Cranial shape predicts arboreal activity of Sigmodontinae rodents

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Abstract

Brain enlargement is an adaptation in animals that use the space in three dimensions and need to integrate information of complex environments. As consequence, this adaptation can result in differences in skull shape among species with different types of locomotion and level of arboreality. We directly tested for the relation between the cranial shape and arboreality (based on field data) of 11 Sigmodontinae rodent species from a Neotropical savanna (Cerrado). For that, we used geometric morphometrics considering both dorsal and lateral views of the cranium. We also accounted for any possible allometric effects (i.e. the relationship between size and shape) and evolutionary constraints (i.e. phylogenetic signal) on the cranial shape. The dorsal view presented strong allometric effects but no phylogenetic signal. On the other hand, the lateral view showed a weak allometric effect and a significant phylogenetic signal. After controlling for size and evolutionary constraints, we found significant relationships between rodent arboreality and cranial shape for both lateral ($r^2 = 0.74$) and dorsal cranium views ($r^2 = 0.40$). Patterns for both views showed that arboreal rodents present more expanded crania, suggesting larger brains. The dorsal view showed a general cranial expansion in arboreal rodents, whereas the lateral view showed a more rounded and posteriorly expanded cranium. This later suggests a larger cerebellum, an important brain portion responsible for orientation and sense of balance. Our study provides evidence that the cranial shape can be used as a proxy to infer arboreal activity of sigmodontine rodents. Moreover, the observed differences in cranial shape between arboreal and terrestrial species are probably related to brain enlargement in the former driven by the need to cope with a three-dimensional environment.

Introduction

The skull is a complex structure that is an outcome of functional adaptations, allometric adjustments and architectural and phylogenetic constraints (Wroe & Milne, 2007; Samuels, 2009; Maestri et al., 2016; McLean et al., 2018), which incorporates features that are essential for manifold functions (e.g. sensory perception, cognition, foraging, food processing and respiration). The understanding of the evolutionary complexity of the skull involves the knowledge of the relationship between morphology and both ecological and functional relevant aspects of organisms, such as diet (Samuels, 2009; McLean et al., 2018), bite force (Maestri et al., 2016), habitat use (Eisenberg & Wilson, 1978; Budeau & Verts, 1986; Shumway, 2008), locomotion (Motani & Schmitz, 2011; Dumont et al., 2016) and brain size (Wroe & Milne, 2007).

Variation in relative brain size (i.e. after the removal of body size influence) in animals have been considered as a result of several factors, such as energetic constraints, life-history patterns, learning, social structure, diet and habitat use (Bennett & Harvey, 1985; Budeau & Verts, 1986; Van Dongen, 1998). However, brain enlargement not necessarily is proportional to the entire brain, and can be concentrated in different parts related to different skills (learning, sociability, sensory and motor-related systems) as consequence of selection pressures (Bennett & Harvey, 1985; Taylor, Nol & Boire, 1995; Van Dongen, 1998; De Winter & Oxnard, 2001). Several studies point to the relation between brain enlargements and the habitat for different vertebrate groups (Bennett & Harvey, 1985; Van Dongen, 1998; De Winter & Oxnard, 2001). For example, certain bat species convergently evolved large brains in complex habitats (i.e. forests), which enhanced their capacity of processing complex information, due to selection for dependence on multisensory inputs to the brain (Eisenberg & Wilson, 1978). A similar pattern was found in chipmunks relative to complexity of their arboreal habitat (Budeau & Verts, 1986) and in lacustrine cichlid fishes in Africa (Shumway, 2008).

Similar to habitat type, locomotor habits also correlate to relative brain size. Aquatic, arboreal and scansorial mammal species commonly have larger brains than terrestrial or fossorial ones (Lemen, 1980; Eisenberg & Wilson, 1981; Pilleri, Gihr & Kraus, 1984; Bernard & Nurton, 1993; Bertrand, Amador-Mughal & Silcox, 2017; Bertrand *et al.*, 2018). A study conducted with extinct and extant sciurids shows that arboreal species present larger brains in comparison to terrestrial ones (Bertrand *et al.*, 2017). Additionally, recent fossorial Aplodontidae rodents present a reduction in relative brain size in comparison to their arboreal ancestors (Bertrand *et al.*, 2018). Larger brains in species that move in three dimensions indicate important adaptations for complex locomotion and exploitation of the environment (Eisenberg & Wilson, 1978; Eisenberg, 1981). In fact, there are evidences of convergences in relative brain sizes even in highly related species with different climbing abilities, as verified, for example, in several rodent species of the genus *Peromyscus* (Lemen, 1980).

Rodents comprise a promising group for the investigation of the relationship of brain size and skull morphology with habitat characteristics and locomotor modes. This group forms the most species-rich mammalian order (at over 2200 species or 44% of the living mammals; Wilson & Reeder, 2005; Wolff & Sherman, 2007), relying on a wide variety of locomotion ways, including aquatic, semi-aquatic, fossorial, semi-fossorial, terrestrial, ricochetal, scansorial, arboreal and gliding habits (Nowak, 1999). Although their morphological variation is considered to be relatively conservative (Hautier *et al.*, 2011), postcranial adaptations (e.g. bone length, robustness and shape and muscular attachment) seem to be more relevant than cranial adaptations for understanding how these species cope with different locomotion types (e.g. Elissamburu & Vizcaíno, 2004; Samuels & Van Valkenburgh, 2008; Astúa, 2009).

