

The humerus of South American caviomorph rodents: shape, function and size in a phylogenetic context

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Abstract

South American caviomorph rodents comprise four major lineages encompassing wide taxonomic and ecological diversity, but the morphological diversity of their postcranial skeleton has not been thoroughly explored using phylogenetic comparative methods. The main goal of this work is to analyze their humerus using geometric morphometrics in a phylogenetic context and attempt to tease apart the influence of locomotory preferences and shared evolutionary history on morphological variation. We examined 28 genera in 9 families representing all major clades. Humeral shape was captured by 13 landmarks and four semilandmarks in 2D. In the morphospace of the first two principal components, most taxa were located near the origin along both axes. Fossorial octodontoids were apart from this central group. Most caviids were separated in one extreme of the morphospace; the specialized digging ctenomyid *Ctenomys*, the fossorial chinchillid *Lagostomus* and the generalized cavioid *Cuniculus* were located at the opposite end. Phylogenetic signal was significant. Regressions of shape on size were not significant; regression of shape on habit was significant for raw data and not significant after phylogenetic control. Humeral shape variation was primarily associated with the phylogenetic structure of the group; additionally, some morphological traits were associated with particular habits and interpreted as functional specializations. This association between humeral shape and both phylogeny and habit at different hierarchical levels suggests early ecomorphological diversification of caviomorphs.

Introduction

As a major component of the forelimb skeleton, the humerus provides much functional information. The strong link between humeral morphology and locomotory and substrate preferences has been widely recognized; for example, runners tend to have longer, gracile humeri with narrow distal epiphyses, while diggers have shorter, more robust humeri with well-developed deltopectoral crest and broad distal epiphyses (see Hildebrand, 1988; Polly, 2007 and literature cited therein). Morphological variation is also expected to reflect shared evolutionary history (Felsenstein, 1985; Losos & Miles, 1994). Thus, humeral shape results from a complex interaction of factors, and phylogenetic context is essential to analyze and understand putative morphological adaptations.

South American hystricomorph rodents (caviomorphs) are an excellent model to analyze such interactions, as the clade encompasses wide taxonomic and ecological diversity, distributed in four major lineages traditionally considered as superfamilies: Erethizontoidea, Chinchilloidea, Octodontoidea and Cavoidea (Woods & Kilpatrick, 2005). They occupy different habitats and present varied habits: climbers such as the ereth-

izontoid *Coendou* (prehensile-tailed porcupine) and the octodontoids *Phyllomys* and *Dactylomys* (spiny rats); cursors, as the cavioids *Dasyprocta* (agouti) and *Dolichotis* (mara); diggers including the chinchilloid *Lagostomus* (viscacha) and the specialized subterranean octodontoids *Ctenomys* (tucutuco) and *Spalacopus* (coruro); as well as generalized epigeal forms that climb, dig and swim to some extent, such as the cavioids *Cuniculus* (paca) or *Microcavia* (mountain cavies). Their size also ranges widely from the small coruros, tucutucos and some spiny rats (with a body mass as low as 80 g) medium-sized forms such as *Myocastor* (coyupu; 7 kg), *Dolichotis* (12 kg) or *Lagostomus* (6.5 kg), to the largest living rodent, the capybara *Hydrochoerus* (53 kg; Nowak, 1991).

The postcranial skeleton of caviomorphs has been analyzed following different approaches (Lehmann, 1963; Biknevičius, 1993; Vassallo, 1998; Rocha-Barbosa *et al.*, 2002, 2007; Weisbecker & Schmid, 2007; Samuels & Van Valkenburgh, 2008; Seckel & Janis, 2008; Morgan, 2009; Morgan & Verzi, 2011; Rocha-Barbosa & Casinos, 2011). In particular, Elissamburu & Vizcaíno (2004) and Candela & Picasso (2008) studied the humerus of a wide taxonomical sample, while Morgan & Verzi (2006), Steiner-Souza, de Freitas &

Cordeiro-Estrela (2010) and Elissamburu & De Santis (2011) focused on the specialized subterranean genus *Ctenomys* and related taxa; in each case, proposing adaptive explanations for the variation found. However, apart from Morgan (2009), there has been no attempt to tease apart the influence of phylogeny and ecological factors on the morphological variation of the caviomorph skeleton. Thus, the main goal of this work is to perform such an analysis of humeral shape using geometric morphometrics in a phylogenetic context. We first generate a phylogenetic hypothesis using molecular data, and then assess the contribution of phylogeny, locomotor mode and size to humeral shape variation in caviomorphs. Possible functional interpretations are discussed in a phylogenetic framework.

Materials and methods

We examined 28 genera in nine families (94 specimens, Table 1), including representatives of the four major lineages of caviomorphs and of their ecological and morphological diversity (more than 80% of living caviomorph families and about 47% of living genera, Table 2).

