Geographical variation and sexual dimorphism in cranial size and shape of *Kannabateomys amblyonyx* (Rodentia: Echimyidae)

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Abstract: The echimyid rodent *Kannabateomys amblyonyx,* also known as the bamboo rat, is restricted to the Atlantic rainforest of Brazil, Uruguay, Paraguay and Argentina. This species feeds exclusively on bamboo stems and leaves, making it a highly specialist species. Due to the lack of studies about this group, partly because of the poor sample sizes available in museums and collections, morphological diversity aspects and intraspecific variation are poorly understood. Our study is a first evaluation of the sexual and geographic variation in the cranial structure of *K. amblyonyx.* To assess this, we used linear (Euclidean distances) and geometric morphometrics (size and shape) approaches. We investigated intraspecific differences using an analysis of variance (ANOVA), and principal component analyses (PCA). We did not find evidence of sexual dimorphism in the skull of *K. amblyonyx,* using both linear distances and landmark analyses. On the other hand, we found a geographical differentiation between populations, with both morphometric methods used. Most differences were observed in populations from the extremes of its distribution, as São Paulo and Espírito Santo states. Our study is the first attempt to elucidate important information about morphological diversity of *K. amblyonyx.*

Key-Words: Bamboo rat; Cranial variation; Echimyidae; Linear and geometric morphometrics.

Resumo: Variação geográfica e dimorfismo sexual no tamanho e forma do crânio de Kannabateomys amblyonyx (Rodentia: Echimyidae). O roedor equimídeo *Kannabateomys amblyonyx,* também conhecido como rato-do-bambu, distribui-se ao longo do bioma Mata Atlântica presente no Brasil, Uruguai, Paraguai e Argentina. Esta espécie se alimenta exclusivamente de folhas e brotos de bambu, sendo assim altamente especialista. Devido à ausência de estudos envolvendo este grupo, parcialmente associada à pequena disponibilidade de amostras em museus e coleções, aspectos de sua diversidade morfológica e variações intraespecíficas permanecem pouco compreendidas. Nesta perspectiva, nosso estudo compreende uma primeira avaliação da variação sexual e geográfica na estrutura craniana de *K. amblyonyx.* Para acessar tais questões, nós utilizamos morfometria linear (distâncias euclidianas) e geométrica (forma e tamanho). Nós investigamos diferenças intraespecíficas utilizando análises de variância (ANOVA) e análise de componentes principais (PCA). Não encontramos evidência de dimorfismo sexual no crânio de *K. amblyonyx,* utilizando ambas as análises das distâncias e marcadores geométricos. Por outro lado, nós encontramos uma diferenciação geográfica entre populações em ambos os métodos morfométricos utilizados. A maioria das diferenças foi observada em populações provenientes de distribuições extremas, como São Paulo e Espírito Santo. Nosso estudo é a uma primeira tentativa de preencher uma lacuna importante no conhecimento da diversidade morfológica de *K. amblyonyx.*

Palavras-Chave: Echimyidae; Morfometria linear e geométrica; Rato-do-bambu; Variação craniana.

INTRODUCTION

The bamboo rat, *Kannabateomys* Jentink, 1891, is a monotypic genus represented by the species *K. amblyonyx* (Wagner, 1845), which is restricted to the Atlantic rainforest of Brazil, Uruguay, Paraguay and Argentina

(Patton *et al.,* 2015). This species is a habitat specialist that only occurs in bamboo vegetation and feed exclusively on bamboo stems and leaves (Olmos *et al.,* 1993; Silva *et al.,* 2008). Due to its highly specialized habitat, there is very little literature about its natural history or evolutionary history. Most of the information available

in the literature addresses aspects regarding its conservation status (Silva *et al.,* 2012) and its reproductive strategy (Silva *et al.,* 2008; Siman *et al.,* 2017). Moreover, Fabre *et al.* (2017) were proposed a hypothesis of the evolutionary trajectory and phylogenetic relationships between *K. amblyonyx* and its closest relative species in a study where they investigated the phylogenetic relationships of the Echimyidae family, the taxonomic family to which *K. amblyonyx* belongs. Although the studies mentioned above provide some insights about the phylogenetic position of *K. amblyonyx* within the Echymidae family, as well as some aspects about its ecology, we know little about its morphological variation and the possible evolutionary mechanisms that might have shaped the phenotype of this highly specialized species. Therefore, our study is a first attempt to fill such gap by describing the geographical and sexual variation in the skull morphology of this species.

