



Original investigation

Craniodental and forelimb specializations for digging in the South American subterranean rodent *Ctenomys* (Hystricomorpha, Ctenomyidae)

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ABSTRACT

We explored the distribution of tooth- and scratch-digging specializations in species of the subterranean rodent *Ctenomys* (tuco-tucos) from diverse environments and representing different clades. Principal component analysis of craniodental and postcranial indexes with functional relevance showed that specializations for tooth-digging on one hand, and scratch-digging on the other, formed two uncorrelated groups of variables; functionally significant enamel traits varied concurrently with the tooth-digging specialization axis. Species occupied all sectors of the morphospace showing that craniodental and forelimb specializations have not been mutually exclusive in the evolution of the genus.

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Introduction

Morphological specializations for digging and subterranean habits have evolved independently in various rodent lineages around the world, and have long appealed to biologists interested in morphological evolution. Among living South American hystricomorph rodents, the most specialized underground dwellers occur in the Octodontoidea and include the coruro *Spalacopus cyanus* (Octodontidae; Honeycutt et al., 2003) and the tuco-tucos of the genus *Ctenomys* (Ctenomyidae; Lessa et al., 2008; Reig, 1970). Most of the Octodontoidea lineages have fossorial habits and build complex burrows with no substantial morphological specialization (Lessa et al., 2008). In contrast, all species of *Ctenomys* are subterranean and have some degree of digging specialization (Lessa et al., 2008; Morgan and Verzi, 2006; Vassallo, 1998; Verzi and Olivares, 2006). As defined in Lessa et al. (2008), fossorial species spend a substantial fraction of their lives outside their burrows, while subterranean ones perform most activities underground. *Ctenomys* includes nearly 65 living species distributed throughout South America in varied habitats with diverse soil and vegetational char-

acteristics (Bidau, 2015; Cook et al., 2000; Freitas, 2016; Reig et al., 1990; Woods and Kilpatrick, 2005). This genus is the only living representative of Ctenomyidae, a family with several extinct genera that encompass considerable disparity associated to the evolution of different degrees of adaptation to life underground (fossorial to subterranean) and digging strategies (scratch- and tooth-digging) at least since the late Pliocene (Fernández et al., 2000; Lessa, 1993; Quintana, 1994; Reig et al., 1990; Reig and Quintana, 1992; Verzi, 2008; Verzi et al., 2010a). In this context, tuco-tucos have been characterized as scratch-diggers that secondarily use their incisors for burrowing (tooth-digging) according to substrate requirements (Ubilla and Altuna, 1990; Camin et al., 1995; De Santis et al., 1998; Giannoni et al., 1996; Lessa, 1993; Lessa et al., 2008; Mora et al., 2003; Vassallo, 1998).

The study of specialized skeletal traits associated to different digging modes in rodents has been heterogeneous in terms of amplitude and range of samples. For dual-mode diggers, a combined approach is particularly interesting to avoid misleading assessments of digging specialization (Lessa, 1990). However, most works have focused on either craniodental or postcranial elements, and to a lesser extent, some have addressed both simultaneously (Hopkins and Davis, 2009; Lagaria and Youtalos, 2006; Lessa and Stein, 1992; Lessa and Thaler, 1989; Lessa et al., 2008; Marcy et al., 2016; Morgan, 2009a; Samuels and Van Valkenburgh, 2009). For *Ctenomys*, previous analyses have focused on only few species (Vassallo, 1998) or partial arrays of traits (cranio-dental: e.g.

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Table 1
Craniodental, forelimb and enamel variables used in this study. References are given for original source of morphofunctional indexes.