Phylogenetic relationships among genera were studied through Bayesian inference methods. Sequences from growth hormone receptor (GHR; 856 bp), transthyretin hormone (TTH; 1142 bp), mitochondrial subunit 12S (12S; 992 bp) and cytochrome *b* (cytb; 1141 bp) genes were obtained from GenBank (accession numbers in Supporting Information Appendix S1). Genes were selected on the basis of their variation in evolutionary rates (mitochondrial vs. nuclear) and their availability for the studied taxa. jModelTest 0.1 (Posada, 2008) was employed to determine the most appropriate model of sequence evolution for each gene; the best fit model for all genes was GTR + G. The Bayesian Inference method was implemented using MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003). Two simultaneous analyses were run using the algorithm MCMC (Markov chain Monte Carlo) with 10 000 000 generations; sampling frequency was 1000 and burn-in was set at 25%.

Morphological variation of the humerus was analyzed through geometric morphometric techniques. Specimens were photographed in anterior view; each specimen was placed with the plane formed by the diaphyseal axis and the transepicondylar axis (Boileau & Walch, 1999) parallel to the camera lens, and with a ruler to record scale. Thirteen landmarks and four semilandmarks (Fig. 1) were digitized using the software tpsDig 2.16 (Rohlf, 2010); all landmarks were digitized by the same person (C. C. M.) and measurement error was assessed by the Procrustes analysis of variance (ANOVA) method (Klingenberg & McIntyre, 1998). The landmark + semilandmark configurations were superimposed by Generalized Procrustes Analysis (Goodall, 1991; Rohlf, 1999) to remove differences in location, orientation and scaling (i.e. nonshape variation); semilandmarks were slid using the minimum bending energy criterion (Bookstein, 1997; Adams, Rohlf & Slice, 2004) using tpsRelw 1.49 (Rohlf, 2010). Centroid sizes were saved for subsequent analyses. A principal component analysis of the aligned Procrustes coordinates averaged by

genus was performed to explore shape variation among caviomorph genera, using the software MorphoJ 1.04a (Klingenberg, 2011).

The influence of phylogeny on shape variation was evaluated using the univariate *K* statistic (Blomberg, Garland & Ives, 2003). We also tested the phylogenetic signal of all shape variation, that is, the Procrustes shape coordinates, through the multivariate tree length test (Laurin, 2004; Klingenberg & Gidaszewski, 2010). Significance of both statistics was assessed through 10 000 permutations. Analyses of phylogenetic signal were performed using the Picante package (Kembel *et al.*, 2010) for R (ver. 2.11.1, R Development Core Team, 2009) and MorphoJ software (Klingenberg, 2011), respectively.

To analyze the association between humeral morphological variation and ecology, we built variables for two factors closely associated with the latter: size and habit (Hildebrand, 1985; Reilly & Wainwright, 1994) and analyzed their relationship with shape variation by ordinary least squares (OLS) regression analyses. As size variable, we used the log-transformed centroid size averaged for each genus; centroid size is used in geometric morphometrics as a measure of size that is uncorrelated with shape for small isotropic landmark variation (Mitteroecker & Gunz, 2009). The habit variable was built from information available in the literature (sources detailed in Table 2), with four habit categories represented by dummy variables. Because habit categories are not exclusive and most caviomorphs are not greatly constrained to any particular locomotor mode (Elissamburu & Vizcaíno, 2004), those genera in which the relative involvement of the forelimb in running (cursorial), digging (fossorial) and/or climbing (scansorial/arboreal) activities is not predominant were classified as generalized ('ambulatory'). The arboreal *Coendou* was pooled with scansorial taxa in a single habit category. The first three principal components (PC) of the shape analysis (84.43% of the total variation) were retained as shape variables, based on both the Kaiser–Guttman and broken-stick criteria (Legendre & Legendre, 1998). Regressions were made using raw data (OLS) and phylogenetic independent contrasts (PICs), to take into account the expected lack of independence among samples resulting from phylogenetic structure (Felsenstein, 1985; Rohlf, 2001). Regression significance was assessed using permutation tests (10 000 rounds). The amount of variation accounted for by each regression model was expressed as a percentage of total variation, computed using the Procrustes metric (Drake & Klingenberg, 2008). All regression analyses were made in MorphoJ. Morphofunctional interpretations were based on previous proposals (Hildebrand, 1985; Price, 1993; Vassallo, 1998; Elissamburu & Vizcaíno, 2004; Morgan & Verzi, 2006; Candela & Picasso, 2008).