Although occurring less-commonly than for postcranial structures, relationships between cranial morphology and locomotion in rodents have been reported and may be relevant for some rodent groups (Lessa & Patton, 1989; Samuels & Valkenburgh, 2009; Bertrand, Schillaci & Silcox, 2016). However, the available studies that tested for this relation were based solely or partially on categorical descriptions available in literature for the species. The use of quantitative field data for testing of hypotheses relating skull measurements describing morphology to the degree of arboreality of small rodents may contribute to the understanding of the relevance of cranial morphology to the relation between environmental characteristics and mammalian locomotion.

In this study, we evaluated the relation between cranium shape and arboreal activity in different species of muroid rodents of the tribe Sigmodontinae in the Brazilian Cerrado, the biologically richest savanna of the world (Klink & Machado, 2005). This biome includes several distinct formations with high variation in arboreal cover, ranging from open grasslands to typical savanna habitats and forest formations (Eiten, 1972). Thus, it harbors rodents with distinct levels of arboreality (Paglia *et al.*, 2012). We used cranial geometric morphometrics and field-based data related to above-ground captures of 11 sigmodontine rodents to test the relationship between cranial shape and arboreality, also accounting for allometric and phylogenetic constraints. Our main hypothesis was that more arboreal sigmodontine rats present more expanded crania in comparison to terrestrial species. More specifically, we expected a positive relation between cranial expansion and arboreal activity inferred through above-ground captures. Our expectation was based on the larger brains already observed in arboreal rodent species in comparison to terrestrial ones in North America (Lemen, 1980; Meier, 1983) and South Africa (Bernard & Nurton, 1993), in addition to comparisons between extant and fossil species (Pilleri *et al.*, 1984; Bertrand *et al.*, 2017, 2018), which could generate cranial shape differences among animals with distinct locomotor modes (Wroe & Milne, 2007; Dumont *et al.*, 2016). Thus, our study brings insights on the use of cranial shape characteristics as predictors of tropical sigmodontine rodents' arboreality.

Materials and methods

Determination of arboreal activity

We sampled eight sites, four of gallery forest and four of savanna woodland. In each site we sampled the ground, understory and canopy of the forests using Sherman® live traps. Canopy traps were placed using the method described in Vieira (1998). Overall sampling effort totaled 43 200 trap nights (21 600 per forest type; for details see *Study area and Methods* in the Data S1). All methods used in this study were approved by the Ethics in Animal Research Committee of the Institute of Biology of the University of Brasília, and also followed guidelines for the use of mammals in research established by the American Society of Mammalogists (Sikes & Gannon, 2011). This study was conducted under authorization of Chico Mendes Institute for Biodiversity Conservation (Permit Nbr. 33092-3).

To obtain the arboreal activity level of each sigmodontine species we calculated the proportion of above-ground captures, this proportion was obtained by dividing the number of above-ground captures (i.e. sum of all captures recorded in arboreal traps, placed in the understory or in the canopy) by the sum of all captures (obtained in all vertical strata) of each species. For that, we considered only the first capture of each individual, insuring the independence of the data. For six sigmodontine species we obtained arboreality data in field. However, in order to include in further analysis other five sigmodontine species from Cerrado for which we were able to obtain cranial morphometric data but were not captured in our fieldwork, we used data on arboreal activity available from other studies also conducted in Cerrado (see Table 1 in results for more details). We followed the taxonomic arrangements and identified rodent species according to Weksler *et al.* (2017) and Gardner *et al.* (2015).

In relation to the arboreal activity of the 11 species analyzed, three sigmodontine rats had high and similar values of arboreal activity, namely *Rhipidomys macrurus* (76% of above-ground captures), *Oecomys cleberi* (70%) and *Oecomys catherinae* (71%). *Oligoryzomys nigripes* (40%) and *Oligoryzomys mattogrossae* (33%) were the species with considerable arboreal activity but that also used the ground frequently (i.e. scansorial species). *Cerradomys scotti* (21%) and *Hylaeamys*

Table 1 Species code, number of crania used in the morphometric analysis, number of individuals captured in the field and proportion of above-ground captures (i.e. arboreality) of sigmodontine rodents from the Cerrado (Brazilian savanna)

Species	Code	Cranium	Captures	Arboreality
Akodon cursor[a]	Acu	18	11	0.00
Calomys expulsus	Cex	18	13	0.00
Calomys tener[b,c]	Cte	20	6/4	0.00
Cerradomys scotti[d]	Csc	24	19	0.21
Hylaeamys megacephalus	Hme	25	31	0.16
Necromys lasiurus[e]	Nla	20	22	0.00
Oecomys catherinae[e]	Oca	15	8	0.71
Oecomys cleberi	Ocl	25	129	0.70
Oligoryzomys mattogrossae	Oma	25	15	0.33
Oligoryzomys nigripes	Oni	23	15	0.40
Rhipidomys macrurus	Rma	25	106	0.76

Arboreality was obtained dividing the number of captures recorded in both the understory and canopy by the total number of captures (i.e. considering the captures from the ground, understory and canopy combined). Letters next to species names indicate arboreality obtained from other studies conducted in the Cerrado. [a]Palma (2002); [b]Bonvicino *et al.* (2014); [c]Carmignotto and Aires (2011); [d]Camargo *et al.* (2012); [e]Nitikman and Mares (1987).

megacephalus (16%) belonged to a third group, composed of mainly terrestrial species but with sporadic arboreal activity. Finally, the species that presented strictly terrestrial activity (i.e. 0% of above-ground captures) were *Akodon cursor*, *Calomys expulsus*, *Calomys tener* and *Necromys lasiurus*.

