



## Inferring adaptation within shape diversity of the humerus of subterranean rodent *Ctenomys*

FRANCISCO STEINER-SOUZA<sup>1,2</sup>, THALES R. O. DE FREITAS<sup>2,3</sup> and PEDRO CORDEIRO-ESTRELA<sup>2,3\*</sup>

<sup>1</sup>Programa de Pós-Graduação em Biologia Animal, Universidade Federal do Rio Grande do Sul (UFRGS), Av. Bento Gonçalves 9500, prédio 43435, 91501-970, Porto Alegre, Rio Grande do Sul, Brazil

<sup>2</sup>Departamento de Genética, Instituto de Biociências, Universidade Federal do Rio Grande do Sul (UFRGS), Av. Bento Gonçalves, 9500 – prédio 43323 M, 91501-970, Porto Alegre, Rio Grande do Sul, Brazil

<sup>3</sup>Programa de Pós-Graduação em Genética e Biologia Molecular, Universidade Federal do Rio Grande do Sul (UFRGS), Av. Bento Gonçalves, 9500 – prédio 43323 M, 91501-970, Porto Alegre, Rio Grande do Sul, Brazil

Received 18 June 2009; revised 27 November 2009; accepted for publication 27 November 2009

In subterranean rodents of the genus *Ctenomys*, excavation activity can be carried out with the claws and forelimbs (scratch-digging) as well as with the skull and incisor teeth (skull-tooth digging). Within the forelimb myoskeletal system, the humerus is a main bone concentrating a large number of muscles and bearing tensions during excavation. The genus *Ctenomys* is considered primarily a scratch-digger and secondarily a skull-tooth digger. We analysed the humerus ( $N = 165$ ) of four species of *Ctenomys* from southern Brazil, in areas ranging from the soft soils of the first lines of coastal dunes (*Ctenomys flamarioni*, *Ctenomys minutus*), through the sandy fields of the coastal plains (*Ctenomys minutus*, *Ctenomys lami*), on to the hard soils of the southern pampas 'gaúchos' fields (*Ctenomys torquatus*). The differences in the form (size + shape) were quantified using geometric morphometrics methods and interpreted in the light of myological descriptions. As expected from a phylogenetic and ecological point of view, *C. flamarioni* had the most divergent shape and larger size among the species analysed, showing a more slender humerus, especially in the head region, than *C. lami*, *C. minutus*, and *C. torquatus*. Crossing the osteology data with the qualitative observations of the musculature, it was possible to detect large differences in the proximal portion of the humerus that could be related to the insertion of important extension muscles of the pectoral–shoulder joints, which could increase force. The comparison of shape differences between the three closely-related species (*C. lami*, *C. minutus*, and *C. torquatus*) revealed unexpected patterns because *C. lami* was the species phenetically more distant from *C. flamarioni* and not *C. torquatus* as expected from ecological data and phylogenetic relationships. A two-step adaptive path to humeral shapes better fit to digging is postulated where the deltoid crest and epicondylar crest increases precede an articular surface area increase. The absence of sexual dimorphism in *C. torquatus* is discussed with regard to the optimal size required to dig in hard soils. © 2010 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2010, 100, 353–367.

**ADDITIONAL KEYWORDS:** life underground – myoskeletal system – phenotypic evolution – statistical shape analysis.

### INTRODUCTION

Living in excavated burrows, the genus *Ctenomys* has different ecological, physiological, and morphological

adaptations to underground life (Nevo & Reig, 1990). The major morphological adaptations to excavation are represented by the robust and cylindrical body, small eyes, reduced hearing flag, and robust limbs, as well as by modifications to the incisors, mouth, and skull (Pearson, 1959; Reig *et al.*, 1990; Casinos,

\*Corresponding author. E-mail: pedroestrela@yahoo.com

Quintana & Viladiu, 1993; Vassallo, 1998; Lacey, Patton & Cameron, 2000; Verzi, 2002 in Morgan & Verzi, 2006). In subterranean rodents, excavation activity depends of soil conditions (i.e. presence of fibrous roots, rocks, hardness of soils) and can be carried out in three different ways: with the claws and forelimbs (scratch-digging) and with the skull and incisor teeth (skull-tooth digging), or a combination of the two forms (Lehmann, 1963; Dubost, 1968; Hildebrand, 1985; Lessa, 1990; Stein, 2000; Mora, Olivares & Vassallo, 2003). In the literature, the genus *Ctenomys* is considered primarily as a scratch-digger (Lehmann, 1963) and, secondarily, as a skull-tooth digger (Dubost, 1968; Ubilla & Altuna, 1990; Vassallo, 1998; Stein, 2000), although knowledge of soil conditions and predominant digging behaviour for most of the *Ctenomys* ( $\pm 60$  species) species is unknown (Ubilla & Altuna, 1990; Vassallo, 1998; Stein, 2000; Lessa *et al.*, 2008). Among the post-cranial bones, the humerus is possibly the bone that contains the larger amount of functional adaptive modifications (Szalay & Sargis, 2001; Morgan & Verzi, 2006) and, in scratch-diggers, the forelimb myoskeletal system of the scapula, humerus, radius, ulna, and the manus and claws forms a mechanical system of great importance in excavation. However, knowledge of this system (i.e. the relationship between muscles, tendons, and bones, or candidate adaptive structures) in living species of *Ctenomys* is insufficient. In the few available studies of humerus structure, the sample size is reduced (Morgan & Verzi, 2006) or only two species are analysed (Vassallo, 1998), which means that the results obtained in such studies, although pioneering, have little conclusive power both statistically and evolutionarily. In addition, all studies analysed the structures with classical morphometric methods (linear measurements), although this does not comprise an analysis of shape *per se* independently of size (Rohlf & Marcus, 1993).

The four species included in the present study, occur in southern Brazil (Freitas, 1990). The first, *Ctenomys minutus*, occurs in the states of Santa Catarina and Rio Grande do Sul and *Ctenomys flamarioni*, *Ctenomys lami*, and *Ctenomys torquatus* only in the latter state. Their habitats range from the first lines of costal dunes (*C. flamarioni*, *C. minutus*) to the sandy fields of the coastal plains (*C. minutus*, *C. lami*), as well as in the harder soils of the pampas (*C. torquatus*). Although a robust published phylogeny of the genus is not yet available, many studies (Lessa & Cook, 1998; Castillo, Cortinas & Lessa, 2005) allow us to establish a synthetic phylogenetic hypothesis for the species investigated in the present study. The species *C. flamarioni* belongs to the monophyletic 'mendocinus-group' (*Ctenomys australis*, *C. flamarioni*, *Ctenomys rionegrensis*, *Ctenomys porteousi*, *Ctenomys azarae*,

*Ctenomys mendocinus*, and *Ctenomys chasiquensis*) (Massarini *et al.*, 1991; Freitas, 1995; Massarini & Freitas, 2005). The three remaining species, *C. torquatus*, *C. minutus* and *C. lami* are part of another monophyletic group ('torquatus-group'; Parada and Lessa unpubl. data), where according to Freitas (2001), *C. minutus* and *C. lami* comprise recently diverged sister species (Gava & Freitas, 2002).