Results

Phylogeny

The phylogeny obtained (Fig. 2) was congruent with previous partial hypotheses (Rowe & Honeycutt, 2002; Honeycutt,

Table 1 Specimens examined for this work

Family	Species	Specimens
Abrocomidae	<i>Abrocoma</i> sp.	MLP 2038
Caviidae	<i>Cavia aperea</i>	MLP 29.XII.00.15, 5.VI.00.8
	<i>Dolichotis patagonum</i>	MLP Prov. 213, 236, 250, 275, 642
	<i>Galea leucoblephara</i>	CML 3430, 3432, 4875, 7136, 7240; MLP 1928, 1929
	<i>Hydrochoerus hydrochaeris</i>	CZV1, CZV2, MACN 14038, 43.43, MLP 1915
	<i>Microcavia australis</i>	MLP 7.VI.99.7, CML 7237
Chinchillidae	<i>Chinchilla</i> sp.	MLP 31.XII.02.37
	<i>Ch. chinchilla boliviana</i>	MACN 13037
	<i>Lagidium</i> sp.	MACN 14.16, 14.17, 34–263, 44.25
	<i>L. viscacia</i>	CML 5099, MACN 7350
	<i>Lagostomus maximus</i>	MACN 21983, 23.14, 39.92
Ctenomyidae	<i>Ctenomys australis</i>	MLP 7.XI.95.6
	<i>C. flamarioni</i>	MLP 28.V.01.5
	<i>C. frater</i>	CML 7241
	<i>C. sylvanus</i>	CML 7235
	<i>C. lewisi</i>	CBF 2280
	<i>C. opimus</i>	CML 7130, 7244
	<i>C. rionegrensis</i>	412, CA 393; EV 1137
	<i>C. talarum</i>	MLP 1.VIII.00.11, 1.VIII.00.14
	<i>Cuniculus paca</i>	MACN 22.5, 49396
	<i>Dasyprocta azarae</i>	MACN 1.11
Dasyproctidae	<i>D. punctata</i>	CML 4695
	<i>Echimyidae</i>	
Echimyidae	<i>Clyomys laticeps</i>	MN 63851, 63853, 68967
	<i>Dactylomys dactylinus</i>	MVZ 190620
	<i>Euryzgomatomys spinosus</i>	MLP 16.VII.02.11
	<i>Isothrix bistrata</i>	MVZ 190629
	<i>Mesomys hispidus</i>	MVZ 190653
	<i>Myocastor coypus</i>	MLP 20.XII.89.15; MACN 14098, 15.20, 30.70
	<i>Phyllomys pattoni</i>	MVZ 183139
	<i>Proechimys breviceuda</i>	MVZ 153623
	<i>P. poliopus</i>	MLP 22.II.00.7, 22.II.00.8
	<i>P. roberti</i>	MVZ 197578
	<i>Thrichomys apereoides</i>	MN 34406, 61659; UNB 190
	<i>Trinomys dimidiatus</i>	MN 62273, 62278
	<i>Coendou</i> sp.	MACN 6.34, 30.243
Erethizontidae	<i>Aconaemys porteri</i>	MLP 17.II.92.07, 17.II.92.2, 17.II.92.3, 17.II.92.4
	<i>A. sagei</i>	MLP 17.II.92.09, 17.II.92.10
Octodontidae	<i>Octodon</i> sp.	MLP 12.VII.88.2, 12.VII.88.3, 12.VII.88.5, 12.VII.88.7
	<i>Octodontomys gliroides</i>	CML 2872, 7137, 7138, 7140, 7144, 7145, 7146, 7148; MMPMa 4027
	<i>Octomys mimax</i>	CM 06847
	<i>Spalacopus cyanus</i>	MLP 10.XI.95.5

Institutional abbreviations: CA, EV, Laboratorio de Evolución, Facultad de Ciencias, Universidad de la República, Uruguay; CML, Instituto 'Miguel Lillo', Tucumán, Argentina; MLP, Museo de La Plata, Argentina; CZV, Cátedra Zoología III Vertebrados, FCNyM, UNLP, Argentina; MACN, Museo Argentino de Ciencias Naturales 'Bernardino Rivadavia', Buenos Aires, Argentina; MMPMa, Museo Municipal de Ciencias Naturales 'Lorenzo Scaglia', Mar del Plata, Argentina; MN, Museu Nacional, Rio de Janeiro, Brazil; MVZ, Museum of Vertebrate Zoology, Berkeley, USA; UNB, Universidade de Brasília, Brazil.

Taxonomic arrangement follows Woods & Kilpatrick (2005) except for *Galea leucoblephara* (Dunnum & Salazar-Bravo, 2010).

Rowe & Gallardo, 2003; Spotorno *et al.*, 2004; Blanga-Kanfi *et al.*, 2009) and with Upham & Patterson's (2012) more encompassing analysis, with some discrepancies probably due to the fact that these authors used a partially different set of genes. The traditionally recognized clades within each superfamily were recovered: Abrocomidae, Octodontidae,

Ctenomyidae and Echimyidae (Octodontoidea), Caviidae, Dasyproctidae and Cuniculidae (Cavioidea), Chinchillidae (=Chinchilloidea in this case) and Erethizontidae (=Erethizontoidea). Posterior probabilities were moderate to high (>0.74); estimated parameters for Bayesian trees are given in Supporting Information Appendix S2.