Geometric morphometrics

We photographed the dorsal and right lateral view (Fig. 2) of the cranium of 238 specimens of 11 sigmodontine species deposited in the Mammal Collection or in the Laboratory of Vertebrate Ecology of the University of Brasília (Table S1). We used only adult specimens (i.e. specimens with functional third molar) to avoid influence of ontogenetic variation. We placed each cranium in a petri dish filled with salt for the accommodation and standardization of the cranium position. This method was used for minimizing any error associated to the cranium displacement in relation to the horizontal plane. All the photographs were taken at 35 cm from the crania using a digital camera (Nikon COOLPIX, Tokyo, Japan; P520 with a 180 mm lens), which was mounted to a tripod to obtain comparable cranium size of each individual.

We stablished 14 two-dimensional anatomical landmarks for dorsal and 12 for lateral views of the cranium (Fig. 1 and Table S2 for landmark definitions). We chose the landmarks based on our main hypothesis of expanded cranium in more arboreal rodents due to larger brains. Therefore, most of established landmarks were placed to evaluate any cranial shape distortion in this sense. For digitalization of the landmarks in each specimen, we used the software TpsDig2 (Rohlf, 2015). For obtaining the variables (i.e. partial warps and uniform components) and visualization of cranial shape from the superimposition of landmarks, we used the program TpsRelw (Rohlf, 2015). The superimposition method is based on the Generalized Procrustes Analysis (GPA) algorithm (Dryden & Mardia, 1998), which removes the information of the landmark coordinates related to position, orientation and size of the crania (Bookstein, 1997; Dryden & Mardia, 1998). The size of

each cranium was obtained by the centroid size (CS). This metric is defined as the square root of the sum of the squares of the distance of each landmark from the centroid of the configuration (i.e. the mean of all coordinates; Bookstein, 1997). From this measurement, we were able to detect any allometric contribution on the cranial shape in further analysis.

Data analysis

Cranial shape and allometry

We run Canonical Variate Analysis (CVA) on the cranial shape variables to obtain new ones (i.e. Canonical Variates [CV]) that summarize the information contained in the original full set of variables. We chose this ordination analysis to obtain each CV maximizing the segregation of groups (i.e. species) according to the cranial shape (as in Astúa, 2009), and performed the CVA independently for each cranium view (dorsal and lateral). For further analysis we selected the first two CV (CV1 and CV2) because they contributed to a relatively high percentage of the total variance of cranial shape (>60% for each cranium view; see Results for more details). To assess the cranial shape variation related with each CV, landmark coordinates were regressed onto CV scores using the software TpsRegr (Rohlf, 2015).

For assessing allometric effects on cranial shape across species, we performed linear regressions using individual scores of each CV as dependent variable and the CS as explanatory variable. This analysis was performed independently for each cranium view considering all species. In case of relevant ($r^2 \geq 0.30$) and significant ($P < 0.05$) detection of allometry (as in Doube *et al.*, 2011), for further analyses we used the standardized residual values as a way to obtain the cranial shape variation without size effects (i.e. non-allometric components of cranial shape). For assessing the cranial shape variation without allometric effects, the landmark coordinates were regressed onto the standardized residuals using the software TpsRegr (Rohlf, 2015).
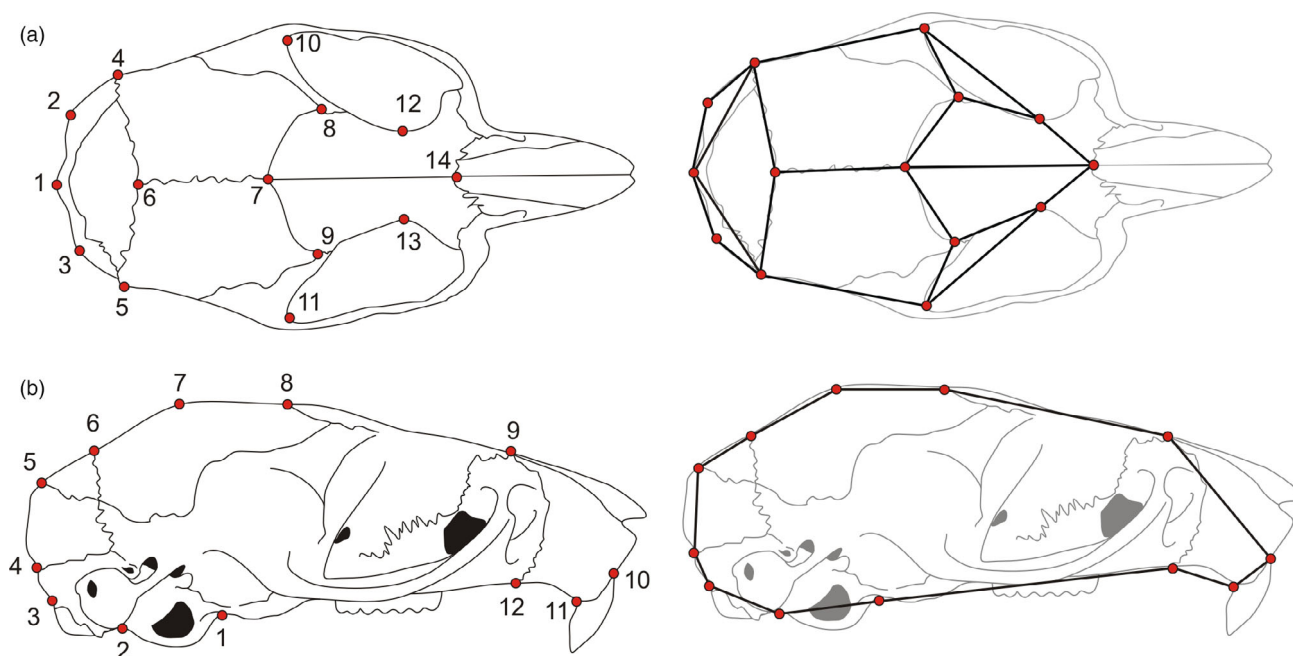
Figure 1 Landmark positions on the cranium of Sigmodontinae rodents for dorsal (a) and ventral (b) views. The wire frame connecting landmarks in the right figures was used to visualize cranial deformations in Figs. 2 and 3. For landmark definitions see Table S2. [Colour figure can be viewed at zslpublications.onlinelibrary.wiley.com]