There are a few studies that have investigated the morphometric variation in Echimyidae, including specimens from *Kannabateomys,* focusing on the tooth (Candela & Rasia, 2012) and the skull morphology (Perez *et al.,* 2009). Those studies gave us a better understanding on the ecological and evolutionary factors leading to the morphological diversification of this group. Furthermore, analyses of skull morphometric using size and shape variables have already been used to investigate sexual and geographic variation in echimid rodents (Monteiro *et al.,* 1999).

The skull is responsible for performing numerous tasks in mammals, and is directly involved in several ecological aspects such as feeding, acquiring and manipulating food, protecting sensorial organs and the brain (Fish, 2017). Due to its multitude of important tasks the skull represents an important structure when trying to elucidate evolutionary aspects within and among populations. Size changes associated with allometric processes are one of the key factors driving cranial diversification in mammalian clades (Cardini & Polly, 2013). In addition, environmental and lifestyle differences, such as diet and locomotion, can also be important drivers of skull morphological variation (Nogueira *et al.,* 2009; Tavares *et al.,* 2010).

In particular, a common phenomenon in mammals is sexual dimorphism, where females are morphologically different from males. These differences can be manifested as difference in size (Lindenfors *et al.,* 2007) or in specific traits (Camargo & Oliveira, 2012; Sebastião & Marroig, 2013). One of the mechanisms behind sexual dimorphism is sexual selection. According to sexual selection theory, the differences between the sexes are a consequence of intrasexual competition for mating opportunities or epigamic selection, in which mating choice is initially influenced by characteristics in the opposite sex that increase the fitness of its offspring (Jones & Ratterman, 2009). Another mechanism that can generate sexual dimorphism is distinct growth trajectories. Irrespective of the mechanism generating this pattern, in echimyid rodents, sexual dimorphism is usually small or absent (Bezerra & Oliveira, 2010; Monteiro *et al.,* 1999).

Local ecological aspects (*e.g.,* competition, climate and resource availability) and phylogenetic history may have an important influence on species distribution and influence geographical variation, as well as in promoting different selective pressures leading to morphological variation within a species (Cardini *et al.,* 2010). In some cases, morphological differences observed along the geographical distribution of a species become the starting point to investigate the presence of a new species (Moratelli *et al.,* 2011) or delimit subspecies (Soisook *et al.,* 2013). In echimyids, the genus *Thrichomys* has been the subject of several studies involving morphometric analyses that aimed to assess the degree and patterns of geographic variation (Duarte *et al.,* 2000; Monteiro *et al.,* 1999; Monteiro *et al.,* 2003; Reis *et al.,* 2002). These analyses were performed based on populations from Central and Northeastern Brazil. In general, the results showed shape differences in regions associated with mastication and correlated with a general latitudinal environmental gradient (Monteiro *et al.,* 2003), as well as the segregation of single populations from all others (Duarte *et al.,* 2000). Perez *et al.* (2009) showed, in species group in Echimyidae, a strong concordance between shape variation of four cranial units (vault, base, oragnathofacial complex and mandible) and their ecological niches.

Knowledge of variations within a species is crucial to investigate functional, evolutionary, ecological and conservation hypotheses in different groups (Des Roches *et al.,* 2018). The main goal of the present contribution is to evaluate the geographic and sexual variation in the skull of *K. amblyonyx,* analyzing those ecological aspects potentially relevant to morphological changes.

MATERIALS AND METHODS

We analyzed 59 skulls of *Kannabateomys amblyonyx* deposited at the following institutions: Instituto Nacional da Mata Atlântica (INMA, Santa Teresa – ES), Museu Nacional (MN, Rio de Janeiro – RJ), Museu de Zoologia da Universidade de São Paulo (MZUSP, São Paulo – SP) and Museu de Zoologia João Moojen (MZUFV, Viçosa – MG). All skulls were assigned to age classes following Silva (2014) and only adult specimens were used to avoid possible ontogenic influences in morphological variation. In an attempt to cover the total geographical distribution of the species, we analyzed skulls from four different Brazilian States in the Southeastern region that contain the Atlantic Forest biome. Our samples came from the States of São Paulo (SP, n = 17), Rio de Janeiro (n = RJ, 5), Espírito Santo (ES, n = 7) and Minas Gerais (MG, n = 20) and included 19 male and 26 female specimens. The list of sampled specimens divided by state and sex is presented in Table S1.