Table 2 Habit categories used in this study for analyzed taxa

Taxon	Habit (for the genus)	Body mass (g)	Taxon	Habit (for the genus)	Body mass (g)
Cavioidea			Octodontoidea		
Caviidae			Abrocomidae		
<i>Cavia aperea</i>	Ambulatory ^a	650 ¹	<i>Abrocoma</i> sp	Ambulatory ^a	148 ²
<i>Microcavia australis</i>	Digging ^{h,i}	275 ¹	Octodontidae		
<i>Galea leucoblephara</i>	Ambulatory ⁱ	235 ¹	<i>Aconaemys sagei</i>	Digging ^a	116 ³
<i>Dolichotis patagonum</i>	Cursorial ^b	12 000 ¹	<i>Spalacopus cyanus</i>	Digging ^a	140 ⁴
<i>Hydrochoerus hydrochaeris</i>	Cursorial ^a	53 000 ¹	<i>Octodon degus</i>	Digging ^a	150 ⁵
Dasyproctidae			<i>Octodontomys gliroides</i>	Ambulatory ^f	90 ³
<i>Dasyprocta azarae</i>	Cursorial ^a	2700 ¹	<i>Octomys mimax</i>	Ambulatory ^a	103 ⁶
Cuniculidae			Ctenomyidae		
<i>Cuniculus paca</i>	Ambulatory ^{a,e}	11 000 ¹	<i>Ctenomys talarum</i>	Digging ^a	140 ⁷
Erethizontoidea			Echimyidae		
Erethizontidae			<i>Clyomys laticeps</i>	Digging ^c	172 ¹⁰
<i>Coendou prehensilis</i>	Scansorial ^a	4500 ¹	<i>Dactylomys dactylinus</i>	Scansorial ^d	750 ¹⁰
Chinchilloidea			<i>Euryzgomatomys spinosus</i>	Digging ^c	185 ¹⁰
Chinchillidae			<i>Isothrix negrensis</i>	Scansorial ^a	410 ¹⁰
<i>Chinchilla lanigera</i>	Ambulatory	435 ¹	<i>Mesomys hispidus</i>	Scansorial ^{a,d}	160 ¹⁰
<i>Lagidium viscacia</i>	Ambulatory	1500 ¹	<i>Myocastor coypus</i>	Digging ^a	7000 ¹
<i>Lagostomus maximus</i>	Digging ^a	6500 ¹	<i>Phyllomys pattoni</i>	Scansorial ^{c,d}	212 ¹⁰
			<i>Proechimys albispinus</i>	Ambulatory ^a	185 ⁸
			<i>Thrichomys apereoides</i>	Ambulatory ^a	340 ⁹
			<i>Trinomys albispinus</i>	Ambulatory ^{a,d}	175 ¹⁰

Sources of habit information: ^aNowak (1991); ^bSeckel & Janis (2008); ^cEisenberg & Redford (1999), ^dBonvincino, de Oliveira & D'Andrea (2008); ^ePérez (1992); ^fLessa *et al.* (2008); ^gSobrero *et al.* (2010); ^hUbilla (2008); ⁱEbensperger & Blumstein (2006).

Sources of body mass information: ¹Canevari & Vaccaro (2007); ²Data from specimen labels (IADIZA); ³Vassallo & Echeverría (2009); ⁴Torres-Mura & Contreras (1998); ⁵Woods & Boraker (1975); ⁶Sobrero *et al.* (2010); ⁷Vassallo (1998); ⁸Pessôa & dos Reis (2002); ⁹dos Reis & Pessôa (2004); ¹⁰Bonvincino, de Oliveira & D'Andrea (2008).

The 'scansorial' category also includes the arboreal *Coendou* (see Materials and Methods). Mean body mass is expressed in grams; values correspond to species included in these analyses (when such data were not available, the value corresponds to a congeneric species). The systematic arrangement follows Woods & Kilpatrick (2005) except for *Myocastor*, which is placed within the Echimyidae following Galewski *et al.* (2005).

Morphometric analyses

Measurement error

According to the Procrustes ANOVA, the level of measurement error is negligible compared to individual variation (Supporting Information Appendix S3).

Principal components analysis

The first two PC summarized 77.43 % of the shape variation (PC1 = 61.04%; PC2 = 16.39%). In the morphospace of these first two PC (Fig. 3), most of the taxa were located near the origin along both axes. Within this central space, the scansorial echimyids *Phyllomys* and *Mesomys* were close to each other and to the cursorial cavioid *Hydrochoerus*. Outside of this sector, the fossorial octodontoids (the echimyids *Clyomys* and *Euryzgomatomys*, and the octodontids *Octodon*, *Spalacopus* and *Aconaemys*) formed a distinct group. Along the main axis of variation (PC1), the caviids (minus *Hydrochoerus*) occupied extreme positive values, while the specialized digging ctenomyid *Ctenomys*, the fossorial chinchillid *Lagostomus* and the generalized cavioid *Cuniculus* were located at

the negative end. *Ctenomys* also occupied the extreme negative values of PC2 along with the semiaquatic occasional digger *Myocastor*, while the extreme positive values along this axis corresponded to *Cuniculus* and another cavioid, the cursorial *Dasyprocta*.

