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Phylogenetic, Allometric, and Ecological Factors Affecting Morphological Variation in the Scapula and Humerus of Spiny Rats (Rodentia: Echimyidae)

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Abstract

Locomotion, as a fundamental function in mammals directly associated with the use of ecological resources, is expected to have anatomical structures functionally committed that evolved under intense selective pressure, possibly carrying specializations for different locomotor habits. Among caviomorph rodents, the family Echimyidae stands out for having the greatest species richness, with relatively well-resolved phylogenetic relationships, wide variation in body mass, and remarkable diversity of locomotor habits, including arboreal, scansorial, semi-aquatic, semifossorial, and terrestrial forms. Thus, Echimyidae constitutes a promising model for understanding how phylogenetic, allometric, and ecological factors affect the evolution of postcranial structures directly linked to locomotor function. We investigated the influence of these three factors on scapular and humeral morphological variation in 38 echimyid species using two-dimensional geometric morphometry and phylogenetically informed comparative methods. Scapular and humeral shape variation had a low correlation with body mass and structure size, conveying a small or negligible allometric effect. Conversely, a significant moderate to strong phylogenetic signal was detected in both structures, suggesting that an important part of their morphometric variation results from shared evolutionary history. Notably, morphological variation of the scapula was extensively structured by phylogeny, without the marked influence of locomotor habits, suggesting that its shape may be a suitable taxonomic marker. Finally, locomotor habits were important in structuring the morphological variation of the humerus. Our results suggest that the morphologies of the scapula and humerus, despite being anatomically and functionally interconnected, were differentially shaped by ecological factors associated with locomotor habits.

Keywords Caviomorpha · Geometric morphometrics · Ecomorphology · Postcranial variation

Introduction

Locomotion is a fundamental ability of animals, necessary for an extensive variety of actions, such as foraging, searching for partners or shelter, and escaping from predators. Locomotion can take several forms, depending on the environment, including swimming, crawling, walking, as well as some more idiosyncratic mechanisms, such as jumping, brachiation, and digging (Ijspeert 2002). Since the variation in the performance of these actions directly affects the fitness of individuals, natural selection is expected to act on any phenotypic traits related to locomotion (Irschick and Garland 2001; Orr 2009). Because of this, the study of locomotion has always played a significant role in adaptation discussions (Bennett and Huey 1990; Garland and Losos 1994; Dickinson et al. 2000; Irschick and Garland 2001; Pough et al. 2008; Shaw 2020). On a microevolutionary scale,

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these discussions helped to understand the causes and consequences of individual variation on animal movement (Scales and Butler 2016; Shaw 2020), while on a macroevolutionary scale, these studies helped to elucidate the relationships between the shape of structures and their functions (Irschick and Garland 2001; Scales and Butler 2016), shedding light on the origin and diversification of specialized locomotor habits.

The emergence of distinct locomotor habits from a generalist ancestor often occurs via natural selection, resulting in improved use of available environmental resources and niche partitioning. It is estimated that terrestrial generalist locomotion was present in the ancestor of rodents (Lovegrove and Mowoe 2014; Hedrick et al. 2020), the largest group of living mammals, and has persisted in most of its descendents (Galewski et al. 2005; Hedrick et al. 2020). Consequently, and also due to their small body mass, most rodents, especially muroids, have only subtle morphological specializations for different locomotion strategies (Elissamburu and Vizcaíno 2004; Weisbecker and Schmid 2007; Coutinho et al. 2013; Coutinho and Oliveira 2017; Hedrick et al. 2020). On the other hand, some lineages have acquired specialized habits, with extreme examples that include gliding in flying squirrels (Pteromyini) and scaly-tailed squirrels (Anomaluridae), burrowing in mole-rats (Bathyergidae), swimming in beavers (Castoridae), and jumping in kangaroo rats (Dipodomyinae). In all these extreme cases, the acquisition of locomotor specializations was associated with the origin of marked behavioral, physiological, and/or morphological adaptations (Irschick and Garland 2001; Edut and Eilam 2003; Samuels and Van Valkenburgh 2008).

Natural selection also seems to have played a crucial role in the locomotor diversification of caviomorphs, the first rodents to colonize South America, in the middle Eocene, when this continent was isolated from the others (Antoine et al. 2012; Boivin et al. 2018; Arnal et al. 2020). In these animals, different bone structures such as skull, mandibles, vertebrae, humeri, carpi, metacarpi, pelvis, femora, tarsi, and metatarsi exhibit signs of adaptation to locomotor niches (Morgan and Verzi 2011; Morgan and Álvarez 2013; Candela et al. 2017; Carvalhaes et al. 2019; Tavares and Pessôa 2020; Ávarez et al. 2021; Netto and Tavares 2021). It is notable that in caviomorphs, the morphological adaptations of the appendicular skeleton to different locomotion strategies have become expressively more pronounced than in muroids, providing valuable ecomorphological markers even for paleontological reconstructions of life habits of already extinct species (Weisbecker and Schmid 2007; Candela and Picasso 2008; Olivares et al. 2020; Tavares and Pessôa 2020).

In addition to reflecting functional specializations (Smith and Savage 1955; Hildebrand and Goslow 2006; Polly 2007; Tague 2020), part of the variation in the shape of the appendicular skeleton is expected to be explained by its phylogenetic history (Morgan 2009; Morgan and Álvarez 2013; Martín-Serra et al. 2014; Gaudioso et al. 2020). It is important to consider that the phylogenetic component of morphological variation does not necessarily carry adaptive traits and may be the result of neutral evolution (Duret 2008; Kern and Hahn 2018). In addition, body size and/or bone structure size may also explain some variation in shape, because of evolutionarily conserved allometric constraints or structural rearrangements required to support body weight (Milne et al. 2009; Campione and Evans 2012; Walmsley et al. 2012; de Oliveira and Santos 2018). Therefore, attempts to identify locomotor adaptations should not disregard the possible existence of phylogenetic structuring and allometric effects on morphological variation. Thus, it is useful to compare forms in unrelated organisms that share similar strategies of environment use due to evolutionary convergence. This approach, when considering the phylogenetic history of the lineages investigated, has bolstered ecomorphological studies as it elucidates the evolutionary associations between forms, their functional properties, and their interactions with the environment (Galewski et al. 2005; Fabre et al. 2013a, 2017; Álvarez et al. 2017; Courcelle et al. 2019).

Given their extensive taxonomic diversity and disparate morphological evolution, associated with disparate morphological evolution associated with different locomotor habits, and availability of a reasonable phylogenetic framework based on molecular data (Fabre et al. 2013a; 2017; Álvarez et al. 2017; Courcelle et al. 2019), the family Echimyidae Gray, 1825 (spiny rats, nutria, and hutias) is an excellent group of caviomorphs to investigate morphological specializations in the appendicular skeleton (Elissamburu and Vizcaíno 2004; Seckel and Janis 2008; Morgan 2009; Morgan and Álvarez 2013; Tavares et al. 2020). There are approximately 100 species and 32 genera of living echimyids (Lacher et al. 2016; Burgin et al. 2018; Emmons and Fabre 2018), currently divided into four subfamilies: Capromyinae Smith, 1842 (exclusively Caribbean), Carterodontinae Courcelle, Tilak, Leite, Douzery, and Fabre, 2019, Euryzygomatomyinae Emmons, 2005 and Echimyinae Gray, 1825, the latter comprising two tribes: Echimyini Gray, 1825 and Myocastorini Ameghino, 1902 (Courcelle et al. 2019). Continental echimyids (all lineages except Capromyinae) probably evolved to both arboreal and semi-terrestrial habitats twice each, as well as once to a semi-aquatic habitat. The presumed ancestral terrestrial habitat is retained as symplesiomorphic in two extant lineages (Galewski et al. 2005; Fabre et al. 2013a; Courcelle et al. 2019). In addition, a clade may explore arboreal and shrub substrates and climb rocks, as well as move frequently on the ground, which is often classified as scansorial (Neves 2003; Hildebrand and Goslow 2006; Weisbecker and Schmid 2007; Karantanis 2017). It has been pointed out that the evolution of these

different modes of locomotion in the Miocene, especially the acquisition of the arboreal habit, was a key factor in the origin of the remarkable echimyid species richness because it allowed access to a wide range of ecological resources previously unused by other rodents in South America, except erethizontids (Fabre et al. 2013a).