Variables	Definition, morphofunctional significance
Amc	Concavity of external auditory meatus (maximum distance between anteroexternal margin of external auditory meatus and posteriormost point of concavity). This variable estimates the fit of the mandibular condyle in the postglenoid joint area (Verzi and Olivares, 2006).
Bl	Basilar length
Dab	Anterior depth of auditory bulla (depth of auditory bulla in front of external auditory meatus, from dorsal contact with squamosal-alisphenoid to ventral margin). Estimates the constraint imposed by the bulla on gape (Verzi and Olivares, 2006).
Pc	Width of base of postcondylean process. This variable estimates the fit of the mandibular condyle in the postglenoid joint area (Verzi and Olivares, 2006).
Pgw	Width of postglenoid fossa (measured at origin of the posterior apophysis of the squamosal). This variable estimates the fit of the mandibular condyle in the postglenoid joint area (Verzi and Olivares, 2006).
lb	Distance between anterior margin of mandibular foramen (mf) and extreme tip of condyle. Estimates depth of the insertion of the lower incisor (Verzi and Olivares, 2006).
Proc	Procumbency of upper incisors (in degrees). Related to angle of attack to soil during tooth-digging (Reig et al. 1965).
DSN	Relative depth of great scapular notch (DSN = Dsn/Tls). Dsn, depth of great scapular notch (maximum distance between scapular blade and scapular spine); Tls, total length of scapula (from margin of glenoid fossa to intersection between scapular spine base and vertebral border of scapula). Estimates relative development of <i>m. infraspinatus</i> ; also related to direction of action lines of trapezius and deltoid muscles (Morgan, 2009a).
HUR	Humeral robusticity (HUR = Hw/HI). Hw, anteroposterior diameter at middle of humeral diaphysis; HI, humeral length. Estimates bone resistance, especially with regards to forces applied on the sagittal plane during scratch-digging (Elissamburu and Vizcaíno, 2004; Hildebrand, 1985).
RWE	Relative width of distal epiphysis at epicondyles (RWE = Ew/HI). Ew, maximum width at humeral epicondyles; HI, humeral length. Estimates development of hand flexor muscles (Hildebrand, 1985).
RDO	Relative development of olecranon (RDO = OI/UI). OI, olecranon length; UI, ulnar length. Estimates mechanical advantage of <i>m. triceps brachii</i> (internal lever arm); also estimates available surface for attachment of hand flexor and extensor muscles (Hildebrand, 1985).
EI	External index (EI = PET/ET). PET, portion externa thickness [radial enamel (RE) + prismless enamel (PLEX)]; ET, total enamel thickness. Higher EI increases resistance to abrasion (Boyde and Fortelius, 1986; Rensberger and Koenigswald, 1980), as does a thick PLEX (Flynn et al., 1987).
HSBi	Hunter–Schreger band inclination, measured as the angle between the HSB and a line perpendicular to the enamel–dentine junction. Increasing inclination reinforces resistance to fracture spread (Koenigswald et al., 1987; Martin, 1999; Pfretzschner, 1988; Rensberger, 1997).

Álvarez et al., 2015; Becerra et al., 2012; Borges et al., 2016; Mora et al., 2003; Verzi and Olivares, 2006; Vieytes et al., 2007; postcranial: e.g. Echeverría et al., 2014; Morgan, 2009b, 2015; Morgan and Álvarez, 2013; Morgan and Verzi, 2006; Steiner-Souza et al., 2010). In this study, we revise craniodental and postcranial traits linked to digging ability in species representing different major clades within *Ctenomys* (Mascheretti et al., 2001; Parada et al., 2011). We do not focus on the phylogenetic pathways of acquisition of such traits, but rather on exploring how tooth- and scratch-digging specializations are combined and distributed through the variation of living *Ctenomys*. Detected patterns are discussed and compared with those described for other digging rodents.

Material and methods

We analyzed craniodental and postcranial morphology in 88 specimens of 18 living species of *Ctenomys* (detailed information in Supplementary material 1). The sample includes species that occupy different environments through diverse areas of South America and spanning major *Ctenomys* clades (i.e. groups ‘*mendocinus*’, ‘*frater*’, ‘*boliviensis*’, ‘*talarum*’, ‘*opimus*’, ‘*Corrientes*’ and the basal species *C. leucodon*; Mascheretti et al., 2001; Parada et al., 2011). Seven craniodental measurements (Fig. 1) with morphofunctional significance were taken following Verzi and Olivares (2006); for analysis, raw values (abbreviations with capitalized first letter) were divided by basilar length to obtain standardised variables (abbreviations in all capitals), except procumbency, which is an angular measurement. To analyze the postcranial skeleton, four indexes (abbreviations in all capitals) were built from seven measurements (Fig. 1), following Hildebrand (1985), Elissamburu and Vizcaíno (2004) and Morgan (2015). Table 1 presents the definition and functional interpretation of these craniodental and forelimb measurements and indexes. Bl, lb and Pc, and all postcranial measurements were taken using digital calipers (0.01 mm). Proc was measured with a protractor on camera lucida drawings (after Reig et al., 1965). The remaining cranial and mandibular measurements were obtained using a reticule eyepiece in a Leica MS5 stereo-