Phylogeny, cranial shape and arboreal activity

Differences among species are potentially affected by the evolutionary history of the clades (Böhning-Gaese, Schuda & Helbig, 2003). For that reason, to further investigation of phylogenetic signals on cranial shape we first produced a sigmodontine phylogeny to obtain phylogenetic distances of the studied species. The phylogenetic hypothesis used in this study was the same of Machado *et al.* (2015), an evaluation of Sigmodontinae relationships at tribal-level. This tree is composed of 78 sigmodontine species, outgroups formed by six species of Muroid rodents from other subfamilies of Cricetidae, and accessed over 2406 base pairs (bp) of concatenated DNA sequence matrix of cytochrome b (Cyt-b; 1140 bp) and Interphotoreceptor Retinoid Binding Protein genes (IRBP; 1266 bp). DNA sequence data from the focus species of this study not accessed in Machado *et al.* (2015) was retrieved from GenBank (Table S3).

DNA sequences were aligned using Muscle (Edgar, 2004) as implemented in MEGA v. 5 (Tamura *et al.*, 2011). Posteriorly, a maximum likelihood analysis was performed using RAXML v.8 (Stamatakis, 2006), and ran on the CIPRES Science Gateway cluster (Miller, Pfeiffer & Schwartz, 2010). This analysis employed a GTRCAT evolutionary model, estimating gamma rate, and was set to perform 1000 bootstrap pseudo replicates. Finally, the phylogenetic tree was pruned in Mesquite v. 3.4 (Maddison & Maddison, 2018) living only the focus taxa (11 species; see Results for more details) and preserving original branch lengths (see Figs S1 and S2 for accessing the phylogenetic tree results).

We used a test of phylogenetic signal to assess phylogenetic effects on cranial shape. We computed the λ statistic (Pagel, 1999) using the function *phylosig* in the *phytools* package (Revell, 2012) of software R (R Development Core Team 2017). This analysis compares the observed phylogenetic signal with a Brownian motion model of trait evolution. A $\lambda = 0$ indicate no phylogenetic bias, whereas $\lambda = 1$ indicate strong bias in accordance with the Brownian motion model. This analysis was conducted using the phylogenetic tree containing the 11 sigmodontine species and performed independently for each cranium view calculating the average scores per species considering the two axes obtained in the CVA. In case of allometric effects detection, we used the standard residuals obtained in the linear regressions.

For assessing the relationship between cranial shape and arboreal activity of the sigmodontine rats, in case of no detection of phylogenetic signal, we performed linear regressions between the mean values of each CV by species or the mean values of the standardized residuals by species (in case of allometry detection) and the proportions of above-ground captures of each species. These proportions were transformed to the arcsin of the square root as recommended for proportion data (Zar, 1999). However, in case of detection of phylogenetic signal we performed Phylogenetic Generalized Least Squares models (PGLS; Grafen, 1989) for accounting to phylogenetic autocorrelation in the residuals of the relationship between cranial shape and values of arboreal activity. For this analysis we assumed the Ornstein-Uhlenbeck model of trait evolution (Martins & Hansen, 1997), which states that traits tend to evolve around a value rather than randomly. PGLS

analyses were performed using the function *pgls* in the *caper* package of the software (R Development Core Team 2017).

Results

Cranial shape and allometry

The CVA analysis shows significant differences in cranial shape for both dorsal (Wilk's lambda < 0.001, $F_{240,1914}$ = 11.525, P < 0.0001) and lateral views (Wilk's lambda < 0.001, $F_{200,1883}$ = 15.760, P < 0.0001).

The first CV of the dorsal view explained 42.9% of the total variance and the second axis explained 21.5%. We found a strong relationship between the CV1 and CS (r^2 = 0.78, d.f. = 236, P < 0.0001) indicating allometry (Fig. 2a), but we found no such relationship considering the CV2 (r^2 < 0.01, d.f. = 236, P = 0.766; Fig 2b). The CV1 showed that small species (i.e. *A. cursor*, *C. expulsus*, *C. tener*, *Ol. mattogrossae* and *Ol. nigripes*), those with negative scores obtained in the CVA, presented more rounded cranial shape with short and wider frontal and parietal bones in comparison to larger species (i.e. *N. lasiurus*, *H. megacephalus*, *C. scotti*, *Oe. catherinae* and *Oe. cleberi*) (Fig. 2a). The CV2 showed that species with negative scores presented wider and longer nasal and parietal bones in comparison to species with positive scores (Fig 2b).