Three-dimensional coordinates were recorded for 32 landmarks in each skull, using a 3D digitizer (MX-Microscribe®) (Figure 1 and Table 1). Whenever one side of the skull was damaged, the measurement and/or landmark information of the other side was used. Each specimen was digitized twice, and repeatability was
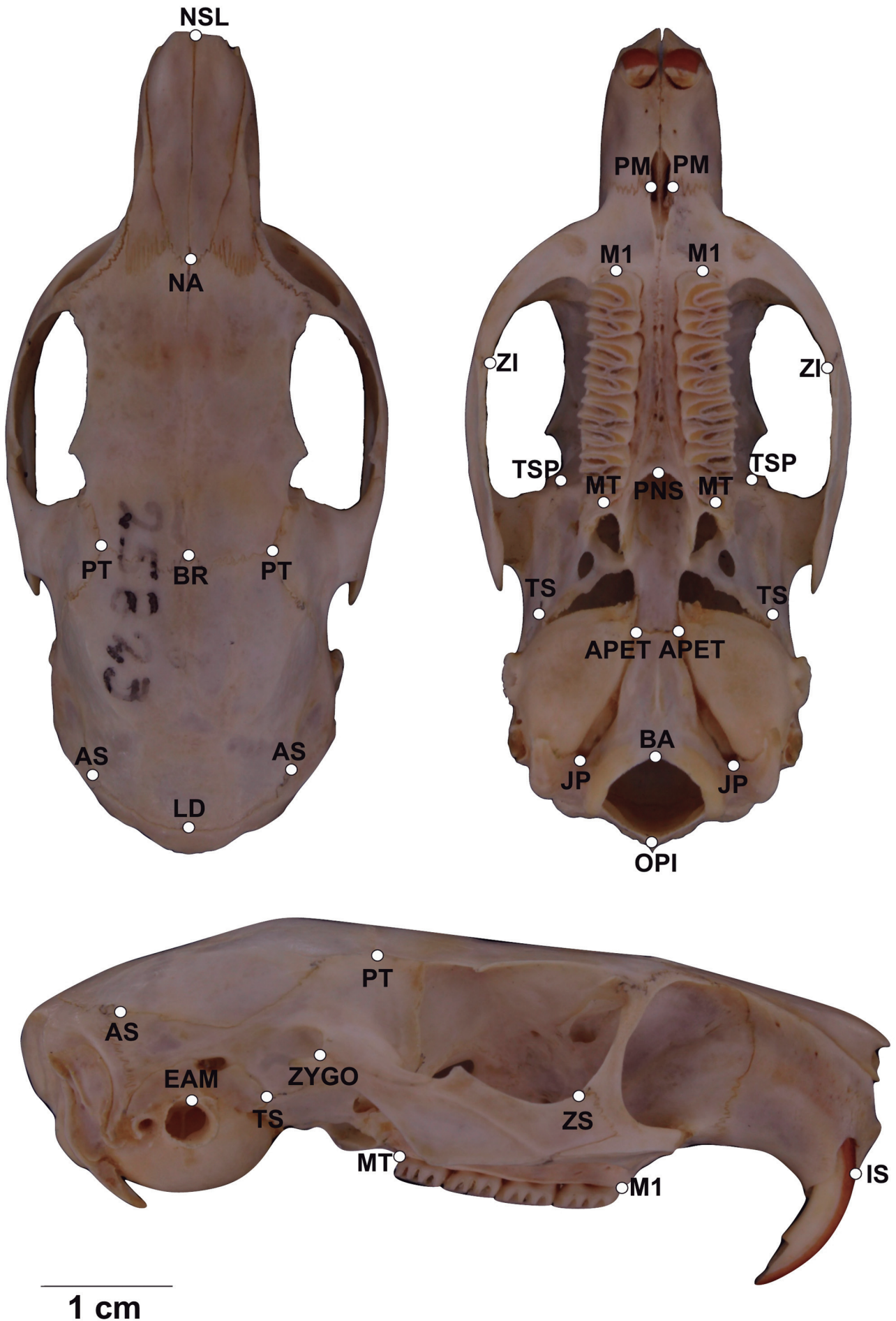
1 cm

Figure 1: Representation of the three views from *K. amblyonyx* skull (dorsal, ventral and lateral) and their respective geometric landmarks.

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Table 1: Description of landmarks used in the *K. amblyonyx* skull according to Figure 1. The sagittal and bilateral positions represent measurements located in left and right size of the skull, respectively.