microscope. The morphometric dataset was explored via Principal Components Analysis (PCA) of a correlation matrix of mean values for each species. Missing data (9.3%) were estimated by the multiple- imputation method using NORM 2.03 (Schafer, 1997). Possible relationship between the indexes used in this study and body mass (evolutionary or interspecific allometry; Cock, 1966; Gould, 1966; Klingenberg and Zimmermann, 1992) was explored by means of lineal regressions of the log-10 transformed species means onto a size estimator (DP4- M2 length) using Model II (standard major axis) in the software package SMATR (Warton et al., 2006). Enamel microstructure was analyzed in three species that span the range of tooth-digging adaptation in the genus: *C. australis*, *C. talarum*, and *Ctenomys* sp. ‘perucho’ (see Vassallo, 1998; Verzi and Olivares, 2006; Vieytes et al., 2007). The incisors were embedded in artificial resin and cut in longitudinal sections from their flat medial surface. Each tooth was ground using sandpaper and 1000 -grit powdered silicon carbide, and etched for 4–5 s with 10% hydrochloric acid to accentuate topographic detail (Flynn and Wahlert, 1978). After rinsing and drying, samples were sputter-coated with gold. Photographs of sections were taken under a Jeol JSM 6360 LV scanning electron microscope (SEM). Table 1 presents the definitions of variables and indexes measured from the photographs (Flynn et al., 1987; Koenigswald, 1985; Korvenkontio, 1934; Wahlert and Koenigswald, 1985). The enamel samples are deposited in the Mammals collection of Museo de La Plata.

Results

Allometry analyses

Among craniodental indexes, only PGW and DAB showed a statistically significant correlation with size, while none of the postcranial indexes showed statistically significant correlation with the size estimator (Table 2). PGW showed strong positive allometry, while DAB was negatively allometric; in any case, both indexes had very low correlation with size. For regressions made using raw measurements, significant correlation with the size esti-

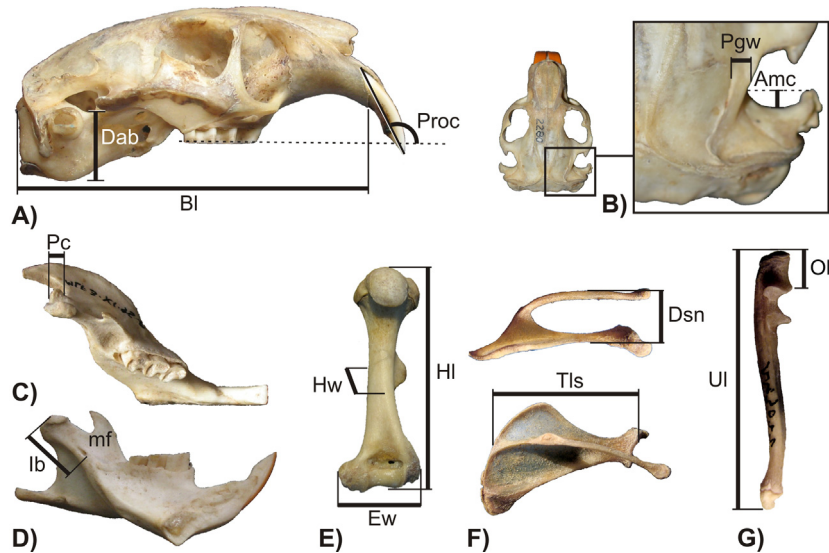


Fig. 1. Measurements in *Ctenomys*. A, lateral and B, dorsal views of skull (with detail of postglenoid region); C, dorsal and D, medial views of mandible; E, posterior view of humerus; F, crano-dorsal and lateral views of scapula; and G, medial view of ulna. Abbreviations: Amc, concavity of external auditory meatus; Bl, basilar length; Dab, anterior depth of auditory bulla; Dsn, depth of great scapular notch; Ew, maximum width at humeral epicondyles; Hl, humeral length; Hw, anteroposterior diameter at middle of humeral diaphysis; Ib, distance between anterior margin of mandibular foramen (mf) and extreme tip of condyle; mf, mandibular foramen; Ol, olecranon length; Proc, procumbency of upper incisors; Pc, width of base of postcondylean process; Pgw, width of postglenoid fossa; Tls, total length of scapula; Ul, ulnar length.

Table 2

Model II regression (SMA, standardised major axis) of cranial and postcranial indexes used in this work on DP4–M2 length (as proxy for size). Asterisk denotes significant regression ($p < 0.05$); bold font denotes significant allometry. Abbreviations: a, intercept; b, slope; r^2 , coefficient of determination.