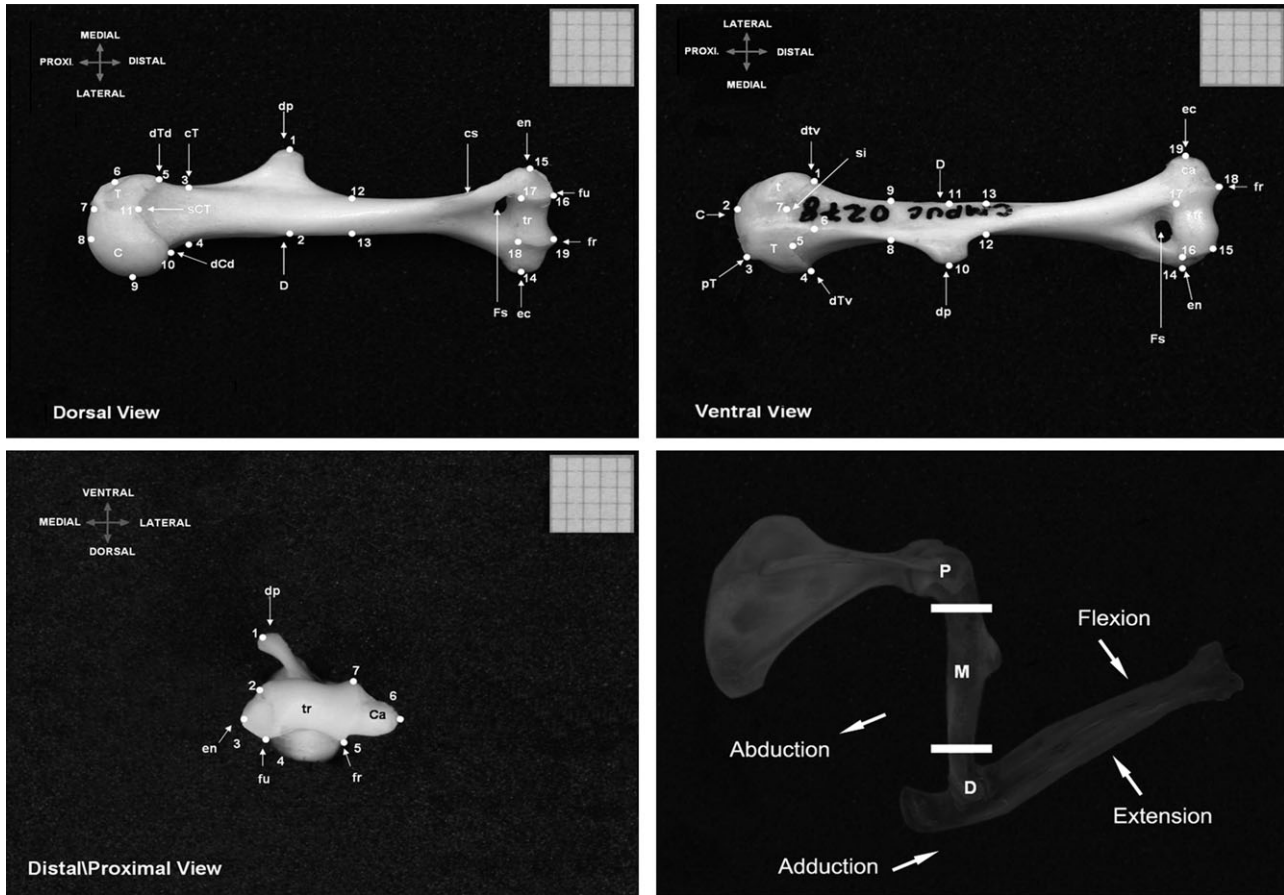
Hypotheses regarding the evolution of the humerus of these four species can be proposed. First, assuming neutral phylogenetic divergence, we expect *C. flamarioni* to be phenetically more distinct, and the sister species *C. lami* and *C. minutus* to be more similar in shape and size. Second, assuming that the humerus shape is selected for optimal excavating performance, *C. flamarioni* and *C. torquatus*, which lie at each extreme of the soil hardness gradient, should have very divergent shapes, whereas the two sister species should again be similar to each other and have intermediate shapes between these extremes. To provide additional support for the adaptive hypotheses based on shape configurations, we provide a detailed qualitative myological analysis. Indeed, selection for particular shapes should be accompanied and/or modified by the muscular system. Finally, the interpretation of shape differences conjointly with the muscular groups implied in these differences allows us to propose hypotheses about the forces and movements that are selected for during excavation.

## MATERIAL AND METHODS

### SAMPLES AND DATA ACQUISITION

We digitized data on 165 samples from four species of the genus *Ctenomys*: *C. minutus* ( $N = 104$ ), *C. torquatus* ( $N = 25$ ), *C. flamarioni* ( $N = 13$ ), and *C. lami* ( $N = 23$ ) of different localities in the state of Rio Grande do Sul. For each sample, we recorded information about sex, weight, and collection locality (only adult specimens were used) (available from authors). All specimens are deposited in the Laboratório de Citogenética e Evolução, Departamento de Genética, Universidade Federal do Rio Grande do Sul, Brazil.

All photos were obtained with a digital camera Canon EOS 400 D [10 mega pixels of resolution, mode = A-DEP, Iso100, One-Shot, AWA with MACRO Lens Canon Normal EF 50 mm f/2.5 (opening of  $-2$ )]. All images were taken from a standard distance (15 cm) with the aid of a tripod. A basis of polyethylene foam with millimeter paper served as background for images and as a scale factor. The photos were organized in lists with the TPSUTIL, version 1.26 (Rohlf, 2004) and the digitization of landmarks was made with TPSDIG, version 1.40 (Rohlf, 2004). We photographed the humerus in dorsal, ventral and



**Figure 1.** The right humerus of *Ctenomys flamarioni*. Dorsal view (upper left), ventral view (upper right), distal/proximal view (lower left) and the position (upper right) of the scapula, humerus, radius, and ulna in the skeletal system of right forelimb of *Ctenomys torquatus*. dtd, distal part of the great tuberosity; cT, lateral crest of the great tuberosity; dp, deltopectoral crest; cs, supinatory crest; en, medial epicondyle; ec, epicondyle; fr, radial facet; fu, ulnar facet; Fs, supratrochlea foramen; D, diaphysis; dCd, distal part of condyle; C, condyle; T, great tuberosity; sCT, groove between condyle and great tuberosity; tr, trochlea; dTv, distal part of great tuberosity; pT, great tuberosity process; dtv, distal part of small tuberosity; si, intertubercular groove; c, capitulum. A definition of the landmarks is provided in the Appendix (Table A2). Scale: 1 cm.

distal/proximal views, where 19, 19, and seven landmarks, respectively, were defined (Fig. 1). A definition of landmarks is provided found in the Appendix (Table A2) and we also provide a summary of interlandmark distances per species (Table A1). These interlandmark distances consist of the distances between landmarks, which are homologous to linear measurements made by Morgan & Verzi 2006.

GEOMETRIC MORPHOMETRIC AND STATISTICAL PROCEDURES

Geometric morphometrics is a set of techniques for the acquisition, analysis, and visualization of the form of objects, which is decomposed into components of size and shape. Unlike traditional morphometrics, the

shape of objects (its geometry) is preserved along all the steps of the analysis and not indirectly inferred from ratios for example. For the analysis of the form of the humerus, we used generalized procrustes analysis (GPA) (Gower, 1975; Dryden & Mardia, 1998). The GPA allows the study of the centroid size and shape (superimposition residuals) as different sets of variables. First, the configuration of landmarks is translated, and then scaled to the same size and, in a last step, rotated to minimize the partial Procrustes distance between configurations by a least squares criterion (Rohlf & Slice, 1990; Bookstein, 1991).

For the analysis of size variation of the humerus, we used the centroid size obtained from the GPA (Rohlf & Slice, 1990; Bookstein, 1991). Uncorrelated with shape, the centroid size is the size measure used

in geometric morphometrics (Monteiro & Reis, 1999; Zelditch *et al.*, 2004) and corresponds to the square root of the sum of squared distances between each landmark and the centroid of the configuration. The presence of interspecific variation, sexual dimorphism, and their interaction for size was tested by a model II analysis of variance (ANOVA) (Sokal & Rohlf, 1995). Pairwise size differences were tested with Tukey's honestly significant difference test.

Shape variables (superimposition residuals) are the difference at each landmark of each configuration to a mean shape after a GPA. With these shape variables, a principal components analysis (PCA) was computed to find the axes of major shape differences. Interspecific differences and sexual dimorphism in shape and their interaction was tested through a multivariate analysis of variance (MANOVA) on shape variables after a correction of minus 4 degrees of freedom lost in the GPA after translation, scaling, and rotation. The linear discriminant analysis (LDA) is a widespread classification technique used to quantify and display variation among groups, in our case the species. We used PCs as shape variables and reduced the dimensionality of the data using the criterion of maximum classification percentage (Baylac & Friess, 2005). To evaluate the performance of classification by LDA, we used the leave-one-out cross-validation. With the values of the three discriminant axes, we visualized the shape differences through multivariate regression of shape variables on discriminant axes. Shape changes along axes are visualized as the shapes obtained at the positive and negative extremes of each axis. This corresponds approximately to an analysis of the weight of variables (i.e. cartesian coordinates of landmarks) on discriminant axes. In a last step, the squared Mahalanobis distances (D2) between the four species were computed on shape variables.

First, all statistical procedures were applied on the three views separately (dorsal, ventral, and distal/proximal), and then on the three views pooled (total view). In the total view, we used the natural logarithms of centroid size values of each view, calculated first for each view separately, and then we summed the logs of centroid size. In the total view, shape variables were

pooled *sensu* Cordeiro-estrela *et al.* (2006). PCs of each view were pooled to calculate a pooled PCA.

All statistical analyses and graphs were made using the 'R' language and environment for statistical computing, version 2.0 (R Development Core Team, 2004; <http://cran.r-project.org/>). Morphometric analyses were performed with the Rmorph library (Baylac, 2007).

## MYOLOGY

Dissectable museum specimens of *Ctenomys* are rare. First, we tried to use animals (carcasses) conserved in alcohol 70% but these specimens were not preserved in correct anatomical position, which made the dissection and a comparison between the specimens impossible. To make a comparative dissection, we used only one forelimb (right) of one specimen of 'similar size' from each of the species *C. flamarioni*, *C. lami*, and *C. torquatus* preserved in 10% formaldehyde solution. The dissections were performed to confirm the region of origin and insertion of the muscles related to the humerus (excluding the intrinsic muscles of the manus) as reported by Lehmann (1963) for some fossorial rodents and by Woods (1972) for Hystricomorph rodents, aiming to test any variation in muscle insertion point between species that might affect shape or biomechanical properties. Other guides used in the observations were based on studies by Walker & Homberger (1997), Vassallo (1998), Fernández, Vassallo & Zárate (2000), and Elissamburu & Vizcaíno (2004).