The taxa distributed in the central portion of the morphospace share a humeral morphology characterized by moderately slender diaphysis, proximal epiphysis with medium-sized tuberosities and humeral head almost level with the greater tuberosity, distal epiphysis with moderate entepicondyle development, and a deltoid process located on the proximal third of the diaphysis. In comparison, the humeri of fossorial octodontoids (*Clyomys*, *Euryzgomatomys*, *Octodon*, *Spalacopus* and *Aconaemys*) are more robust, with relatively broader proximal and distal epiphyses. The scansorial *Phyllomys* and *Mesomys* also show more robust humeri, with the deltoid process extending more distally along the diaphysis. The humeri of the taxa located at extreme positive values of PC1 have narrow epiphyses, a high greater tuberosity that surpasses the level of the articular head, and a relatively more proximal deltoid process. The taxa with the most negative values along PC1 have broader epiphyses, especially the entepicondyle, and a more distally extended deltoid process.

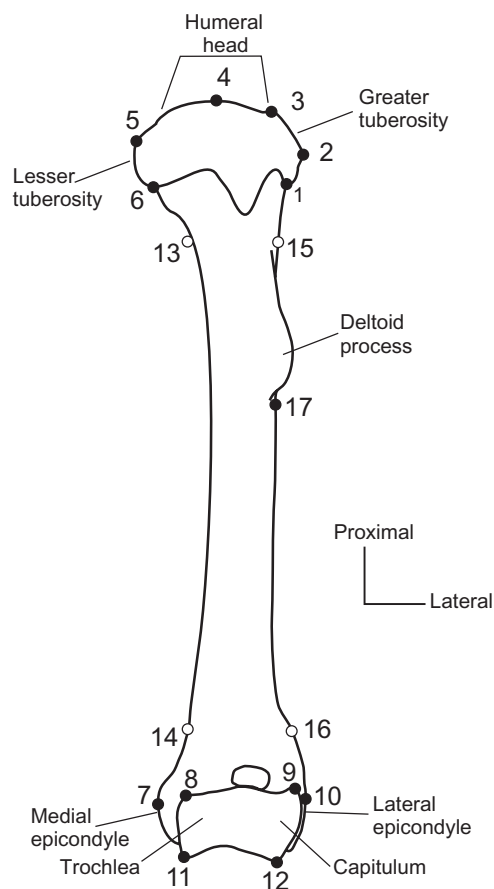


Figure 1 Schematic representation of humerus in anterior view showing placement of landmarks (full circles) and semilandmarks (empty circles). Major anatomical features discussed in the text are indicated. Definition of landmarks: 1, suture between diaphysis and proximal epiphysis on the lateral side; 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, suture between diaphysis and proximal epiphysis on the medial side; 7, most extreme point on medial epicondyle; 8, dorsomedial extreme of trochlea; 9, dorsolateral extreme of capitulum; 10, most extreme point on lateral epicondyle; 11, ventromedial extreme of trochlea; 12, ventrolateral extreme of capitulum; 13–16, semilandmarks along curvature of humeral diaphysis; 17, end of deltoid process.

These traits are associated with two distinct morphologies: one corresponds to *Ctenomys*, *Myocastor* and *Lagostomus*, with markedly more robust humeri (negative scores on PC2), and the other to *Cuniculus*, with a more slender humerus (extreme positive values on PC2).

Phylogenetic signal

Shape data displayed significant phylogenetic signal ($P = 0.002$); the three PCs had significant phylogenetic signal

(Table 3). Consequently, subsequent analyses were made using both raw data and PICs to account for phylogenetic structure.

Regression analyses

The regressions of the first three PC on logCS were not significant, neither for raw values (OLS, % predicted variation = 6.58%, $P = 0.155$) nor for independent contrasts (PIC, % predicted variation = 10.95%, $P = 0.072$). In contrast, the regression of raw shape variables on habit was significant (OLS, % predicted variation = 26.70%, $P = 0.021$), but not after phylogenetic control (PIC, % pred. variation = 24.07%, $P = 0.057$).

Discussion

The considerable humeral shape variation found in caviomorphs was primarily associated with their phylogenetic structure (Supporting Information Fig. S1); nevertheless, beyond this, some morphological traits could be associated with particular habits and therefore interpreted as specializations. Thus, it is possible to characterize the typical humeral morphology of each lineage while recognizing variations linked to adaptive differences.