Variables	r^2	b (95% confidence interval)	a
AMC	0.025	-3.28 (-5.42 to -1.98)	1.24
DAB	0.277*	-1.44 (-2.25 to -0.93)	0.59
IB	0.189	-1.95 (-3.09 to -1.23)	0.91
PC	0.053	-3.63 (-5.96 to 2.21)	1.87
PGW	0.359*	2.97 (1.96–4.48)	-4.31
Proc	0.21	0.63 (0.39–0.99)	1.45
DSN	0.019	-0.59 (-0.98 to -0.36)	0.04
HUR	0.142	0.86 (0.54–1.38)	-1.79
RDO	0.125	0.79 (0.46–1.34)	-1.42
RWE	0.04	0.64 (0.39–1.05)	-1.07

Table 3

Factor loadings of variables for the first two PCs. Loadings > 0.7 in bold.

Variables	PC 1	PC 2
PC	-0.87	0.39
IB	-0.94	0.19
PGW	0.73	0.49
AMC	0.73	-0.43
DAB	-0.85	0.24
Proc	0.87	0.02
DSN	0.45	-0.64
HUR	0.52	0.68
RWE	0.45	0.69
RDO	0.34	0.82
Eigenvalue	4.96	2.68
Total variance	49.56%	26.77%

mator was found only for Pgw among cranial variables, and for all postcranial variables (Supplementary Material 2).

Principal component analysis

The first two principal components (PC1 and PC2) summarized 76.33% of the variation in the sample (Fig. 2, Table 3). PC1 (49.56% of explained variation) summarized mainly the variation of craniodental variables (Proc, IB, PGW, AMC, DAB, and PC). Positive scores

Table 4

Measurements and indexes of the upper incisor enamel of ctenomyids examined. EI, external index; ET, total enamel thickness; HSBi, Hunter-Schreger band inclination; PET, thickness of portio externa. EI, ET, PET in μm ; HSBi in degrees. Values represent mean \pm SD, or range in HSBi.

Species	ET	PET	EI	HSBi	n
<i>C. australis</i>	226.34 \pm 27.1	31 \pm 3.46	0.14 \pm 0.01	30–40	3
<i>C. talarum</i>	187.13 \pm 19.6	31.5 \pm 2.88	0.17 \pm 0.01	30–45	8
<i>Ctenomys</i> sp. 'perucho'	230	50	0.24	40–48	1

on PC1 are associated to higher upper incisor procumbency (Proc) and depth of implantation of the lower incisor (IB), greater development of postglenoid fossa width (PGW), increase in concavity of the external auditory meatus (AMC), lesser development of the postcondyle process (PC), and decrease in anterior depth of the bulla (DAB); these craniodental indexes are related to tooth-digging ability (Table 1). In turn, PC2 (26.77% of explained variation) was highly correlated with morphological specializations of the scapula (DSN), humerus (HUR and RWE) and the forearm (RDO). Positive scores on PC2 were associated to higher development of the olecranon (RDO), greater width of the distal humeral epiphysis (RWE) and humeral robusticity (HUR), and lesser depth of the great scapular notch (DSN); these postcranial indexes are related to scratch-digging ability (Table 1).

In this morphospace, *Ctenomys* species were distributed in all four quadrants; those with stronger craniodental specializations (e.g. *C. lewisi*, *C. leucodon*, *Ctenomys* sp. 'perucho') were located at positive PC1 scores, while those with more marked forelimb specializations (e.g. *C. frater*, *C. steinbachi*, *Ctenomys* sp. 'mármol'), at positive PC2 scores (Fig. 2). As expected from the allometric analyses of indexes (and even raw cranial measurements), their distribution was not related to size.

Enamel microstructure

Table 4 summarizes the measurements and indexes that characterize the enamel microstructure of incisors in three *Ctenomys* species with different digging modes. Further details of incisor enamel of the scratch-digger *C. australis* (Fig. 3A) and the occa-