Generally, among mammals, the scapular appendicular skeleton concentrates some of the osteological structures that most intensely reflect locomotor specializations (Szalay and Sargis 2001; Polly 2007; Steiner-Souza et al. 2010; Morgan and Álvarez 2013; Pérez et al. 2021). Previous studies on caviomorphs, including some genera of echimyids, have shown that the morphology of part of their forelimbs, specifically humeri, ulnae, carpals, and metacarpals (Candela and Picasso 2008; Morgan and Verzi 2011; Morgan and Álvarez et al. 2013; Tavares et al. 2020), carries strong specializations associated with different locomotor habits. Contrarily, the shape of the scapulae of caviomorphs mostly reflects the phylogenetic history of the species, with no evidence of marked functional specializations (Morgan 2009). Considering the functional interdependence between scapula and humerus, it is notable that the evolution of these structures responds so differently to the functional demands imposed by different locomotor habits.

In the present study, our main goal was to understand how historical, allometric, and functional factors associated with

locomotion shaped the evolution of the scapula and humerus morphology throughout the extraordinary phylogenetic and ecological diversification of the Echimyidae. Indirectly, our investigation will be a resource for studies that require functional and phylogenetic markers in the morphology of the postcranial skeleton of echimyids, with potential utility for other caviomorphs and rodents.

Material and Methods

Specimens and Taxa Examined

For this study, we captured images of a total of 186 scapulae and 181 humeri of 236 echimyids, belonging to 38 species, distributed in 15 genera (Figure 1, Online Resource 1). All specimens analyzed were intact and without damage or abnormalities that could prevent the digitalization of anatomical landmarks. Since none of the structures used presents sexual dimorphism in rodents (Coutinho et al. 2013; Coutinho and Oliveira 2017), we grouped individuals of both sexes in all analyses. Only adult individuals were used, all identified by having the fourth premolar and the three molars erupted, thus avoiding the inclusion of ontogenetic variation (Pessôa and Reis 1991; Leite 2003; Tavares and Pessôa 2010; Tavares et al. 2016). The species examined
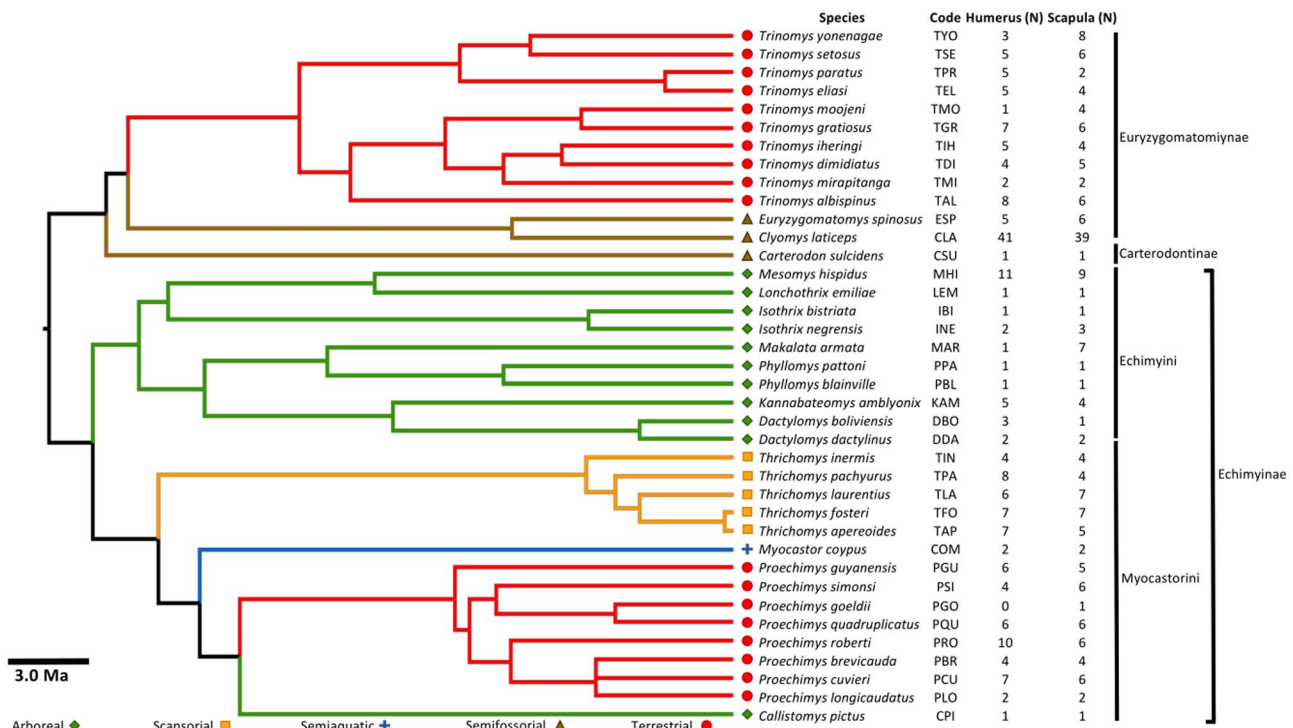

Species	Code	Humerus (N)	Scapula (N)	
Trinomys yonenagae	TYO	3	8	
Trinomys setosus	TSE	5	6	
Trinomys paratus	TPR	5	2	
Trinomys eliasi	TEL	5	4	
Trinomys moojeni	TMO	1	4	
Trinomys gratiosus	TGR	7	6	Euryzygomatomiynae
Trinomys iheringi	TIH	5	4	
Trinomys dimidiatus	TDI	4	5	
Trinomys mirapitanga	TMI	2	2	
Trinomys albispinus	TAL	8	6	
Euryzygomatomys spinosus	ESP	5	6	
Clyomys laticeps	CLA	41	39	
Carterodon sulcidens	CSU	1	1	Carterodontinae
Mesomys hispidus	MHI	11	9	
Lonchothrix emiliae	LEM	1	1	
Isothrix bistriata	IBI	1	1	
Isothrix negrensis	INE	2	3	
Makalata armata	MAR	1	7	Echimyini
Phyllomys pattoni	PPA	1	1	
Phyllomys blainville	PBL	1	1	
Kannabateomys amblyonix	KAM	5	4	
Dactylomys boliviensis	DBO	3	1	
Dactylomys dactylinus	DDA	2	2	
Thrichomys inermis	TIN	4	4	
Thrichomys pachyurus	TPA	8	4	
Thrichomys laurentius	TLA	6	7	Echimyinae
Thrichomys fosteri	TFO	7	7	
Thrichomys apereoides	TAP	7	5	
Myocastor coypus	COM	2	2	
Proechimys guyanensis	PGU	6	5	
Proechimys simonsi	PSI	4	6	
Proechimys goeldii	PGO	0	1	
Proechimys quadruplicatus	PQU	6	6	Myocastorini
Proechimys roberti	PRO	10	6	
Proechimys brevicauda	PBR	4	4	
Proechimys cuvieri	PCU	7	6	
Proechimys longicaudatus	PLO	2	2	
Callistomys pictus	CPI	1	1	

Arboreal ◆   Scansorial ▇   Semiaquatic ✚   Semifossorial ▲   Terrestrial ●

Fig. 1 Phylogenetic tree and species list used as a framework for comparative analysis, highlighting locomotor habits and main clades. Green = arboreal, yellow = scansorial, blue = semiaquatic, brown = semi-fossorial, red = terrestrial. Adapted from Álvarez et al. (2017)

were allocated into five locomotor habits (terrestrial, semi-fossorial, semi-aquatic, arboreal, and scansorial) as per the literature (Galewski et al. 2005; Fabre et al. 2013a; Emmons et al. 2015a; Hannibal et al. 2019). Although some authors classify *Thrichomys* Trouessart, 1880 species as terrestrial (Galewski et al. 2005; Neves and Pessôa, 2011; Olivares et al. 2012; Tavares et al. 2018; Carvalhaes et al. 2019; Courcelle et al. 2019), we considered them as scansorial, following other studies (Lacher and Alho 1989; Weisbecker and Schmid 2007; Patterson and Velasco 2008) and because *Thrichomys* has also been captured in trees (Hannibal et al. 2019). The specimens examined are deposited in the collections of the Laboratório de Biologia e Parasitologia de Mamíferos Reservatórios Silvestres (LABPMR), the Museu Nacional da Universidade Federal do Rio de Janeiro (MN/UFRJ), the Museu de Zoologia da Universidade de São Paulo (MZUSP), and the Universidade Federal da Paraíba (UFPB) (Appendix 1).

Obtaining Morphometric Data

Morphological variation was assessed by two-dimensional morphometry based on digital photographs. The images of the structures were captured only by one author (JGC), with a SONY P520 digital camera with 16-megapixel resolution, positioned at a standard distance of 5 centimeters from the photographic plane. The structures were placed in a container containing dark sand to minimize shadows, and a grid-scale of 1x1 centimeter, subdivided into millimeters, was added.