## RESULTS

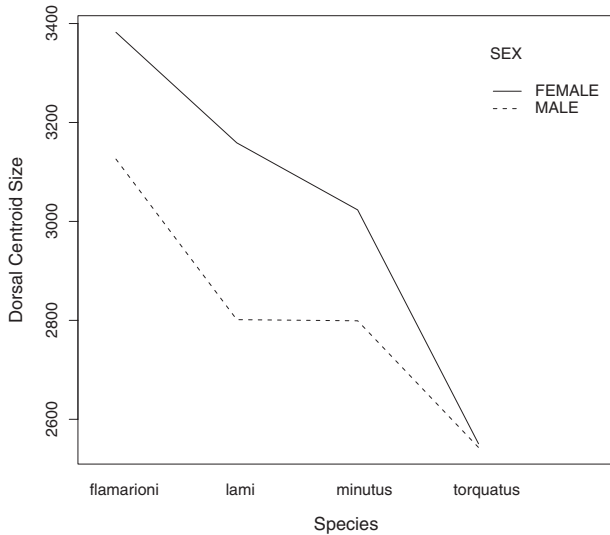
### SIZE

The results obtained for the centroid size between sexes and among species for the four views revealed similar results (including total view using logarithms). The ANOVAs of centroid size yielded highly significant *F*-values (of similar magnitude) among species and sexes (Table 1). The interactions were also significant. In Figure 2, the mean of centroid size for species/sexes is shown in an interaction plot,

**Table 1.** Analysis of variance of centroid size (*F*- and *P*-values) among sex and species in four species of *Ctenomys*

	Dorsal view		Ventral view		Distal/proximal view		Total view	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Sex	38.00	7e-09***	34.94	2.56e-08***	37.00	1.05e-08***	25.84	1.21e-06***
Species	35.00	2.2e-16***	44.68	2.2e-16***	38.31	2.2e-16***	36.79	2.2e-16***
Sex : Species	3.10	0.027*	2.82	0.041*	2.56	0.056	2.78	0.043*

Significance: \*\*\**P* < 0.00; \*\**P* < 0.001; \**P* < 0.05.



**Figure 2.** Interaction plot with mean centroid size by sex and species.

**Table 2.** Adjusted probabilities of Tukey's pairwise honest significant test for size (\*\* $P < 0.01$ ; \*\*\* $P < 0.001$ )

	$P$ (adjusted)
<i>lami</i> – <i>flamarioni</i>	0.0053877**
<i>minutus</i> – <i>flamarioni</i>	0.0003887***
<i>torquatus</i> – <i>flamarioni</i>	0.0000000***
<i>minutus</i> – <i>lami</i>	0.9984392
<i>torquatus</i> – <i>lami</i>	0.0000004***
<i>torquatus</i> – <i>minutus</i>	0.0000000***

where sexual dimorphism is absent for *C. torquatus*. The results of Tukey's test from the total view (Table 2) show that *C. flamarioni* was the species with the larger humerus, followed by *C. lami* and *C. minutus* (without significant difference among them,  $P = 0.99$ ). *C. torquatus* have the smallest humerus and an absence of sexual dimorphism (Fig. 2).

#### SHAPE

Data from any of the four views considered shows similar results. In all cases, MANOVAs of shape variable were significant for sexes and species (Table 3), except for the distal/proximal view, which did not show any sexual dimorphism ( $P = 0.21$ ). For the four views, the interaction term between sex and species was not significant.

For the four views in PCA, the main axes of variation (PC1 and 2) are not equivalent to interspecific patterns of shape differences because no clear specific grouping was detected (data not shown). Only the PC1 of the dorsal view revealed some clustering

among *C. flamarioni* and the other three species (not shown). To measure the association between shape variables (PC1, PC2) and size (allometry), we made a correlation test, although no significant correlation was detected.

The classification and the percentage of reclassification for the four views by the LDA using leave-one-out cross-validation is shown in Table 4.

Analysed separately, the dorsal view was the one that best discriminated the species globally. *Ctenomys flamarioni* have the higher percentage of reclassification and *C. lami* have the smaller and, as expected from their close phylogenetic proximity, all misclassified *C. lami* were considered as *C. minutus*. The ventral view gives similar results to the dorsal view. In the distal/proximal view, we obtained the smaller correct classification percentages, including a low percentage for *C. lami*. In the total view, we obtained the highest discrimination percentages (100% for *C. flamarioni* and *C. minutus*).

The dorsal and ventral views gave similar results for LDA (Figs 3, 4). In dorsal view (Fig. 3), the first axis (44% of variance) discriminates *C. torquatus* and *C. flamarioni* from *C. lami* and *C. minutus*. The shape changes inferred from this axis demonstrate a difference in robustness of the head (namely small tuberosity and condyle), a more enlarged supinatory crest region, and a small difference in the area and direction of deltopectoral crest. *Ctenomys flamarioni* was separated from the other species by the second axis (41% of variance), for which inferred shape differences are a slender head and neck (proximal diaphysis) of the humerus compared to the other three species. Other perceptible differences are detected in the small robustness of medial diaphysis and a slim and elongated supinatory crest. Finally, we observed a difference in the distal part in the region of the epicondyles, which consequently affects the orientation of the trochlea. The third axis (15% of variance) discriminates *C. lami* from the others, and highlights a slightly more robust humerus, with a difference in the tip of the deltopectoral crest which appears to be relatively smaller.

The results of first and second axis for the ventral view (Fig. 4) gave results similar to those for the dorsal view. In the first axis (60% of variance), we obtained *C. flamarioni* with most extreme negative values and *C. torquatus* with extreme positive values, whereas *C. minutus* and *C. lami* had intermediate values. For this view, the most perceptible differences in shape are observed again in the head of humerus, the deltopectoral crest orientation, a 'relative size' of trochlea and medial epicondyle, and the shape and orientation of the ulnar facet. In the second discriminant axis (28% of variance), as in the first axis of the dorsal view, we observed a shape differentiation

**Table 3.** Multivariate analysis of variance (approximate *F*- and *P*-values) of shape variables (residuals of superimposition) for sex and species in four species of *Ctenomys*

	Dorsal view		Ventral view		Distal/proximal view		Total view	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Sex	2.51	0.0001***	2.20	0.001**	1.35	0.209	2.22	0.0008***
Species	8.85	0***	7.58	0***	7.27	0***	5.43	0***
Sex : Species	1.19	0.123	0.83	0.855	0.93	0.563	0.98	0.553

Significance: \*\*\**P* < 0.00; \*\**P* < 0.001.

**Table 4.** The number of correctly classified specimens of each species and percentage of correct reclassification for the four views by the linear discriminant analysis (LDA) using leave-one-out cross-validation

	<i>Ctenomys flamarioni</i>	<i>Ctenomys lami</i>	<i>Ctenomys minutus</i>	<i>Ctenomys torquatus</i>	%
Dorsal view					
<i>Ctenomys flamarioni</i>	14	0	0	0	100
<i>Ctenomys lami</i>	0	19	3	0	86
<i>Ctenomys minutus</i>	0	1	71	2	96
<i>Ctenomys torquatus</i>	0	1	1	51	96
Ventral view					
<i>Ctenomys flamarioni</i>	14	0	0	0	100
<i>Ctenomys lami</i>	0	15	6	0	71
<i>Ctenomys minutus</i>	0	2	70	1	96
<i>Ctenomys torquatus</i>	0	0	1	53	98
Distal/proximal view					
<i>Ctenomys flamarioni</i>	12	0	2	0	86
<i>Ctenomys lami</i>	0	13	9	0	59
<i>Ctenomys minutus</i>	3	5	57	11	75
<i>Ctenomys torquatus</i>	1	1	13	39	72
Total view					
<i>Ctenomys flamarioni</i>	14	0	0	0	100
<i>Ctenomys lami</i>	0	20	1	0	95
<i>Ctenomys minutus</i>	0	0	70	0	100
<i>Ctenomys torquatus</i>	1	0	1	49	96

