Pleistocene. To determine climatically stable regions, we selected areas with suitability higher than the 95% percentile, which is a conservative threshold (Fig. 1B). Next, we defined continuous polygons for each climatically stable region previously identified, and retained only those polygons with at least 500 pixels. A polygon size of 500 pixels is a conservative value because populations in small habitat patches have a high chance of becoming locally extinct due to stochastic variation in food availability and biological processes such as reproduction and mating. For each selected polygon we calculated the centroid and extracted longitude and latitude coordinates of stable areas (Fig. 1C).

In addition to calculating climate stability on a species-by-species basis, one could also model habitat stability of ecoregions, as in the habitat stability map for South America developed by Costa et al. (2018). We built habitat stability maps from palaeo-projections of the random forest model for distribution of present-day conditions for South American biomes (Costa et al., 2018). The advantage of using a habitat stability map is that, instead of having independent stable areas for each species, we can identify, through time, stable habitat areas that possibly harbored greater species diversity and endemism (Graham et al., 2006). Therefore, we also used the stable habitat areas from Costa et al. (2018) to test our hypotheses. We selected areas with stability higher than 95% from the stability raster available in the supporting information of Costa et al. (2018). Because of the large area South America occupies, we only kept stable area polygons that were equal to or greater than 1000 pixels. We then calculated the centroid of each 1000-pixel or bigger polygon and extracted coordinates.

Testing the Niche Variation and Spatial Sorting Hypotheses.—Our hypotheses posit that morphological variability in head measurements and hind limb size increases with distance from climatically stable areas. However, it is well known that individuals do not disperse randomly, and that landscape heterogeneity likely modulates how species disperse (McRae, 2006). Under such a scenario, Euclidian distance is likely not a reasonable proxy for distance between sampled localities and climatically stable areas. We used resistance distance as a proxy of movement. Resistance distance measures relationships between random walk times and effective resistances in electronic networks (McRae, 2006). Because historical climate has been shown to influence dispersal in the study region (Oliveira et al., 2018; Vasconcellos et al., 2019), we used the ENMs to calculate resistance distance between sampled localities and the centroid of climatically stable areas for each species. Also, we calculated resistance distance between sampled localities and habitat-stable areas selected based on the Costa et al. (2018) habitat stability map. Specifically, we calculated the least-cost path from the closest centroid of habitat or climatically stable areas to each sampled locality. Resistance distance was calculated using the stability raster derived from three different time slices using the R package “gdistance” (van Etten, 2017). We hypothesized that the closest stable areas acted as the source of individuals to a given locality outside these climatically stable areas.

To test our hypothesis of increased morphological variability in head depth, length, and width (HD, HL, and HW) as distance from habitat or climatically stable areas in-

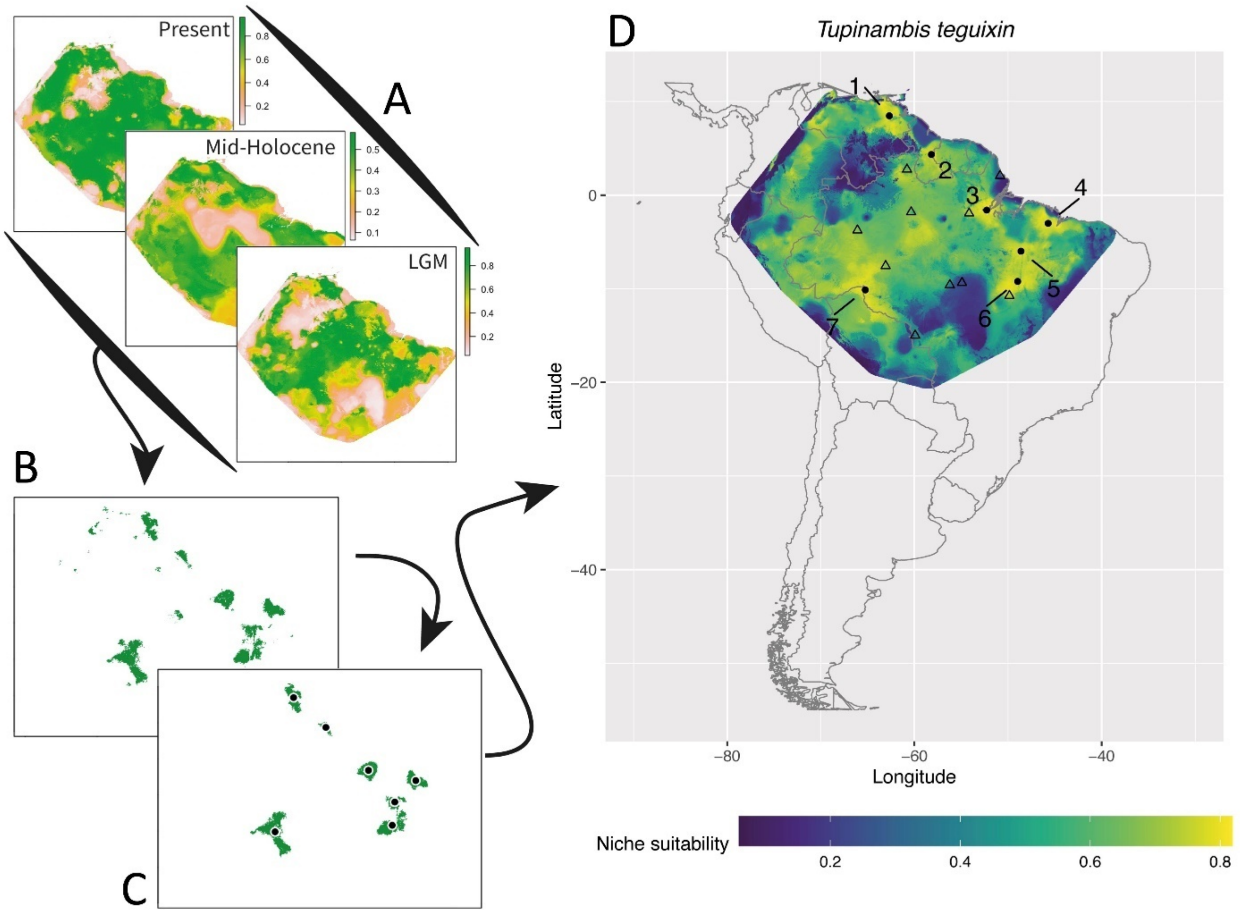

FIG. 1. An example of steps used for production of stability maps for our focal species: (A) combination and average of three time slice maps; (B) separation of 95th percentile of high suitability regions; (C) exclusion of areas with less than 500 pixels of size and estimation of high stability areas' centroids, and (D) final map showing niche suitability, with areas of high climatic stability through time in yellow, and areas with low climatic stability in dark blue. Black circles and their associated numbers are climatically stable areas, triangles are occurrence points of *T. teguixin* which have associated morphological data.

crease, we used linear models using the function *lm* from the 'stats' package (R Core Team, 2022). For the analysis, we only used localities with more than one sampled individual.