The position of octodontoids in the morphospace reflects their moderately to very robust humeri with well-developed tuberosities and medial epicondyle. The generalized taxa ('ambulatory' category, e.g. *Proechimys*, *Octomys*) present a more gracile humerus with relatively less developed processes. The scansorial taxa (e.g. *Phyllomys*, *Isothrix*), as well as the fossorial genera (e.g. *Octodon*, *Spalacopus*; *Euryzygomatomys*, *Myocastor*), show greater robustness and more developed sites for muscle attachment, especially *Ctenomys*, the living caviomorph with greatest digging specialization (Lessa *et al.*, 2008; Morgan & Verzi, 2011). The position of the deltoid process also varies from being located on the proximal third of the diaphysis in the generalized octodontoids, relatively more distal in *Mesomys*, *Phyllomys* and *Isothrix* (scansorial) and *Myocastor* (fossorial), and almost reaching the middle of the diaphysis in *Ctenomys*. Greater robusticity provides resistance to the loads imposed by muscular action and substrate resistance during scratch-digging (Stein, 2000). Similarly, well-developed humeral tuberosities reflect powerful stabilizing shoulder muscles to withstand the forces applied across this joint during digging (Argot, 2001; Sargis, 2002). In any case, the humeral tuberosities of octodontoids never surpass the level of the humeral head, thus allowing wide range of shoulder movements (Szalay & Sargis, 2001), especially in the generalized and scansorial taxa. In both digging and scansorial taxa, the expanded medial epicondyle provides a more extensive surface for carpal and digital muscles that provide more powerful flexion of the wrist and digits during scratch-digging and ensure a strong grasp during climbing (Hildebrand, 1985; Van Valkenburgh, 1987; Argot, 2001; Sargis, 2002). Similarly, the distal extension of the deltoid process observed in scansorial and some fossorial taxa enhances mechanical advantage by increasing the in-lever arm (i.e. distance from muscle

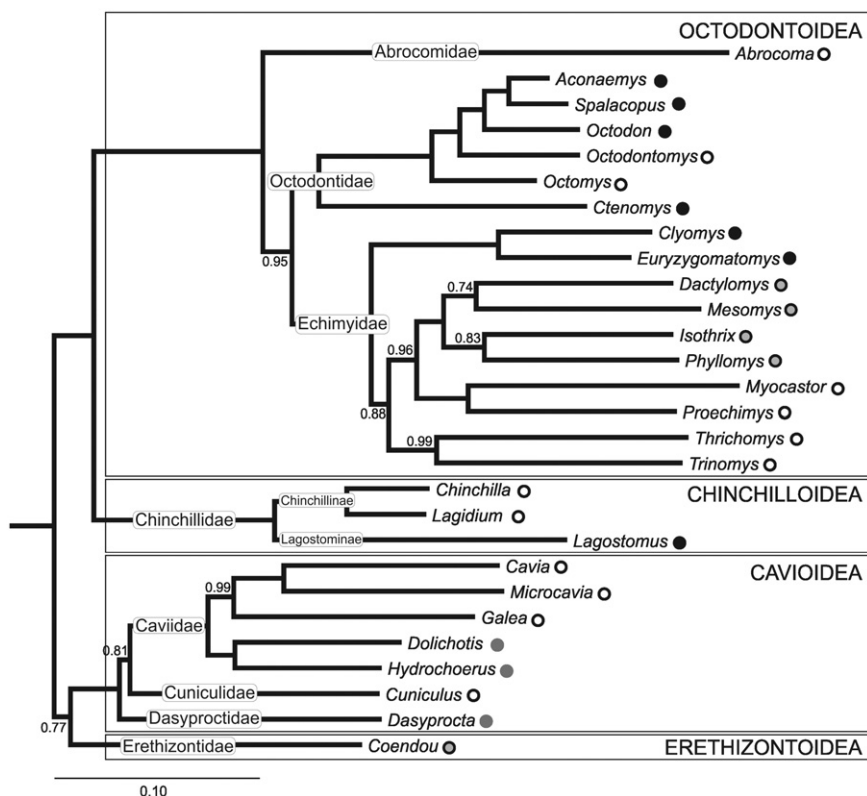


Figure 2 Phylogenetic relationships of extant South American caviomorph rodents included in this study. Superfamilial and familial clades are indicated. Branch lengths represent molecular substitutions. Posterior probabilities different from 1 are indicated on corresponding nodes. Topology is congruent with previous analyses (Huchon & Douzery, 2001; Rowe & Honeycutt, 2002; Honeycutt *et al.*, 2003; Ledesma *et al.*, 2009). Circles beside each taxon indicate habit: full gray, cursorial; full black, digging; full white, ambulatory; black border with gray fill, scansorial (including arboreal). Scale: substitutions per site.

attachment to joint) of the deltoid and pectoral muscles that contribute to forelimb retraction (Hildebrand, 1985; Fernández, Vassallo & Zárate, 2000; Stein, 2000).