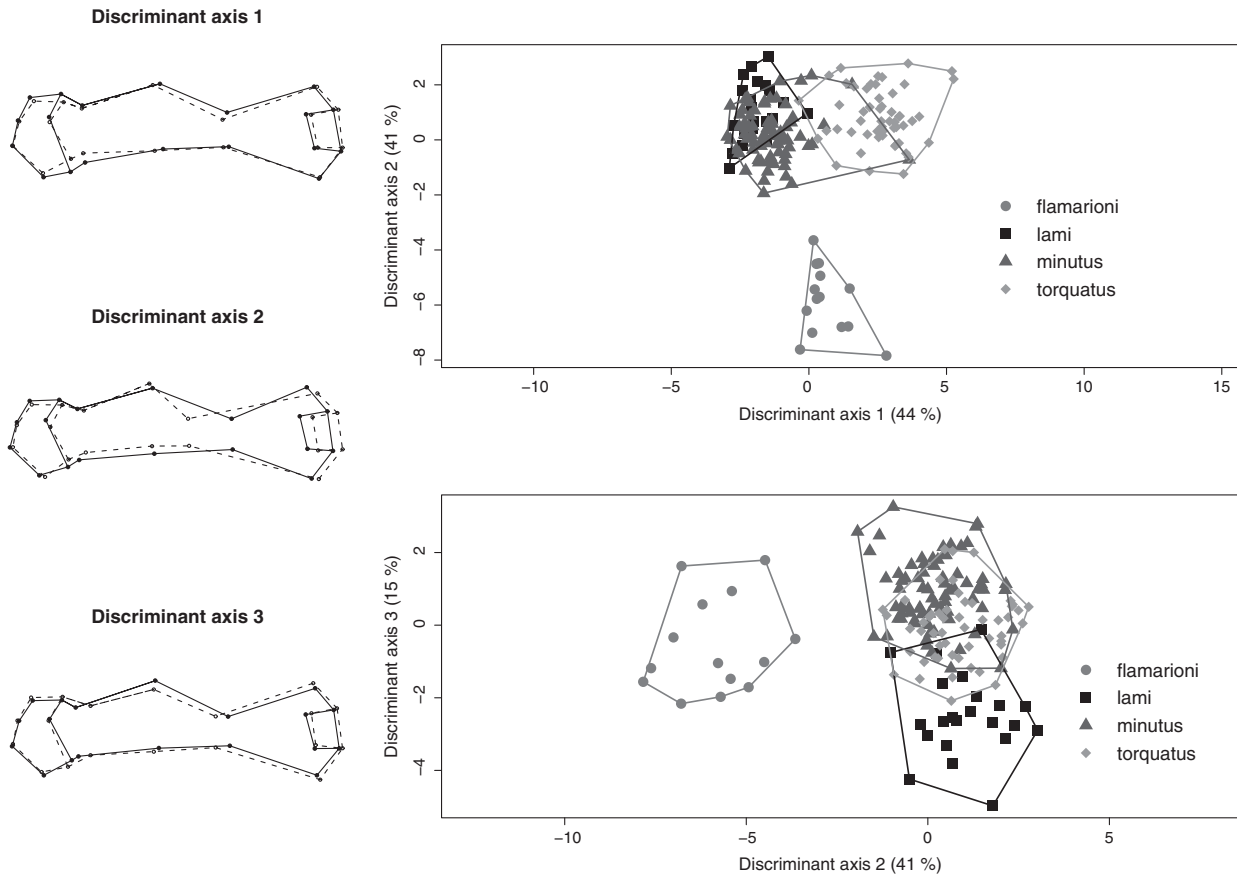
Rows indicate original classification and columns the classification by LDA.

between the *C. flamarioni*–*torquatus* group and the *C. lami*–*minutus* group. The shape difference inferred is observed in the region between the distal part of great tuberosity and the deltopectoral crest, with other small differences occurring in the proximal part of the condyle (as well as greater tuberosity), the medial diaphysis and the medial epicondyle of humerus. The third axis (12% of variance) discriminates *C. lami* from the other species, and again by the orientation and a more pronounced deltopectoral crest.

The distal/proximal view does not show clear results by LDA (results not shown), as attested by lower classification percentages. Finally, in the total view (not illustrated), the first axis (39.2% of

variation) discriminates *C. flamarioni* from the other species and, for the second discriminant axis (35.6% of variation), we have *C. flamarioni*, *C. minutus* and *C. lami* in the positive values and in the negative ones, a great amount of individuals from *C. torquatus*. The shape visualization obtained from total view gave similar results, but less pronounced shape differences compared to the shape visualization of the three views analysed separately.

The quantification of shape difference of the humerus between the species was examined through the squared Mahalanobis distances (*D*<sup>2</sup>) (Table 5). In all views, *C. flamarioni* has the most different shape compared to the three other species, and *C. minutus*



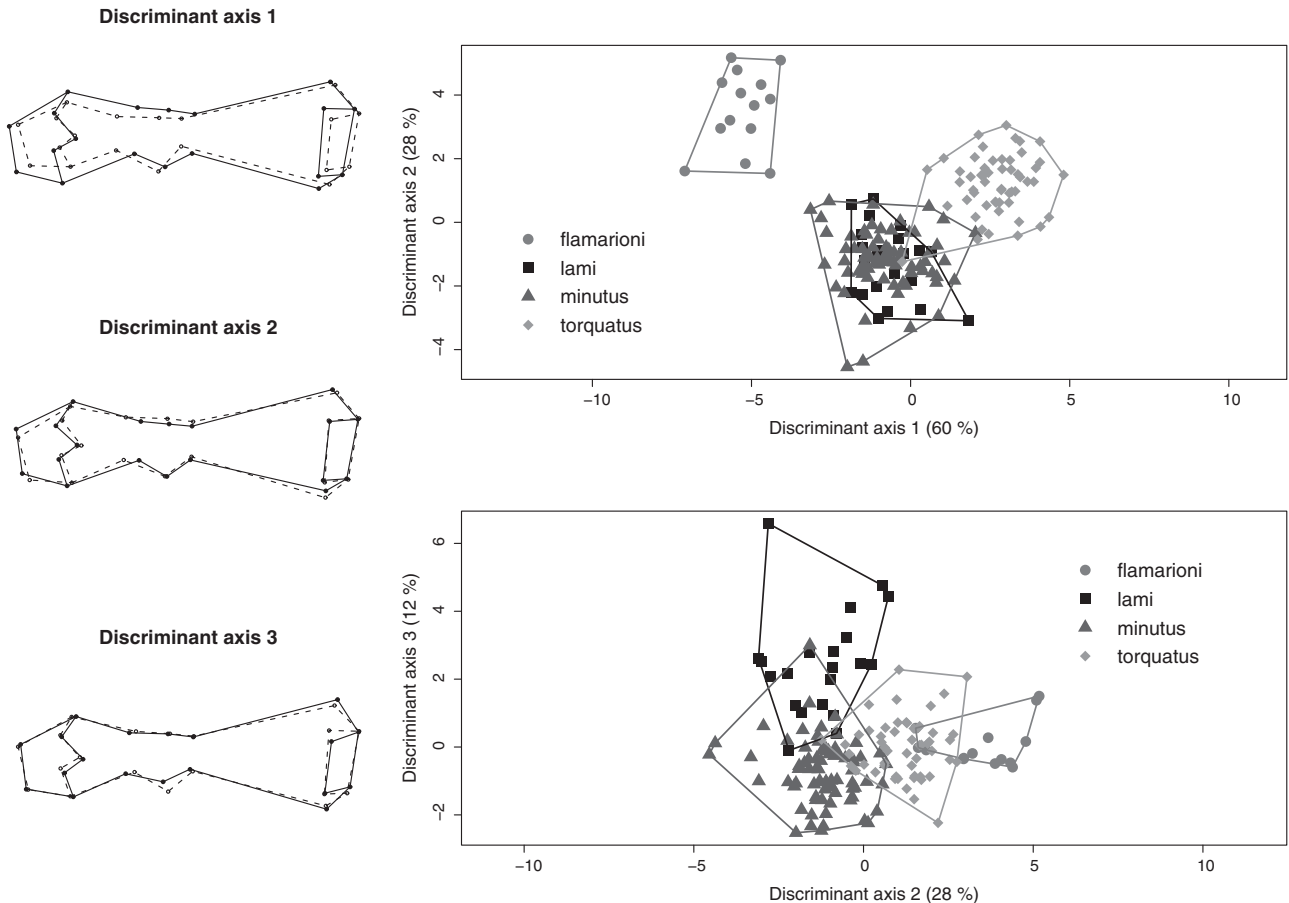
**Figure 3.** Shape changes (left) and projection of individuals (right) for the three axes of the linear discriminant analysis of the dorsal view. The first and second axes (upper right), second and third axes (lower right) are shown with the percentage of variance indicated by each axis in parenthesis and convex hulls delimiting species. The shapes inferred at the extremes of each of the axes are shown as solid and dotted lines, for the positive and negative extremes, respectively.

and *C. lami* are closer phenetically, followed by *C. torquatus*, which is more adjacent to *C. minutus* than to *C. lami*. However, the three views do not follow the same pattern; for example, in the dorsal view, *C. flamarioni* and *C. lami* have the largest distance, whereas, in the ventral view, *C. flamarioni* and *C. torquatus* are the most different. The distal/proximal view proceeds similarly to the ventral view and the total view proceeds similarly to the dorsal view, although the two have a significant difference in magnitude compared to the other views.

#### MYOLOGY

On the basis of the avialbale literature and our own observations, we found nineteen muscles (Table 6) attached to the humerus (Fig. 5). Thirteen were part of the extensor system and six were part of the flexor system. The origin and insertion of the muscles attached to the humerus are similar in all the three species examined and, as pointed out for other species

by Vassallo (1998), the major difference observed in the dissection between *C. flamarioni*, *C. lami*, and *C. torquatus* was muscle robustness. The forelimb myological system (excluding the intrinsic muscles of the manus) can be divided into two groups: shoulder-pectoral and elbow-arm muscles (including *M. coracobrachialis* and *M. brachialis* with more than one origin and insertion in the humerus). *Sensu* Lehmann (1963) and Woods (1972), we separated the muscles inserted in the humerus into three different groups: proximal part (condyle, lesser and greater tuberosity), medial part (medial diaphysis and deltopectoral crest region), and distal part (supinatory crest, epicondyle, medial epicondyle, and trochlea). In the proximal part, we found ten muscles, eight of them belonging to the shoulder-pectoral group and two of them belonging to the elbow-arm group; in the medial part, we found seven muscles (including complex muscles with more than one origin or insertion), with three making part of shoulder-pectoral group and four belonging to the elbow-arm group; and, finally, in the distal part,



**Figure 4.** Shape changes (left) and projection of individuals (right) for the three axes of the linear discriminant analysis of the ventral view. The first and second axes (upper right), second and third axes (lower right) are shown with the percentage of variance indicated by each axis in parenthesis and convex hulls delimiting species. The shapes inferred at the extremes of each of the axes are shown as solid and dotted lines, for the positive and negative extremes, respectively.

the six muscles exclusively comprise the elbow–arm group.