The two-dimensional morphological landmarks were mostly based on previous studies of the scapula (Morgan 2009) and the humerus of caviomorph rodents (Steiner-Souza et al. 2010; Morgan and Álvarez 2013), seeking to capture the maximum morphological variation observed during specimen handling and respecting the criteria of homology, consistency of relative position, repeatability, and coplanarity (Zelditch et al. 2004). We digitized 31 morphological characters for the scapula, including 14 landmarks and 17 semilandmarks; we digitized 23 morphological characters for the humerus, including 19 landmarks and 4 semilandmarks (Fig. 2; Table 1). All landmarks and semilandmarks were digitized by the same person, the author JGC (Online Resources 2 and 3). Specimens and their respective data used in analyses with the MorphoJ program can be found in Online Resource 4.

The digitized images were gathered and organized using the program tps-Util v. 1.78 (Rohlf 2019), and the anatomical landmarks were digitized with the program tps-Dig v. 2.31 (Rohlf 2017). The coordinates obtained from the anatomical landmarks were processed in the MorphoJ program (Klingenberg 2011), where General Procrustes Analysis Superimposition
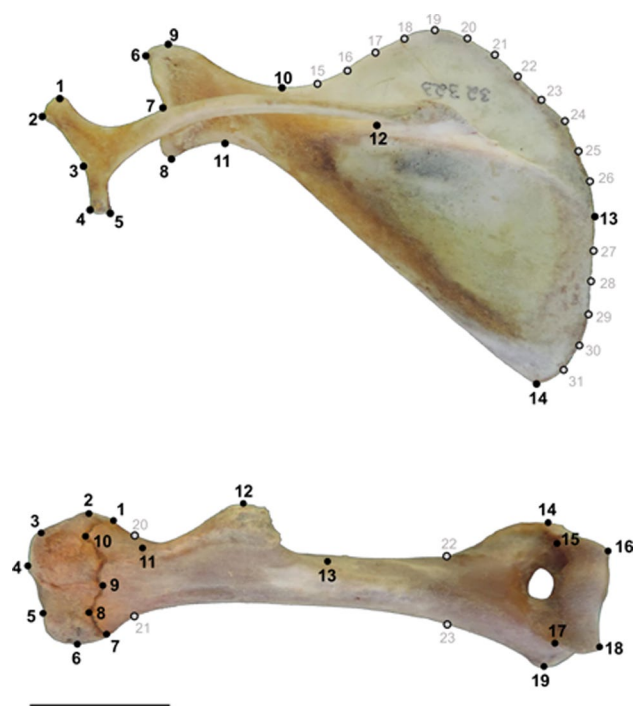

Fig. 2 Anatomical structures of this work: scapula and anterior view of the humerus showing the location of landmarks in the main anatomical features. Scale bars = 2 cm

was performed to remove variation not corresponding to shape (size, position, and orientation; Debat et al. 2000).

Mean centroid size and Procrustes coordinates were calculated for each species examined and used in subsequent analyses. In addition, to each species, we assigned a mean body mass value obtained from the label of the examined specimens or the literature (Bonvicino et al. 2008).

Phylogenetically-informed Comparative Analyses

Despite the robust phylogeny proposed by Courcelle et al. (2019), in this study we used the phylogenetic framework of the calibrated phylogenetic tree obtained by Álvarez et al. (2017), through Bayesian Inference based on four mitochondrial and five nuclear genes, as it includes a more extensive taxonomic coverage. Using the program TreeGraph 2 (Stöver and Müller 2010), we made manual edits to the tree of Álvarez et al. (2017) to include four species not included in it (Fig. 1, Online Resource 5). While including species within the genus *Proechimys* Allen, 1899, we assumed that the currently recognized species groups are reasonable proxies to their phylogeny (Patton and Leite, 2015). Thus, *P. cuvieri* Petter, 1978 was included in a polytomic clade containing *P. brevicauda* (Günther, 1876) and *P. longicaudatus* (Rengger, 1830) and split at 5.39 Ma (Alvarez et al. 2017), as they are all allocated in the *longicaudatus* group. *Proechimys goeldii* Thomas, 1905 was inserted as sister to *P. quadruplicatus* Hershkovitz, 1948, as both belong to the *goeldii* group (Patton and Leite 2015).

Table 1 Description of the landmarks used on the scapula and humerus in this work

Landmark	Scapular landmarks
1	Upper tip of the coracoid process
2	Dorsal tip of metachromium or most ventral point along tip of scapular spine when no metachromium is evident
3	Central point between the upper and lower coracoid process
4	Ventral tip of metacromion or ventralmost point along scapular spine tip when no metacromion is evident
5	Lower tip of the coracoid process
6	Anterior tip of the coracoid process
7	Central point between the tip of the coracoid process and the most ventral point at the tip of the glenoid fossa
8	Ventral most point on tip of glenoid fossa
9	Posterior tip of the coracoid process
10	Point of maximum curvature along upper scapular neck margin
11	Point of maximum curvature along lower scapular neck margin
12	Union between spine and blade (point of max. concavity on greater scapular notch)
13	Intersection between vertebral border and base of scapular spine
14	Lowest point of the lower angle of the scapula
15–26	Semilandmarks that outline the upper margin of the scapula
27–31	Semilandmarks that outline the medial margin of the scapula
Landmark	**Humeral landmarks**
1	Suture between diaphysis and proximal epiphysis on the lateral side
2	Extreme ventrolateral point of greater tuberosity
3	Dorsalmost point of greater tuberosity
4	Proximal extremity of humeral head
5	Dorsalmost point of lesser tuberosity
6	Most extreme point of the minor tuberosity
7	Suture between diaphysis and proximal epiphysis on the medial side
8	Proximal extremity of the contact of the great tuberosity with the diaphysis
9	Distal extremity of the contact of the great tuberosity with the small tuberosity
10	Meeting of the proximal extremity of the intertubercular sulcus with the small tuberosity
11	Beginning of the deltoid process
12	Most extreme point of the deltoid process
13	End of deltoid process
14	Most extreme point on lateral epicondyle
15	Dorsolateral extreme of capitulum
16	Ventrolateral extreme of capitulum
17	Dorsomedial extreme of trochlea
18	Ventromedial extreme of trochlea
19	Most extreme point on medial epicondyle
20–23	Semilandmarks that delimit both ends of the diaphysis

Given the absence of an estimated date for the divergence of *P. goeldii*, its branch was arbitrarily inserted halfway along the branch length separating its sister taxon, *P. quadruplicatus*, and its next most closely related taxon included in the backbone phylogeny, *P. simonsi*. We included *Trinomys mirapitanga* Lara, Patton, and Hingst, 2002 as sister to the clade formed by *T. iheringi* (Thomas, 1911) and *T. dimidiatus* (Günther, 1876) following Lara et al. (2002). Given the absence of an estimate date for the divergence of *T. mirapitanga*, its branch was arbitrarily inserted halfway along the branch length separating its sister taxa, *T. iheringi* and *T. dimidiatus*, and their next most

closely related taxa included in the backbone phylogeny, *T. gratiosus* (Moojen 1948). We inserted *T. moojeni* (Pessôa, Oliveira, and Reis, 1992) as sister to *T. gratiosus*, diverging at 5.9 Ma (Tavares et al. 2015).

Aiming to understand the allometric effect on scapular and humeral shape in Echimyidae, phylogenetically informed regressions (phylogenetic generalized least squares – PGLS) was employed to test the correlation between body mass, centroid size, and Procrustes coordinates (Freckleton et al. 2002; Revell 2009, 2010). Residuals from regressions between centroid size and Procrustes variables were

obtained and used in subsequent analyses to ensure the absence of allometric effect on analyses of shape variation (Revell 2009, 2010). Given the exceptionally large size of *Myocastor coypus* Molina, 1782 compared to other echimyids, the PGLS tests of correlation between body mass, centroid size and shape were run with two sets of data: including *Myocastor* and excluding this taxon.

Phylogenetically informed principal component analyses (pPCA) based on matrices of variance and covariance were implemented on the residuals of the Procrustes variables enabling the identification of the major sources of variation in the shape of the scapula and humerus of echimyids (Revel 2012). A broken-stick model was applied to determine the number of pPCs to be used in the subsequent analysis. The figure containing the pPC values resulting from the brocken stick model is available as Online Resource, (6). For each structure examined – i.e., scapula and humerus – the scores of each species along the informative pPCs according the broken-stick model were projected onto phylomorphospaces reconstructed by Maximum Likelihood.

Seeking to estimate the phylogenetic effect on the morphometric variation, the phylogenetic signal in shape variation was quantified and tested using Blomberg's method (Blomberg et al. 2003; Adams 2014) with 10,000 permutations (Klingenberg and Gidaszewski 2010). Blomberg's K-statistic quantifies the intensity of phylogenetic signals with values equal to or close to 1.0 when phenotypic variation is well explained by the Brownian motion model; with values greater than 1.0 when phenotypic attributes are phylogenetically more structured than expected by Brownian motion, and with values close to 0 in the absence of phylogenetic structure in phenotypic variation. K-statistics were estimated for the variation in the shape of each structure, as a whole using the multivariate approach of Adams (2014; K_{multi}), and separately for each informative pPC using the conventional approach of Blomberg et al. (2003).