For the lateral view, the CV1 explained 40.0% of the total cranial shape variance, whereas the CV2 explained 25.3%. For the CV1 we found a weak but significant allometry effect (r^2 = 0.10, d.f. = 236, P < 0.0001; Fig. 2c) in comparison to the CV2 (r^2 = 0.33, d.f. = 236, P < 0.0001; Fig. 2d). For smaller rodents in the CV1 (positive scores), the posterior portion of the cranium is less rounded and expanded in addition to a flatter top of the cranium in comparison to larger species. Since we found weak allometric effects on cranial shape, we conducted further analysis using the scores of the CV1 without size corrections (i.e. not using standard residuals). For smaller sigmodontine in the CV2 (negative scores), our results showed a more rounded and expanded posterior portion of the crania and an arched rostrum in comparison to larger species.

Cranial shape and phylogeny

The analysis investigating for phylogenetic signal on cranial shape considering the dorsal view showed no significant results for both CV axis (CV1 after removal of effect size and CV2 without correction for size), showing no phylogenetic constraints on cranial shape (CV1: λ = 0.669, P = 0.322; CV2: λ < 0.0001, P = 1.000). For the lateral view, we found that the CV1 (without correction for size effects), showed phylogenetic constraints on cranial shape (λ = 0.999, P = 0.017) but for the CV2 after the removal of size effect we detected no phylogenetic signal (λ = 0.999, P = 0.111).

Cranial shape and arboreal activity

For the dorsal view of the cranium we found a positive significant relationship between the CV1 (after removal of size effect) and arboreal activity of the sigmodontine rats (linear regression; r^2 = 0.40, d.f. = 9, P = 0.035). Highly arboreal sigmodontine rats (e.g. *R. macrurus* and *Oecomys* species) clearly presented a general cranial expansion in all directions in comparison to the terrestrial ones (e.g. *A. cursor*, *N. lasiurus* and *Calomys* species) (Figs 3a and S3). The same analysis using the CV2 without removal of size effects showed no significant relationship (linear regression; r^2 = 0.01, d.f. = 9, P = 0.722) (Figs 3b and S3; for descriptions regarding VC2 see Cranial shape and allometry).

For the lateral view of the cranium our results showed a relationship between the CV1 and arboreal activity of the sigmodontine rats (PGLS; r^2 = 0.74, d.f. = 9, P < 0.001). Arboreal rodents present a posterior portion of the cranium more rounded and expanded in addition to a rounder top of the cranium in comparison to terrestrial rodents (Fig. 3c). After removal of size effects, the CV2 showed no relation with arboreal activity (linear regression; r^2 < 0.01, g.l. = 9, P = 0.896). Species with negative residual values presented a flatter top of the cranium with a more arched rostrum in comparison to species with positive residual values (Fig. 3d).

Discussion

In this study, we showed that cranial shape of sigmodontine rodents is dependent of both allometric (i.e. species with similar size tend to present similar cranial shape) and phylogenetic (i.e. phylogenetic closer species tend to present similar cranial shape) effects. However, even accounting for these constraints, our study supported the hypothesis of a relation between cranial shape and arboreal activity of sigmodontine rodent species considering both dorsal and ventral cranium views. Although some studies have related brain size and arboreal activity in rodents (e.g. Lemen, 1980; Meier, 1983; Bernard & Nurton, 1993), to our knowledge, no studies directly investigated this relationship using cranial shape (as a proxy for brain size) and field data on arboreal activity of several sigmodontine rodents. The latter is particularly important for the accurate evaluation of arboreality levels.

