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Morphological integration and cranial modularity in six genera of echimyid rodents (Rodentia: Echimyidae)

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Rodents of the family Echimyidae present a wide variety of life histories and ecomorphological adaptations. This study evaluated morphological integration patterns, modularity, and evolutionary flexibility in six Echimyid genera representing ecomorphological extremes within the family. The relationships between traits were evaluated by comparing estimated covariance and correlations matrices of populations. The presence of modules was investigated by comparing the patterns of integration between traits and using hypothetical matrices based on shared development/function and masticatory stress. The results point to a common covariance and correlation pattern among the six echimyid genera, suggesting a conserved pattern of covariation (associations among traits) throughout the evolution of this group. The overall magnitude of integration, however, varied greatly. We also found a high degree of modularity in all six echimyid genera. Finally, we observed a clear association between flexibility, i.e., the ability of a species to respond to the direction of selection, with the overall magnitude of integration and degree of modularization. The results of this study provide hypotheses concerning the underlying effects of the association among traits, which may have facilitated or constrained the evolution of morphological variation in the diverse family Echimyidae.

Keywords: *Caviomorpha*, *morphometrics*, *natural selection*, *phenotypic covariance matrix*

Os roedores da família Echimyidae apresentam uma ampla variedade de histórias de vida e adaptações ecomorfológicas. O objetivo deste estudo foi investigar a variação craniana nesta família, fundamentando-se na genética quantitativa e integração morfológica. Nós avaliamos os padrões de integração morfológica, modularidade e flexibilidade em seis táxons de Equimídeos representando extremos ecomorfológicos dentro da família. As relações entre os traços foram avaliadas comparando matrizes de covariância e correlação estimadas para as populações. A presença dos módulos foi investigada comparando os padrões de integração entre os traços e utilizando matrizes hipotéticas baseadas no desenvolvimento/função compartilhado e estresse mastigatório. Os resultados apontam para um padrão de covariância e correlação comum entre os equimídeos, sugerindo um padrão de covariação (associação entre traços) conservado ao longo da evolução desse grupo. A magnitude de integração geral, por outro lado, variou significantemente. Além disso, nós demonstramos que o crânio dos equimídeos é amplamente modular. Finalmente, observamos uma clara associação entre flexibilidade, ou seja, a capacidade de uma espécie em responder na direção da seleção, com a magnitude de integração e o grau de modularização. Os dados obtidos nesse estudo fornecem hipóteses relativas aos efeitos subjacentes da associação entre as características que podem facilitar ou restringir a evolução da variação morfológica na diversa família Echimyidae.

Palavras-chave: Caviomorpha, Morfometria, seleção natural, matriz de covariância fenotípica

Rodents of the family Echimyidae present a striking pattern of morphological variation. This family comprises approximately 28 extant genera and 90 species distributed throughout South America, southern Central America, and the Caribbean islands (Emmons et al. 2015). The high speciation rate, high ratio of occurrence of homoplastic characters, and heterogeneous rates of molecular evolution have contributed to evolutionary complexity in the group (Fabre et al. 2013). Phylogenetic relationships within Echimyidae are complex and have been the focus of several studies (Leite and Patton 2002; Galewski et al. 2005; Fabre et al. 2013; Fabre et al. 2017). Most recently, nuclear DNA exon data resolved most nodes within echimyids which were classified into four subfamilies: Euryzygomatomyinae, Echimyinae, Capromyinae, and Carterodontinae (Courcelle et al. 2019). Euryzygomatomyinae comprises the terrestrial genus *Clyomys*, inhabitant of grassland regions (Cerrado), the semi-fossorial genus *Euryzygomatomys*, and the terrestrial genus *Trinomys,* inhabitants of the forested (Atlantic forest). Echimyinae is subdivided into an arboreal tribe (Echimyini) and a terrestrial tribe (Myocastorini). The tribe Echimyini comprises 11 genera from the Amazon Basin (*Dactylomys*, *Ollalamys*, *Isothryx*, *Echimys*, *Makalata*, *Mesomys*, *Lonchothrix*, *Toromys*, and *Pattonomys*) and the Atlantic Forest (*Kannabateomys* and *Phyllomys*). The genus *Callistomys* (Atlantic forest) is exception between the arboreal echimyid rodents as it belongs in Myocastorini, which includes the semiaquatic genus *Myocastor* (an inhabitant of the humid and dry Chaco, Patagonian steppe, and Pampas grassland) and the terrestrial genera *Proechimys* (both cis- and trans-Andean lowland rainforest extending into southern Central America), *Thrichomys* (a dry forest inhabitant of the Caatinga, Cerrado, Pantanal, Chaco), and *Hoplomys* (lowland rainforest of Central America). The subfamily Capromyinae comprises living hutias from the West Indies: *Plagiodontia*, *Geocapromys*, *Capromys*, *Mysateles,* and *Mesocapromys*. Carterodontinae comprises the monotypic *Carterodon*, represented by the semi-fossorial rodent *Carterodon sulcidens*, inhabitant of the Cerrado biome, in the central and western parts of Brazil.

Phylogenetic analyses recovered a terrestrial state for ancestral echimyids (Fabre et al. 2017), and the arboreal and semi-fossorial states emerged approximately 12 million years ago (Ma), followed by the origin of the semi-aquatic habit (~8 Ma). These authors suggested that geographic opportunities derived from vicariant and dispersal events were the principal driver of morphological divergence (Perez et al. 2009; Fabre et al. 2017).

Environmental factors act as important selective pressures that promote morphological differentiation among populations (Mahler et al. 2010; Yoder et al. 2010). However, equally important in determining morphological evolution are the genetic and developmental patterns of a species, which can facilitate or constrain evolution (Marroig and Cheverud 2004). This is because the shared functional and developmental relationships that form a morphological structure are often not independent (Olson and Miller 1958). Therefore, the patterns of association among morphological elements can significantly influence how each trait evolves through time (Cheverud 1996; Marroig et al. 2009). Estimating covariance and correlation matrices between morphological elements gives us an assessment of how these patterns are structured among different species (Porto et al. 2009). The magnitude of the association among the elements of a structure is equally important to morphological diversification (Porto et al. 2009).

Morphological traits are often structured in modules, wherein traits inside the module have greater correlation with each other than with traits outside the module. The pioneering study by Berg (1960) was among the first studies to demonstrate empirically the existence of these discrete groups of highly correlated traits, the modules that she termed "correlation pleiades" (see also Olson and Miller 1958). Modules are a widespread pattern in biological systems and can be found at different biological levels, from protein interactions and regulatory gene networks to morphological structures (Wagner et al. 2007). A modular architecture may facilitate adaptation, allowing evolutionary changes aligned to selection or differentiation in several traits within a module without disrupting function with other parts (Marroig et al. 2009). In contrast, integration (a high degree of correlation among the elements) can constrain an organism's adaptation if the selection acts in opposing directions on each trait of the same module. Therefore, the higher the integration of an organism, the higher the potential constraint on selection (Porto et al. 2013). The ability to respond in a manner aligned to natural selection can be evaluated through several metrics intended to gauge the evolutionary potential of a population. One such index is evolutionary flexibility, which quantifies a population's ability to respond in the same direction as the selective pressures (Hansen and Houle 2008; Marroig et al. 2009).

Understanding how complex morphological structures evolve requires estimating the magnitudes of morphological association between traits and the characterization of modular patterns of these structures. The family Echimyidae contains ecomorphologically diverse species with a complex evolutionary history, but we know little about how this group's cranial morphological integration patterns are structured. The few existing studies investigated morphological integration in the mandible (Monteiro et al. 2005) or as a single species representative in a larger taxonomic context (Porto et al. 2009; Álvarez et al. 2015). Based on these studies, it is possible to raise the hypothesis for the influence of environmental factors, such as niche occupation, in structuring covariation patterns between cranial bones. We expect that the morphological integration between cranial characters is related to functional demands derived from different environmental occupations, and that the patterns of association become even more divergent when we evaluate genera with specialized performance (e.g., *Kannabateomys*).

The mammalian skull is a complex structure with multiple functional and developmental regions associated with sensorial, skeletal, and muscular systems. The skull performs several different tasks, including the acquisition and initial processing of food, protection of the brain and sensory organs, and participation in water and temperature regulation (Schmidt-Nielsen 1970; Smith 1997). The highly specialized gnawing incisors

and associated diverse muscular system are probably some of the features responsible for the high diversification and evolutionary success of rodents (Cox et al. 2012). The mammalian skull has evolved under diverse selective pressures, and the patterns of association among traits have likely played an essential role in its evolution. Large variation in the patterns of trait relationship among taxa can be generated by high variation in ecological factors (e.g., diet) and different functions, which can promote an increase in morphological variation (Makedonska et al. 2012).