Our second hypothesis predicts increased hind limb sizes with increased distance from climatically stable areas. Again, we used resistance distance as a proxy of species movement. We used the function *lmer*, implemented in the R package "lmerTest" (Kuznetsova et al., 2017), to fit models for each species. Because individuals in the same locality are not independent due to shared recent evolutionary history, we included localities as a random variable in the model. All regression models were conducted after removal of outliers.

RESULTS

Number of Localities Sampled, Habitat, and Climatically Stable Areas.—We selected five lizard species that are widely distributed in an environmental forest-savanna gradient with available morphological and georeferenced data (in parenthesis: number of localities; number of total samples): *Iguana iguana* (13; 32), *Micrablepharus maximiliani* (7; 195), *Notomabuya frenata* (9; 45), *Tropidurus oreadicus* (12; 363), *Tupinambis teguixin* (10; 28). Final sample sizes used for analyses of each species and each trait varied after removing outliers (see Table S1). Number of climatically stable areas varied among species (5.6 ± 2.0 SD; range: 3–8 areas; Figs. 2–6), with *T. oreadicus* showing the lowest number of climatically stable areas (three). Conversely, *I. iguana* had the highest number of stable areas (eight). Climatically stable areas for *T. oreadicus* (three total) were clustered in the northeastern portion of its distribution. Likewise, climatically stable areas for *M. maximiliani* (six) were concentrated in the northern portion of its distribution, but more spread out than areas for *T. oreadicus*. We found a total of four and seven climatically stable areas for *N. frenata* and *T. teguixin*, respectively. Conversely, these species had the climatically stable areas distributed throughout their distributions (Figs. 4, 6). *Iguana iguana* had eight climatically stable areas distributed in both the border and center of its distribution (Fig. 2). Number of localities sampled was slightly larger than number of stable areas for each species. Number of stable habitat areas was larger than number of climati-

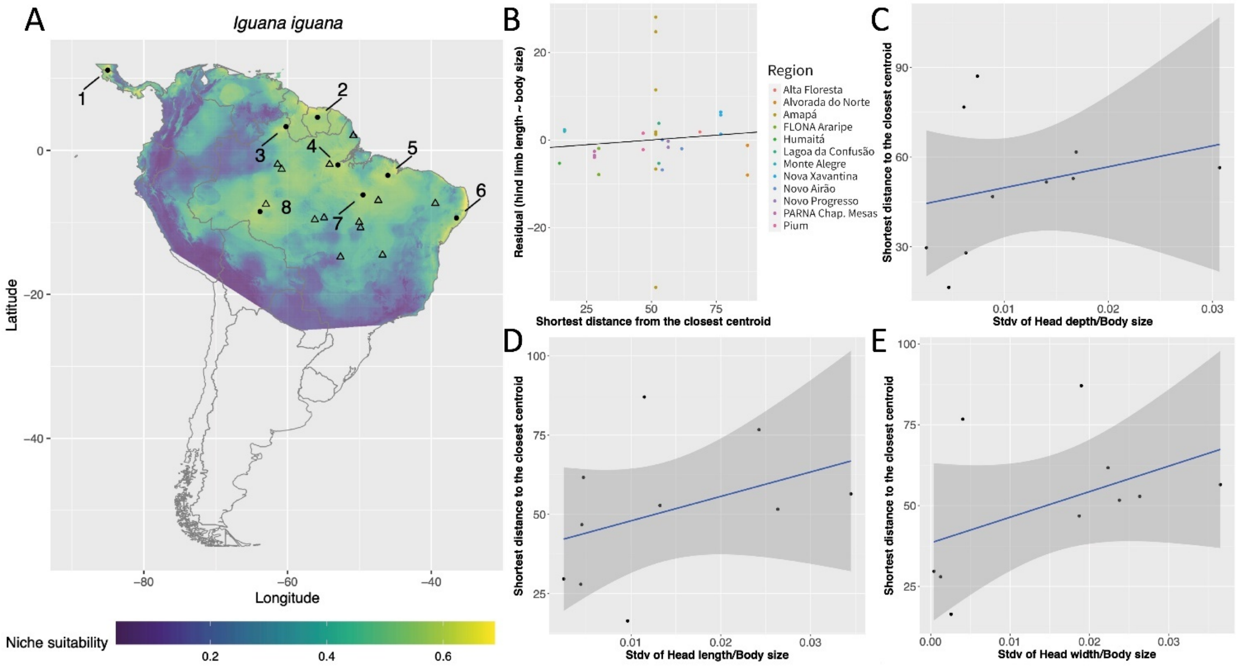

FIG. 2. *Iguana iguana*. Map of stability (A); numbers 1–8 correspond to centroids of stable areas; triangles correspond to localities at which we have morphological data. Plots (B–E) correspond to linear mixed-effects models and linear models for each trait: (B) residual from HLS and SVL linear model (*P*-value: 1); (C) variation of HD (*P*-value: 1); (D) variation of HL (*P*-value: 1); (E) variation of HW (*P*-value: 0.744). Abbreviations: HLS (hind limb size); SVL (snout-vent length); HD (head depth); HL (head length); HW (head width); Stdv (standard deviation).

cally stable areas per species (13 stable habitat areas; Fig. 7).