In the proximal part, precisely in the lesser tuberosity, we have insertions of the shoulder flexor muscle *M. pectoralis abdominalis* and some important extensor muscles such as *M. latissimus dorsi*, *M. teres major*, and *M. subscapularis*; these muscles help in arm retraction, a necessary movement during the digging movement of the scratch-diggers (Lehmann, 1963; Woods, 1972; Walker & Homberger, 1997; Lagaria & Youlatos, 2006). In the greater tuberosity, we located the insertion of one part of the flexor *M. pectoralis minor* and shoulder extensor muscles *M. teres minor*, *M. infraspinatus* and *M. supraspinatus* and the origin of two important elbow muscles: the forearm extensor *M. triceps brachii lateralis* and a ‘part’ of the flexor *M. brachialis* (Lehmann, 1963; Woods, 1972). The *triceps brachii* group is involved in the dissociation of soil particles during digging (Lagaria & Youlatos, 2006). In the medial part, we

find an equal concentration of shoulder–pectoral and elbow–arm muscles; for the deltoid group, we have the insertion of extensor muscle *M. clavodeltoideus* in the dorsal margin of the tip of the deltopectoral crest and, in the ventral margin, we have the *M. spinodeltoideus* and *M. acromiodeltoideus* (Woods, 1972). *M. clavodeltoideus* protracts the humerus and *M. spinodeltoideus* has an important role in arm retraction and movement of shoulder joints (Fernández *et al.*, 2000; Elissamburu & Vizcaíno, 2004; Lagaria & Youlatos, 2006). Still in the shoulder–pectoral muscles of the medial part, we have, in the deltoid crest region, the inserts of flexor *M. pectoralis major* and a part of the complex *M. cutaneos maximus*; as a primary function, these two muscles involve the retraction of the arm (Walker & Homberger, 1997). In the elbow muscles of the medial part, we have two important muscles: first, the inserts of *M. coracobrachialis* (small adductor of the humerus) in the medial diaphysis and in the proximal part of the epicondyle

**Table 5.** Mahalanobis distances (D2) between the four species calculated from shape variables using the group averages of linear discriminant analysis (LDA) for the distal, ventral, distal/proximal and total view

	<i>Ctenomys flamarioni</i>	<i>Ctenomys lami</i>	<i>Ctenomys minutus</i>
Dorsal view			
<i>Ctenomys lami</i>	57.81	–	–
<i>Ctenomys minutus</i>	42.93	12.48	–
<i>Ctenomys torquatus</i>	49.09	26.14	16.95
Ventral view			
<i>Ctenomys lami</i>	54.51	–	–
<i>Ctenomys minutus</i>	46.58	10.48	–
<i>Ctenomys torquatus</i>	72.08	24.41	18.67
Distal/proximal view			
<i>Ctenomys lami</i>	13.89	–	–
<i>Ctenomys minutus</i>	9.88	3.63	–
<i>Ctenomys torquatus</i>	15.49	7.02	2.56
Total view			
<i>Ctenomys lami</i>	95.72	–	–
<i>Ctenomys minutus</i>	88.95	27.34	–
<i>Ctenomys torquatus</i>	90.00	44.05	27.89

Dorsal and ventral views on principal components (PCs) 1–34, distal/proximal view PCs 1–10 and total view PCs 1–55.

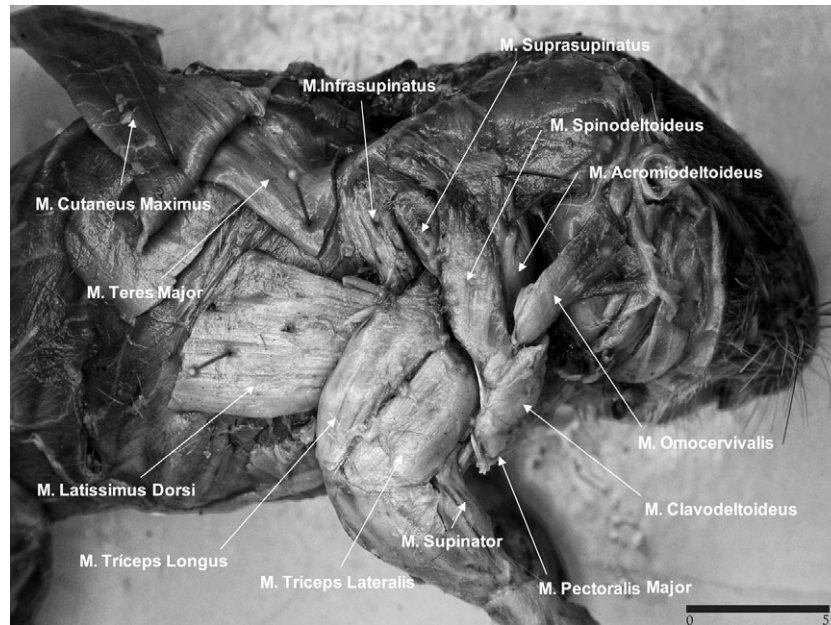
**Table 6.** The list of the shoulder–pectoral and elbow–arm muscles of the extensor and flexor system attached to the humerus based in Lehmann (1963) and Woods (1972)

Muscles/system	
<i>Shoulder–pectoral muscles</i>	
Extensor system	<i>M. latissimus dorsi</i> <i>M. teres major, minor</i> <i>M. subscapularis</i> <i>M. clavodeltoideus</i> <i>M. spinodeltoideus</i> <i>M. acromiodeltoideus</i> <i>M. suprasupinatus</i> <i>M. infrasupinatus</i>
Flexor system	<i>M. pectoralis major, minor,</i> <i>and abdominalis</i> <i>M. cutaneos maximus</i>
<i>Elbow–arm muscles</i>	
Extensor system	<i>M. triceps brachii lateralis</i> <i>M. triceps brachii medialis</i> <i>M. anconeus</i> <i>M. supinator</i>
Flexor system	<i>M. coracobrachialis</i> <i>M. brachialis</i>

(Woods, 1972; Walker & Homberger, 1997) as well as the origin of the second part of *M. brachialis* in the tip of the deltopectoral crest (Woods, 1972). For the distal part, we have only elbow muscles: four of the extensor group and two of the flexor group (*M. coracobrachialis* and *M. brachialis*) (Lehmann, 1963; Woods, 1972). The principal extensor group is formed by *M. triceps medialis*, *M. anconeus* and *M. supinator*. The *M. t. medialis* and *M. anconeus* originate in the four-fifths of the distal part of humerus (supinatory crest region) and have an important role in the extension of the arm (Woods, 1972; Thorington, Darrow & Betts, 1997; Walker & Homberger, 1997). Another important extensor muscle is the *M. supinator*, with an origin in the supinatory crest and in the epicondyle and medial epicondyle areas, and it is considered as a good indicator of fossoriality (Hildebrand, 1985; Lessa & Stein, 1992; Elissamburu & Vizcaíno, 2004).

DISCUSSION

The influence of phenotypic evolution in the process of diversification is a broad question in evolutionary biology that can be tackled at very different taxonomic levels and through different research designs. Speciose groups with functional structures whose variations are straightforward to interpret in terms of performance, as a proxy for fitness, comprise valuable cases for achieving this goal. An understanding of how natural selection acts on the phenotypic traits as well as an understanding of the relationship between phenotype and performance (i.e. the link between morphology and ecology) are the realm of functional morphology, evolution, and ecology (Arnold, 1983; Wainwright, 1994; Kingsolver & Huey, 2003). The humerus is an appropriate structure for studying adaptive patterns of locomotion among terrestrial vertebrates. In the diverse tuco-tucos, the humerus may impart how selection could contribute to the speciation process. Indeed, most of its diversity has been attributed to its low mobility, underground habitat, and chromosomal variability, which produce structured and semi-isolated populations prone to allopatric speciation. However, the ability to successfully dig and forage in different types of soils has been overlooked as a potential force driving speciation. The present study has shown significant variation, most probably of adaptive origin not only for different types of soils, but also between closely-related species inhabiting similar soil types. The adaptive nature of these variations can be postulated based on qualitative biomechanical inferences suggested below. Furthermore, we discuss how constraints may have acted on size and heterogeneous sexual dimorphism variation.