In this study, we used morphometric analyses to explore if patterns of integration and modularity among cranial characters and their evolutionary potential vary among six genera of Echimyidae. These six genera represent two of four echimyid subfamilies and extremes of ecomorphological diversity, including semi-fossorial, terrestrial, and arboreal species. The pattern of association between characters is expected to be similar due to a shared basis of development and function among mammals. On the other hand, the magnitude of integration between cranial traits will reflect the different ways of life of the studied genera.

Materials and Methods

Specimens.—We digitized landmark data from 372 crania deposited at the following institutions: Instituto Nacional da Mata Atlântica (Santa Teresa-ES), Museu Nacional (Rio de Janeiro-RJ), Museu de Zoologia da Universidade de São Paulo (São Paulo-SP), Museu de Zoologia João Moojen (Viçosa-MG), and Coleção de mamíferos da Universidade Federal do Espírito Santo (Vitória-ES). The complete list of examined specimens is available at the Supplementary Data SD1. Our dataset comprises six genera and seven species distributed in four different biomes and includes representatives of two of the four echimyid subfamilies (Emmons et al. 2015). We evaluated two species of the subfamily Euryzygomatominae: *Euryzygomatomys spinosus* (Fischer 1814) and *Trinomys paratus* (Moojen 1948); and three of the subfamily Echimyinae: Echimyini tribe, *Kannabateomys amblyonyx* (Wagner 1845), *Phyllomys blainvilii* (Jourdan 1837), and *Phyllomys pattoni* (Emmons et al. 2002), Myocastorini tribe, *Proechimys roberti* (Thomas 1901), and *Thrichomys apereoides* (Lund 1839). This set of species was chosen to include as many different ecomorphological states as possible while also maximizing the number of specimens available in scientific collections. The sample size for each species can be viewed in Supplementary Data SD2.

Data acquisition, covariance, and correlation matrix estimation.—We recorded three-dimensional coordinates for 31 landmarks on both sides of the skull (Fig. 1, Supplementary Data SD3) using a 3D digitizer (X-Microscribe). We estimated a set of 35 Euclidean distances from these landmarks, which were then grouped into nine functional/developmental and masticatory stress subgroups (Table 1). Only adult individuals were measured in this study. The ontogenetic evaluation of specimens was made using the available literature (Supplementary

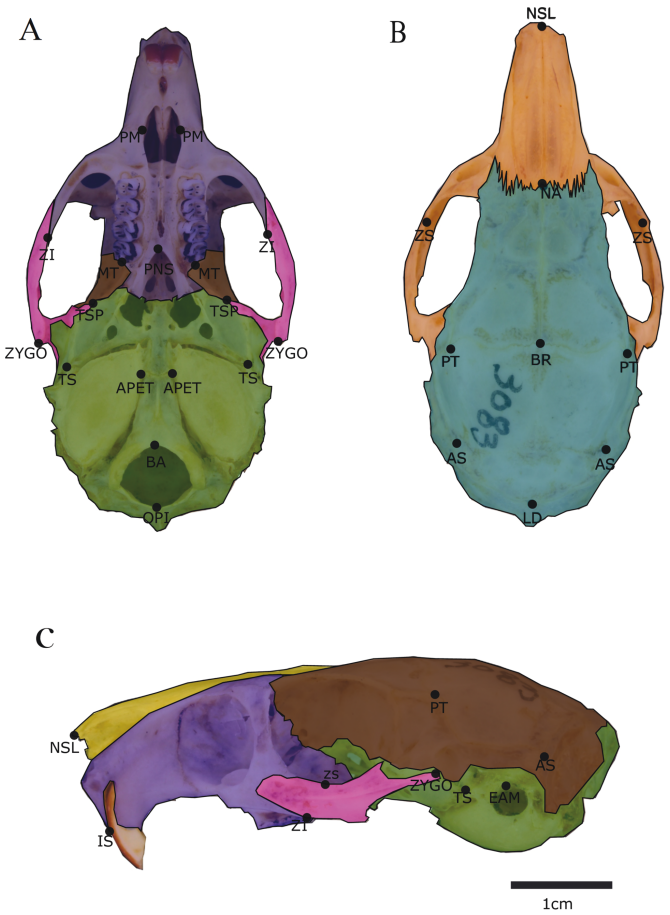

Fig. 1.—Representation of *Thrichomys apereoides* cranium (dorsal, ventral, and lateral) and the landmarks digitized in this study. (A) Purple indicates the oral module; brown: cranial vault; Pink: zygomatic; green: skull base; (B) orange indicates the face module and blue the neurocranium module. (C) Yellow indicates the nasal module; purple: oral; brown: cranial vault; pink: zygomatic; green: skull base. The description of landmarks and respective functional/developmental regions/subregions can be viewed in Supplementary Data SD3 and Table 1, respectively.

Data SD2). Each specimen was digitized twice on both sides of the skull and repeatability of the linear measurements was estimated to assess measurement reliability. Whenever one side of the skull was damaged, the measurement of the other side was used as a substitute. All species showed high values of repeatability for most measurements (Supplementary Data SD4), indicating a higher variance among specimens than between the first and second measurements. Repeatability was estimated according to Lessells and Boag (1987), where the variance within (different measurements of the same specimen) and between each group (different specimens) is assessed using the mean squares of an ANOVA:

$$r = \frac{S_A^2}{S^2 + S_A^2}$$

where S_A^2 represents the variance among groups and S^2 the variance within groups. The repeatability presented an overall

Table 1.—Euclidian distances calculated from 31 anatomical landmarks obtained from echimyid skulls and their respective functional/developmental regions/subregions. The definition of each landmark is presented in Supplementary Material SD3. The positions of landmarks are shown in Fig. 1.

Distance	Functional subregion	Region
IS-PM	oral, gnaw	Face
IS-NSL	nasal, gnaw	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, chew	Face
PT-TSP	cranial vault, zygomatic, chew	Neurocranium/face
ZS-ZI	oral, zygomatic, gnaw, chew	Face
ZI-MT	oral, chew	Face
ZI-ZYGO	zygomatic	Face
ZI-TSP	zygomatic, chew	Face
MT-PNS	oral	Face
PNS-APET	cranial base	Neurocranium
APET-BA	cranial base	Neurocranium
APET-TS	cranial base, gnaw	Neurocranium
BA-EAM	cranial base	Neurocranium
EAM-ZYGO	zygomatic	Face
ZYGO-TSP	zygomatic, chew	Face
LD-AS	cranial Vault	Neurocranium
BR-LD	cranial Vault	Neurocranium
OPI-LD	cranial Vault	Neurocranium
PT-AS	cranial Vault	Neurocranium
JP-AS	cranial base	Neurocranium
BA-OPI	cranial base	Neurocranium

average of 0.96, with a minimum value of 0.57 and a maximum value of 0.99. The lowest value corresponds to MT-PNS distance, which is one of the smallest distances and where a very small error in absolute scale will represent a substantial part of the total variation. All subsequent analyses were carried out using the average of replicated measurements and averages between the bilateral distances.

We estimated a phenotypic variance-covariance matrix (**P**-matrix) for each genus from the Euclidean distances. Prior to each matrix estimation, we controlled for additional sources of variation that are not directly related to the genotype-phenotype map per se (ontogeny, sexual dimorphism, and geographic variation) by using the residuals of a Multivariate Analysis of Variance (MANOVA), whenever the level of the Wilk's lambda statistic was significant (alpha level of significance, $\alpha = 0.05$). We intended to control differences in population averages while maintaining a suitable representation of the covariance structure for each genus. However, on a few species (e.g., age classes for *Euryzygomatomys spinosus* and *Phyllomys pattoni*), and despite our efforts to include as many specimens as possible covering the breadth of species distribution, sex, and ontogenetic stages, the low sample sizes available may have reduced power to detect a significant effect (see this detailed information in Supplementary Data SD5). Both *Phyllomys* species (*Phyllomys pattoni* and *Phyllomys blainvilii*) were grouped due to their small sample size and the species variation were removed prior to the estimation of the phenotypic covariance matrix. The P-matrices (var/cov) were estimated using the residual matrix of a general linear model, including the 35 distances as dependent variables and significant sources of variation as independent ones. Conversely, the cov/var matrices were estimated directly from the raw data when no effect was detected. More importantly, whether or not these sources of variation were controlled did not impact the general results of this study since the similarity between raw and controlled matrices was high, with values uniformly above 0.9 (Supplementary data SD6).