Correlation Between Body Size and Other Morphometric Measurements.—Morphological data varied among species, but the variation pattern was similar for all measurements (Fig. 8). *Iguana iguana* had the most variation, followed by *T. teguixin*, *T. oreadicus*, *N. frenata*, and then *M. maximiliani*. Linear mixed-effects model results showed significant correlations between body size (SVL) and HLS for all species (Table 1), with all species showing significant *P*-values. All slope values from these correlations were greater than zero, indicating a positive correlation between body size and limb size, varying from 0.28 (for *M. maximiliani*) to 0.67 (for *I. iguana*). Likewise, correlations between body size and each head measurement (HD, HL, HW) were also significant (Table 1). For HD, the significant *P*-value was lower than 0.001 for all species. Similarly, regressions between HL and body size and between HW and body size were significant for all five species ($P < 0.001$). Like the regression between HLS and body size, regressions between each head measurement and body size showed slope values higher than zero for all species. Slope values for the HD regression varied from 0.06 (for *N. frenata*) to 0.14 (for *T. oreadicus*). Slope values for the HL regression varied from 0.12 (for *N. frenata*) to 0.25 (for *T. teguixin*), and for the HW regression, they varied from 0.09 (for *I. iguana*) to 0.20 (for *T. oreadicus*) .

Niche Variation Hypothesis.—To use standard deviation values as response variables, we were unable to use all localities included in HLS models. To calculate standard deviation, the mean of a collection locality is required, so our analysis was limited to those localities where at least two individuals were sampled. The final number of localities per species and per head measurement were: 10 for HD, HL and HW for *I. iguana*, 9 for HD, HL and HW for *M. maximiliani*, 6 for HD, HL and HW for *N. frenata*, 12 for HD and HW, and 11 for HL for *T. oreadicus*, and 7 for HD and HW, and 6 for HL for *T. teguixin*.

Correlations between standard deviation of each head measurement and shortest distance from the closest centroid (SDC) of climatically stable areas were not significant for all species. We found positive correlations, although not significant, between SDC and variation of all head measurements for *I. iguana* (*P*; HD: 1, HL: 1, and HW: 0.74; Slope; HD: 0.00010, HL: 0.00019, and HW: 0.00026), *N. frenata* (*P*; HD: 1, HL: 1, HW: 1; Slope; HD: 1.41e-5, HL: 0.00015, HW: 3.50e-5), and *T. teguixin* (*P*; HD: 1, HL: 1, HW: 1; Slope; HD: 1.28e-4, HL: 7.46e-5, HW: 1.42e-5). For *M. maximiliani* and *T. oreadicus* we found positive correlations between SDC and HD (*P*: 1; Slope: 2.17e-5), and SDC and HL (*P*: 1; Slope: 7.08e-6), respectively. All other correlations between SDC and head measures for these two species were negative (*M. maximiliani* – *P*; HL: 0.98, HW: 0.26, Slope; HL: -7.52e-5, HW: -9.49e-5; *T. oreadicus P*; HD: 1, HW: 1, Slope; HD: -2.38e-5, HW: -1.27e-5) (Table 2).

As for what was found in correlation between standard deviation of each head measurement and distance from closest centroid of climatically stable habitat areas, correlations for most species were not significant (Table 3). Except for the correlation between SDC of stable habitat areas and HD for *M. maximiliani* (*P*: 0.72, Slope: -4.85e-5), HW for *T. oreadicus* (*P*: 0.13; Slope: 8.29e-5), and HW for *T. teguixin*

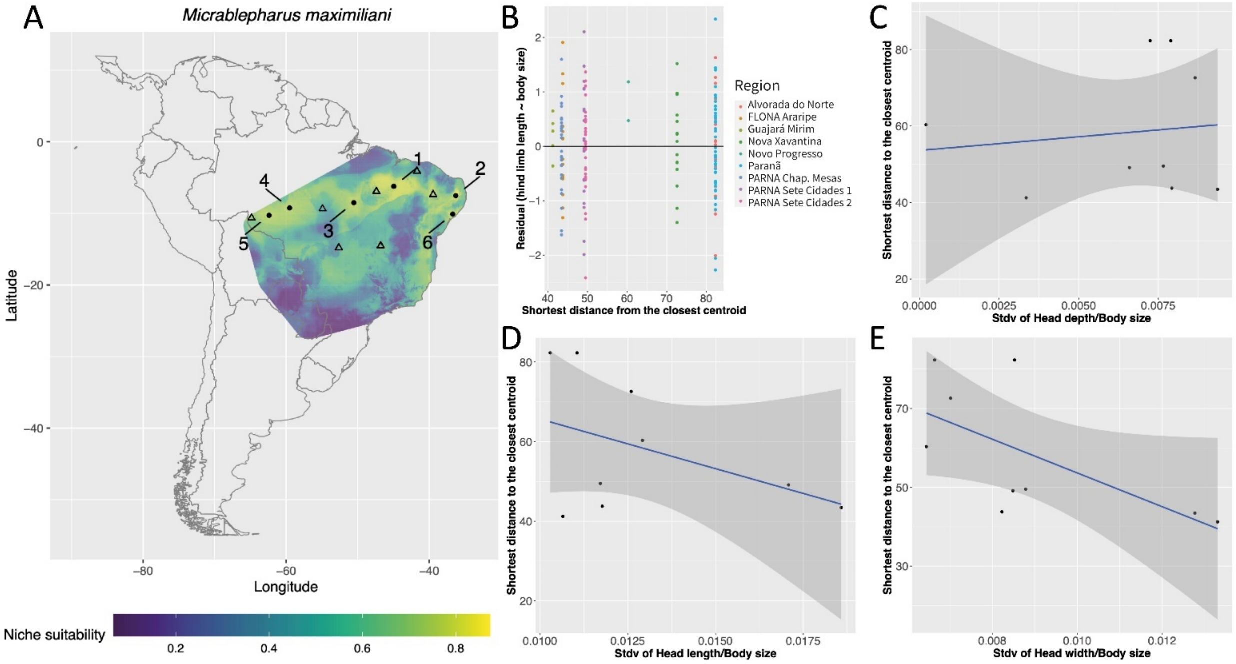

FIG. 3. *Micrablepharus maximiliani*. Map of stability (A); numbers 1–6 correspond to centroids of stable areas; triangles correspond to localities at which we have morphological data. Plots (B–E) correspond to linear mixed-effects models and linear models for each trait: (B) residual from HLS and SVL linear model (*P*-value: 1); (C) variation of HD (*P*-value: 1); (D) variation of HL (*P*-value: 0.97852); (E) variation of HW (*P*-value: 0.259228). Abbreviations: HLS (hind limb size); SVL (snout-vent length); HD (head depth); HL (head length); HW (head width); Stdv (standard deviation).