Morphometric differentiation among locomotor habits considering the phylogenetic history of taxa, reflecting possible morpho-functional specializations, was tested using phylogenetic ANOVA and MANOVA (phyANOVA and phyMANOVA) on species scores informative pPCs (Garland et al. 1993), run with 10,000 permutations. The phyANOVA,

phyMANOVA, pPCA, PGLS, and phylomorphospace reconstruction were employed using the packages Geomorph 4.0 (Adams et al. 2021) and phytools (Revell 2012) in R computational environment R Core Team version 4.1.2 (R Core Team, 2021).

Results

Correlations Between Body Mass, Scapular and Humeral Size and Shape

Body mass was strongly correlated with humeral and scapular centroid sizes but not with the shapes of these structures (Table 2), regardless of whether *Myocastor* was included in the analysis. The centroid size of the scapular and humerus varied between *Carterodon sulcidens* Lund, 1838, *Lonchothrix emiliae* Thomas, 1920, and *Mesomys hispidus* Desmarest, 1817, with the smallest values, and *Myocastor coypus* with the largest value.

Variation in the Shape of the Scapula

The overall variation of scapular shape had low although significant phylogenetic signal ($K_{multi-scapula}$ = 0.488; p < 0.001), with no significant influence of locomotor habit (F < 4.6; p > 0.321; Table 3). The broken-stick model found that only the first four pPCs summarized more information than expected at random, accounting altogether for 79.4% of all scapular measured variation (Table 3). Strong and highly significant phylogenetic signal was found in species distribution along $pPC1_{scapula}$ and $pPC2_{scapula}$, while weaker phylogenetic signal was found in $pPC4_{scapula}$ and non-significant phylogenetic signal was reported for $pPC3_{scapula}$. Although along $pPC1_{scapula}$ and $pPC4_{scapula}$ species with different locomotor habits tended to occupy different regions of morphospace, phyANOVA did not find significant, phylogenetically independent differences between locomotor habits in any of the four major axes of variation. The variation summarized by the first two main components is described in detail below, as they are the only ones to present a strong and highly significant phylogenetic signal. The distribution

Table 2 Allometric analyses with phylogenetic generalized least squares (PGLS) for the scapula and humerus performed including and excluding the genus *Myocastor*

		Centroid size vs. body mass		Procrustes coordinates vs. centroid size		Procrustes coordinates vs. body mass	
		R^2	p	R^2	*p*	R^2	*p*
Including *Myocastor*	Scapula	**0.846**	**< 0.001**	0.046	0.116	0.039	0.200
	Humerus	**0.886**	**< 0.001**	0.013	0.818	0.026	0.446
Excluding *Myocastor*	Scapula	**0.619**	**< 0.001**	0.026	0.427	0.041	0.199
	Humerus	**0.722**	**< 0.001**	0.024	0.575	0.019	0.744

Table 3 Comparative analyses phylogenetically informed with pPC scores, including phylogenetic signal, MANOVA (without phylogenetic information), and phyMANOVA (with phylogenetic information)

	Explained variance	Phylogenetic signal		ANOVA/MANOVA		
		K	*p*	F	*p*	p (phylo)
all.shape.scapula	100%	0.488	**< 0.001**	2.631	**< 0.001**	0.983
pPC1$_{scapula}$	29.808%	0.769	**< 0.001**	4.546	**0.005**	0.321
pPC2$_{scapula}$	22.159%	1.063	**< 0.001**	2.211	0.089	0.628
pPC3$_{scapula}$	19.389%	0.304	0.455	0.544	0.704	0.960
pPC4$_{scapula}$	8.019%	0.625	**0.005**	4.644	**0.004**	0.311
all.shape.humerus	100%	0.908	**< 0.001**	8.759	**< 0.001**	**0.014**
pPC1$_{humerus}$	54.693%	1.673	**< 0.001**	15.845	**< 0.001**	**0.012**
pPC2$_{humerus}$	16.858%	0.880	**< 0.001**	8.013	**< 0.001**	0.090

Phylogenetic signal estimates and F-statistics are provided for the most expressive phylogenetic principal components of each analyzed dataset. Abbreviations: K = Blomberg's K; F = F-statistics; *p* = p-value

of taxa along the morphospace formed by pPC3$_{scapula}$ and pPC4$_{scapula}$ can be found in Online Resource, (7).

The high pPC1$_{scapula}$ scores represented scapulae with narrow supraspinous and infraspinous fossae, long scapular spine, cranially projected coracoid process and infraglenoid tubercle, elongated coracoid process, and a narrow region between acromion and metacromion (Figure 3). Low pPC1$_{scapula}$ scores represented the opposite morphological conformation for these characters. The highest pPC1$_{scapula}$ value was found in *Callistomys* Emmons and Vucetich,

1998, while the lowest one was in *Kannabateomys* Jentink, 1891. It is noteworthy that most major clades were cohesively distributed along the pPC1$_{scapula}$, reflecting strong phylogenetic signal (K = 0.769; *p* < 0.001). Echimyini had lower scores, while Euryzygomatomyinae had higher scores. Myocastorini exhibited a wide distribution, with *Callistomys* and *Myocastor* sharing the highest scores; most Proechimys and *Thrichomys* had intermediate scores, except for *P. goeldii* and *T. apereoides* Lund, 1839, with low scores.
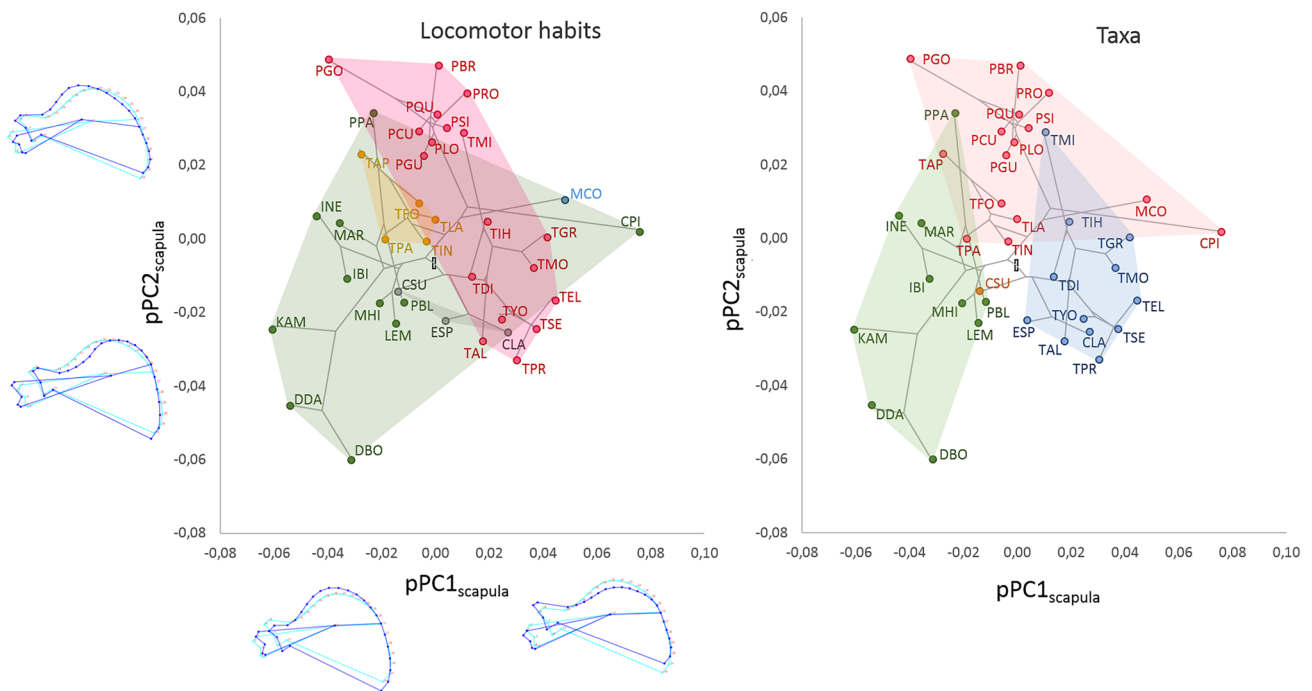
Fig. 3 Phylogenetic principal components analysis (pPCA) of the scapulae. Dark blue wireframes show changes in shape, while light blue wireframes show the average shape. Minimal convex polygons: Left, the distribution of each locomotor habit in morphospace: green = arboreal, yellow = scansorial, blue = semi-aquatic, gray = semi-fossorial, red = terrestrial. Right, the distribution of each taxon in morphospace: green = Echimyini, blue = Euryzygomatomyinae, red = Myocastorini, orange = Carterodontinae

Carterodon Waterhouse, 1848 overlapped with *Thrichomys* and *Echimyini*, with intermediate scores.