Our results showed strong allometric effects on cranial shape considering the dorsal view of the studied species, since the variation along the CV1 was related to cranium size. The same pattern, although weaker, was noted for the lateral cranium view considering both CV1 and CV2. As result, before controlling for any allometric effects, rats with similar size but with different levels of arboreal activity (e.g. the arboreal *Rhipidomys macrurus* and the terrestrial *Cerradomys scotti*) presented similar cranial shape. This could indicate that, for maintenance of basic functions (e.g. masticatory function) shared among these rodent species, the cranium present morphological adjustments according to size (Michaux, Chevret & Renaud, 2007; Samuels, 2009). For a large structure to remain functional, certain morphological aspects must be proportionately larger relative to body size. For instance, an enlargement of muscles would be required for more massive crania, ultimately affecting bone development, and thus, cranial shape (Lightfoot & German, 1998; Samuels, 2009).
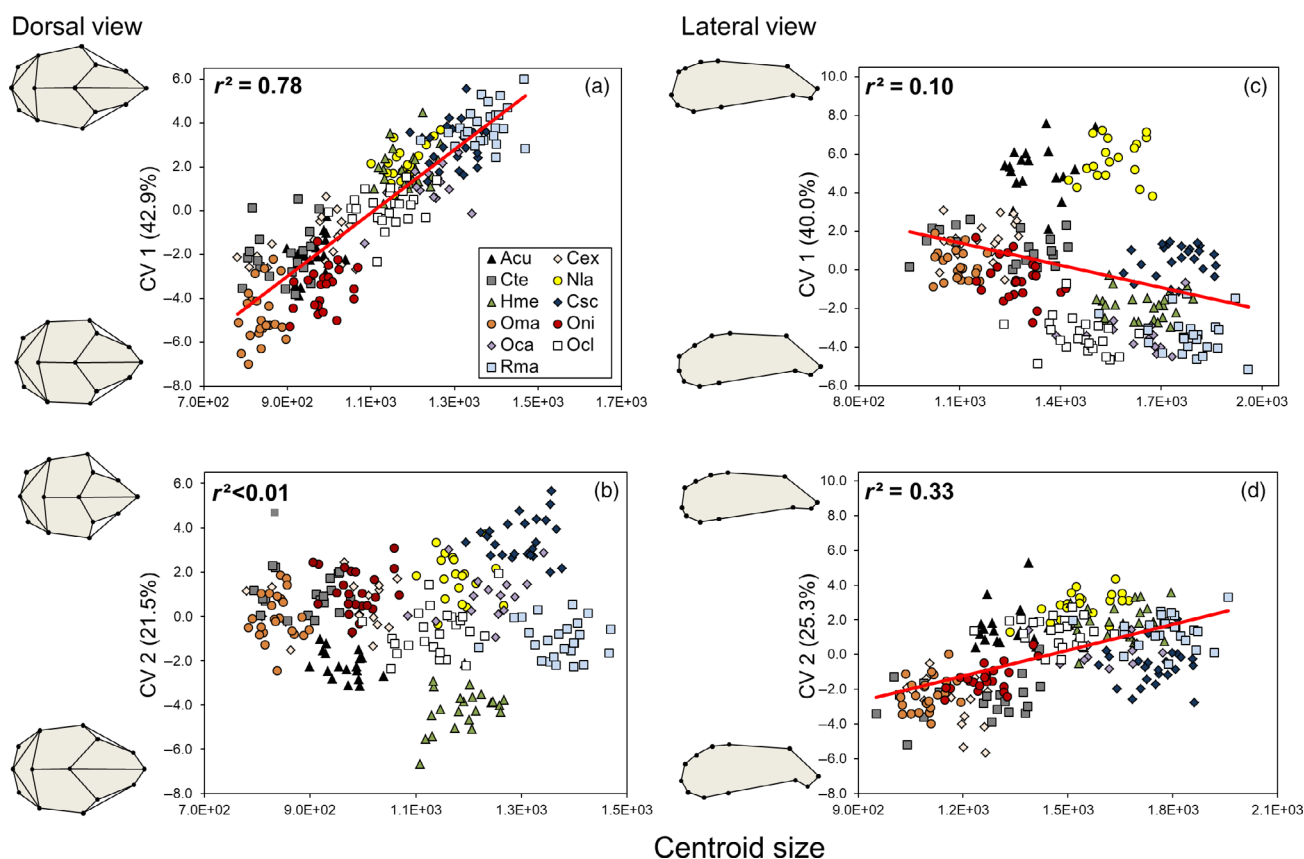
Figure 2 Relationship (linear regressions) between centroid size and the first and second axes obtained with the Canonical Variate Analysis (CV1 and CV2) that reduced the original shape variables of dorsal (a and b) and ventral (c and d) cranium views for 11 species of Sigmodontinae rodents. The percentage of the total variance explained by each CV is indicated between parentheses. Figures in the vertical axes indicate the main morphological changes of the crania. Trend lines are shown only for the significant relationships ($P < 0.05$). [Colour figure can be viewed at zslpublications.onlinelibrary.wiley.com]

Our results indicated a phylogenetic signal on cranial shape, which is in agreement with other studies investigating the role of phylogeny on skull morphology (e.g. Wroe & Milne, 2007; Samuels, 2009; Hautier, Lebrun & Cox, 2012; McLean *et al.*, 2018). However, we detected this evolutionary constraint only in the CV1 of the lateral cranium view. Although there were clear cranial shape similarities among non-related phylogenetic rodents, such as *Rhipidomys macrurus* (Thomasomyini tribe) and *Oecomys* species (Oryzomyini tribe), there were also several similarities among phylogenetic-related species, such as the Oryzomine *Oligoryzomys* species, *Hylaeamys megacephalus* and *Cerradomys scotti*; and the Akodontine *Akodon cursor* and *Necromys lasiurus*. Additionally, our study relied on three pairs of congeneric species (from the genera *Calomys*, *Oecomys* and *Oligoryzomys*), which probably contributed to the phylogenetic signal found. The lack of phylogenetic signal in the dorsal cranium view, however, suggests that features of this cranium view may be more related to ecological factors (e.g. Wroe & Milne, 2007; Samuels, 2009; Maestri *et al.*, 2016) than to the inherited characteristics of each group (evolutionary history). In any case, after controlling for

phylogenetic and allometric effects on both cranial shape views, a strong relation between cranial morphology and arboreal activity arose.