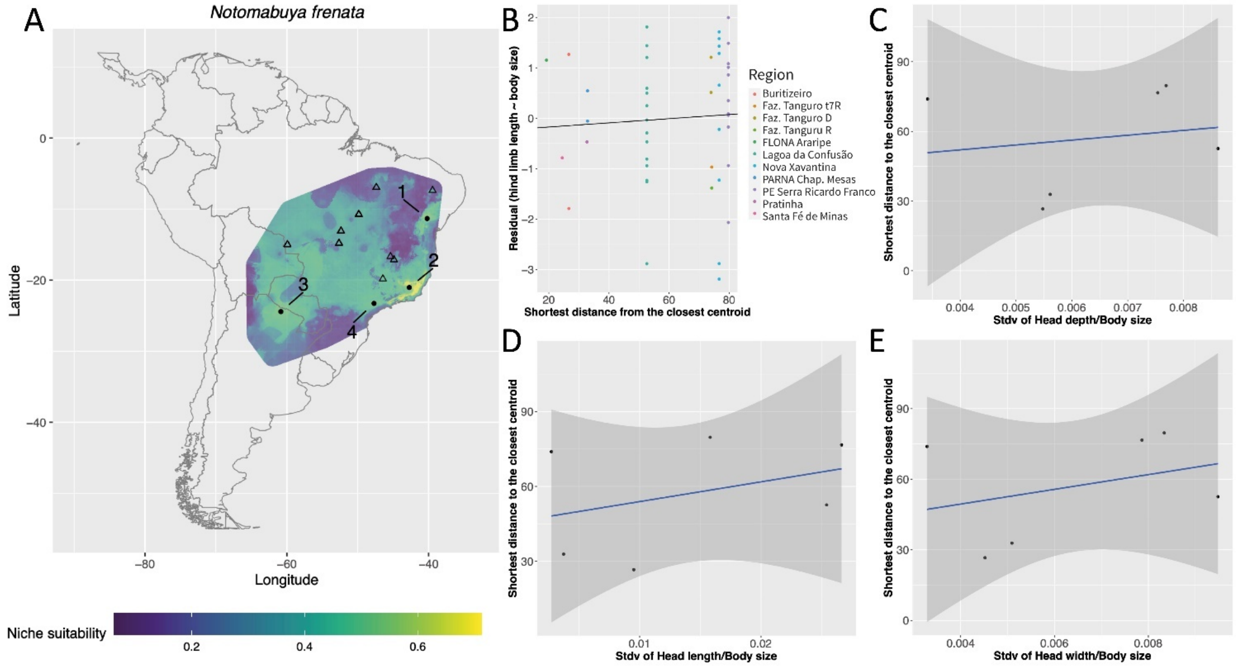

FIG. 4. *Notomabuya frenata*. Map of stability (A); numbers 1–4 correspond to centroids of stable areas; triangles correspond to localities at which we have morphological data. Plots (B–E) correspond to linear mixed-effects models and linear models for each trait: (B) residual from HLS and SVL linear model (*P*-value: 1); (C) variation of HD (*P*-value: 1); (D) variation of HL (*P*-value: 1); (E) variation of HW (*P*-value:1). Abbreviations: HLS (hind limb size); SVL (snout-vent length); HD (head depth); HL (head length); HW (head width); Stdv (standard deviation).

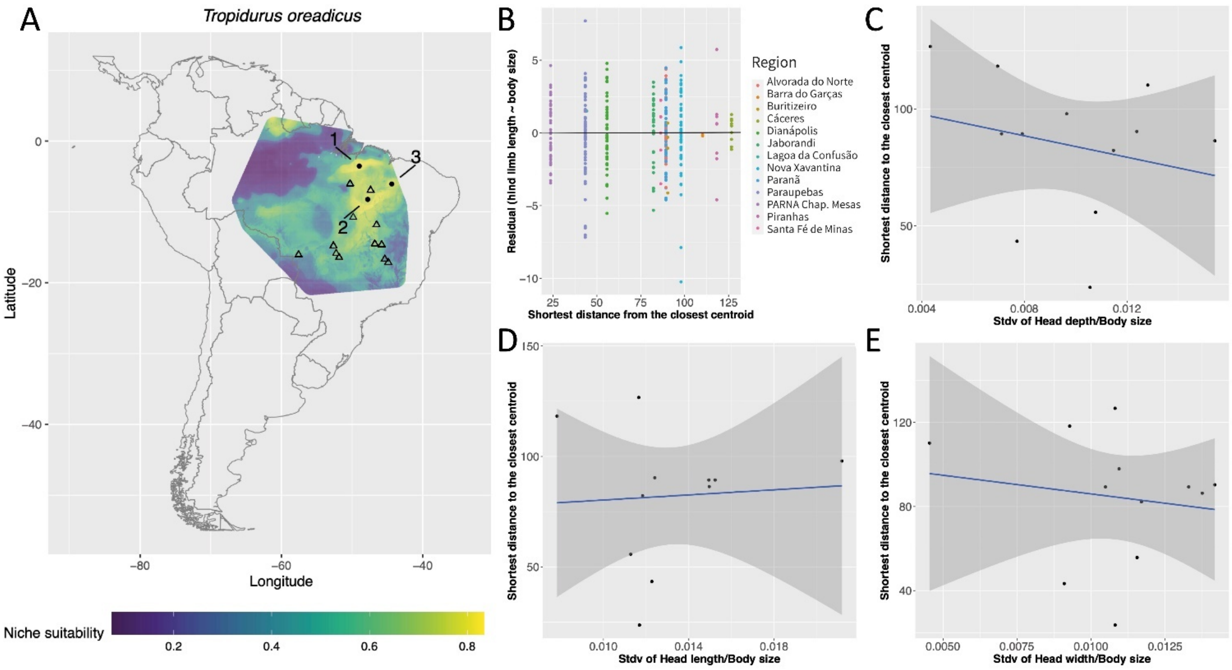

FIG. 5. *Tropidurus oreadicus*. Map of stability (A); numbers 1–3 correspond to centroids of stable areas; triangles correspond to localities at which we have morphological data. Plots (B–E) correspond to linear mixed-effects models and linear models for each trait, (B) residual from HLS and SVLe linear model (*P*-value: 1), (C) variation of HD (*P*-value: 1), (D) variation of HL (*P*-value: 1), (E) variation of HW (*P*-value: 1). Abbreviations: HLS (hind limb size); SVL (snout-vent length); HD (head depth); HL (head length); HW (head width); Stdv (standard deviation).