The correlation matrix was estimated from the var/cov matrix generated for each genus. All morphological integration and modularity analyses were done in the R statistical environment (R Development Core Team 2017) using the package evolqg (Melo et al. 2015).

Matrix comparisons and matrix repeatability.—To evaluate the similarity of patterns of covariance among the six genera, we compared the estimated var/cov matrices using the Random Skewers method (*RS*). This method is based on Lande's (1979) multivariate response to the natural selection equation, where each phenotypic matrix is multiplied by a set of random natural selection vectors (in this study 10,000) and the evolutionary responses between each pair of matrices are compared through the correlation of the resulting normalized pair of vectors (angle cosine). The response to selection equation is defined as

$$\Delta z = G\beta$$

where Δz represents the evolutionary response in relation to the gradient selection vector (β) applied to the additive genetic variance-covariance matrix **G**. In our study, we replaced the **G**-matrix by its phenotypic counterpart, the **P**-matrix. This analysis will return a measure of how similar two matrices are in their structure.

Correlation matrices were compared using Krzanowski's projection method (*KRZ*; Krzanowski 1979). This tool compares two matrices by calculating the angles among the corresponding orthogonal axis pairs (Principal component, PC) in a subspace of dimensionality k (where k contains the first 16 of 35 PCs extracted for each observed matrix). This technique results in an **S** projection matrix, representing the cosines of minimum angles among a group of **A** matrix vectors, which is closest to the other vectors in **B** matrix subspace. The **S** matrix can be defined as

$$S = A^T BB^T A$$

where **A** would be the 16 PCs extracted from the first matrix and **B** would be the 16 PCs extracted from the second matrix. The ratio of the sum of eigenvalues of matrix **S** over the maximum value of dimensions used (in this case, k = 16) will determine the similarity index. This index varies from 0 to 1, where

the closer to zero an observed value is, the more dissimilar the matrices will be. Additionally, the var/cov matrices were also compared using the *KRZ* method.

To account for the effects of sampling on the estimation of covariance matrices resulting in the underestimation of similarity between matrices, we estimated the matrix repeatability index (Cheverud 1996) and adjusted the observed similarity by sample size. This adjustment is calculated through a resampling method to see how much the sample represents the real population, where matrix repeatability values close to 1 indicate that samples represent a high degree of reliability (Marroig and Cheverud 2001). In this case, the adjusted correlation (r_{adj}) will be given as

$$r_{adj} = \frac{r_{obs}}{\sqrt{(t_1 + t_2)}}$$

where r_{obs} represents the correlation value between two observed matrices, and t_1 and t_2 represent the repeatability of each matrix comparison.

We performed rarefaction analysis using the var/cov matrix of *T. apereoides* (the species with the largest sample size, $n = 80$) to verify the impact of sample size in the estimation of var/cov and correlation matrices. This approach differs from repeatability (resampling with fixed sample size) because it provides the mean values of correlation from resampling the same population in different sample sizes and therefore gives us a benchmark of the sample size that could represent a sufficient number of individuals to estimate the var/cov matrices confidently. Each matrix was compared to the original using the *RS* (Marroig and Cheverud 2001) and *KRZ* (Krzanowski 1979) methods, and the correlation values between them were estimated. The result of rarefaction analyses can be visualized in Supplementary Data SD7 and SD8. In general, the similarity values were high (>0.80) for the comparisons above $n = 30$ in the two methods.

Divergence and similarity among the covariance matrix characters.—After obtaining the degree of similarity among the matrices, we used an extension of the *RS* method to disentangle which traits affect the differences and similarities among matrices. *RS* provides an overall similarity measure between two covariance matrices, whereas selection response decomposition method (*SRD*) identifies outlier traits that are most different and/or most similar between matrices. The *SRD* is an exploratory tool capable of identifying which distances are related to the specific trait covariance divergence among species (Marroig et al. 2011). In this case, covariance matrices are multiplied by 10,000 random selection vectors and the response vectors (Δz) are decomposed into their subcomponents. Then, we evaluated similarities and differences for each trait by performing pairwise comparisons between these trait-specific vectors for each genus (Marroig et al. 2011). The *SRD* score is obtained as the average correlation of the trait-specific response vectors obtained for each 10,000 random selective vectors. The correlation of the response vectors obtained from the comparison between two matrices will indicate whether they are similar. The higher the similarity among the traits, the higher the *SRD* average value will be, and the variance of the responses will be smaller. In contrast, higher divergence among traits results in a lower *SRD* average value and higher variance. Here, we also calculated an *SRD* index that represents the proportion in which one trait appears significantly divergent in all pairwise comparisons between genera. The *SRD* index varies from 0 to 1 with values close to 1 reflecting the most divergent traits among all comparisons.

Modularity in echimyid skulls.—Here we investigated the presence of modules in the skulls of six echimyid genera by comparing our empirical matrices with theoretical matrices constructed based on hypotheses of expected association of traits derived from shared developmental/functional relationships and/or stress generated from chewing. These hypotheses (Table 1) followed those proposed for other mammal species (Cheverud 1995; Porto et al. 2009). The modularity hypothesis tested represents two major regions, Face and Neurocranium, and five hypothetical cranial modules: Oral, Nasal, Cranial Vault, Zygomatic, and Base. In addition, we also tested two new modularity hypotheses related to the force distribution during chewing in Hystricomorph rodents (Gnaw and Chew), based on the regions of greatest stress detected by Cox et al. (2012) during the act of gnawing (incisors) and chewing (molars). These hypotheses and their adjacent measures are presented in Table 1. Finally, we tested a composite hypothesis, the Total matrix, that combines all five sub regions into one matrix. These theoretical matrices (i.e., a priori modularity hypothesis) were composed of 1 and 0, representing the traits present inside and outside the module, respectively.

The hypothetical modules matrices were then correlated with the observed correlation matrices for each genus using a Pearson matrix correlation procedure, and significance was assessed by a Mantel test (Cheverud 1995; Garcia et al. 2014). A significant correlation between the observed correlation matrix and one modularity hypothesis matrix implied support for the existence of a particular module in the genus. Note that this test simply compares the average correlation of two groups of traits: one group hypothesized as a module against all the rest. Thus, it is similar to a t-test with two groups where the significance is adjusted to account for the non-independence of correlations among traits (Mantel's test).

Moreover, we estimated the ratio between the average correlations within modules (avg+) and the average correlations between modules (avg−). Because we expect the average correlations within modules (avg+) to be higher than the average correlations between modules (avg−), we expect a ratio higher than 1.0.

Allometric size variation strongly influences the overall level of association between morphological traits of a structure, particularly in mammals (e.g., Costa 2013; Porto et al. 2013). This source of variation is an overall integration factor since all traits are affected, increasing the correlations between cranial traits or regions, and thus to some extent masking modular signals (Marroig and Cheverud 2004; Mitteroecker and Bookstein 2007). Therefore, detection of the modularity patterns underlying traits can be obscured by size variation (e.g., Porto et al. 2013).

To circumvent this, we removed allometric size from the matrices to evaluate modularity, i.e., using matrices with allometric size excluded. In order to do this, the first eigenvector of each original covariance matrix was obtained, since in all groups, the first principal component (PC1) was associated with allometric size. This eigenvector is an allometric size-related vector since, in all cases, the first eigenvector showed values oriented in the same direction. The residual matrices were obtained using the following relationship:

$$\mathbf{R} = \mathbf{P} - \mathbf{V}\mathbf{V}^{\mathrm{T}}$$

where $\mathbf{P}$ is the original var/cov phenotypic matrix and $\mathbf{V}^{\mathrm{T}}$ denotes the transposed first eigenvector of $\mathbf{P}$. Correlation matrices for each taxon were obtained again from the residual covariance matrices (Shirai and Marroig 2010) and compared to the hypothesized modular matrices. These residual correlation matrices usually present negative values for correlations between traits outside modules (AVG–). Thus, the use of the avg+/avg- ratio as a tool for module detection becomes compromised. Because of this, the modularity index without size was calculated as the difference between avg+ and avg– divided by the overall magnitude of the integration coefficient (r^2, see description of r^2 below) for residual matrices. This calculation is termed the Modularity Hypothesis Index (*MHI*).

Magnitude of integration.—The overall magnitude of integration was obtained by calculating the mean determination coefficient (r^2) from the correlation matrices. This coefficient is calculated as the mean of the squared correlation coefficients and represents the global level of integration among all cranial traits (Marroig et al. 2009). In order to generate a confidence interval for the r^2 index, we performed a Monte Carlo resampling of the correlation matrices. It is noteworthy that this is a scale-independent index, thus allowing the comparison among species (Porto et al. 2009).