**Figure 5.** Myological description of right forelimb of *Ctenomys flamarioni*.

#### QUALITATIVE BIOMECHANICS AND SELECTIVE SHAPE PATHS

Digging activity has caused diverse burrowing adaptations in subterranean rodents (Bou, Casinos & Ocanã, 1987; Castiella & Casinos, 1990; Reig *et al.*, 1990; Casinos *et al.*, 1993; Quintana, 1994; Fernández *et al.*, 2000; Verzi, 2002; Lessa *et al.*, 2008). Compared to nondigging mammals, the humerus bone of subterranean rodents has a well developed 'head', a stout diaphysis with a pronounced deltopectoral crest, and elongated epicondyles. The muscles are wider and their origins and insertions are relatively farther away from the articulations (Hildebrand, 1985; Lessa & Stein, 1992; Vassallo, 1998; Fernández *et al.*, 2000; Morgan & Verzi, 2006; Lessa *et al.*, 2008). These alterations (i.e. the relationships between bone structures, origin, and inserts of the muscles) can result in a mechanical advantage for muscles and an increased resistance of bone to muscular actions (Morgan & Verzi, 2006; Lessa *et al.*, 2008). In scratch-diggers, the forelimb system is adapted to produce strong forces of extension of the shoulder and elbow joint (Vassallo, 1998). In almost all comparative studies of diggers and nondiggers, bone resistance is demonstrated by the robustness of the diaphysis and the proximal part (shoulder joint), and by a roughened and triangular deltopectoral crest for the fixation of important extensor muscles of the pectoral and deltoid. Finally, a wider epicondyle process tends to increase the force in the manus (Lehmann, 1963; Casinos *et al.*, 1993; Vassallo, 1998; Fernández *et al.*, 2000; Elissamburu &

Vizcaíno, 2004; Morgan & Verzi, 2006). In a study of the myoskeletal system of two sympatric *Ctenomys* species, Vassallo (1998) found major differences in the humerus located only in pronounced epicondylar processes and a relatively larger forelimb extensor muscles in *Ctenomys talarum*. Morgan & Verzi (2006), using classical morphometric methods (linear measurement) to compare living and extinct species, found that the major trends of variation comprised the robustness of the diaphysis, a distalization of the deltopectoral crest region, and, *sensu* Vassallo (1998), more pronounced epicondylar processes and an extensive trochlear area.

A robust published phylogeny of the genus is unavailable, although it is known that *C. flamarioni* is a species more distantly related to the other three species, comprising the recently diverged sister species *C. minutus* and *C. lami* (Freitas, 2001). With respect to the Mahalanobis distances (Table 5) obtained in the present study, *C. flamarioni* has a more differently shaped humerus compared to *C. torquatus*, *C. lami*, and *C. minutus*, which are much closer phenetically (Table 5). The difference observed between *C. flamarioni* and the other species can be attributed either to phylogenetic or adaptive divergence. Indeed, *C. flamarioni* belongs to another monophyletic clade, the 'mendocinus group', and is the only species exclusively living in sand dunes in the present study. Therefore, the large differences observed could have arisen through a gradual random accumulation of differences or by the action of natural selection.

However, musculoskeletal analysis indicates that most of the changes observed in the present study have been described in previous studies as being functionally meaningful, as noted above. In comparative works between diggers and nondigging caviomorph rodents, the clearest distinction lies in the epicondyles, the deltopectoral crest, the relative width of the distal articular surface, and muscle robustness (Hildebrand, 1985; Lessa & Stein, 1992; Vassallo, 1998; Fernández *et al.*, 2000; Morgan & Verzi, 2006; Lessa *et al.*, 2008). In the present study, although we compared only diggers, most of the observed changes occur in these same areas that serve as the fixation of important extensor muscles of shoulder–pectoral joints; these changes can give a greater mechanical advantage to the forelimb movements and fixation of the scapula–humerus joint. According to Morgan & Verzi (2006), the most different species analysed are *C. flamarioni* and *C. lewisi*, which, despite being similar for all morphological indices, differ with respect to their humeral robustness (i.e. length of epicondyles divided by humeral length). *Ctenomys lewisi* (with *Ctenomys frater*) showed the most specialized humeral morphology and inhabits deep soils, often near creeks and rivers (Morgan & Verzi, 2006). These results corroborate the differences observed in the present study (i.e. major shape differences in the proximal and medial part of the humerus) between *C. flamarioni* and *C. torquatus*, independently of size variation. In the dorsal and ventral view, the major difference between the species was observed in the region of insertion of a major number of extensor muscles of the shoulder–pectoral joints (distal and medial part) of the humerus, with *C. torquatus* having a stouter humerus compared to *C. flamarioni*. In an analysis of small mammal limb kinematics, Fischer *et al.* (2002) concluded that more than 50% of its humeral displacement results from scapular retraction alone and only 25 % of its forearm displacement is achieved in the elbow joint, indicating the predominance of scapular retraction in the forelimb. Elissamburu & Vizcaíno (2004), in a study of limb proportion and adaptations in caviomorph rodents, argue that functional specialization for speed occurs in the proximal part of the forelimb and for force in the medial segments; when analysing indexes involving the shoulder, differences in the musculoskeletal system in this region can increase the force, especially in digging forms. We found differences in the area of the trochlea and epicondyle and a great difference in condyles and in the supinatory crest. These musculoskeletal interpretations lead us to propose the hypothesis that differences can originate from adaptive components for the recognized difference in the type of soils (costal dunes and fields), indicated by a more robust and ‘specialized’ hard soil digger for *C.*

*torquatus* and a slender humerus for soft soils for *C. flamarioni*. However it should be noted that complex forces and tensions must be acting in the humerus. For example, *C. flamarioni* despite having a relatively less enlarged deltopectoral crest, has an articular surface of the head of the humerus compatible with a hard soil digger (Fig. 3, discriminant axis 1). This could indicate that, in some aspects, dunes are not as ‘soft’ as they appear. Globally, the differences found were in total accordance with previous studies and predictions.

Between the three closely-related species (*C. lami*, *C. minutus*, and *C. torquatus*), the shape difference was less pronounced compared to *C. flamarioni* (approximately half the magnitude). However, dorsal discriminant axis 1 clearly show that the main trend of shape difference between these three species is the area of the articular region of the head of the humerus. One of the advantages of geometric morphometrics is to pinpoint the pattern of covariation. If global changes corresponding to previous observation have been confirmed and are precise, specific patterns within the three closely-related species have been highlighted.

On the basis of the present study, we might conclude that there appears to be an adaptive path in shape changes towards structures that are more adapted to hard soils. First, a global shape change is required, involving a deltoid and epicondylar crest increase, as observed between *C. flamarioni* and the other three species (Fig. 2, axis 1). Second, as observed between *C. lami*, *C. minutus*, and *C. torquatus*, an increase in articular area (Figs 1, 2 axis 1) is required. As pinpointed in most previous studies (Szalay & Sargis, 2001), articular surface is an important shock absorption area, especially in harder soils. Most importantly, these differences are statistically independent of size.

#### SIZE VARIATION: BURROWING ADAPTATION OR SEXUALLY SELECTED?

The body size in *Ctenomys* has a great diversity, in the range 100–1000 g (Vassallo, 1998). Of the four species, the larger *C. flamarioni* has the larger mean size humerus, with *C. lami* and *C. minutus* being intermediate, and *C. torquatus* having the smallest size. Fernández *et al.* (2000) note that the biggest living subterranean rodents live in very friable ‘sand like’ soils because their body size increases the energy expense during long periods of excavation (Vleck, 1979, 1981; Arnold, 1983; Du toit, Jarvis & Louw, 1985; Lessa & Stein, 1992; Lessa *et al.*, 2008). Is the reduction of size an adaptation to harder soils? Sexual size dimorphism might indicate the beginning of an answer.

The sexual dimorphism of larger males is recognized for *Ctenomys* in *C. minutus* (Gastal, 1994;

Freitas, 2005; Marinho & Freitas, 2006), *C. opinus* (Pearson, 1959; Cook, Anderson & Yates, 1990), *C. talarum* (Pearson *et al.*, 1968; Zenuto, Malizia & Busch, 1999), *C. flamarioni* (Bretschneider, 1987), and, inclusively, *C. torquatus* (Travi, 1983). In the present study, the closely-related species *C. lami* and *C. minutus* did not demonstrate a significant difference in mean size of the humerus, although the sexual dimorphism shows a different pattern, with *C. lami* males having a larger humerus, whereas females are of similar size, and *C. minutus* and *C. flamarioni* demonstrated a similar pattern of dimorphism. *Ctenomys lami* have a narrow occurrence compared to the sister species *C. minutus* and *C. flamarioni*. *Ctenomys torquatus* occupies a large area of pampas (hard soils) and demonstrates a smaller humerus and an absence of a clear sexual dimorphism, which could indicate selection for smaller size. This selection for small size could have at least two explanations. First, it may be imposed by a mechanical limit to digging in hard soils. If there is an optimum size for digging, selection could be selecting for smaller animals overall or, if the optimum is between an average male and female size, it could be selecting for smaller males or larger females. Second, the absence of sexual dimorphism could arise from differences in behaviour between the sexes compared to other species. For example, sexual selection could play a stronger role by selecting for bigger males in 'narrow area' species such as *C. lami* or *C. flamarioni*. Finally, these hypotheses need to be tested over many populations because Zenuto *et al.* (1999) and Zenuto, Vassallo & Busch (2002) have shown that sexual selection can be strong enough at the population scale to drive different sexual dimorphism patterns.