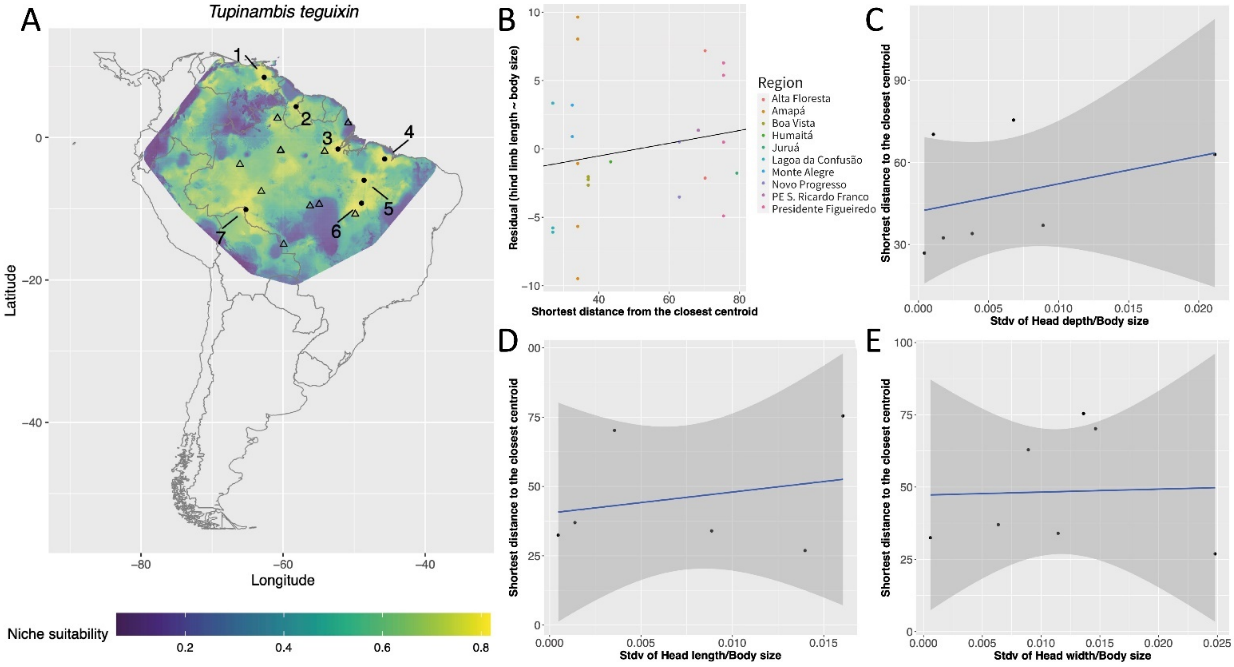

FIG. 6. *Tupinambis teguixin*. Map of stability (A); numbers 1–7 correspond to centroids of stable areas; triangles correspond to localities at which we have morphological data. Plots (B–E) correspond to linear mixed-effects models and linear models for each trait, (B) residual from HLS and SVL linear model (*P*-value: 1), (C) variation of HD (*P*-value: 1), (D) variation of HL (*P*-value: 1), (E) variation of HW (*P*-value: 1). Abbreviations: HLS (hind limb size); SVL (snout-vent length); HD (head depth); HL (head length); HW (head width); Stdv (standard deviation).