Evolutionary potential.—The evolutionary potential is the potential of a population to evolve in the direction of selection. We evaluated this potential using the multivariate response to selection equation (Lande 1979). We multiplied the var/cov matrix for each genus by 10,000 normalized random vectors of selection (β), sampled from a normal distribution, with zero mean and standard deviation of one. After obtaining for each genus the response vectors to the selection vectors, we estimated the evolutionary flexibility index (Marroig et al. 2009) as the mean correlation (angle cosine) among the 10,000 β-vectors and its corresponding response vectors (Δz). All vectors were rescaled to have a norm = 1 prior to computing the correlation. In this way, the higher the correlation between the response and selection vectors, the higher the evolutionary flexibility implied (the response to selection was in a similar direction as the selection). We applied a Monte Carlo resampling of 1,000 matrices for each genus to get a confidence interval for the evolutionary flexibility index.

The structuring of patterns and magnitude of integration in a population can potentially have a strong influence on its evolutionary trajectory, as these aspects would influence how it responds to selection. In this way, to understand the relationship between the covariance structure and the evolutionary potential (flexibility), we calculate the observed percentage of variation in the first principal component (%PC1) of var/cov matrix (since the first eigenvectors oriented in the same direction were related to allometric size in all genera). The higher the percentage of variation explained by the first principal component, the higher the influence of this axis of variation on the evolutionary response. Therefore, high levels of PC1 variation tend to bias the response to selection in this direction (Schluter 1996). We also used the morphological integration index (r^2) since the magnitude of association will imply the degree of interdependence between characters and their ability to respond to selection. The parameters were correlated to the flexibility index and the relation between them was evaluated.

Results

Similarity among the correlation and covariance matrices.—In general, the var/cov and correlations matrices were similar for all comparisons between genera, irrespective of the pairwise comparison method used (*RS* and *KRZ*, Tables 2 and 3). This result indicates that all genera share a common covariance and correlation pattern. In the comparisons using the Random Skewers method (*RS*), the highest similarity values for the adjusted var/cov matrices was 0.94 between *Thrichomys* and *Trinomys*, whereas the lowest value was 0.79 between *Euryzygomatomys* and *Thrichomys*. Using the *KRZ* method, the highest similarity for the adjusted var/cov matrices was observed between *Thrichomys* and *Trinomys* (0.87) and the lowest was observed between *Euryzygomatomys* and *Kannabateomys* (0.76). Comparisons using the correlation matrices (Table 4) also presented high similarity values, with the highest between *Kannabateomys* and *Phyllomys* (0.84) and the lowest between *Euryzygomatomys* and *Kannabateomys* (0.77). Even using the raw matrices (without controlling factors) of *Phyllomys*, *Proechimys*, and *Thrichomys* (Supplementary Material SD6) the similarity patterns remained high.

Similar and divergent traits.—The most divergent traits among all genera were the measurements APET-TS, BA-OPI (cranial base), BR-PT (cranial vault), and MT-PNS (oral). This result indicates that these four measurements had a larger variance in the selective response in all pairwise comparisons (Fig. 2). Those traits were considered dissimilar in covariance patterns in most pairwise comparisons (values close to 1, indicating that the particular distance was considered dissimilar in almost 100% of the cases). On the other hand, several distances were not significantly divergent in any comparison, meaning that the percentage of times these distances were considered dissimilar were zero or close to zero. Non-divergent traits in all comparisons were those of the oral region (IS-PM, PM-ZS, PM-ZI, and PM-MT), nasal region (IS-NSL, NSL-NA, NSL-ZS, and NA-PNS), oral/nasal region (IS-PNS and NSL-ZI), cranial vault region (BR- APET, PT-BA, PT-EAM, BR-LD, and PT-AS), zygomatic region (PT-ZYGO, ZI-ZYGO, and ZI-TSP), and at the cranial base region (PNS-APET, and BA-EAM).

Table 2.—Estimates of similarity among covariance matrices based on the *RS* method. Repeatability values for each matrix are given in bold, above the diagonal are values adjusted by the matrices repeatability (corrected) values, and below the diagonal are values between the non-adjusted covariance matrices. All genera presented high values of repeatability, indicating that the matrices were robustly estimated.

Covariance matrices comparison—RS method						
	Euryzygomatomys	*Kannabateomys*	*Phyllomys*	*Proechimys*	*Thrichomys*	*Trinomys*
Euryzygomatomys	**0.90**	0.82	0.83	0.82	0.79	0.80
Kannabateomys	0.76	**0.94**	0.88	0.88	0.89	0.89
Phyllomys	0.78	0.84	**0.95**	0.91	0.89	0.87
Proechimys	0.76	0.83	0.87	**0.96**	0.93	0.93
Thrichomys	0.73	0.85	0.86	0.90	**0.96**	0.94
Trinomys	0.74	0.84	0.82	0.88	0.90	**0.94**

Table 3.—Estimates of similarity among covariance matrices based on the *KRZ* method. Repeatability values for each matrix are given in bold, above the diagonal are values adjusted by the matrices repeatability (corrected) values, and below the diagonal are values between the non-adjusted covariance matrices. All genera presented high values of repeatability, indicating that the matrices were robustly estimated.

Covariance Matrices Comparison—KRZ Method						
	Euryzygomatomys	*Kannabateomys*	*Phyllomys*	*Proechimys*	*Thrichomys*	*Trinomys*
Euryzygomatomys	**0.81**	0.76	0.78	0.81	0.77	0.82
Kannabateomys	0.65	**0.89**	0.82	0.81	0.81	0.80
Phyllomys	0.67	0.74	**0.90**	0.83	0.84	0.81
Proechimys	0.69	0.72	0.74	**0.89**	0.84	0.84
Thrichomys	0.65	0.73	0.75	0.75	**0.88**	0.87
Trinomys	0.69	0.72	0.72	0.74	0.77	**0.88**

Table 4.—Estimates of similarity among correlation matrices based on the *KRZ* method. Repeatability values for each matrix are given in bold, above the diagonal are values adjusted by the matrices repeatability (corrected) values, and below the diagonal are values between the non-adjusted covariance matrices. Most genera presented high values of repeatability, indicating that the matrices were robustly estimated.

Correlation Matrices Comparison—KRZ Method						
	Euryzygomatomys	*Kannabateomys*	*Phyllomys*	*Proechimys*	*Thrichomys*	*Trinomys*
Euryzygomatomys	**0.79**	0.77	0.80	0.83	0.79	0.83
Kannabateomys	0.65	**0.90**	0.84	0.80	0.78	0.80
Phyllomys	0.68	0.76	**0.90**	0.82	0.81	0.82
Proechimys	0.69	0.71	0.74	**0.88**	0.83	0.82
Thrichomys	0.65	0.70	0.72	0.73	**0.88**	0.82
Trinomys	0.69	0.71	0.73	0.72	0.72	**0.87**

In the pairwise comparisons, *SRD* score distributions range from 0 to 1, where more dissimilar characters present values close to 0. The terrestrial genera (*Proechimys, Thrichomys,* and *Trinomys*) shared the highest overall average similarity scores in skull traits, with similarity coefficients above 0.9 for all traits in all pairwise comparisons (Supplementary Data SD9). The most divergent traits in this ecological group were associated with the cranial base region (BA-OPI and MT-PNS) and cranial vault (BR-PT), the same traits identified as divergent when comparing all genera. In the pairwise comparisons between arboreal genera (*Kannabateomys* and *Phyllomys*, Supplementary Data SD10), the most divergent traits were associated with the cranial vault (BR-PT), the cranial base (MT-PNS, BA-OPI, and APET-TS), and the zygomatic region (ZS-ZI).

When we compared the arboreal (*Kannabateomys* and *Phyllomys*) with terrestrial group (*Proechimys, Thrichomys*, and *Trinomys)*, the cranial base (MT-PNS and BA-OPI) and cranial vault regions (BR-PT) were divergent in their covariation patterns when *Phyllomys* was included (Supplementary Data SD11). In contrast, when the arboreal *Kannabateomys* was compared with terrestrial species, the greatest divergences were those of the oral/zygomatic region (ZS-ZI), cranial vault (BR-PT), and cranial base (BA-OPI, APET-TS and MT-PNS) (Supplementary Data SD12).