#### ACKNOWLEDGEMENTS

We thank Dr Sueli Hoff Reckziegel for help with the myological descriptions, as well as Dr Larissa R. de Oliveira, Dr Aldo Vassallo, Dr Richard Farinã, and two anonymous reviewers for the valuable comments that improved the manuscript. This research was supported by the Projeto tuco-tuco and CAPES. Francisco Steiner de Souza was supported by the CAPES. Pedro Cordeiro Estrela was supported by CNPq (grant number 151887/2005-5) and by the CNPq/CAPES-PROTAX program for taxonomy.

#### REFERENCES

- Arnold SJ. 1983.** Morphology, performance and fitness. *American Zoologist* **23**: 347–361.
- Baylac M. 2007.** *Rmorph: a geometric and multivariate morphometrics library for R*. Available from the author.
- Baylac M, Friess M. 2005.** Fourier descriptors, Procrustes superimposition and data dimensionality. In: Slice DE, ed. *Modern morphometrics in physical anthropology*. New York, NY: Kluwer Academic/Plenum Publishers, 145–162.
- Bookstein FL. 1991.** *Morphometric tools for landmark data*. London, United Kingdom: Cambridge University Press.
- Bou J, Casinos A, Ocanã J. 1987.** Allometry of long bones of insectivores and rodents. *Journal of Morphology* **192**: 113–123.
- Bretschneider DS. 1987.** Alguns aspectos da biologia e ecologia de *Ctenomys flamarioni* Travi, 1981 (Rodentia: Ctenomyidae). Dissertação de Mestrado, UFRGS.
- Casinos A, Quintana C, Viladiu C. 1993.** Allometry and adaptation in the long bones of a digging group of rodents (Ctenomyinae). *Biological Journal of the Linnean Society* **107**: 107–115.
- Castiella MJ, Casinos A. 1990.** Allometry of leg muscles in insectivores and rodents. *Annales des Sciences Naturelles, Zoologie* **11**: 165–178.
- Castillo A, Cortinas M, Lessa EP. 2005.** Rapid Diversification of South American tuco-tucos (*Ctenomys*; Rodentia; Ctenomyidae): contrasting mitochondrial and nuclear intron sequences. *Journal of Mammalogy* **86**: 170–179.
- Cook JA, Anderson S, Yates TL. 1990.** Notes on Bolivian mammals 6: the genus *Ctenomys* (Rodentia: Ctenomyidae) in the highlands. *American Museum Novitates* **2980**: 1–27.
- Cordeiro-Estrela P, Baylac M, Denys C, Marinho-Filho J. 2006.** Interspecific patterns of skull variation between sympatric Brazilian vesper mice: geometric morphometrics assessment. *Journal of Mammalogy* **87**: 1270–1279.
- Dryden IL, Mardia KV. 1998.** *Statistical shape analysis*. London: Wiley.
- Du Toit JT, Jarvis JUM, Louw GN. 1985.** Nutrition and burrowing energetics of the Cape mole-rat *Georchus capensis*. *Oecologia* **66**: 81–87.
- Dubost G. 1968.** Les mammifères souterrains. *Revue d'Ecologie et Biologie du Sol* **5**: 99–197.
- Elissamburu A, Vizcaíno SF. 2004.** Limb proportions and adaptations in caviomorph rodents (Rodentia: Caviomorpha). *Journal of Zoology (London)* **262**: 145–159.
- Fernández ME, Vassallo AI, Zárate M. 2000.** Functional morphology and paleobiology of the Pliocene rodent *Actenomys* (Caviomorpha: Octodontidae): the evolution to a subterranean mode of life. *Biological Journal of the Linnean Society* **71**: 71–90.
- Fischer MS, Schilling N, Schmidt M, Haarhaus D, Witte H. 2002.** Basic limb kinematics of small therian mammals. *Journal of Experimental Biology* **205**: 1315–1338.
- Freitas TRO. 1990.** *Estudos citogenéticos e craniométricos em três espécies do gênero Ctenomys*. PhD Thesis, UFRGS.
- Freitas TRO. 1995.** Geographic distribution and conservation of four species of the genus *Ctenomys* in southern Brazil. *Studies on Neotropical Fauna & Environment* **1**: 53–59.

- Freitas TRO. 2001.** Tuco-tucos (Rodentia, Octodontidae) in southern Brazil: *Ctenomys lami* spec. nov. separated from *C. minutus* Nehring 1887. *Studies on Neotropical Fauna and Environment* **36**: 1–8.
- Freitas TRO. 2005.** Analysis of skull morphology in 15 species of the genus *Ctenomys*, including seven karyologically distinct forms of *Ctenomys minutus* (Rodentia: Ctenomyidae). In: Lacey EA, Myers P, eds. *Mammalian diversification from chromosomes to phylogeography (a celebration of the career of James L. Patton)*. Berkeley: University of California, 131–154.
- Gastal ML. 1994.** Sistema de túneis e áreas de vida de *Ctenomys minutus* (Nehring, 1887) (Rodentia, Caviomorpha, Ctenomyidae). *Inheringia, Serie Zoológica* **77**: 35–44.
- Gava A, Freitas TRO. 2002.** Characterization of a hybrid zone between chromosomally divergent populations of *Ctenomys minutus* (Rodentia: Ctenomyidae). *Journal of Mammalogy* **83**: 843–851.
- Gower JC. 1975.** Generalized Procrustes analysis. *Psychometrika* **40**: 33–50.
- Hildebrand M. 1985.** Digging of Quadrupeds. In: Hildebrand M, Bramble DM, Liem KF, Wake DB, eds. *Functional vertebrate morphology*. Cambridge: MA: Belknap Press of Harvard University Press, 89–109.
- Kingsolver JG, Huey RB. 2003.** Introduction: the evolution of morphology, performance and fitness. *Integrative and Comparative Biology* **43**: 361–366.
- Lacey EA, Patton JL, Cameron NG. 2000.** *Life underground: biology of subterranean rodents*. London: University of Chicago Press.
- Lagaria A, Youlatos D. 2006.** Anatomical correlates to scratch digging in the forelimb of european ground squirrels (*Spermophilus citellus*). *Journal of Mammalogy* **87**: 563–570.
- Lehmann WH. 1963.** The forelimb architecture of some fossorial rodents. *Journal of Morphology* **113**: 59–75.
- Lessa EP. 1990.** Morphological evolution of subterranean mammals: integrating structural, functional, and ecological perspectives. In: NEVO E, REIG OA, eds. *Evolution of subterranean mammals at the organismal and molecular levels*. New York, NY: Allan Liss, 211–230.
- Lessa EP, Cook JA. 1998.** The molecular phylogenetics of tuco-tucos (genus *Ctenomys*, Rodentia: Octodontidae) suggests an early burst of speciation. *Molecular Phylogenetics and Evolution* **9**: 88–99.
- Lessa EP, Stein BR. 1992.** Morphological constraints in the digging apparatus of pocket gophers (Mammalia, Geomyidae). *Biological Journal of the Linnean Society* **47**: 439–453.
- Lessa EP, Vassallo AI, Verzi DH, Mora MS. 2008.** Evolution of morphological adaptations for digging in living and extinct ctenomyid and octodontid rodents. *Biological Journal of the Linnean Society* **95**: 267–283.
- Marinho JR, Freitas TRO. 2006.** Population structure of *Ctenomys minutus* (Rodentia, Ctenomyidae) on the coastal plain of Rio Grande do Sul, Brazil. *Acta Theriologica* **51**: 53–59.
- Massarini AI, Barros MA, Ortells MO, Reig OA. 1991.** Evolutionary biology of fossorial Ctenomyine rodents (Caviomorph: Octodontidae). Chromosomal polymorphism and small karyotypic differentiation in Central Argentina population of tuco-tucos. *Genética* **83**: 131–144.
- Massarini AI, Freitas TRO. 2005.** Morphological and cytogenetics comparison in species of the *mendocinus*-group (genus *Ctenomys*) with emphasis in *C. australis* and *C. flamarioni* (Rodentia-ctenomyidae). *Caryologia* **58**: 21–27.
- Monteiro LR, Reis SF. 1999.** *Princípios de Morfometria Geométrica*. São Paulo: Holos editora.
- Mora M, Olivares AI, Vassallo AI. 2003.** Size, shape and structural versatility of the skull of the subterranean rodent *Ctenomys* (Rodentia, Caviomorpha): functional and morphological analysis. *Biological Journal of the Linnean Society* **78**: 85–96.
- Morgan CC, Verzi DH. 2006.** Morphological diversity of the humerus of the south american subterranean rodent *Ctenomys* (Rodentia, Ctenomyidae). *Journal of Mammalogy* **87**: 1252–1260.
- Nevo E, Reig OA. 1990.** *Evolution of subterranean mammal at the organismal and molecular levels: proceeding of the fifth International Theriological Congress held in Rome, Italy, 22–29 August 1989*. New York, NY: Wiley-Liss.
- Pearson OP. 1959.** Biology of subterranean rodents, *Ctenomys*, in Peru. *Memorias Museo Natural 'Javier Prado'* **9**: 1–56.
- Pearson O, Binsztein N, Boiry L, Busch C, Di Pace M, Gallopin G, Penchaszadeh P, Piantanida M. 1968.** Estructura social, distribución espacial y composición por edades de una población de tuco-tucos (*Ctenomys talarum*). *Investigaciones Zoológicas Chilenas* **13**: 47–79.
- Quintana CA. 1994.** Sistemática y anatomía funcional del roedor Ctenomyinae *Praectenomys* (Caviomorpha: Octodontidae) del Plioceno de Bolivia. *Revista Técnica de Yacimientos Petrolíferos Federales de Bolivia* **15**: 175–185.
- R Development core team. 2004.** *R: a language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing.
- Reig OA, Busch C, Ortells MO, Contreras JR. 1990.** An overview of evolution, systematics, molecular biology and speciation in *Ctenomys*. In: Nevo E, Reig OA, eds. *Evolution of subterranean mammals at the organismal and molecular levels*. New York, NY: Wiley-Liss, 71–96.
- Rohlf FJ. 2004.** *Tpsutil*, Version 1.26; *tpsdiag*, Version 1.40; *tpsregr*, Version 1.29. Stony Brook, NY: State University of New York at Stony Brook.
- Rohlf FJ, Marcus LF. 1993.** A revolution in morphometrics. *Trends in Ecology and Evolution* **8**: 129–132.
- Rohlf FJ, Slice DE. 1990.** Extensions of the Procrustes method for the optimal superimposition of landmarks. *Systematic Zoology* **39**: 40–59.
- Sokal RR, Rohlf FJ. 1995.** *Biometry: the principles and practice of statistics in biological research*. New York, NY: WH Freeman.