Landmark	Position	Description
IS	SAGITAL	Intradentale superior
NSL	SAGITAL	"Nasale" – Anterior extremity of nasal bone
NA	SAGITAL	"Nasion" – Posterior extremity of nasal bone
BR	SAGITAL	"Bregma" – Suture between frontal and parietal
ZS	BILATERAL	Zygomaxillare superior
PT	BILATERAL	"Pterion" – Suture between frontal and parietal
TSP	BILATERAL	Temporo-pheno-parietal suture
PM	BILATERAL	Pre-maxillare-maxillare suture at the alveolus
ZI	BILATERAL	Zygomaxillare inferior
MT	BILATERAL	Maxillary tuberosity
PNS	SAGITAL	Posterior nasal spine
ZYGO	BILATERAL	Suture between zygomatic and temporal
TS	BILATERAL	Temporo-sphenoidal junction at petrous
EAM	BILATERAL	Anterior external auditory meatus
JP	BILATERAL	Jugular process
APET	BILATERAL	Anterior petrous temporal
BA	SAGITAL	"Basion" – Ventral point of foramen magnum
OPI	SAGITAL	"Opistion" – Dorsal point of foramen magnum
LD	SAGITAL	Lambda
AS	BILATERAL	"Asterion" – Suture between parietal and occipital

Table 2: Euclidean distances estimated from 32 markers obtained from skull and their respective functional/development groups. Distances were chosen to represent evolutionary important traits that could have impacted the biological phenomenon that we were interested in (sexual dimorphism and geographical variation).

Distances	Functional Subregion	Region
IS-PM	Oral	Face
IS-NSL	Nasal	Face
IS-PNS	Oral/Nasal	Face
PM-ZS	Oral	Face
PM-ZI	Oral	Face
PM-MT	Oral	Face
NSL-NA	Nasal	Face
NSL-ZS	Nasal	Face
NSL-ZI	Oral/Nasal	Face
NA-BR	Cranial Vault	Neurocranium
NA-PNS	Nasal	Face
BR-PT	Cranial Vault	Neurocranium
BR-APET	Cranial Vault	Neurocranium
PT-APET	Cranial Vault	Neurocranium
PT-BA	Cranial Vault	Neurocranium
PT-EAM	Cranial Vault	Neurocranium
PT-ZYGO	Zygomatic	Face
PT-TSP	Cranial Vault/Zygomatic	Neurocranium/Face
ZS-ZI	Oral/Zygomatic	Face
ZI-MT	Oral	Face
ZI-ZYGO	Zygomatic	Face
ZI-TSP	Zygomatic	Face
MT-PNS	Oral	Face
PNS-APET	Skull Base	Neurocranium
APET-BA	Skull Base	Neurocranium
APET-TS	Skull Base	Neurocranium
BA-EAM	Skull Base	Neurocranium
EAM-ZYGO	Zygomatic	Face
ZYGO-TSP	Zygomatic	Face
LD-AS	Cranial Vault	Neurocranium
BR-LD	Cranial Vault	Neurocranium
OPI-LD	Cranial Vault	Neurocranium
PT-AS	Cranial Vault	Neurocranium
JP-AS	Skull Base	Neurocranium
BA-OPI	Skull Base	Neurocranium

estimated to assess measurement reliability according to Lessels & Boag (1987). All specimens showed high values of repeatability for all measurements (see Table S2). Therefore, all subsequent analyses were carried out using the average replicated measurements and were performed in the R statistical environment (R development Core Team, 2015).

We used a combination of geometric and linear morphometrics to analyze geographic variation and sexual dimorphism in skulls of *K. amblyonyx*. Using geometric morphometrics, we analyzed isometric size changes using centroid size, which is a shape independent variable (Bookstein, 1991) and the skull shape, defined as "all the geometric information that remains when location, scale and rotational effects are filtered out from an object" (Kendall, 1977; Zelditch *et al.,* 2004). The geometric morphometric framework represents a powerful tool to assess shape variation between groups, as it removes effects of scaling (isometric size) from the analyses. The shape variables were obtained by scaling, aligning and transforming the landmark coordinates for each specimen through General Procrustes Alignment (GPA) using the R packages "AMP", "OSymm", "unifyVD" (Haber *et al.,* 2015) and "Geomorph" (Adams & Otarola-Castillo, 2013). In addition, we also analyzed skull morphology using linear morphometric. From the landmarks, we estimated a set of 35 Euclidean distances (Table 2). Those distances were chosen because they represent important functional aspects of the mammalian skull (Cheverud, 1982; Marroig & Cheverud 2001).