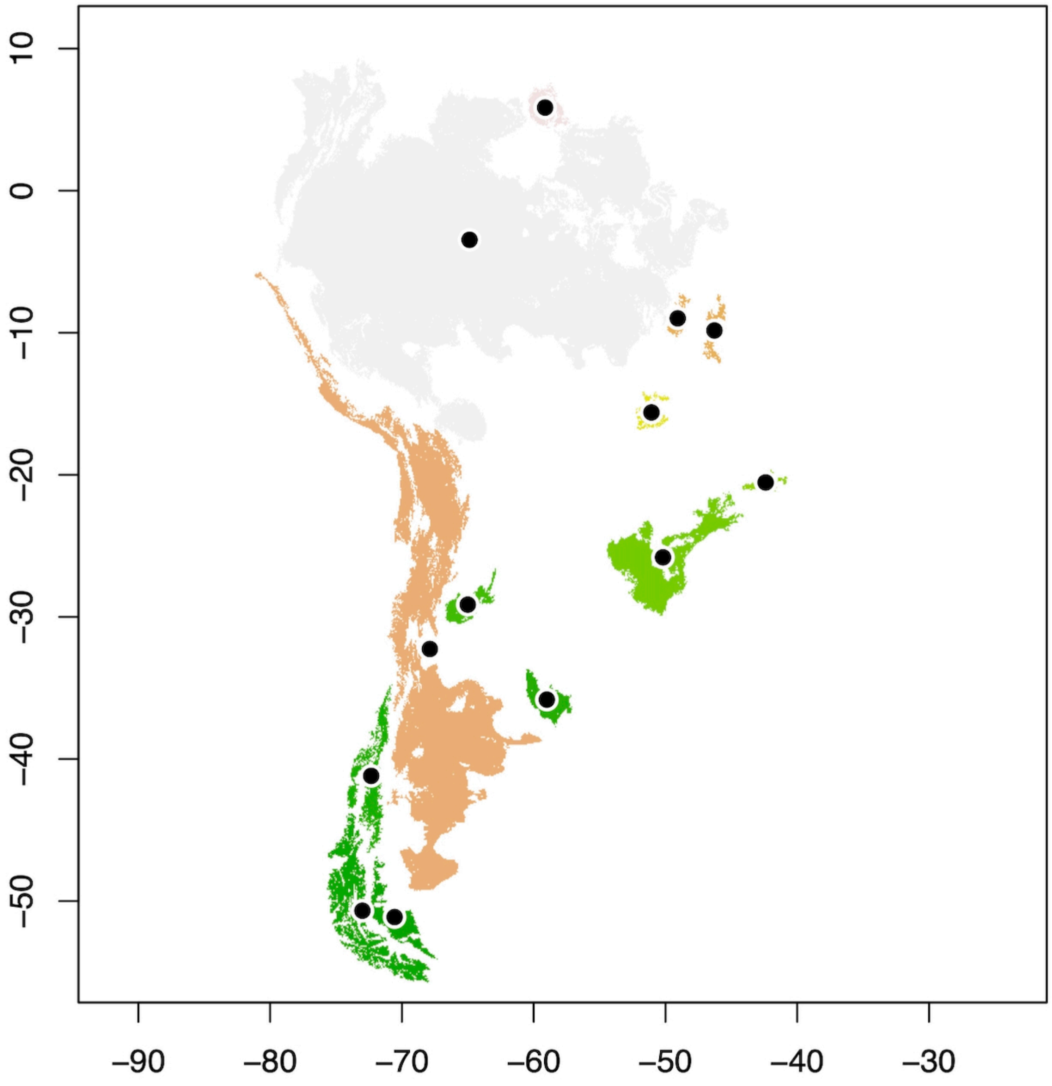

FIG. 7. Map showing stable habitat areas (13 areas) in South America, selected using the Costa et al. (2018) stability map, which had more than 95% stability and were bigger than 1000 pixels. X-axis denotes longitude in degrees, y-axis represents latitude in degrees. Each color represents one continuous area; black circles are stable habitat area centroids.

(*P*: 0.67, Slope: -0.00017), all other correlations presented a corrected *P*-value equal to 1.

Spatial Sorting Hypothesis.—Our results did not support the spatial sorting hypothesis. For all species, the regression between HLS and SDC of climatically stable areas (Table 2, Figs. 2–6), and between HLS and SDC of stable habitat areas (Table 3, see Figs. S1–S5), were not significant. All *P*-values were equal to 1 after Bonferroni correction (Tables 2, 3, Figs. 2–6, Figs. S1–S5). Although slopes of regression analyses were positive for all species, except for *T. oreadicus* (Slope: -0.0014), they were not significantly different from zero, implying that individuals in the leading edge of the species distribution do not have larger hind limb sizes (Tables 2, 3).

DISCUSSION

Pleistocene climatic oscillations have been proposed as one of the main drivers of evolutionary change and diversity patterns across the globe (Fonseca et al., 2023; Hewitt, 2000; Woodman, 1995). However, most investigations have revealed impacts of these oscillations by interrogating, for example, contemporary and/or ancient genetic variation (Fonseca et al., 2021; Marchi et al., 2022; Seersholm et al., 2020) or community-level information (Malhi et al., 2016; Rangel et al., 2018) rather than morphological data. In this study, we aimed to evaluate effects of Pleistocene climatic oscillations on morphological traits with known ecological functions in five Neotropical lizards, such as those related to dispersal abilities. Specifically, we evaluated two ecological hypotheses: (i) niche variation and (ii) spatial sorting. The first predicts that species with broader ecological niches will have greater morphological variability than species with narrower ecological niches. Meanwhile, the spatial sorting hypothesis predicts that HLS is longer in regions on the leading edge of an expanding range and increases as individuals get farther from climatically stable areas or stable habitat areas (center of dispersal). However,