Pairwise comparisons involving *Trinomys* identified OPI-LD and LD-AS distances, revealed that both are related to the cranial vault region, suggesting that the patterns of covariance in *Trinomys* were different from other taxa in these specific traits. Moreover, lower overall values of similarity were found in pairwise comparisons involving the semifossorial *Euryzygomatomys* (Supplementary Data SD13). This genus was distinguishable in the cranial vault region (NA-BR and BR-PT), cranial base (APET-TS, BA-OPI, and MT-PNS), and zygomatic region (EAM-ZYGO and ZYGO-TSP), in comparisons with both terrestrial and arboreal groups. The lowest value of similarity, 0.76, was observed between *Euryzygomatomys* and *Trinomys*.

Modularity.—The modularity index (AVG+/AVG- ratio) calculated from the raw matrices (Table 5) showed that the nasal region was the only functional/developmental

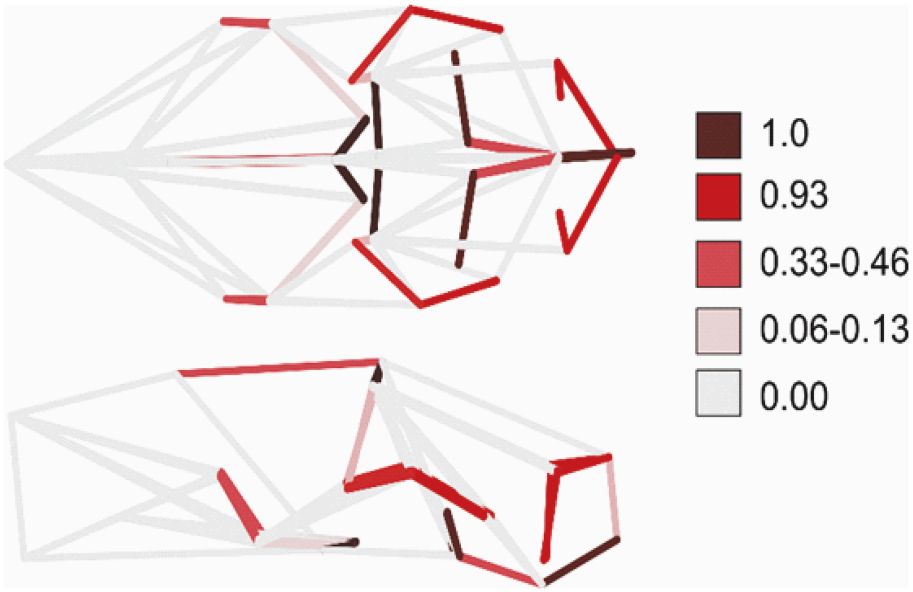

Fig. 2.—Representation of the cranial distances (as seen in dorsal and lateral views) and their respective variance in selection responses (0 to 1) obtained in the pairwise comparisons among the six echimyid genera. Higher variance is indicated by darker shades of red, suggesting greater divergence of these distances among genera.

Table 5.—Modularity indices obtained according to the functional/development and masticatory stress hypotheses for the matrices observed (raw matrices). Significant values for the Mantel test are in bold. The values were calculated using the ratio between avg+ and avg–.

Modularity—Observed Matrices										
Species	Oral	Nasal	Cranial vault	Zygomatic	Cranial base	Gnaw	Chew	Neurocranium	Face	Total
Euryzygomatomys	1.70	**2.26**	1.36	0.98	0.36	0.76	1.13	0.99	0.67	**1.39**
Kannabateomys	**1.70**	**2.81**	0.39	–0.07	–1.68	0.58	0.89	-0.30	**0.89**	**1.25**
Phyllomys	0.55	**1.93**	0.14	–0.11	–1.09	0.78	0.92	-0.29	0.47	**1.09**
Proechimys	0.27	**2.49**	0.36	–0.12	–1.16	0.76	0.92	-0.18	0.26	1.04
Thrichomys	**1.18**	**2.78**	–0.34	–0.07	–0.95	1.18	0.94	-0.60	**0.80**	**1.11**
Trinomys	**1.27**	**2.48**	–0.23	0.10	–0.31	0.95	1.01	-0.33	**0.75**	**1.19**

group significantly detected as a module in all genera, with *Thrichomys* showing the highest index (2.31) and *Phyllomys* showing the lowest value (2.01). The oral module was identified in *Kannabatebomys* (1.74), *Thrichomys* (1.55), and *Trinomys* (1.54). Cranial vault, zygomatic, cranial base, neurocranium, and masticatory force distribution modules (gnaw and chew) were not significantly detected as a module in any species. Only *Thrichomys* (1.41) and *Trinomys* (1.38) exhibited modularity in the face. Total integration, including all five subregions (oral, nasal, zygomatic, cranial vault, and cranial base) combined into one matrix, was significantly identified in *Euryzygomatomys* (1.39), *Kannabatebomys* (1.25), *Phyllomys* (1.09), *Thrichomys* (1.11), and *Trinomys* (1.19).

After allometric size removal (Table 6), the *MHI* index showed that the zygomatic module was significantly detected as a module for all genera. The highest value was 0.97 observed in *Kannabateomys* and the lowest value in *Phyllomys* (0.45). The cranial vault module was significant in all genera, with the highest value observed in *Kannabateomys* (1.79) and the lowest value in *Phyllomys* (0.48). The neurocranium region was detected for all genera, once again with *Kannabateomys* having the highest index (1.22) and *Phyllomys* the lowest (0.30). The face module was evident only in *Euryzygomatomys* (0.58). Total integration was significant in *Euryzygomatomys* (0.94), *Kannabateomys* (0.51), *Phyllomys* (0.21), and *Trinomys* (0.47).

Overall magnitude of integration.—Figure 3A illustrates the mean values of the magnitude of integration and their confidence interval. Our support for these results can be seen in the description of the quantiles (first quartile Q1 (2.5%) and third quartile Q3 (97.5%)) obtained through Monte Carlo resampling for each genus. *Kannabateomys* (Q1 = 0.08 and Q3 = 0.13) had the lowest value of integration with respect to the other taxa ($r^2 = 0.09$). *Euryzygomatomys* (Q1 = 0.13 and Q3 = 0.23), *Phyllomys* (Q1 = 0.14 and Q3 = 0.22) and the terrestrial group of *Proechimys* (Q1 = 0.12 and Q3 = 0.19), *Thrichomys* (Q1 = 0.11 and Q3 = 0.20), and *Trinomys* (Q1 = 0.12 and Q3 = 0.21), shared similar values of r^2 (about 0.15).

Evolutionary potential.—Figure 3B illustrates mean values of flexibility, and their confidence limits, as well as the measure of the overall magnitude of integration. *Kannabateomys* (Q1 = 0.41 and Q3 = 0.48) was the most evolutionarily flexible in comparison with the other studied genera (mean value = 0.46). *Euryzygomatomys* (Q1 = 0.31 and Q3 = 0.40 = 0.37), *Phyllomys* (Q1 = 0.33 and Q3 = 0.39 = 0.38) and the terrestrial genera, *Proechimys* (Q1 = 0.35 and Q3 = 0.40), *Thrichomys* (Q1: 0.33 and Q3: 0.42 = 0.38) and *Trinomys* (Q1 = 0.36 and

Table 6.—Modularity indices obtained according to the functional/development and masticatory stress hypotheses for adjusted matrices (allometric size excluded). Significant values for the Mantel test are in bold. The values were calculated using the *MHI* index, e.g., ratio between the absolute difference between avg+ and avg– and r^2.