The two subfamilies of Chinchillidae show divergent morphologies: the humerus of chinchillines *Lagidium* and *Chinchilla* is gracile and shares the morphospace of the generalized octodontoids, while that of the lagostomine *Lagostomus* is more similar to that of the digging octodontoids. This disparity between the clades also agrees with their different habits: chinchillines use a half-bounding gait to move across rocky terrain (Lammers & German, 2002) that is not likely to exert strong demand on the humerus, while the fossorial viscachas construct communal burrow systems presumably using their forelimbs (Fernández, 1949), and are thus under similar mechanical requirements as other scratch-diggers.

The distinctive humeral morphology of cavioids is reflected by their position in the morphospace, mostly separated from other caviomorphs. In general, their humeri are comparatively slenderer, with narrow epiphyses and strongly developed greater tuberosity that surpasses the level of the humeral head. The position of the deltoid process varies from markedly proximal in the caviine caviids (*Cavia*,

Microcavia and *Galea*) to similar to that of most other caviomorphs in *Dasyprocta*, *Dolichotis* and *Hydrochoerus*. *Cuniculus* is quite different from the remaining cavioids: the humerus is more robust, with a markedly distal deltoid process. In morphofunctional terms, the large greater tuberosity restricts the range of shoulder movements to the parasagittal plane; such restriction is characteristic of cursorial species (Argot, 2001; Sargis, 2002; Salton & Sargis, 2008). Concurrently, the proximally located deltoid process optimizes speed over force production, as expected for cursorial forms (Hildebrand, 1985; Polly, 2007). However, this structure is relatively more distal in *Hydrochoerus*; this may be related to different mechanical requirements linked to the large size of this rodent. The robust humerus of *Cuniculus*, with well-developed distal epiphysis and more distally extended deltoid process, may reflect the wide functional spectrum of this genus, which includes running, swimming and digging (Pérez, 1992). Likewise, the greater robusticity observed in *Microcavia* compared to other caviines could also be associated with its digging habit.

The only erethizontoid included in this analysis, the arboreal *Coendou*, was close in the morphospace to the scansorial