The High pPC2$_{scapula}$ scores represented scapulae with a relatively expanded supraspinous fossa and reduced infraspinous fossa, supraspinous fossa more developed in its cranial region and retracted at the vertebral border, scapular spine short and caudally inclined, coracoid process and infraglenoid tubercle cranially projected, elongated coracoid process, and narrowing of the region between the acromion and metacromion (Figure 3). The low pPC2$_{scapula}$ scores represented the opposite morphological conformation. The highest pPC2$_{scapula}$ value was found in *P. goeldii,* while the lowest was in *Dactylomys boliviensis* Anthony, 1920. It is noteworthy that most major clades were cohesively distributed along pPC2$_{scapula}$, reflecting the strong phylogenetic signal (KPC2$_{scapula}$ = 1.063; p < 0.001). Echimyini, especially the bamboo specialist clade, Carterodontinae, and Eurygygomatominae shared mostly low scores, while Myocastorini exhibited mostly high scores, with *P. goeldii* and *P. brevicauda sharing* the highest scores. However, most *Thrichomys* had intermediate scores, except for *T. apereoides*. (Fig. 3).

Variation in the Shape of the Humerus

The overall variation of humeral shape had strong and significant phylogenetic signal (K$_{multi-humerus}$ = 0.908; p < 0.001), and strong and significant influence of locomotor habits (F = 8.759; p = 0.014). The broken-stick model found that the first two pPCs summarized more information than expected at random, accounting altogether for 71.5% of all humeral measured variation (Table 3). Strong and highly significant phylogenetic signal was found in species distribution along both axes. Although along both pPC1$_{humerus}$ and pPC2$_{humerus}$ species with different locomotor habits tended to occupy different regions of morphospace, phyANOVA found significant, phylogenetically independent differences between locomotor habits only along pPC1$_{humerus}$.

The high pPC1$_{humerus}$ scores represented humeri with an elongated diaphysis, reduced epiphyses, poorly developed and proximally positioned deltoid tuberosity (Fig. 4). The low pPC1$_{humerus}$ scores represented the opposite morphological conformation. The highest pPC1$_{humerus}$ value was found in *Euryzygomatomys spinosus* Fischer, 1814 while the lowest was in *Proechimys cuvieri*. Despite the overlap between tribes and subfamilies along pPC1$_{humerus}$, the variation of scores along this axis denoted a strong signal (K = 1.673; p < 0.001). Notably, some taxa were strongly cohesive along with this axis: *Proechimys*, with low scores in most species; Echimyini, with high scores in most species; *Thrichomys*, with intermediate scores in most species; *Trinomys* Thomas, 1921, with intermediate to low scores; and the clade formed by *Clyomys* Thomas, 1916 and *Euryzygomatomys* Goeldi, 1901, with some of the highest scores. It is also noteworthy that some locomotor habits differed along pPC1$_{humerus}$, showing that the distribution of species in the morphospace
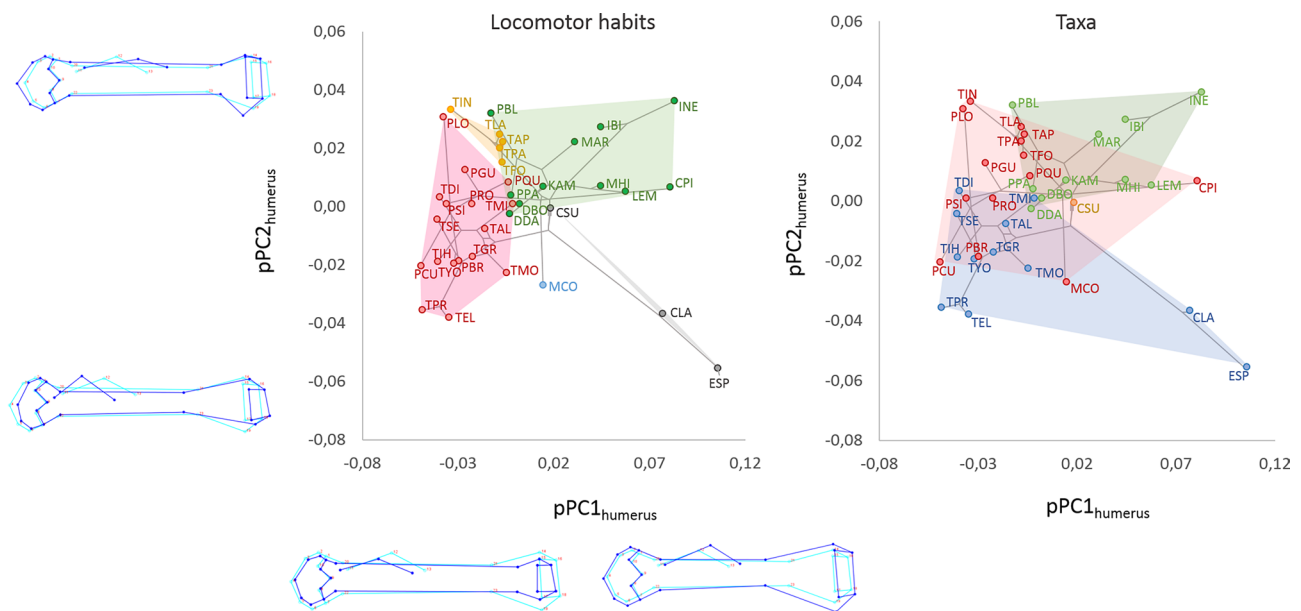

Fig. 4 Analysis of phylogenetic principal components (pPCA) of the humerus. Dark blue wireframes show changes in shape, while light blue wireframes show the average shape. Minimal convex polygons: Left, the distribution of each locomotor habit in morphospace: green = arboreal, yellow = scansorial, blue = semi-aquatic, gray = semi-fossorial, red = terrestrial. Right, the distribution of each taxon in morphospace: green = Echimyini, blue = Euryzygomatomyinae, red = Myocastorini, orange = Carterodontinae

is strongly structured by locomotor habits, as found in the phyANOVA results (F = 15.845; p = 0.012). The semi-fossorial and most arboreal echimyids had high $pPC1_{humerus}$ values while terrestrial echimyids had low scores. Most scansorial scores were intermediate between arboreal and terrestrial along $pPC1_{humerus}$. The high $pPC2_{humerus}$ scores represented humeri with a rounded and proximally projected head, deltoid tuberosity retracted and positioned in the medial region of the diaphysis, short distal epiphysis, a more elongated mid-laterally trochlea, more developed medial epicondyle, and less developed lateral epicondyle. The low $pPC2_{humerus}$ scores represented the opposite morphological conformation. The highest $pPC2_{humerus}$ value was found in *Isothrix negrensis* Thomas, 1920 while the lowest was in *Euryzygomatomys*. It is noteworthy that some of the major clades have differentiated along $pPC2_{humerus}$, reflecting strong phylogenetic signal (K = 0.880; p < 0.001), with Echimyini showing high scores, Euryzygomatomyinae low scores, Carterodontinae intermediate score, and Myocastorini with a wide distribution along this axis. The distribution of scores along $pPC2_{humerus}$ was not significantly structured by locomotor habits according to phyANOVA (F = 8.014; p = 0.090); however, it was noticeable that some arboreal and scansorial species stood out by having relatively high scores, while the semi-fossorial genera *Euryzygomatomys* and *Clyomys* stood out with some of the lowest scores. Part of the terrestrial echimyids, mainly *Proechimys*, overlapped the arboreal ones with intermediate scores. Most species of the genus *Trinomys* had lower scores than the *Proechimys* species. The semi-aquatic *Myocastor* also had low $pPC2_{humerus}$ scores (Fig. 4).

Discussion

Allometric Effects on the Shape of the Scapula and Humerus

The evolution of body size and its allometric consequences on shape have played an important role in ecomorphological diversification in different mammalian taxa, such as artiodactyls, carnivores, primates, and rodents (Marroig and Cheverud 2005; Renaud et al. 2006; Meloro and Raia 2010; Raia et al. 2010; Meloro et al. 2015). Indeed, locomotor specialization through adaptive evolution may be achieved through an allometric change of shape and proportions (Schmidt-Kittler 2002, 2006; Meloro et al. 2015; Sansalone et al. 2018). Often body size and mass are correlated with several morphological, physiological, and life history characteristics in mammals (Biknevicius et al. 1993; Biknevicius 1999; Millien and Bovy 2010; Tavares and Pessôa 2020; Netto and Tavares 2021) and variation in body size is usually associated with biomechanical properties that influence

the morphology and function of the appendicular skeleton (Biewener 2000; Morgan 2009). However, our results show a weak or negligible allometric effect on the shape of the scapula and humerus of echimyids (Table 3).