The dorsal shape view of the cranium and correspondent arboreal activity showed that arboreal sigmodontine present wider and expanded crania. This suggests an overall enlargement of the brain, as expected for arboreal rodents (as seen in Pilleri *et al.*, 1984; Bernard & Nurton, 1993; Bertrand *et al.*, 2017, 2018). On the other hand, the lateral shape view showed that arboreal sigmodontine present more rounded and posteriorly expanded cranium. This later could be due to an enlargement of the cerebellum (as seen in arboreal anurans and primates; Taylor *et al.*, 1995; De Winter & Oxnard, 2001), which is related to certain skills expected to be increased in rodents with high arboreal activity, such as sense of balance, orientation and the ability to synchronize visual information with physical movement (Reeber, Otis & Sillitoe, 2013). Therefore, these increased skills would allow species to cope with the adversities of exploring the habitat in three dimensions (e.g. movement in trees and exploitation of resources; Eisenberg & Wilson, 1981). This pattern was also observed for squirrels. In addition to positive relation between arboreal activity
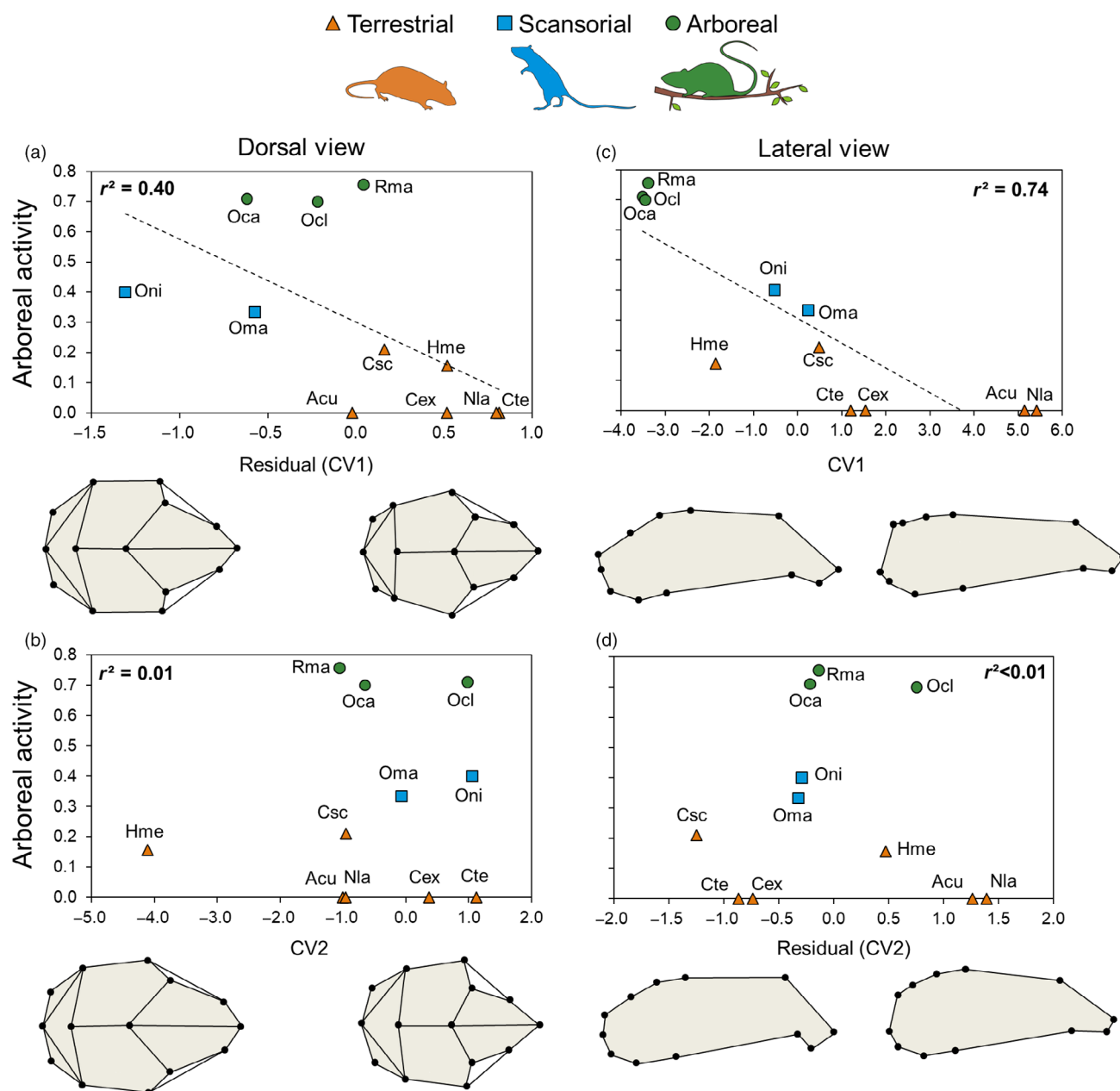
Figure 3 Relationship between arboreal activity (proportion of arboreal captures) and the two axes obtained with Canonical Variate Analysis (CV1 and CV2) that reduced the original shape variables of dorsal (a and b) and ventral (c and d) cranium views for 11 species of Sigmodontinae rodents. For the CV1 of the lateral view we performed a Phylogenetic Generalized Least Squares models (PGLS) since we found phylogetic signal on cranial shape. For the other CVs we performed simple linear regressions. Figures in the horizontal axes indicate the main morphological changes of the crania. For obtaining cranial shapes without allometric effects, we used the residuals of the regressions between cranial shape and centroid size (Fig. 2) for the CV1 of the dorsal view and the CV2 of the lateral view. Trend lines are shown only for the significant relations ($P < 0.05$). Acu = *Akodon cursor*, Cex = *Calomys expulsus*, Cte = *Calomys tener*, Csc = *Cerradomys scotti*, Hme = *Hylaeamys megacephalus*, Nla = *Necromys lasiurus*, Oca = *Oecomys catherinae*, Ocl = *Oecomys cleberi*, Oni = *Oligoryzomys nigripes*, Oma = *Oligoryzomys mattogrossae*, Rma = *Rhipidomys macrurus*.[Colour figure can be viewed at zslpublications.onlinelibrary.wiley.com]

and brain size reported for these animals (Meier, 1983; Bertrand *et al.*, 2017), arboreal squirrels also have expanded and rounded vaults, whereas the cranium of terrestrial species is more flattened (Lu *et al.*, 2014; Bertrand *et al.*, 2016).

The positive relation between brain size and habitat has already been observed in different mammal orders including rodents (Lemen, 1980; Meier, 1983; Pilleri *et al.*, 1984; Budeau & Verts, 1986; Bernard & Nurton, 1993; Bertrand *et al.*, 2018), marsupials (Eisenberg & Wilson, 1981) and bats (Eisenberg & Wilson, 1978), indicating that species occupying more structurally complex environments present larger brains than those occurring in more simple habitats (e.g. forests in comparison to open habitats). For rodents, the relationship between arboreal activity and brain size was observed in different groups in Africa (Bathyergidae, Gliridae, Hystricidae, Muridae, Sciuridae and Thryonomyidae; Bernard & Nurton, 1993) and North America (Cricetidae [*Peromyscus* genus] and Sciuridae; Lemen, 1980; Meier, 1983; Pilleri *et al.*, 1984; Bertrand *et al.*, 2017). In fact, this seems to be a general pattern for different vertebrate groups such as fish, frogs and birds (Bennett & Harvey, 1985; Van Dongen, 1998).