We checked for normality using *"stem and leaf"* display to evaluate the distribution of values in a graphical data (Tukey, 1977). To evaluate sex and geographic variation, we performed a Principal Component Analysis (PCA) to detect potential cranial variations between individuals of *K. amblyonyx* related to skull linear distances, centroid size and geometric morphometric shape. To test if these differences were statistically significant, we performed an analysis of variance (ANOVA, p-value of 0.05) for centroid size and mean shape. For multiple tests (35 linear distances), we correct the level of significance using a Bonferroni correction (*i.e.,* p-value of 0.05/35 = 0.001). Visual information related to changes in cranial shape regarding the first and second principal component axes of variation was analyzed through deformation grids (*Thin-plate spline, TPS*).

RESULTS

Tests for sexual dimorphism on linear distances were not significant, indicating the absence of absolute dimorphism. On the other hand, in the geographical variation analyses, only one linear measurement was

Table 3: Linear and geometric morphometric ANOVA results for geographic differentiation analyses representing the variance (*F*), values of probability (*p-value*) and descriptive statistics, with maximum and minimum values, means and standard deviations (SD) for localities.

Variables	F	*p-value*	São Paulo		Rio de Janeiro		Espírito Santo		Minas Gerais	
			Min/Max	Mean/SD	Min/Max	Mean/SD	Min/Max	Mean/SD	Min/Max	Mean/SD
PTAPET	7.85	0.000	18.06/20.81	19.26/0.82	17.98/19.24	18.80/0.51	16.52/18.53	17.78/0.72	17.71/19.75	18.57/0.60
Shape	2.50	0.009	—	—	—	—	—	—	—	—

significant (PT-APET). Individuals from RJ and SP showed higher mean values for this measurement than those from other localities (Table 3).

The Euclidean measurements were compared using a Principal Component Analysis (PCA) and the projection of each individual in the first and second principal component space (Figure 2). The first principal component axis summarizes 36% of the total variation and the second principal component axis 13% of the variation. In the principal component analysis, the individuals of both sexes overlap along the first and second principal components, demonstrating that the major axes of variation do not separate sexes. On the other hand, the different sampling localities showed a pronounced segregation in the principal component space, mostly between SP and

Figure 2: Scores of each specimen in the first and second principal component space based on the values of 35 linear distances, showing differentiation among localities. Males and females remain overlapping in subspace.

ES, while the other localities (MG and RJ) presented an intermediary, partially overlapping, position (Figure 2).

There were no statistically significant differences in centroid sizes between sexes (p = 0.561, Figure 3). There were no statistically significant differences in centroid sizes between localities as well (p = 0.4, Figure 4). The Procrustes ANOVA showed no statistical differences between sexes (p = 0.217). In contrast, the Procrustes ANOVA demonstrated significantly shape differences among localities (Table 3). In the Principal component multivariate shape space, the first principal component axis summarized 14% of the variation and the second principal component represented 13% of the variation. Males and females presented a large overlap in projection of the first two principal components, whereas the localities presented a similar segregation pattern as the linear distances results (Figure 5).