Modularity—Adjusted matrices										
Species	Oral	Nasal	Cranial vault	Zygomatic	Cranial base	Gnaw	Chew	Neurocranium	Face	Total
Euryzygomatomys	0.70	0.35	**0.57**	**0.93**	0.54	–0.40	0.21	**0.66**	**0.58**	**0.94**
Kannabateomys	–0.22	–0.84	**1.79**	**0.97**	–0.48	–0.98	–0.26	**1.22**	–0.43	**0.51**
Phyllomys	0.02	–0.55	**0.48**	**0.45**	–0.13	–0.42	–0.14	**0.30**	0.00	**0.21**
Proechimys	–0.46	–0.30	**0.67**	**0.71**	0.51	–0.50	–0.15	**0.70**	–0.40	0.16
Thrichomys	–0.32	–0.48	**0.53**	**0.89**	0.14	0.40	–0.11	**0.49**	–0.23	0.16
Trinomys	0.29	–0.01	**0.87**	**0.85**	0.99	–0.08	0.09	**0.98**	–0.26	**0.47**

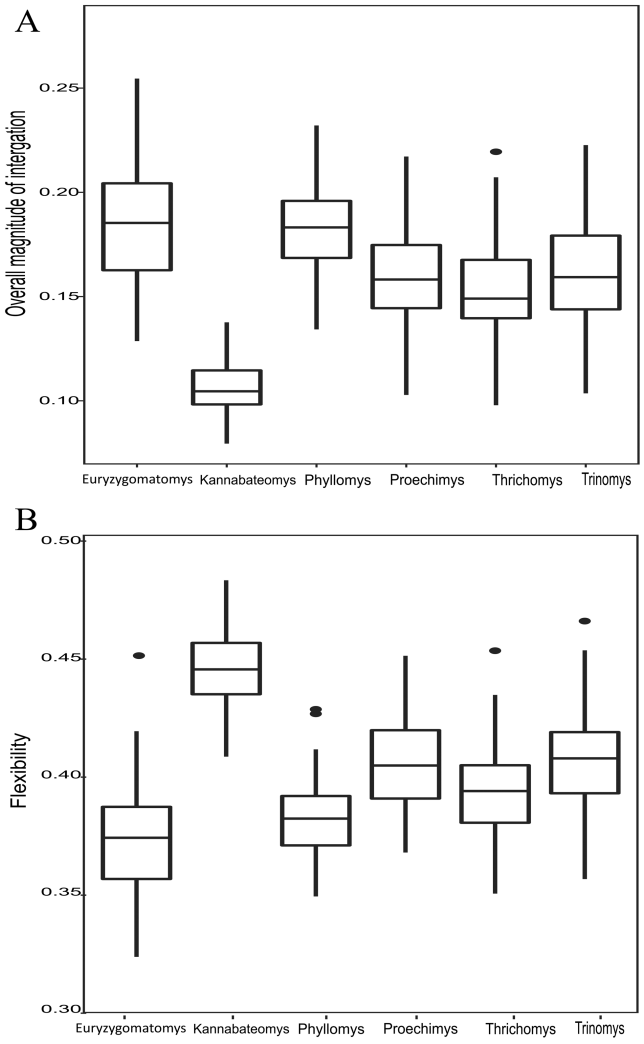

Fig. 3.—Boxplots of the estimated overall magnitude of integration (A) and evolutionary flexibility (B) for each genus; medians are represented by the horizontal bars; the box represents the 50% distribution and whiskers 75%. Confidence intervals for this index for each genus are represented by a Monte Carlo resampling of 100 matrices.

Q3 = 0.43 = 0.41) presented a similar pattern of selection responses.

For a better understanding of evolutionary potential, we also examined the percentage of variation explained by the first principal component (%PC1). The % PC1 was variable among genera, highest (Q1 = 0.47 and Q3 = 0.67 = 53%) in *Phyllomys*, followed successively by *Thrichomys* (Q1 = 0.45 and Q3 = 0.61 = 52%), *Euryzygomatomys* (Q1 = 0.36 and Q3 = 0.59 = 48%), *Proechimys* (Q1 = 0.41 and Q3 = 0.55 = 48%), *Trinomys* (Q1 = 0.39 and Q3 = 0.54 = 46%), and *Kannabateomys* (Q1 = 0.30 and Q3 = 0.44 = 37%). Flexibility was negatively correlated with the overall magnitude of the integration coefficient (r^2) and the percentage of variation explained by PC1 (Fig. 4A and B). The percentage of variation explained by PC1 was positively correlated with the r^2 index (Fig. 4C).

Discussion

In this study, we investigated the patterns of integration and modularity of cranial morphology in six genera of rodents representing three of four extremes in ecomorphological form in the family Echimyidae (i.e., terrestrial, arboreal, and semifossorial). Despite their ecomorphological variation and phylogenetic breadth, all genera studied shared an overall pattern of similarity of their var/cov and correlation matrices. The stability of covariance and correlation patterns has been reported for mammals in general (Porto et al. 2009), evidenced by a common developmental pattern of cranial bones (Goswami 2006; de Oliveira et al. 2009; Shirai and Marroig 2010). According to Smith (1997), the main differences in developmental process in mammals are associated with comparisons involving eutherians and metatherians, where features like heterochrony and/or changes in developmental sequences constitute the principal causes of variations in skull formation and its patterns of variance/covariance.

Although the overall cranial variance/covariance structure has remained stable across Eutherian mammals, including the six genera of echimyids in this study (Table 2), we found that some individual cranial traits showed high divergence in covariance pattern amongthe studied echimyid genera (Fig. 2). The four most divergent traits detected in pairwise comparisons were located on the cranial base (APET-TS and BA-OPI), cranial vault (BR-PT), and oral region (MT-PNS). These distances represent local variations and do not affect the overall covariance structure (see *RS* results), nor our shared development/function hypothesis in the overall covariance pattern. The divergence in the variance/covariance patterns involving the cranial base region (BA-OPI, APET-TS, and OPI-LD) was also detected comparing between the marsupial genera *Caenolestes*

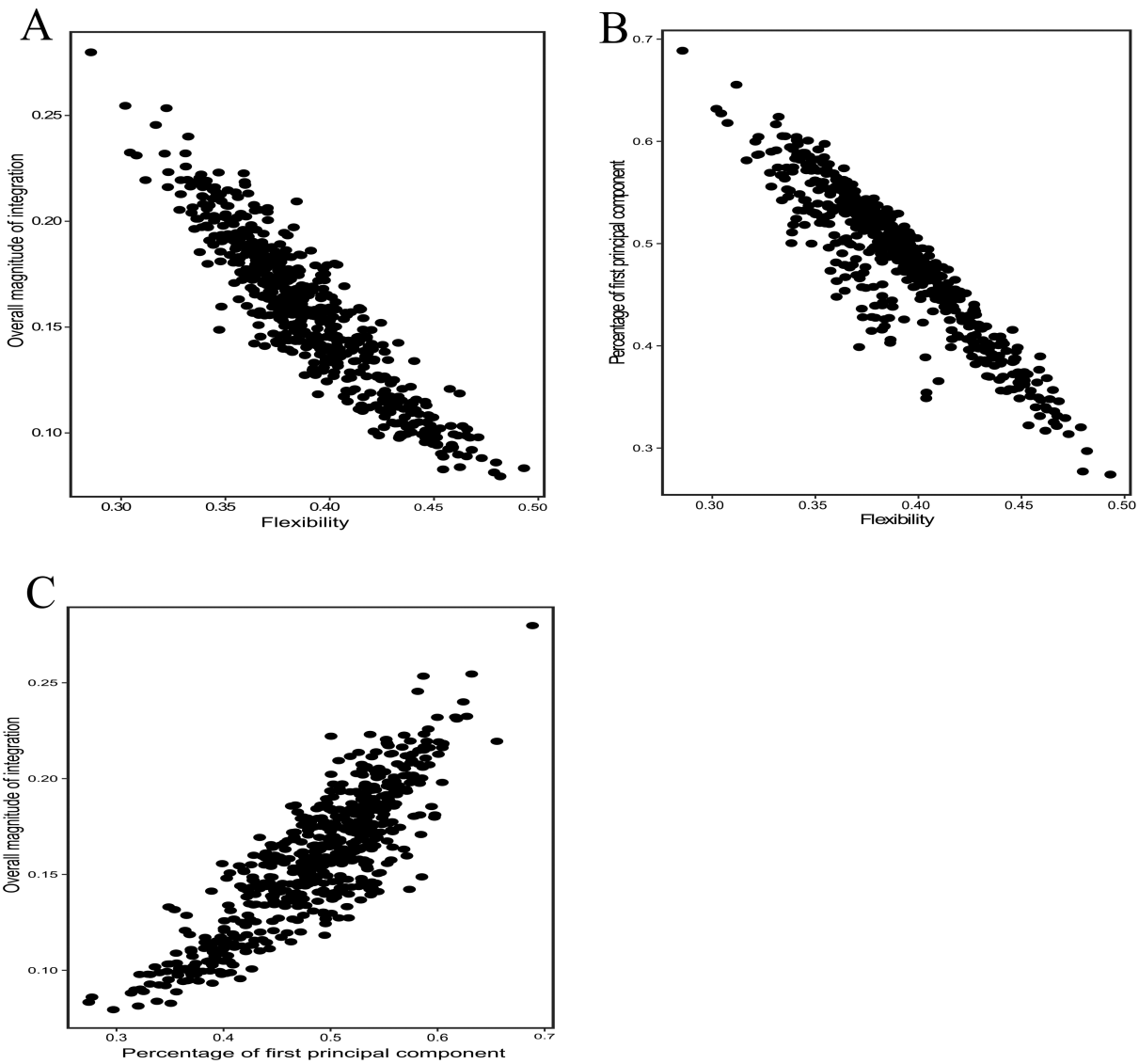

Fig. 4.—Scatterplots showing correlation among evolutionary flexibility, percentage of variation in the first principal component (%PC1), and overall magnitude of integration (r^2). (A and B) Represent a negative correlation of the flexibility index with r^2 and % PC1. (C) Indicates a positive correlation between r^2 and % PC1.