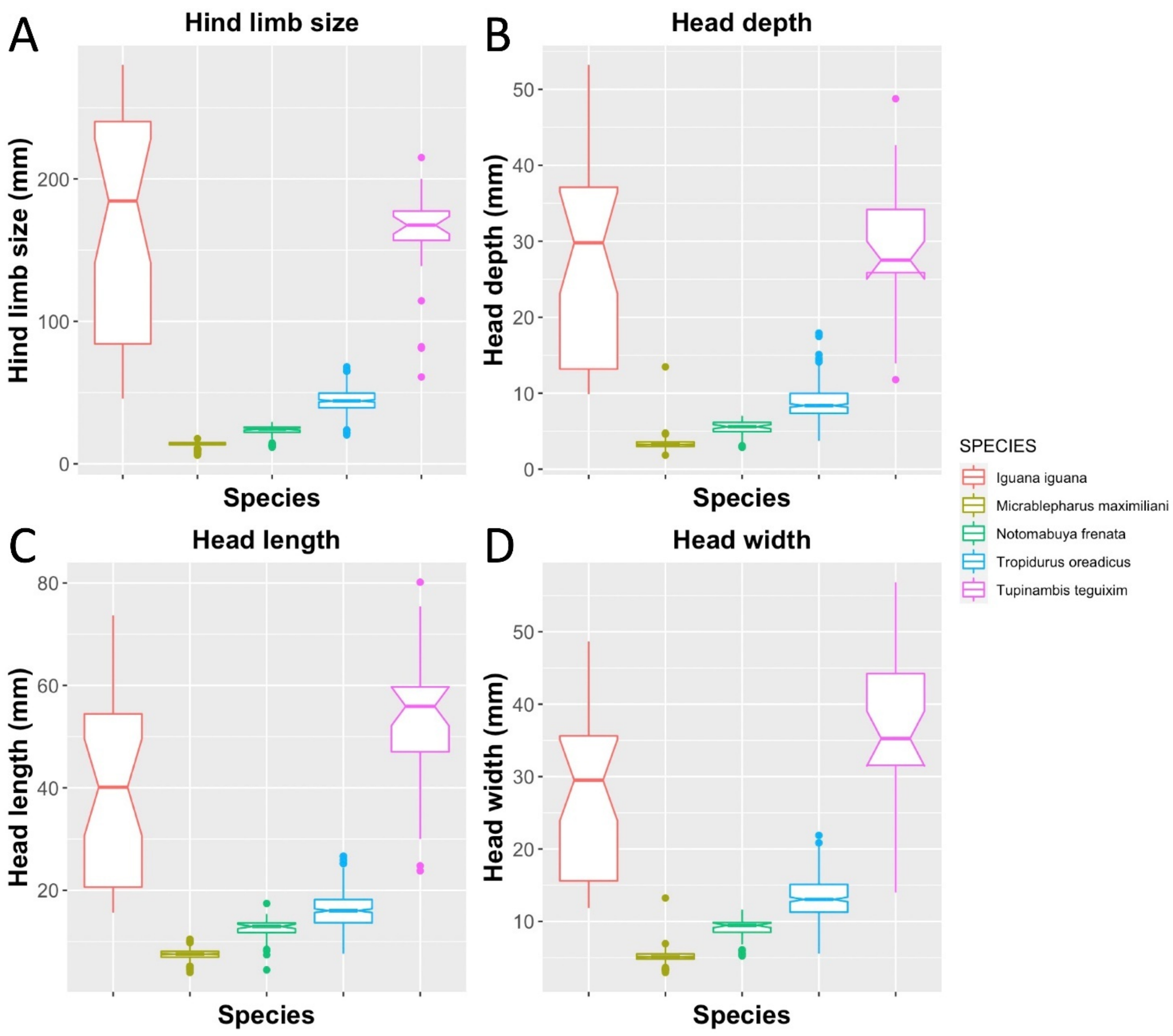

FIG. 8. Boxplots illustrating the distribution of morphological traits across different species. The plots depict (A) hind limb size (HLS), (B) head depth (HD), (C) head length (HL), and (D) head width (HW). Each species is represented by a unique color, as indicated in the legend. In each boxplot, the central line represents the median, the box encompasses the interquartile range (IQR), and whiskers extend to 1.5 times the IQR. Data points beyond the whiskers are considered outliers and are shown as individual dots.

the data collected here did not support either hypothesis. There are several possible explanations for our results, including species-specific factors, difficulties in modeling historical habitat stability, and a lack of statistical power caused by low sample sizes. We used two approaches as proxies of degree of niche variation across species ranges: environmental niche models for each species and stable habitat areas based on Costa et al. (2018). Stable areas have been shown to be centers of species' genetic, functional, and phylogenetic diversity, as well as phylogeographic endemism (Carnaval et al., 2009; Huxley & Spasojevic, 2021; Mastrogianni et al., 2019). We expected that higher species diversity in those stable areas (both climatically stable areas and stable habitat areas) would lead to higher interspecific competition and, consequently, to narrow niche width. Thus, to coexist locally in stable areas, species should increasingly partition their niche, leading to a narrow niche width. However, our findings did not support this model. We found that head variability does not increase as resistance distance increases from the centroid of stable areas to unstable areas. Although heavily criticized in the past (see Meiri et al., 2005; Simberloff et al., 2000), the niche variation hypothesis has been supported by many recent investigations (Costa, Mesquita, et al., 2008; Jesmer et al., 2020; Maldonado et al., 2017). Importantly, Van Valen (1965) proposed this hypothesis to test for morphological variability (as we implemented here). However, Bolnick et al. (2007) pointed out that increased use of resources does not necessarily lead to increased morphological variability. While many investigations have tested the niche variation hypothesis in the context of "mainland" versus "island" (e.g., Bolnick et al., 2007; Costa, Mesquita, et al., 2008), our approach is notably different in one aspect. We used a continuous approach to test this hypothesis, in which we expected

Table 1. Correlation between body size and each of the features analyzed here. HLS (hind limb size); HD (head depth); HL (head length); HW (head width).

Species	HLS			HD			HL			HW		
	t	Slope	*P*	*t*	Slope	*P*	*t*	Slope	*P*	*t*	Slope	*P*
Iguana iguana	41.794	0.66744	8e-16	19.562	0.09887	8e-16	20.792	0.15495	8e-16	17.473	0.08580	8e-16
Micrablepharus maximiliani	17.522	0.28168	8e-16	12.09	0.06572	8e-16	15.855	0.17038	8e-16	15.43	0.09496	8e-16
Notomabuya frenata	17.96	0.31331	8e-16	11.397	0.06198	3.58e-13	10.507	0.11953	2.61e-12	19.782	0.11434	8e-16
Tropidurus oreadicus	59.751	0.61161	8e-16	50.132	0.13552	8e-16	62.94	0.2214	8e-16	65.225	0.19699	8e-16
Tupinambis teguixin	23.07	0.55919	8e-16	10.707	0.10680	2.34e-09	19.893	0.24548	1.50e-13	9.402	0.14860	1.38e-07

Table 2. Correlation between shortest distance from the closest centroid of the climatically stable areas and the residuals resulting from a regression between hind limb size (HLS) and body size (i.e., snout-vent length; SVL), the variation of head depth (HD) divided by SVL, the variation of head length (HL) divided by body size (SVL), and the variation of head width (HW) divided by SVL.

Species	HLS ~ SVL residuals			HD/SVL variation			HL/SVLvariation			HW/bSVL variation		
	t	Slope	*P*	*t*	Slope	*P*	*t*	Slope	*P*	*t*	Slope	*P*
Iguana iguana	0.452	0.04375	1	0.791	0.00010	1	1.196	0.00019	1	1.445	0.00026	0.744
Micrablepharus maximiliani	0.004	1.28e-5	1	0.333	2.17e-5	1	-1.27	-7.52e-5	0.97852	-2.189	-9.49e-5	0.25922
Notomabuya frenata	0.386	0.004129	1	0.349	1.41e-5	1	0.760	0.00015	1	0.702	3.50e-5	1
Tropidurus oreadicus	0.104	4.59e-4	1	-0.76	-2.38e-5	1	0.193	7.08e-6	1	-0.481	-1.27e-5	1
Tupinambis teguixin	0.893	0.04657	1	0.864	1.28e-4	1	0.49	7.46e-5	1	0.086	1.42e-5	1

TABLE 3. Correlation between shortest distance from the closest centroid of the stable habitat areas and the residuals resulting from a regression between hind limb size and body size (i.e., snout-vent length; SVL), the variation of head depth divided by body size, the variation of head length divided by body size, and the variation of head width divided by body size. HLS (hind limb size); SVL (snout-vent length); HD (head depth); HL (head length); HW (head width).