Our results are congruent with previous studies showing a low or negligible allometric effect on scapular shape variation of small eutherian mammals, such as bats (Gaudioso et al. 2020) and sciuromorph (Wölfer et al. 2019) and caviomorph rodents (Morgan 2009). Even in sciuromorph rodents, where this effect is shown to be somewhat more pronounced than in bats and caviomorphs, only 8.4% of the total variation in scapular shape is explained by variation in body mass (see Table S3 in Wölfer et al. 2019). The allometric effect on the scapula of didelphid marsupials is greater than that found in the eutherian small mammals, being more pronounced in larger species (Astúa 2009). The infraspinous fossa tends to be disproportionately more expanded in didelphids of greater body mass (Astúa 2009). Unlike didelphids, echimyids, as well as other caviomorph and sciuromorph rodents (Morgan 2009; Wölfer et al. 2019), tend to have a relationship between body size and infraspinous and supraspinous fossae sizes close to isometric. All these observations suggest absence of a generalized and conserved allometric pattern in the scapula of small therian mammals, although further studies are needed to verify this hypothesis.

Regarding the humerus, the relationship between body mass and diaphysis circumference remains highly conserved among the major clades of Mammalia, suggesting that the shape of the diaphysis of this weight-bearing bone is only weakly influenced by compressive forces on the limbs (Campione and Evans 2012). Concurrently, Christiansen (1999) found that mammals, on average, do not have limb bones that are disproportionately thicker or shorter than predicted for their body mass. On the other hand, studies restricted to medium and large sized mammals, such as felids and xenarthrans, show that the effect of body size and mass on the architecture of the humerus, especially its epiphyses, can be more evident (Milne et al. 2009; Walmsley et al. 2012; de Oliveira and Santos 2018). In contrast to these findings, among small mammals, the allometric effect is less common among small mammals. Although this effect has already been reported for moles (Sansalone et al. 2018), it should be considered that this the latter is a group with a highly specialized burrowing habit, and thus, the allometric effect may be enhanced by interaction with biomechanical demands of digging (Sansalone et al. 2018). Among muroid and geomyoid rodents, which are far more generalist than moles, this effect was shown to be small, although some sampling bias may have existed (Hedrick 2020). Similarly, in caviomorph rodents, the allometric effect on humerus shape has repeatedly been shown to be very low or negligible (Casinos et al. 1993; Fernández et al. 2000; Steiner-Souza et al. 2010; Morgan and Álvarez 2013). An exception is reported for octodontids, in which the diaphysis length and

thickness scale isometrically, but the position and size of the deltoid tuberosity scale with positive allometry, although the correlation between deltoid tuberosity and forelimb size is low (Pérez et al. 2021).

The allometric effect concentrated in the humeral epiphyses of large mammals may be the result of specific functional demands for support and propulsion of their large body mass since these regions are among the main areas of insertion and muscle origin of the forelimb (Christiansen 1999; Biewener 2000; Campione and Evans 2012; Walmsley et al. 2012). On the other hand, the lack of allometric effect on humeral shape reported here for Echimyidae is congruent with those that have been observed for most other small mammals, as discussed above. Corroborating the assumption that low body mass imposes few structural constraints on the shape of supporting structures, it is noticeable that, similarly to what we observed for the humerus in echimyids and other small mammals, the effect of body size on the shape of lumbar vertebrae is negligible (Álvarez et al. 2013; Netto and Tavares 2021), contrasting with the strong effect on the same structures in large-sized mammalian taxa (Chen et al. 2005; Jones 2015; Randau et al. 2016).

Phylogenetic Effects on Scapular and Humeral Morphology

Our results demonstrated that the morphological variation in scapula and humerus of echimyids reflects the phylogenetic structure of the family. Moreover, the morphology of the humerus also reflects the functional demands associated with different locomotor habits, which was not evident in the scapula.

The significant value of *K*, notably high in the two major variation axes, and the absence of significant differences in the shape of the scapula associated with locomotor habits suggest that, in the spiny rats, this structure evolved approximately according to Brownian motion, without strong selective pressures associated with locomotion being necessary to explain its current variation. This evolutionary scenario is reflected in the unique morphology of some echimyid clades, independently of the locomotor habits of the species that constitute them.

As an example, the myocastorines *Callistomys pictus* Pictet, 1843 and *Myocastor coypus*, recovered as sister taxa by some studies (Fabre et al. 2017; Courcelle et al. 2019), share similar scapula shapes, despite having very distinct body sizes, external morphology, and ecology. The scapular shape of *Callistomys* (Fig. 5o), an arboreal taxon, differs greatly from the scapula of the other echimyids with similar locomotor habits. The other arboreal echimyids form the tribe Echimyini and share a cohesive scapular morphology (Fig. 5e-k). In addition, the terrestrial myocastorines (*Proechimys* spp.; Fig. 5n) have scapulae more
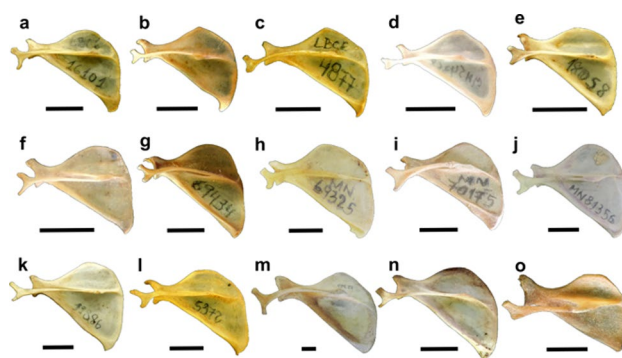

Fig. 5 Echimyidae scapula specimens organized according to the taxa examined. Subfamily Euryzygomatomyinae: **a**, *Trinomys iheringi* LBCE 16,101; **b**, *Euryzygomatomys spinosus* MN 75,752; **c**, *Clyomys laticeps* LBCE 4877. Subfamily Carterodontinae: **d**, *Carterodon sulcidens* MN 54,368. Tribe Echimyini: **e**, *Mesomys hispidus* LBCE 18,058; **f**, *Lonchothrix emiliae* LBCE s/n (no identification); **g**, *Isothrix negrensis* MN 69,434; **h**, *Makalata armata* MN 69,325; **i**, *Phyllomys pattoni* MN 70,175; **j**, *Kannabateomys amblyonyx* MN 81,356; **k**, *Dactylomys boliviensis* LBCE 19,886. Tribe Myocastorini: **l**, *Thrichomys fosteri* LBCE 5372; **m**, *Myocastor coypus* MZUSP 32,353; **n**, *Proechimys quadruplicatus* MN 69,197; **o**, *Callistomys pictus* MZUSP 31,404. Scale bars = 1 cm

like those of the scansorial myocastorines (*Thrichomys* spp.; Fig. 5l) than the other terrestrial echimyids (*Trinomys* spp.; Fig. 5a). Consistently, the scapulae of the semifossorial euryzygomatomyines (*Euryzygomatomys* and *Clyomys*; Fig. 5b-c) are more like those of the terrestrial euryzygomatomyines (*Trinomys*; Fig. 5a) than to that of the semi-fossorial carterodontine (*Carterodon*; Fig. 5d).

Within Echimyini, the small clade of the bamboo rats (*Dactylomys* I. Geoffroy St.-Hilaire, 1838 and *Kannabateomys*; Fig. 5 j-k), previously recognized as the subfamily Dactylomyinae (Emmons et al. 2015b), can also be recognized for exhibiting unique and cohesive morphologies, with an exceptionally well-developed infraspinous fossa. This general pattern suggests strong phylogenetic conservatism in the shape of the scapulae of spiny rats. It is necessary to recognize, however, that sampling of *Callistomys*, *Carterodon* and *Myocastor*, taxa with postcranial skeleton scarcely represented in scientific collections, was limited to few specimens. A larger sample would be needed to verify the robustness of our interpretations regarding phylogenetic or ecological effects on their morphology.

Despite the striking evidence of a strong phylogenetic structure of the scapular shape, it must be pointed out that in many cases (as in Fig. 1) locomotor habits accompany the phylogeny and, therefore, it is not easy to distinguish the influence of these factors to explain the scapular shape. That is, the acquisition of a certain habit early in a clade's history may have constrained the evolution of the scapular form, and later a particular scapular shape (clearly conservative in many mammals) may constrain ecological diversification.