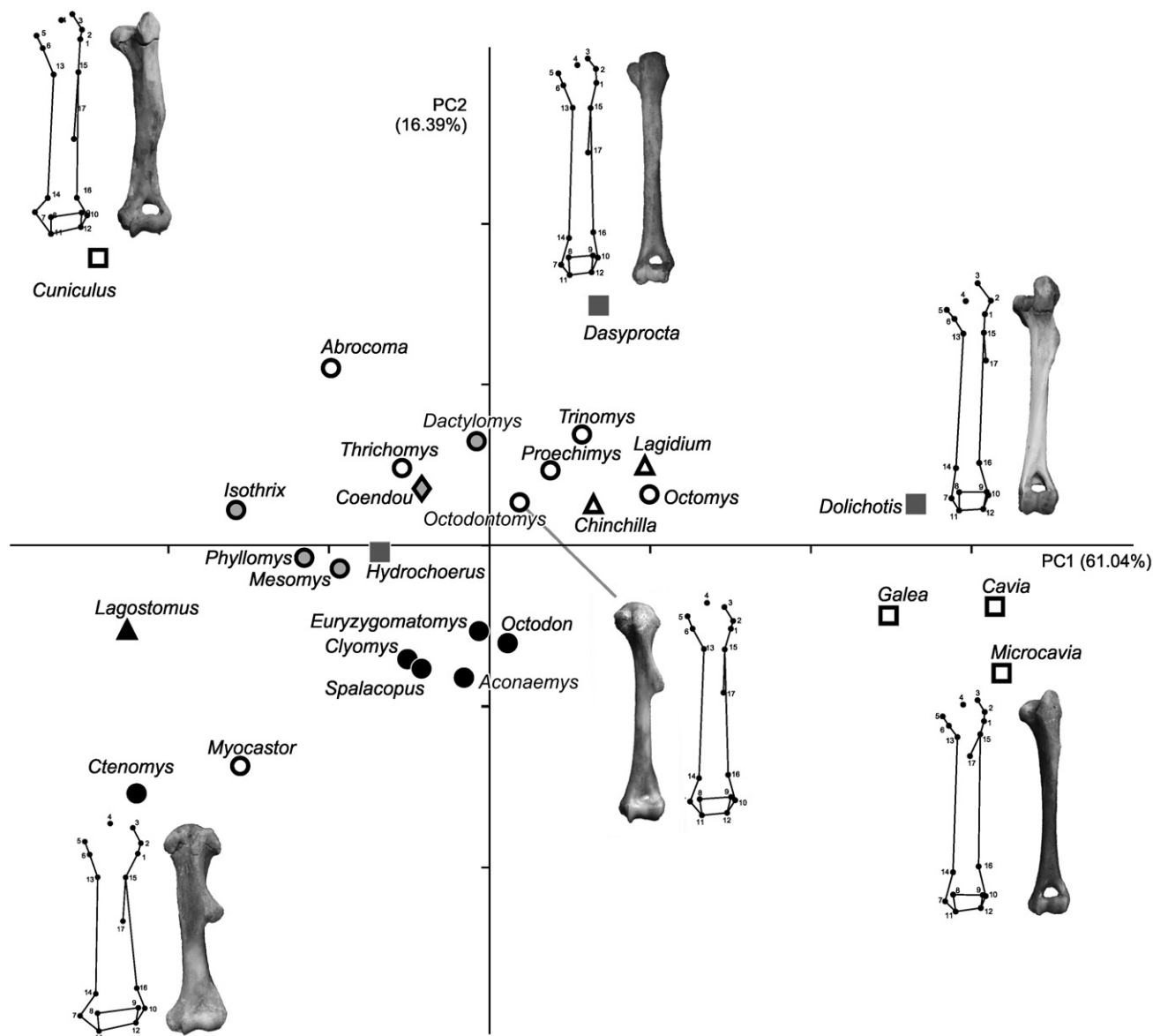


Figure 3 Ordination of 28 extant caviomorph genera in the morphospace defined by the first two principal components (PCs). Symbols represent caviomorph superfamilies: triangles, Chinchilloidea; circles, Octodontidae; diamonds, Erethizontidae; squares, Caviidae. Colors indicate habit: full gray, cursorial; full black, digging; full white, ambulatory; black border with gray fill, scansorial (including arboreal).

Table 3 Eigenvalues, per cent of explained variation and Blomberg’s *K* statistic (Blomberg *et al.*, 2003) of phylogenetic signal for the first three principal components

PC	Eigenvalue	% explained variation	Cumulative %	Blomberg’s <i>K</i>
1	0.00207	61.04	61.04	0.77 ($P < 0.01$)
2	0.00055	16.39	77.43	0.87 ($P < 0.05$)
3	0.00024	7.01	84.43	0.89 ($P < 0.01$)

and some generalized taxa. Its humerus shows low tuberosities, moderately developed medial epicondyle and deltoid process on the proximal third of the diaphysis. *Coendou* is a tree dweller, with a prehensile tail that reflects its arboreal specialization (Nowak, 1991; see also Candela & Picasso, 2008), but at least in this analysis, it did not occupy a distinct portion of the morphospace. Because this was the only erethizontid included in our sample, it is not possible to infer characteristic morphological traits for this superfamily without the inclusion of additional representatives of the group.

Conclusions

The association between humeral shape and both phylogeny and habit suggests an early ecomorphological diversification of caviomorph rodents accompanying their phylogenetic divergence. Our analysis showed that the major lineages of the clade could be distinguished by characteristic humeral features, even those taxa that were not so close in the morphospace. Similar phylogenetic constraints on morphological patterns have been observed for several mammalian groups (e.g. scandentians, Sargis, (2002); carnivorans, Meloro & O'Higgins, 2011, Meloro *et al.*, 2011; New World platyrrhines, Perez *et al.*, 2011). At the same time, in a context of characteristic suprafamilial morphologies, habits were associated with particular morphological traits interpreted as specializations, in agreement with previous qualitative and quantitative analyses (Elissamburu & Vizcaino, 2004; Candela & Picasso, 2008). Interestingly, Samuels and Van Valkenburgh's (2008) analysis of locomotor adaptations in rodents showed that fossorial taxa had more robust humeri with well-developed muscular attachment sites, in agreement with our results; however, they did not find major differences in humeral morphology among other locomotor habit categories. This discrepancy with the present results could be attributed to different methodological approaches (indexes from linear measurements vs. geometric morphometrics) and also probably to different taxonomical range of the respective samples.

To date, only a few studies have focused on macroevolutionary processes driving the morphological variation of caviomorph rodents. Craniomandibular shape variation has been shown to have significant phylogenetic signal and simultaneously a strong allometric component (Álvarez, 2012). In contrast, variations in scapular shape agree with the phylogenetic pattern and are not associated with the different habits of these rodents (Morgan, 2009). It seems evident that complex factors have disparate influence on different skeletal elements among caviomorphs, and may facilitate or constrain ecomorphological evolution within each lineage (Miles & Dunham, 1993; Losos & Miles, 1994). These results emphasize the importance of performing further comparative analyses to achieve a better perspective regarding the evolution of morphological disparity in South American caviomorphs.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Ordination of 28 extant caviomorph genera in the morphospace defined by the first two principal components (PCs) with the obtained phylogeny superimposed according to reconstructed ancestral values.

Appendix S1. GenBank accession numbers for the 12S, cytb, GHR and TTH gene sequences for caviomorph rodents analysed in this work.

Appendix S2. Parameters from the Bayesian analysis of gene sequences used in this work.

Appendix S3. Procrustes ANOVA test for measurement error. Individual effect represents overall variation, and Error 1 is the measurement error calculated for repeat measurements. SS: sum of squares; MS: mean squares, d.f.: degrees of freedom; F: *F* statistic; p: *P*-value.