For a better visualization of shape change we performed a deformation grid analysis (Figure 5). The first principal shape component represents an axis of strong deformation in the cranial vault and skull base region towards the positive (PC1max) and negative (PC1min) axis of PC1. Specimens with higher loading values in PC1 space (PC1max) presented an extension of those regions, while PC1min values are associated with retraction of those regions. Individuals from ES (PC1max) and SP (PC1min) are positioned at such extremes. The second principal component axis also represents deformations related to the cranial vault and skull base, as well as with the addition of the nasal region. In general, there was a narrowing of the skull as a whole toward the PC2max. The individuals from ES, MG (PC2max) and SP (PC2min) are positioned near the positive and negative axis of the second principal component.
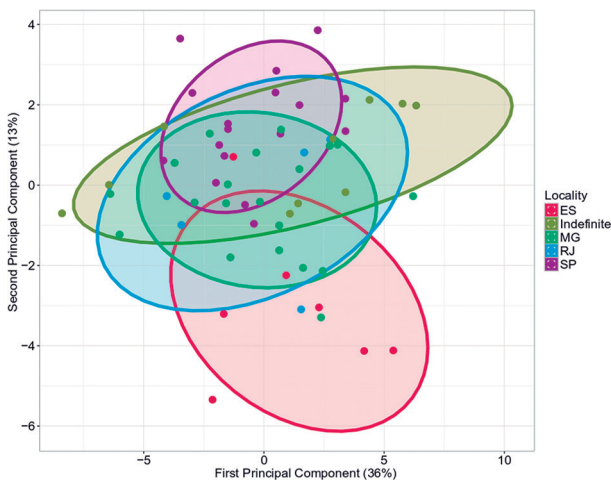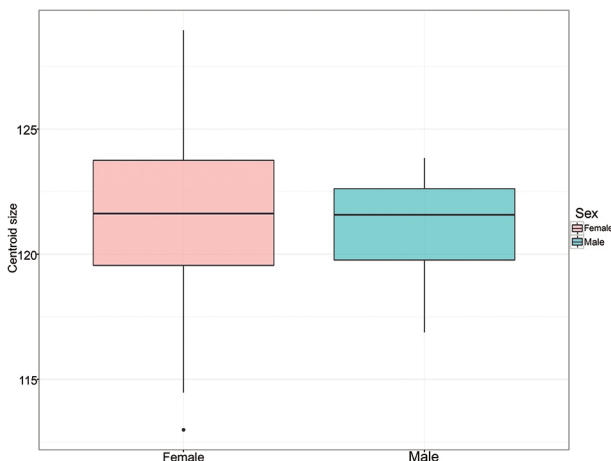

Figure 3: Boxplot indicating the compared values of centroid size obtained for males and females.
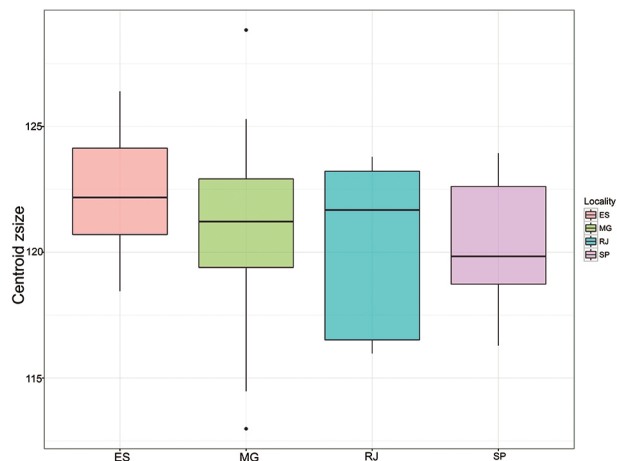
Figure 4: Boxplot indicating the compared values of centroid size obtained for geographic localities.