- Stein BR. 2000.** Morphology of subterranean rodents. In: Lacey EA, Patton JL, Cameron NG, eds. *Life underground: biology of subterranean rodents*. Chicago, IL: The University of Chicago Press, 19–61.
- Szalay FS, Sargis EJ. 2001.** Model-based analysis of postcranial osteology of marsupials from the Palaeocene of Itaboraí (Brazil) and the phylogenetics and biogeography of Metatheria. *Geodiversitas* **23**: 139–302.
- Thorington RW, Darrow K, Betts AD. 1997.** Comparative myology of the forelimb of squirrels (Sciuridae). *Journal of Morphology* **234**: 155–182.
- Travi VH. 1983.** *Etologia de C. torquatus Lichtenstein, 1830 (Rodentia, Ctenomyidae) na Estação Ecológica do Taím, Rio Grande do Sul, Brasil*. Master Thesis, UFRGS.
- Ubilla M, Altuna CA. 1990.** Analyse de la morphologie de la main chez des especes de *Ctenomys* de l'Uruguay (Rodentia: Octodontidae). Adaptations au fouissage et implications e'volutives. *Mammalia* **54**: 107–117.
- Vassallo AI. 1998.** Functional morphology, comparative behaviour, and adaptation in two sympatric subterranean rodents genus *Ctenomys* (Caviomorpha: Octodontidae). *Journal of Zoology (London)* **244**: 415–427.
- Verzi DH. 2002.** Patrones de evolucion morfológica en Ctenomyinae (Rodentia, Octodontidae). *Mastozoología Neotropical* **9**: 309–328.
- Vleck D. 1979.** The energy cost of burrowing by the pocket gopher *Thomomys bottae*. *Physiological Zoology* **52**: 122–134.
- Vleck D. 1981.** Burrow structure and foraging cost in the fossorial rodent, *Thomomys bottae*. *Oecologia* **49**: 391–396.
- Wainwright PC. 1994.** Functional morphology as a tool in ecological research. In: Wainwright PC, Reilly SM, eds. *Ecological morphology: integrative organismal biology*. Chicago, IL: University of Chicago Press, 42–59.
- Walker WF, Homberger DG. 1997.** *Anatomy and dissection of the rat, 3rd edn*. New York, NY: WH Freeman and Company.
- Woods CA. 1972.** Comparative myology of jaw, hyoid, and pectoral appendicular regions of new and old world hystricomorph rodents. *Bulletin of the American Museum of Natural History* **147**: 115–198.
- Zelditch ML, Swiderski DL, Sheets HD, Fink WL. 2004.** *Geometric morphometrics for biologists, a primer*. New York, NY: Elsevier Academic Press.
- Zenuto RR, Malizia AI, Busch C. 1999.** Sexual size dimorphism, testes size and mating system in two populations of *Ctenomys talarum* (Rodentia: Octodontidae). *Journal of Natural History* **33**: 305–314.
- Zenuto RR, Vassallo AI, Busch C. 2002.** Comportamiento social reproductivo del roedor subterráneo solitario *Ctenomys talarum* (Rodentia: Ctenomyidae) en condiciones de semicautiverio. *Revista Chilena de Historia Natural* **75**: 165–177.

## APPENDIX

**Table A1.** Summary of interlandmark distances in millimeters and statistics per species

Summary	2–18	1–4	3–12	14–19	15–18	17–18
Landmarks	2–18	1–4	3–12	14–19	15–18	17–18
Minimum	37.62	8.96	17.28	11.28	6.56	2.91
Maximum	61.85	14.91	30.77	19.22	10.70	5.63
Mean	49.23	11.49	23.89	14.91	8.78	4.25
Mean per species (std)						
<i>Ctenomys flamarioni</i>	56.41 (14.5)	11.80 (0.4)	26.10 (3.83)	16.38 (1.13)	9.12 (0.36)	4.60 (0.15)
<i>Ctenomys lami</i>	50.80 (13.7)	11.60 (0.95)	24.47 (5.93)	15.88 (2.5)	8.75 (1.05)	4.36 (0.37)
<i>Ctenomys minutus</i>	50.71 (14.65)	11.46 (0.88)	24.35 (6.1)	15.25 (1.01)	9.06 (0.31)	4.41 (0.23)

**Table A2.** Key to the landmarks, with numbers and locations, indicated in the dorsal, ventral, and dista/proximal views of the humerus of the *Ctenomys* species shown in Figure 1.

---

<i>Dorsal View</i>	In the dorsal view, the humerus was naturally laid upon the photographic plane in such a way that its dorsal part faced the camera and was equilibrated laterally by the deltopectoral crest. Landmarks: 1, end of medial deltopectoral crest. 2, end of lateral diaphysis, parallel to landmark 1. 3, encounter of the lateral crest of the great tuberosity with the deltopectoral crest. 4, end of the lateral diaphysis, parallel to landmark 3. 5, meeting of the distal extremity of the great tuberosity with the lateral crest of the great tuberosity. 6, groove in the great tuberosity, most lateral part. 7, groove in the meeting of the great tuberosity with the condyle, most lateral part. 8, proximal end of the condyle. 9, lateral extremity of the condyle. 10, meeting of the distal part of the condyle with the diaphysis. 11, groove between condyle and the great tuberosity, most proximal part. 12, meeting of the most distal part of the deltopectoral crest and supinatory crest with the diaphysis. 13, extremity of the lateral diaphysis, parallel to landmark 12. 14, lateral extremity of the epicondyle. 15, medial extremity of the medial epicondyle. 16, distal extremity of the ulnar facet. 17, proximal extremity of the articulated part of the ulnar facet. 18, proximal extremity of the articulated part of the radial facet. 19, distal extremity of the radial facet.
<i>Ventral View</i>	In the ventral view, the ventral part of the humerus faced the camera and was naturally laid on the support and equilibrated naturally by the trochlea. Landmarks: 1, meeting of the distal part of the great tuberosity with the diaphysis. 2, proximal extremity of the condyle. 3, proximal extremity of the distal process of the great tuberosity. 4, meeting of the distal extremity of the small tuberosity with the diaphysis. 5, proximal extremity of the encounter of the great tuberosity with the diaphysis. 6, distal extremity of the encounter of the great tuberosity with the small tuberosity. 7, meeting of the proximal extremity of the intertubercular sulcus with the small tuberosity. 8, meeting of the proximal part of the deltopectoral crest with the medial diaphysis. 9, extremity of the lateral diaphysis, parallel to landmark 8. 10, medial extremity of the deltopectoral crest. 11, extremity of the lateral diaphysis, parallel to landmark 10. 12, meeting of the distal part of deltopectoral crest with the medial diaphysis. 13, lateral extremity of the diaphysis, parallel to landmark 12. 14, medial extremity of the medial epicondyle. 15, distal extremity of the ulnar facet. 16, proximal extremity of the articular part of the ulnar facet. 17, proximal extremity of the articular part of the radial facet. 18, distal extremity of the radial facet. 19, lateral extremity of the epicondyle.
<i>Distal/Proximal View</i>	In the distal/proximal view, the proximal part of the humerus (condyle) was inserted in a hole in a polyethylene base in such a way that its major axis was perpendicular to the photographic plane, and the distal part (trochlea) faced the lens. Landmarks: 1, medial-ventral extremity of the deltopectoral crest. 2, ventral extremity of the ulna facet. 3, medial extremity of the medial epicondyle. 4, ventral extremity of the ulnar facet. 5, ventral extremity of the radial facet. 6, lateral extremity of the epicondyle (capitulum). 7, ventral extremity of the radial facet.

---