and *Macropus* (Marroig et al. 2011). The APET-TS distance spans the auditory bulla, a highly variable structure in rodents (Perez et al. 2009; Álvarez et al. 2013). Auditory bullae have a large variation in size, which is not necessarily linked to the variation observed in other traits. This might explain why the APET-TS distance presented a difference in covariance pattern, as the largest auditory bullae are from fossorial species, followed by arboreal and then terrestrial species. Size variation in auditory bullae allows detecting different frequencies of acoustic signals (Perez et al. 2009). The divergence of covariation pattern for the trait BA-OPI, which measures the diameter of the foramen magnum, might be related to the high variation in this region among rodent species, probably as a consequence of the connection of different structures of the postcranial skeleton. This statement is supported in part by the evidence that genes associated with the development of the axial skeleton are related to developmental control in the basioccipital region, suggesting that the region of the foramen magnum is more genetically integrated with the axial skeleton than with the rest of the skull (Kessel et al. 1990). Thus, variation in the postcranial skeleton related to different locomotor adaptations is more likely to be realized in the foramen magnum than other skull parts. This supports our hypothesis that ecological aspects, such as locomotion, may be acting in the structuring of the variance/covariance pattern in the studied echimyids, since the divergence in BA-OPI may reflect differences in the axial skeleton. The cranial vault was also detected as divergent through the BR-PT trait, which measures the breadth of the cranium at the suture of the frontal and parietal bones. This measure comprises areas responsible for accommodating the brain. Differences in the encephalization process may cause the variability observed among mammals in the var/cov patterns between the vault compared with the rest of the skull (Ackermann and Cheverud 2004; Gowswami 2006). Of the consistently divergent traits,

MT-PNS, which measures the distance from the distal end of the molar row to the posterior of the palate (posterior nasal spine), is the most likely to be involved in mastication and diet, because it is associated with the different dental configurations observed between the genera studied. Differentiation in masticatory apparatus, including dental morphology, is related to dietary variation. In rodents, graminivorous diets are associated with large differences in tooth rows compared to rodents with other diets (fruits and seeds; Hautier et al. 2012). Hence, the divergence in covariance pattern of MT-PNS observed for all echimyid genera that we examined is probably related to their dietary differences, which range from ingestion of bamboo shoots and leaves (*K. amblyonyx*) to fruits, leaves, and insects (*Phyllomys, T. apereoides*, and *P. roberti*) (Olmos et al. 1993; Leite 2003; Hautier et al. 2012).

The six echimyid genera evaluated showed a strong integration in facial region modules (nasal and oral), particularly the nasal module that was evident in all genera with the highest avg+/avg− ratio of all hypotheses (average = 2.45). The oral module was only detected for *Kannabateomys, Trinomys*, and *Thrichomys*. These modules can favor morphological disparity in these regions and provide a coordinated response between the bones within the complex and its associated structures (Goswami and Polly 2010; Renaud et al. 2012). The nasal area is also important for both water and temperature balance and chemical communication, encompassing traits related to the olfactory sense in rodents (Eisenberg and Kleiman 1972). The predominance of the nasal module has also been reported for other Eutherians like Neotropical marsupial groups (Porto et al. 2009; Shirai and Marroig 2010), sigmodontine rodents (Costa 2013), and several other mammalian groups, such as carnivores and monotremes (Goswami 2006). On the other hand, in Neotropical primates, the oral region was the most distinctive module, not the nasal group (Shirai and Marroig 2010). The predominance of facial elements being identified by modularity analyses of mammals (i.e., oral or nasal) may be related to developmental processes in skull formation. In contrast to the neurocranium region, the facial region develops late in ontogeny, possibly due to a posterior action of genetic factors and growth hormones (Ackermann and Cheverud 2004; Porto et al. 2013). Therefore, the high level of integration found in facial elements may result from late influence of growth factors.

Across the six genera, removing allometric size revealed modules for the zygomatic functional subregion and neurocranium region. For five genera (i.e., excluding *Thrichomys*), removing allometric size also revealed a module for the cranial vault subregion. Also, by removing size, we identified a facial module in *Euryzygomatomys*. Allometric size impacts the detection of modularity patterns since it is associated with the developmental process, influencing the growth of all traits, and promoting the overall integration of traits (Marroig et al. 2004; Mitteroecker and Bookstein 2007; Shirai and Marroig 2010; Porto et al. 2013). Although the modular pattern was stronger after removing allometric size, the cranial base region still was not recognized since it presented a non-significant result (Mantel test) for the modularity hypothesis index (*MHI*). This complex provides the scaffold upon which the rest of the skull will develop and protect brain connections with the face and rest of the body (Lieberman et al. 2000). The lack of modularity signal at the base of the skull may be related to it serving as a foundation in developing the rest of the skull, including the face and vault regions (Hallgrímsson et al. 2007). The masticatory modules were not detected in any modularity test (raw and size-free matrices). Notably, we also failed to detect any modules related to mastication (i.e., gnaw and chew subregions). The orbital bones play an important role in the distribution of stress during chewing (Cox et al. 2012), and unfortunately, the measures in this study did not comprehend this region in a representative way. Additional research is still needed to understand the functional relationship between tensions caused by chewing and covariation patterns in the skull.

We used the ability to respond in the direction of selection (flexibility index), the percentage of variation on PC1, and the magnitude of total integration (r^2) to explore the evolutionary potential among the echimyid genera we examined. They presented similar values to the observed flexibility indices for representatives of Lagomorpha and Carnivora (between 0.3 and 0.4). Except for *Kannabateomys*, which exhibited the highest flexibility values, close to the values obtained for *Cebus*, *Alouatta*, and *Mazama gouazoubira* (0.4 to 0.5) (Marroig et al. 2009). Furthermore, the magnitude of integration results was also consistent with those found for the taxa *Callithrix*, *Cebus*, *Akodon cursor*, *Tupaia glis*, *Tapirus terrestris*, and *Mazama gouazoubira* (range from 0.09 to 0.16; Porto et al. 2009). We found a strong positive association between overall integration and the percentage of variation on PC1. On the other hand, these two indices were negatively correlated with the ability to respond to selection (flexibility index), consistent with other mammal species (Marroig et al. 2009; Porto et al. 2013). Therefore, taxa with higher levels of flexibility are also those with lower rates of r^2 and lower percentages of variation in PC1 (i.e., *Homo*, *Callithrix*, and *Pan*), whereas taxa with lower flexibility also have higher rates of r^2 and higher percentages of variation in PC1 (e.g., marsupials; Marroig et al. 2009). The first principal component (in this case, the allometric vectors) can greatly influence the direction of evolutionary paths of a population. In a phenotypic matrix, PC1 represents the "evolutionary line of least resistance", i.e., the direction in which evolutionary changes would be favored (Schluter 1996). Therefore, allometric size can act as a force that facilitates the adaptive process rather than an obstacle to evolution, depending on the relationship between the covariance matrix and the adaptive landscape (Schluter 1996; Marroig and Cheverud 2010; Melo et al. 2016).