Species	HLS ~ SVL residuals			HD/SVL variation			HL/SVLvariation			HW/SVL variation		
	t	Slope	*P*	*t*	Slope	*P*	*t*	Slope	*P*	*t*	Slope	*P*
Iguana iguana	0.507	0.02584	1	-0.019	-1.77e-6	1	0.727	8.55e-5	1	-0.127	-1.75e-5	1
Micrablepharus maximiliani	0.482	0.00121	1	-1.489	-4.85e-5	0.72068	-0.531	-1.94e-5	1	0.942	2.83e-5	1
Notomabuya frenata	0.883	0.00875	1	0.385	1.86e-5	1	0.066	1.76e-5	1	0.512	3.15e-5	1
Tropidurus oreadicus	-0.152	-0.0014	1	-0.331	-1.67e-5	1	0.246	1.51e-5	1	2.498	8.29e-5	0.12618
Tupinambis teguixin	1.105	0.04950	1	-0.696	-8.43e-5	1	-0.421	-5.03e-5	1	-1.610	-0.0001	0.6732

that morphological variability increased continuously from stable areas, because the potential habitat occupied by lizard species was not partitioned into discrete units. A side effect was producing unequal numbers of samples in the stable and less-stable habitat because specimens were collected through sporadic field expeditions over many years and deposited in biological collections.

Our findings did not support the spatial sorting hypothesis in any species. One possible explanation for our result is that spatial sorting is an ephemeral process that is expected to occur at the edge of an expanding range, but the signal should dissipate after several generations of assortative mating once range size reaches stability (Shine et al., 2011). Another explanation is that life history of some species might not be conducive for identifying effects of spatial sorting. For example, in *M. maximiliani*, our result is not unexpected given the ecology and morphology of this species. *Micrablepharus maximiliani* moves mainly using the vertebral axis, and limbs play only a secondary role in locomotion (Silva et al., 2021). For the other four species, however, there is no evidence for the main locomotion to be from a different part of the body. We analyzed species with different ecology and natural history. For example, whereas *M. maximiliani* is terrestrial, an active forager, and oviparous, *N. frenata* is arboreal and terrestrial, a sit-and-wait and active forager, and viviparous. Other species, such as *I. iguana*, are herbivorous and oviparous. Thus, even species with different ecologies did not show evidence of spatial sorting.

It is important to highlight that scale-dependence is a key concept in ecology and refers to the idea that different processes occur at specific spatial and/or temporal dimensions (Levin, 1992; Schneider, 2001). For example, dispersal usually occurs at shallow temporal (few hundreds to few thousand years) and fine spatial scales. In contrast, historical events, such as range expansion and establishment, usually occur over deep-time (few thousand to few million years) and broad spatial scales. Therefore, Pleistocene climatic fluctuations that occurred at deep time scales might not be a good proxy for dispersal that happened over shallow scales (i.e., scale mismatch). The issue of scale is particularly important given our reliance on modeling to identify stable areas. Our analyses assumed that we correctly identified climatically stable areas, which represented the center of dispersion, but this assumption is difficult to verify given the lack of available fossils from this region and these taxonomic groups. It remains possible that the processes that form the basis of our hypotheses act on short temporal or limited spatial scales and as such were not detectable here.

Another factor that could influence our results is sexual dimorphism, which is common in many species of lizards (Garda et al., 2012; Pinto et al., 2005; Vitt, 1993; Vitt & Cooper, 1985), particularly in body size and coloration (Pinto et al., 2005; Vitt & Cooper, 1985). Determining the sex of lizards presents a challenging task due to lack of distinctive external characteristics but is still of great importance to understand implications of sexual dimorphism. On the other hand, sexual dimorphism in food resources used by lizards have been shown to be absent (Costa, Mesquita, et al., 2008). In that case, differences in body size between sexes would not influence prey size preference as much as variation in body size within a population. To avoid the influence of body size in our analyses, and consequently sexual dimorphism in body size, we used residuals of the linear model between body size (SVL) and hind limb size (HLS) and a proportion of head measurements divided by SVL as response variables in linear models.

Finally, it is possible that lack of evident phenotypic variation among locations was a function of our small sample sizes. Number of localities at which we sampled for morphometric measurements was similar to the number of stable areas. Number of individuals measured per species varied considerably (from 28 to 363 individuals), but the number of localities from which those individuals were collected did not vary much (from 7 to 13). Regression analyses were conducted using the number of localities as either a fixed effect or a random effect in a mixed-effects model, and as the response variable when analyzing head measurement variation. Therefore, number of localities is important to be considered and it is small even for the species with more individuals collected. Based on simulations, Jenkins and Quintana-Ascencio (2020) recommended sample sizes greater than or equal to 25 in comparable regression-based analyses. Considering that our sample sizes are generally smaller than the suggested number, they could be the most important factor for lack of significance in our results.

We expected to find more HLS variation among individuals from different localities than among individuals from the same locality. When removing effects of body size, residuals varied more within localities than among localities (Figs. 2A–6A), at least for some localities. High levels of variability in traits may mask variance among localities and contribute to the results found here. Even though we found positive correlations between shortest distance from the closest centroid of stable areas (SDC) and limb size corrected for body size (better dispersers in edges of occurrence areas) for three of five species, the correlation was not significant. Similarly, we found negative correlations between SDC and two of three head measurements for two of five species, showing increases in head size variation in areas closer to the center of distribution, but again, the correlations were not significant. Therefore, we did not support either hypothesis using our data from these five lizard species.

In summary, our results did not support either niche variation or spatial sorting hypotheses. It appears that populations close to climatically stable areas and stable habitat areas are as variable as those far from such areas. Similar patterns of morphological variation could reflect genetic constraints, because it has been shown that many species experienced population bottlenecks during the LGM (e.g., Fonseca et al., 2021; Gehara et al., 2017; Prates et al., 2016), which may have purged their genetic diversity and the standing genetic variation that natural selection acts upon. We argue that future sampling should be conducted to increase sample size and collect morphological information in presumed stable areas to provide more robust information to test our hypotheses. Also, future investigations would benefit from including direct measures of resource availability (e.g., prey stomach contents) rather than morphological variability to test whether niche variation increases from stable to unstable areas.

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SUPPLEMENTARY DATA

Supplementary data associated with this article can be found online alongside the manuscript.

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