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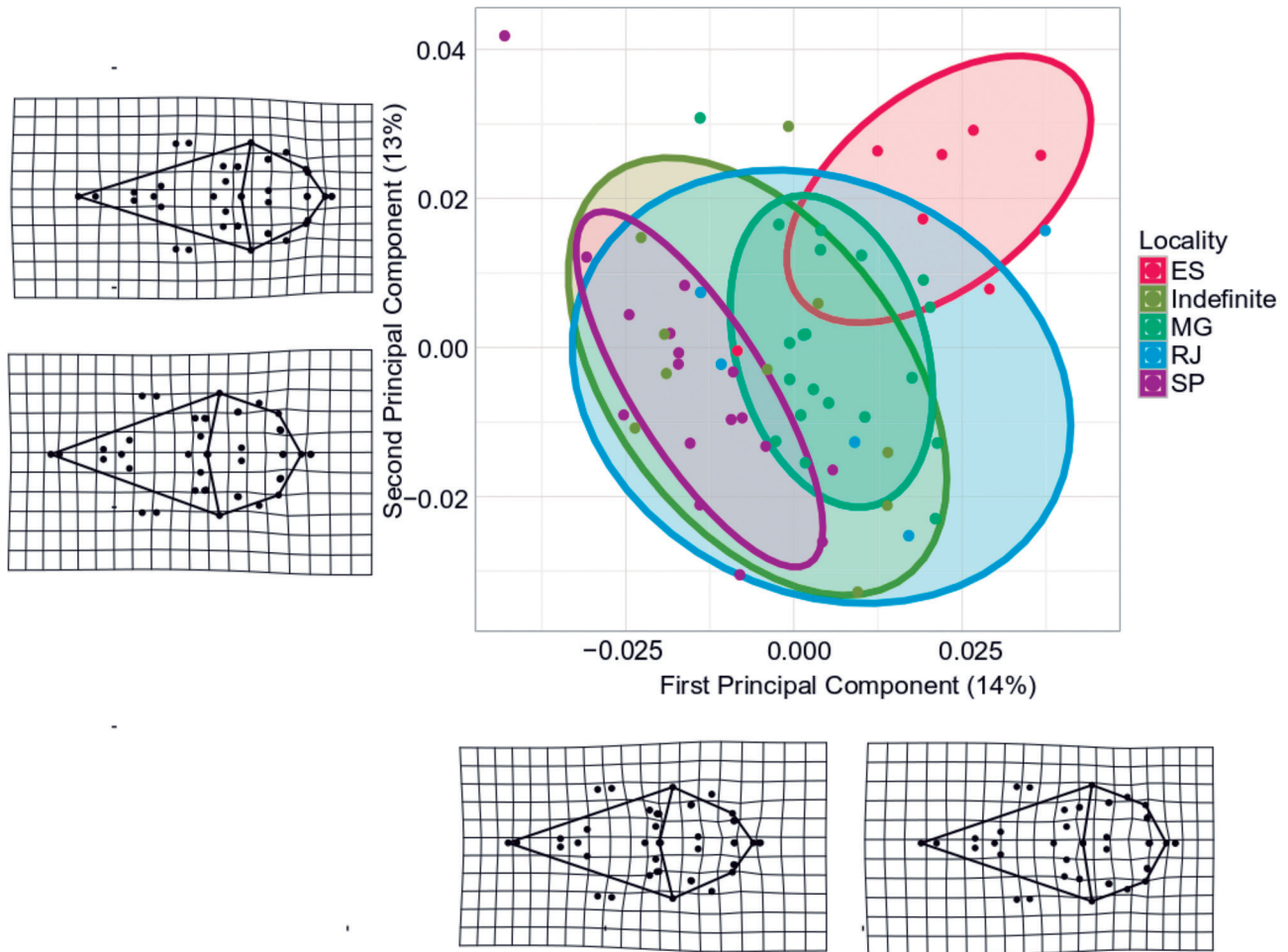
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Figure 5: Scores of each specimen in the first and second principal component space based on the shape coordinates, showing differentiation among localities. Males and females remain overlapping in subspace. Deformation grids representing skull shape differences for *K. amblyonyx* observed in first and second principal component towards the positive and negative axis for dorsal view of the cranium. Variations related to cranial vault region can be observed.

DISCUSSION

Our study investigated morphological variation in the highly specialist species *Kannabateomys amblyonyx.* We found that most intraspecific morphological variation was due to geographical differentiation. We found no evidence of sexual dimorphism in this species, in any data analyzed, linear measurements, centroid size or shape variables, indicating the absence of this phenomenon in *K. amblyonyx.* Sexual monomorphism was also described by Silva *et al.* (2008) in body mass of specimens from Southern Brazil.

On the other hand, sexual dimorphism has already been reported for other echimyids taxa. For example, the genus *Proechimys* presents size sexual dimorphism, with males larger than females (Corti *et al.,* 2001). In contrast, *Thrichomys apereoides* are not dimorphic in shape or size of skull (Monteiro *et al.,* 1999). In mammals, the presence of sexual dimorphism is most often attributed to a polygamous mating system, where individuals (usually males) compete for sexual partners (Andersson, 1994; Lu *et al.,* 2014). Alternatively, intersexual morphological differences, such a habitat use, may be associated with environmental factors. In this sense,

with each sex using different resources, we would expect different selective pressures acting upon each sex leading to a pattern of sexual dimorphism (Butler *et al.,* 2007). Despite the scarcity of ecological data, Olmos *et al.* (1993) described social and habit characteristics of *K. amblyonyx,* such as feeding and calling behavior, showing no clear sexual segregation, which might help explain the lack of sexual dimorphism pattern found.

The absence of sexual dimorphism can be explained by several factors related to specific aspects of *K. amblyonyx*'s natural history and ecology (Silva *et al.,* 2008). This species is monogamous with biparental care (Silva *et al.,* 2008). Moreover, food resource distribution is homogenous across space, since this species is extremely dependent on bamboo shoots as a food resource (Oliveira & Bonvicino, 2006; Silva & Vieira, 2006; Silva *et al.,* 2008; Silva *et al.,* 2012). In addition, another factor that may explain this result is the low occurrence of sexual dimorphism generally found in small mammals (Lu *et al.,* 2014), and particularly in histricognats rodents (Bezerra & Oliveira, 2010, Lessa & Pessôa, 2005; Pessôa & Reis, 1991; Pessôa & Reis, 1992; Pessôa & Strauss, 1999).