Phylogenetic conservatism in the shape of the scapula, as well as in the muscles attached to it (Arnold et al. 2017), is remarkable in several mammalian groups, including marsupials, primates, rodents, carnivores, and bats (Young 2008; Morgan 2009; Martín-Serra et al. 2014; Gaudioso et al. 2020). Strong phylogenetic signal was also recognized for scapular morphology in other Neotropical small mammals, as didelphid marsupials and sigmodontine rodents (Astúa 2009; Coutinho et al. 2013; Coutinho and Oliveira 2017), being stronger in the former group (Bubadué et al. 2019). Despite conservatism of the scapula, Monteiro and Abe (1999) found a mixed contribution between function and phylogeny in xenarthrans, depending on the observed level. Young (2008) demonstrates that functional demands have a greater impact than the phylogenetic signal in primates.

Unusually, in some very specialized groups of burrowing species, such as Myrmecophagidae, Talpidae, Dasypodidae, Chlamyphoridae, and Chrysochloridae, scapular morphology seems to have been shaped by strong natural selection, showing evolutionary convergence in the elongation of the acromion, the expansion of the caudal angle and other conspicuous modifications (Smith and Savage 1955; Hildebrand and Goslow 2006). However, we did not observe shape changes in the scapulae of the semi-fossorial echimyids as significant as those found in the aforementioned families.

Similar to the scapula, shape variation in the humerus of echimyids exhibited strong phylogenetic signal, in congruence with previous studies with several mammalian groups, including marsupials, primates, rodents, carnivores and bats (O'Neill and Dobson 2008; Walmsley et al. 2012; Fabre et al. 2013b, 2019; Holliday and Friedl 2013; Morgan and Álvarez 2013; Martín-Serra et al. 2014; Janis et al. 2020; López-Aguirre et al. 2021). Even within less inclusive subclades, the phylogenetic structure could be recovered. As an example, within Echimyini, an exclusively arboreal taxon, the subclade formed by *Isothrix* Wagner, 1845 (Fig. 6g) and *Lonchothrix* Thomas, 1920 (Fig. 6f) shared humeri with especially short diaphysis and well-developed distal epiphysis, whereas the clade formed by *Kannabateomys* (Fig. 6j), *Dactylomys* (Fig. 6k), *Phyllomys* Lund, 1839 (Fig. 6l), and *Makalata* Husson, 1978 (Fig. 6h) had longer diaphysis and relatively smaller distal epiphysis. If this morphological difference is also reflected in different biomechanical abilities, it is possible to conjecture that the subclades of Echimyini have distinct locomotor behaviors, which can be further investigated with field and laboratory studies. However, certain biomechanical capacities associated to scapular and humeral shape and thus to particular clades, cannot be ruled out, and may be important to understand the ecomorphological diversification within each clade. Phylogenetic history and adaptive events occurred during that history are not mutually exclusive.

In other mammalian groups, the phylogenetic structure is important to explain even variation in internal humeral
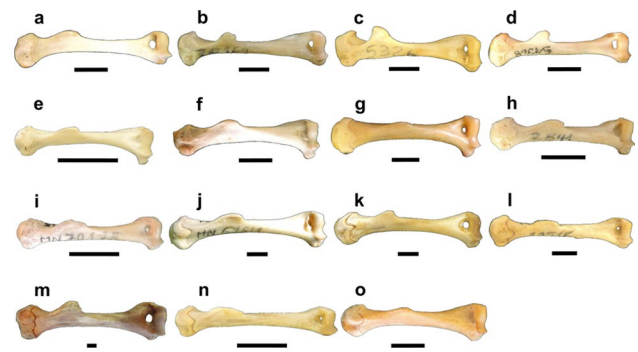

Fig. 6 Echimyidae humerus specimens organized according to the taxa examined. Subfamily Euryzygomatomyinae: **a**, *Trinomys iheringi* LBCE 16,101; **b**, *Euryzygomatomys spinosus* MN 75,752; **c**, *Clyomys laticeps* LBCE 5326. Subfamily Carterodontinae: **d**, *Carterodon sulcidens* MN 54,368. Tribe Echimyini: **e**, *Mesomys hispidus* LBCE 19,843; **f**, *Lonchothrix emiliae* LBCE s/n (no identification); **g**, *Isothrix negrensis* MN 56,811; **h**, *Makalata armata* MN 70,179; **i**, *Phyllomys pattoni* MN 70,175; **j**, *Kannabateomys amblyonyx* MN 61,811; **k**, *Dactylomys boliviensis* LBCE 19,878. Tribe Myocastorini: **l**, *Thrichomys apereoides* LBCE 11,516; **m**, *Myocastor coypus* MZUSP 32,353; **n**, *Proechimys quadruplicatus* LBCE 14,994; **o**, *Callistomys pictus* MZUSP 31,404. Scale bars = 1 cm

architecture, including blood vessel distribution and bone density (Houssaye and Prévoteau 2020; Amson and Bibi 2021). These results warn us that analyses seeking to understand the morpho-functional factors guiding forelimb evolution must consider that phylogenetic history alone can explain a large amount of interspecific morphometric variation. Even forelimb structures strongly associated with functional specializations, such as the humerus, may have much of their interspecific variation explained by shared evolutionary history (Pérez et al. 2021). This consideration becomes especially important when we analyzed the placement of scansorial echimyids in the recovered morphospaces (Fig. 4). For both the humerus and scapula, scansorial echimyids occupied a region intermediate between most terrestrial and arboreal species, which could suggest that their shapes correspond to locomotor specializations, achieved and maintained via natural selection, that allow them to run and to climb. However, even though there are scansorial representatives in the subfamily Capromyinae (not analyzed), it should be noted that the scansorial echimyids in our sample belong to only one genus, *Thrichomys*. Meaning that, in our analysis, the scansorial habit conceivably resulted from a single evolutionary event, making it elusive to identify evolutionary convergences implying that natural selection shaped the form of *Thrichomys*. In addition, it should also be noted that in both recovered morphospaces, *Thrichomys* species were adjacent to *Proechimys*. Considering this, even if it is not possible to rule out an adaptive hypothesis for the shape of the humerus and scapula of *Thrichomys,* it could be argued that its shape is explained by phylogenetic history.

Similar caution should be applied to explain the shape of the semi-aquatic habit in Echimyidae, limited to *Myocastor*.

Ecological Effects on Humerus Morphology

Unlike scansorial and semi-aquatic echimyids, there is consistent evidence that the humeral forms of terrestrial, arboreal, and semi-fossorial echimyids carry morpho-functional specializations partly shaped by natural selection. In these cases, similar locomotor habits have arisen more than once independently within Echimyidae and have been associated with evolutionary convergences or conspicuous changes in morphology, in line with previous studies that point to the humerus as possibly the postcranial structure containing the greatest amount of functional adaptive modifications (Morgan and Verzi 2006; Steiner-Souza et al. 2010; Tavares et al. 2020).

The terrestrial echimyids (*Trinomys* and *Proechimys*; Fig. 6a and n, respectively) have been characterized by elongation and tapering of the diaphysis, in addition to having small epiphyses, and a reduced deltoid tuberosity close to the proximal epiphysis. These features are the same as reported in other terrestrial or cursorial mammals, such as extinct kangaroos (Janis et al. 2020), carnivores (Taylor 1974; Heinrich and Rose 1997; Martín-Serra et al. 2014), felines (Walmsley et al 2012), marsupials (Argot 2001; Szalay and Sargis 2001), procyonids (Tarquini et al. 2017, 2019), rodents (Candela and Picasso 2008; Steiner-Souza et al. 2010; Morgan and Álvarez 2013; Coutinho and Oliveira 2017), tenrecoids (Salton and Sargis 2008), tupaids (Sargis 2002), and various small therian mammals (Janis and Martín-Serra 2020). The elongation of the humeral diaphysis results in a long stride, enabling greater travel speed, which is selectively favored in terrestrial mammals, for example, during an escape from predators (Wilson et al. 2015). In addition, the favoring of speed over strength is associated with the relatively underdeveloped epiphyses in terrestrial echimyids. These regions are the main insertion areas for muscles responsible for stabilizing joints under high stress and for flexing the digits (Woods 1972). These functions require relatively little strength and consequently small muscle insertion area in terrestrial species (Steiner-Souza et al. 2010; Morgan and Álvarez 2013). At the distal epiphysis, terrestrial echimyids tended to have the articular surface mediolaterally compressed, with the capitulum poorly developed, relatively to arboreal echimyids. This feature has also been reported as a locomotor specialization of terrestrial marsupials (Szalay and Sargis 2001), giving them more stability and amplitude of the parasagittal movements of the limbs (Candela and Picasso 2008).