Despite the stability in the calculated metrics, some aspects were variable among genera. *Thrichomys* has a broad distribution, inhabiting xeric and rocky environments within the Brazilian Cerrado and Caatinga biomes (Emmons et al. 2015). This species presents a high intraspecific variation, including the oral region (Reis et al. 2002; Monteiro et al. 2003). This variation may also be associated with different configurations of nasal turbinate elements related to water balance,

previously noted for groups living in desert regions (Schmidt-Nielsen et al. 1970). The modular structure in the oral region of *Thrichomys* can potentially facilitate the selection of traits capable of improving the performance in environments with extreme differences in water availability. *Euryzygomatomys* is the only semi-fossorial genus in this study. Semi-fossoriality is characterized by several morphological specializations, such as very robust zygomatic arches and thus an expansion for the area of muscle insertion (Agrawal 1967). Therefore, it is not surprising that the measurements in the zygomatic region, such as ZYGO-TSP and EAM-ZYGO, were detected as divergent in comparisons involving this species. In all pairwise comparisons with *Trinomys*, the distances OPI-LD and LD-AS had divergent covariance patterns, indicating that the occipital bone area of *Trinomys* has a divergent covariance pattern with the rest of the skull in relation to the other species. Variations related to these distances have not yet been investigated for *Trinomys*, indicating the importance of new approaches associated with the study of the occipital bone in this taxon. The oral complex was also detected in *Trinomys* and is consistent with differences related to the oral and facial region, such as variation in the incisive foramen, that have been reported across the geographic distribution of the genus (Tavares and Pessôa, 2010; Dalapicolla and Leite, 2015). Thus, the oral complex in this genus constitutes a set of widely variable measurements, suggesting the relative independence of that module for the rest of the skull. Higher modularization leads to higher evolvability, given that a modular structure allows the remodeling of a structure without changing other structures (Clune et al. 2013). *Kannabateomys* constitutes an example of ecological specialization that diverges from other studied echimyids. The high divergence in covariance patterns for ZS-ZI (a trait located in the zygomatic arch) was only detected in this genus. It might be associated with its dietary specialization on young bamboo shoots and leaves (Olmos et al. 1993). The ZS-ZI trait represents the depth of the zygomatic bone, the origin region of the masseter muscle. This muscle is specialized for chewing and the zygomatic bone is responsible for relieving facial tensions derived from movements in that area (Dechow and Wang 2016). Like *Thrichomys* and *Trinomys*, the oral module was detected for *Kannabateomys,* which supports our inference that the oral module might have facilitated the ecomorphological form and specialized feeding habits of *Kannabateomys* (bamboo shoots and leaves). Additionally, the higher evolutionary potential of *Kannabateomys*, evident in its greater flexibility index and lower level of general magnitude compared to other genera, suggests that it has a greater capacity to respond in line with selection, which may be related to its dietary and ecomorphological specialization.

Although the analyses here are based on only 6 of 28 genera in Echimyidae, they are consistent with morphological integration of the skull being involved in the diversification of echimyids and warrant further examination from a more complete set of taxa. On the one hand, the modular structure, observed mainly in the cranial vault, in the nasal and zygomatic regions of the skull in all genera, and the reduced overall magnitude of integration in *Kannabateomys* may be associated with ecological variables, such as feeding strategies. This relationship has already been reported by Rossoni et al. 2017 during the evolutionary history of phyllostomid bats, where the evolution of morphological integration was influenced by diet specializations and roosting ecology, evidencing an important role of ecological variables in the structuring of covariance in mammals. The difference in the overall magnitude of integration detected when comparing *Kannabateomys* with the other genera has the potential to alter the course of evolution, as an equal selection gradient can produce a very different evolutionary response (Melo et al. 2016). Steppan et al. (2002), using two theoretical populations with different magnitudes of integration between them, showed that divergent evolutionary outcomes could be obtained, reinforcing our findings for *kannabateomys* and the relationship of its divergent magnitude of integration and evolutionary flexibility with the occupation of a specialized niche. Empirical data for this statement can be observed on a macroevolutionary scale in the study by Marroig et al. (2009), where differences in the magnitudes of integration observed in mammalian groups had an important impact on the evolutionary potential of the taxa. In this perspective, Fabre et al. (2013) argued that the ecomorphological diversity observed in Echimyidae could have resulted from the occupation of alternative evolutionary peaks within an adaptive landscape. Fabre et al. (2017) reinforced this assumption by arguing that the broad availability of ecological niches, vicariance events and dispersal, were determinants in the lineage diversification. The capacity to occupy new environments suggests that populations presented an efficient performance in this trajectory. Based on the results obtained, we assume that the low integration observed in echimyids played a fundamental role in morphological diversification, as this aspect has an important impact in terms of restricting or facilitating the evolutionary capacity of a population. Are differences in magnitude associated with the occupation of new environments? To discuss this issue, it will be necessary to add new samples in order to robustly represent the Echimyidae family. Thus, it will be possible to investigate a possible phylogenetic signal in the structuring of covariance and how feeding specialization events can influence this aspect.

It was possible to detect here that the origin of a diet specialized in *Kannabateomys* is characterized by a divergent structuring of covariance in relation to the other genera. Furthermore, the modularity and *SRD* results pointed out some particularities in the integration pattern that could be attributed to differences in diet and locomotion, as they comprise traits related to these functionalities. The presence of modules and the low overall magnitude of integration detected here possibly made the potential for individual trait variation among the studied echimyids more flexible. In this scenario, populations would respond more aligned to the selection regimes resulting from the occupation of new environments. These results point to a possible influence of ecological aspects on the covariance pattern of cranial traits. The addition of new representatives of the Echimyidae family will better evaluate this hypothesis.

Data Availability

Upon acceptance, data (covariance and correlation matrices; Euclidean distances of each species) will be archived on Dryad data repository and the Data Accessibility statement completed.
Orcid *Carolline Raidan*: https://orcid.org/0000-0002-7837-8113

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Authors' Contributions

CR measured the specimens, carried out the statistical analyses, and wrote the first draft of the manuscript. BMAC and APAA designed the methodology, created models (R scripts), and reviewed the manuscript. GM provided critical review and editing. RP managed and coordinated responsibility for the research activity planning/execution and manuscript review. This research was supervised by RP and APAA. All authors gave final approval for publication.

Conflict of Interest

The authors declare no conflict of interest.

Supplementary Data

Supplementary data are available at *Journal of Mammalogy* online.

Supplementary Data SD1.—List of measured specimens and their respective scientific collections.

Supplementary Data SD2.—Sample sizes for each species, including numbers of identified males and females, and references used to classify the age of individuals. Total sample size includes specimens with and without defined sex.

Supplementary Data SD3.—Abbreviations, positions, and description of landmarks used in this study. The positions of landmarks are shown in Fig. 1.

Supplementary Data SD4.—Repeatability estimates for each linear distance by species (*Euryzygomatomys spinosus*, *kannabateomys amblyonyx, Phyllomys blainvilii, Phyllomys pattoni, Proechimys roberti, Thrichomys apereoides*, and *Trinomys paratus*).

Supplementary Data SD5.—Controlled intraspecific variations from multivariate analysis of variance. NA is for individuals without available information. Tested models include the comparison among geographic areas (G), ontogeny (O), sexes (S), and species (Spp).

Supplementary material SD6.—Comparison between raw and controlled effects matrices of *Phyllomys*, *Proechimys*, and *Thrichomys*. We used the Random Skewers method.

Supplementary Data SD7.—Similarity values according to the sample size compared in the rarefaction analysis using the RS method for the *T. apereoides* sample.

Supplementary Data SD8.—Similarity values according to the sample size compared in the rarefaction analysis using the KRZ method for the *T. apereoides* sample.

Supplementary Data SD9.—Representation of SRD scores obtained in comparisons involving terrestrial genera, being (A) *Proechimys* and *Thrichomys*; (B) *Proechimys* and *Trinomys;* and (C) *Thrichomys* and *Trinomys*. The mean values and standard deviation are represented for 35 cranial measurements. Dotted line represents the SRD overall mean.

Supplementary Data SD10.—Representation of SRD scores obtained in comparisons involving arboreal genera, being (A) *Kannabateomys* and *Phyllomys*. The mean values and standard deviation are represented for 35 cranial measurements. Dotted line represents the SRD overall mean.

Supplementary Data SD11.—Representation of SRD scores obtained in comparisons involving *Phyllomys*, being (A) *Phyllomys and Proechimys;* (B) *Phyllomys* and *Thrichomys*; and (C) *Phyllomys* and *Trinomy*s. The mean values and standard deviation are represented for 35 cranial measurements. Dotted line represents the SRD overall mean.

Supplementary Data SD12.—Representation of SRD scores obtained in comparisons involving K*annabateomys*, being (A) K*annabateomys* and *Proechimys*; (B) *Kannabateomys* and *Thrichomys*; and (C) K*annabateomys* and *Trinomys*. The mean values and standard deviation are represented for 35 cranial measurements. Dotted line represents the SRD overall mean.

Supplementary Data SD13.—Representation of SRD scores obtained in comparisons involving *Euryzygomatomys*, being (A) *Euryzygomatomys* and *Thrichomys*; (B) *Euryzygomatomys* and *Kannabateomys;* (C) *Euryzygomatomys* and *Phyllomys;* (D) *Euryzygomatomys* and *Proechimys;* and (E) *Euryzygomatomys* and *Trinomys*. The mean values and standard deviation are represented for 35 cranial measurements. Dotted line represents the SRD overall mean.

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