Although we found a considerable relationship between cranial features and the arboreal activity of sigmodontines, the skull is a very complex structure representing multifunctionality means and shaped by multiple evolutionary forces (McLean *et al.*, 2018). Other potentially relevant factors that we did not account for (e.g. function, architectural constraints, epigenetics and evolutionary integration; Mora, Olivares & Vassallo, 2003; Hallgrímsson *et al.*, 2007; Wroe & Milne, 2007; Monteiro & Nogueira, 2010; McLean *et al.*, 2018), possibly contribute to cranial morphology and could explain the weak (although significant) association of the dorsal cranium (in comparison to the lateral view) with sigmodontine arboreality.

Previous studies have showed that skull morphology of different mammal groups, including rodents, is associated with diet (Wroe & Milne, 2007; Samuels, 2009; Maestri *et al.*, 2016; McLean *et al.*, 2018). However, this influence seems not to be preponderant for the rodents evaluated in our study, especially considering the dorsal view of the cranium. Some species with similar diet presented very different cranial shape (the frugivorous/granivorous species of the *Oligoryzomys* and *Calomys* genera; Paglia *et al.*, 2012), or species with different diets presented similar cranial shape (the frugivorous/seed predator *R. macrurus* and the insectivore/omnivore *A. cursor*; Paglia *et al.*, 2012).

The results indicated that *Ol. nigripes* have morphological adaptations for higher arboreal activity levels, taking in consideration its expanded dorsal view of the cranium. In fact, experimental evaluation of locomotor performance indicated that this species presents an arboreal walking ability similar to highly arboreal species (Camargo *et al.* 2016). This sigmodontine rat, however, presents a typical scansorial pattern of vertical space use in the Cerrado (Camargo, Sano & Vieira, 2018). Moreover, *Ol. nigripes* is able to vary its proportional use of vertical strata across different environments (Vieira & Monteiro-Filho, 2003; Camargo *et al.*, 2018). This variation in the use of vertical space by *Ol. nigripes* is related to ecological factors such as structure and availability of vertical space (Hannibal & Caceres, 2010; Camargo *et al.*, 2018), competition (Meserve, 1977; Miles, Souza & Povoa, 1981), or

predation (Bonvicino & Bezerra, 2003). Although this plasticity could explain the expanded cranial shape of *Oligoryzomys* species considering the dorsal view of the cranium, they were positioned along the CV1 axis of the lateral view of the cranium as expected for scansorial species (between terrestrial and arboreal rodents).

Assessing the composition of small mammal assemblages with captures in different vertical strata is recommended (Camargo *et al.*, 2018), but not always achievable (Vieira, 1998). In such cases, considering morphological features as a proxy for inferring arboreal activity of sigmodontine rodents can be useful, since even highly arboreal species are occasionally captured on the ground (Umetsu, Naxara & Pardini, 2006; Camargo *et al.*, 2012). Additionally, the use of morphometric methods on cranial shape also can be helpful for understanding ecological adaptations of extinct rodents (as in Bertrand *et al.*, 2016, 2017, 2018), especially considering that postcranial fossil material is commonly absent or highly fragmented (Rose & Chinnery, 2004).

Conclusions

Our study showed that although allometric (dorsal and lateral view of the cranium) and phylogenetic (lateral view of the cranium) effects play an important role on the cranial shape of sigmodontine rodents, arboreal activity is also relevant for evolution of cranial morphology. Our study indicated that cranial shape is a good predictor for arboreal activity of sigmodontine rodents. Arboreal species presented more expanded cranial shapes, which suggests a selection for relative larger brains, allowing the animals to better exploring the space in a three-dimensional way. The dorsal view of their cranium presented an overall cranial expansion suggesting brain enlargement. Moreover, the observed posterior cranial expansion considering the lateral view suggests that arboreal sigmodontine rats present a large cerebellum, which could enhance orientation and sense of balance during locomotion.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Study area and methods.

Table S1. List of visited specimens sampled for geometric morphometrics in the Mammal Collection (UNB, LM and AP) and Laboratory of Vertebrate Ecology (ECOVERT) at the University of Brasília.

Table S2. Definition of landmarks placed in the dorsal, ventral and lateral skull views of all sigmodontine specimens (Fig. 1 in the main paper).

Table S3. Accession number of Cytochrome b (Cyt-b) and Interphotoreceptor Retinoid Binding Protein (IRBP) gene sequences of sigmodontine species retrieved from GenBank and not analysed in Machado *et al.* (2015).

Figure S1. Maximum likelihood (ML) phylogram inferred using the concatenated IRBP and Cyt-b DNA sequence dataset. Numbers by nodes represent bootstrap proportion values. Only values above 70 are showed.

Figure S2. Pruned phylogenetic tree representing only the 11 taxa for which we assessed skull shape.

Figure S3. Examples of sigmodontine rodent species showing the main modifications of the cranium according to the arboreal activity: a general expansion of the cranium considering the dorsal view of arboreal species in comparison to terrestrial ones; and a more rounded top of the cranium in addition to an expansion of the posterior portion of the cranium in arboreal species in comparison to terrestrial ones.