Although less evident than in fossorial species, most species of *Proechimys* and *Trinomys* also shared reduced, flatter

humeral heads compared to arboreal species, as indicated by their low scores along pPC2h$_{umerus}$. This recurrent feature in the humerus of terrestrial mammals restricts and stabilizes humeral movements along a parasagittal plane (Morgan and Álvarez 2013; Janis and Martín-Serra 2020).

The remarkable overlap of *Proechimys* and *Trinomys* in the humeral morphospace highlights that these two phylogenetically distant lineages share similar morpho-functional characters, in agreement with what has been previously reported in external, cranial, dental, femoral, pelvic, and lumbar morphology (Moojen 1948; Carvalho and Salles 2004; Perez et al. 2009; Tavares and Pessôa 2020; Netto and Tavares 2021), probably being thus reached and maintained by occupying similar ecological niches.

The humerus of arboreal echimyids, including *Callistomys* (Fig. 6o) and the Echimyini (Fig. 6e-k), was characterized by a wide diaphysis, intermediate in length between fossorial and terrestrial, well-developed epiphyses, with a larger and more rounded head than in other species, a well-developed medial epicondyle, and a medially wide distal articular surface, with an expanded capitulum approaching the apex of the lateral epicondyle, and the deltoid tuberosity poorly projected in most taxa. In general, these features are convergent with several other groups of arboreal mammals, including caviomorphs (Candela and Picasso 2008; Morgan and Álvarez 2013), sigmodontines (Coutinho and Oliveira 2017), tenrecoids (Salton and Sargis 2008), primates (Rose 1989; Szalay and Dagosto 1980), marsupials (Argot 2001; Szalay and Sargis 2001), and other arboreal therian mammals (Janis and Martín-Serra 2020). The rounded and well-developed humeral head in these animals gives them greater amplitude and stability of movement at the glenohumeral joint, necessary for climbing (Candela and Picasso 2008; Szalay and Sargis 2001; Rose and Chinnery 2004). The highly developed distal epiphysis and the expressive protrusion of the medial epicondyle allow for the insertion of robust forearm pronator and hand flexor muscles, necessary for grasping and climbing movements (Argot 2001; Szalay and Sargis, 2001; Candela and Picasso 2008; Janis and Martín-Serra 2020). The well-developed capitulum likely enhances rotational movements of the radial head during forearm flexion (Candela and Picasso 2008; Szalay and Dagosto 1980; Sargis 2002).

Remarkably, *Callistomys pictus* and *Isothrix negrensis* occupy extreme positions in the humeral morphospace (high pPC2$_{humerus}$ scores; Fig. 4), indicating that morphological characters typical of arboreality are well developed in these species. This result is congruent with previous findings, showing that the femur and lumbar vertebrae of *Callistomys* are among the most specialized within arboreal echimyids (Tavares and Pessôa 2020; Netto and Tavares 2021). At the other extreme among arboreal species are *Phyllomys* and the bamboo rats *Dactylomys* and *Kannabateomys*, whose

humeral morphology was more like that of terrestrial species, especially by having a relatively elongated diaphysis and reduced epiphyses. Congruently, the femurs of bamboo rats and *Phyllomys lamarum* Thomas, 1916 also stood out for having elongated diaphysis and proximal epiphysis more like those of terrestrial species (Tavares and Pessôa 2020). *Dactylomys* and *Kannabateomys* display highly specialized cranial, dental, and external morphology for life into bamboo groves (Perez et al. 2009; Tavares et al. 2016; Candela et al. 2017) and their locomotion differs from other arboreal echimyids, by using the second and third digits to hold firmly onto thin bamboo branches, along which they move smoothly and cautiously (Emmons 1981; Candela et al. 2017). Although it cannot be ruled out that elongated humeri and femora, and scapulae with a wide infraspinous fossa, are phylogenetic traits in *Dactylomys* and *Kannabateomys*, it is reasonable to assume that such features are adaptations for their specialized locomotion, a proposition that should be investigated in the future.

Despite having an intermediate morphology between terrestrial and arboreal echimyids, the humerus of the scansorial *Thrichomys* had more general morphological similarities with that of terrestrial echimyids, overlapping with the latter along the axis of greatest variation ($pPC1_{humerus}$). This greater similarity was concentrated in the elongated diaphysis. The similarity of *Thrichomys* with arboreal echimyids was concentrated in the broad, rounded head. These features suggest that *Thrichomys* species are capable of long and fast steps, favored by terrestrial locomotion, as well as wide movements of the glenohumeral joint, useful for climbing branches and rocks, a habit reported for this genus (Mares and Ojeda 1982; Hannibal et al. 2019).

The humerus of semi-fossorial echimyids had a well-developed deltoid tuberosity, besides having a short, although not markedly thick diaphysis, large and robust epiphyses, especially the distal epiphysis, with both epicondyles well projected. These features are congruent with those found by other authors in burrowing rodents (Candela and Picasso 2008; Steiner-Souza et al. 2010; Morgan and Álvarez 2013; Coutinho and Oliveira 2017; Tavares et al. 2020). A larger deltoid tuberosity is linked to the locomotor habit providing greater attachment of important muscles involved in arm protraction, retraction, and extension (acromiodeltoid, clavodeltoid, and spinodeltoid) and humeral flexion (pectoralis major and pectoralis minor) (Rabey et al. 2015). The shortening of the diaphysis favors strength over velocity and is congruent with the condition in several other fossorial rodents, which increases their resistance to bending and shear stresses during digging, increasing robustness and reducing humerus length (Samuels and Van Valkenburgh 2008; Hopkins and Davis 2009; Coutinho et al. 2013; Morgan and Álvarez 2013). To remove soil while tunneling with their claws, semi-fossorial rodents rely on well-developed muscles of the shoulder and arm retractors, elbow extensors, arm pronators, and carpal and digital flexors (Hildebrand and Goslow 2006; Lagaria and Youlatos 2006; Tavares et al. 2020). Their wider epicondyles suggest a larger area available for the origins of the flexor, pronator, and supinator muscles of the forearm and fingers (Woods 1972; McEvoy 1982; Coutinho et al. 2013; Tavares et al. 2020).

Strikingly, all the aforesaid characteristic traits of semi-fossorial echimyids were more prominent in *Clyomys* and *Euryzygomatomys* than in *Carterodon*, whose humeral shape somewhat resembles that found in arboreal echimyids. This result taken alone may suggest that *Carterodon* is less specialized for digging than semi-fossorial euryzygomatomyines. On the other hand, previous studies have shown that other morphological specializations associated with subterranean life, such as elongation of the olecranon process and enlargement of the auditory bulla, may be more prominent in *Carterodon* than in *Euryzygomatomys* (see Fig. 11 in Verzi et al. 2016; Fig. 4 in Tavares et al. 2020). In addition, the pelvis and penultimate lumbar vertebra of *Carterodon* present morpho-functional characters associated with fossoriality in a more pronounced way than in *Euryzygomatomys* (see Fig. 4 in Tavares and Pessôa 2020; Fig. 5 in Netto and Tavares 2021). This suggests that the different lineages of fossorial rats, Carterodontinae and the tribe Euryzygomatomyini Emmons, 2005 (i.e., *Euryzygomatomys* and *Clyomys*), evolved fossoriality through distinct morphological specializations, despite important evolutionary convergences. It should also be considered that *Carterodon* is poorly sampled in collections and all analyses carried out so far with its postcranial skeleton are based only on a single individual. Therefore, a more detailed understanding of the evolution of fossoriality in Echimyidae will require a larger sample to understand the limits of variation in this monospecific genus.

Conclusion

Our analyses showed that allometry is not an important active factor in the morphological evolution of the scapula and humerus of echimyids, similar to what has already been reported for other caviomorphs and other small mammals. Furthermore, the present study evidenced that phylogeny and locomotor habits are reflected in different ways on the scapular and humeral morphology. While phylogeny proved important in structuring the morphometric variation of both structures, locomotor habits seem to have been a relevant factor only in the differentiation of the humerus. The strong phylogenetic effect and the absence of the influence of locomotor habits on scapular morphology could make this structure a relevant taxonomic and phylogenetic marker in future studies. On the other hand, the presence of

morpho-functional markers on the humerus shape makes it remarkably useful for studies that seek to reconstruct life habits from postcranial morphological data, especially paleontological investigations aiming at a better perspective on the evolution of ecomorphological disparity in caviomorphs. It is also worth emphasizing the importance of preserving postcranial structures to obtain larger sample sizes, thus increasing the robustness of these results with additional comparative analyses.

Data Availability The datasets generated and analyzed during the current study are not publicly available as they are being continuously analyzed for further studies and are available from the corresponding author upon reasonable request.

Declarations

Conflicts of Interest/Competing Interests The authors declare no conflict of interest.

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