On the other hand, we did find geographical differentiation in the cranial structures of *K. amblyonyx.*

These differences were detected using linear measurements (PT-APET) and geometric morphometric, with the exception of centroid size, where no statistical differences between localities were found. The shape deformation grids showed that the axes with greatest variation were related to the cranial vault, skull base and nasal region. These regions exert several different functional demands on the skull, with the cranial vault responsible for protecting the superior and lateral portions of the brain, whereas the skull base is associated with protecting the cerebral connection with the face/body and also provides a platform under which the rest of skull develops (Lee *et al.,* 2017). Finally, the nasal region is associated with respiratory and olfactory systems and with water balance regulation in mammals (Schimidt-Nielsen *et al.,* 1970).

Therefore, the regions where most morphological changes were observed are associated with lifestyle tasks, such as protection and feeding. The feeding habits of *K. amblyonyx* only include bamboo shoots and leaves. However, studies involving anatomical variations among Atlantic Forest bamboo are scarce, and therefore we cannot say that the feeding does not vary, as regards the hardness and thickness of seeds and leaves. The existence of geographic variation could be a result of ecological aspects, involving biotic (interspecific competition) and physical aspects (climatic conditions) (Bornholdt *et al.,* 2008). The Atlantic forest region is characterized by its highly heterogeneous environmental conditions with large altitudinal range, complex topography and strong seasonality (Morellato & Haddad, 2000). Additionally, despite the lack of information, behavioral data described by Olmos *et al.* (1993) for *K. amblyonyx* reported the existence of vocalization between individuals for territorial and defensive functions. Following this reasoning, it is known that the vocal tract is formed by anatomical structures of the upper respiratory pathway (*e.g.,* throat, laryngeal, oral and nasal cavity) and communicational differences can promote vocal tract specializations (Frey & Gebler, 2009). Variation in the form of the bulla is also associated with acoustic signals, and can promote different configurations in the skull base region (Perez *et al.,* 2009). Intraspecific variation of these behavioral characteristics was already reported for Neotropical singing mice *Scotinomys teguina,* however, cranial variations were not investigated (Campbell *et al.,* 2010). In this way, potential vocalization differences among populations of *K. amblyonyx* could be influencing the geographic variation in cranial structure, although further studies are needed to test this hypothesis.

Moreover, the differences observed in the functional regions of the skull (cranial vault, skull base and nasal region) might be related to the isolation-by-distance model (Wright, 1943). This model proposes that an increase in the geographic distance results in a decrease in the total gene flow among populations, favoring the genetic, and consequently the morphological, and divergence between populations. For echimyids, isolation-by-distance has already been proposed as the mechanism explaining skull variation in *Thrichomys apereoides*

(Reis *et al.,* 2002). In our study, the principal component analyses indicate differences between populations from Espírito Santo and São Paulo, which constitute the most extreme locations analyzed. Therefore, the geographic isolation is also a viable mechanism to explain the morphological differentiation observed in our analyses. Unfortunately, due to the small number of localities sampled in our study, we are unable to test this hypothesis.

Future studies are needed to elucidate the specific mechanisms proposed here that are responsible for the morphological differentiation observed in *K. amblyonyx.* The present study represents a descriptive first attempt to characterize intraspecific morphological variation in the skull of *K. amblyonyx.* However, it is important to note that our samples were limited due to the rarity of specimens in museum collections. Therefore, even though our study represents a first step in characterizing morphological variation in *K. amblyonyx,* more studies (including field sampling efforts) are necessary to corroborate our findings. Even with the scarcity of data, the geometric morphometric approaches used were able to detect geographic variation, filling gaps in the morphological evolutionary history of this species. Moreover, we present evidence for the lack of sexual dimorphism in this species, in both shape and size variation. In the future, we intend to investigate aspects of morphological integration and their evolutionary consequences, comparing the very specialized lifestyle of *K. amblyonyx* to other echimyids rodents.

The supplementary information is available in: https://sbmz.org/publicacoes. Legends: **Table S1.** List of measured specimens and their respective scientific collections. Sex information is indicated as F (female) and M (male); **Table S2.** Representation of the values obtained in the repeatability analysis of the measurements for *K. amblyonyx* species; **Table S3.** Pairwise ANOVA comparing the linear measurement difference between pairs of populations. This table only shows the statistically different populations after Bonferroni correction ($p < 0.001$).

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