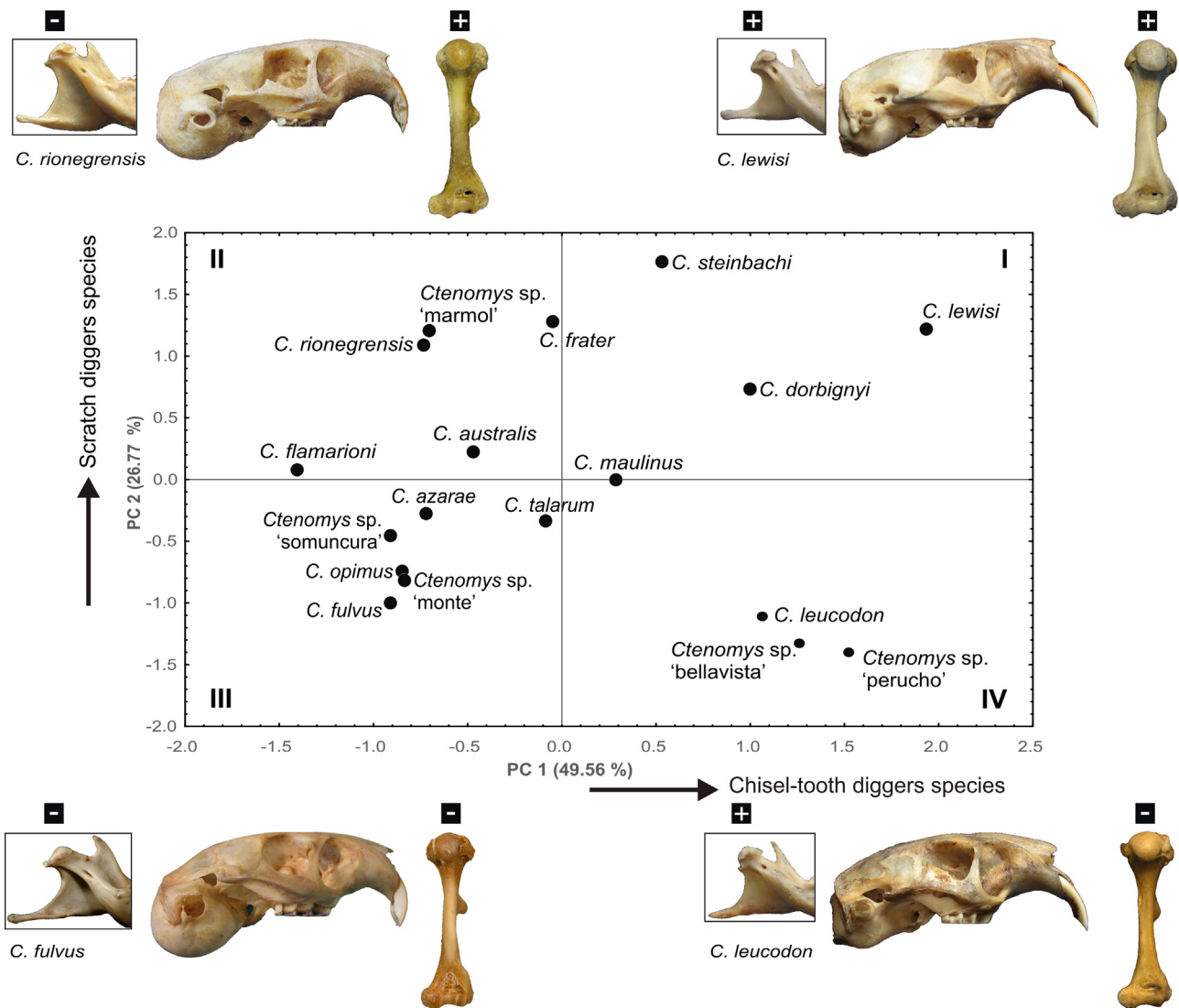


Fig. 2. Scatterplot of scores on principal component axes 1 (PC 1) and 2 (PC 2) from principal component analysis of skull, jaw, dental and postcranial variables of *Ctenomys* species. Extremes of variation illustrated by selected species; in each case, medial portion of the alveolar incisor in the mandible, lateral view of skull, and posterior view of humerus is shown. I–IV: quadrants.

sional tooth-digger *C. talarum* (Fig. 3B) were published by Vieytes et al. (2007). *Ctenomys* sp. 'perucho' (Fig. 3C) has a total enamel thickness (ET) of 230 μm with a thick layer of radial enamel (RE: 24% of ET); the inclination of Hunter-Schreger bands (HSBi) is high, greater than 45°. Although the observed between-taxa disparity in enamel traits is low (as expected for an intrageneric comparison), *Ctenomys* sp. 'perucho' shows interesting differences in the studied traits: it has the highest values of HSBi, RE, External Index (EI) and portio externa thickness (PET).

Discussion

As mentioned above, when compared to epigeal and fossorial non-specialized octodontoids, all species of *Ctenomys* exhibit some degree of craniodental and forelimb specialization, which has been described in a comprehensive literature (see literature cited in Álvarez et al., 2015 and Morgan, 2015). As primary scratch-diggers, they have robust arm bones with large crests and processes, and well-stabilized articulation surfaces (Echeverría et al., 2014; Morgan and Álvarez, 2013; Morgan and Verzi, 2006, 2011; Vassallo, 1998), traits that provide larger surface areas for muscle attachment and greater resistance to the forces gener-

ated during digging, and occur in many scratch-digging mammals (Dubost, 1968; Hildebrand, 1985; Stein, 2000; Polly, 2007; Samuels and Van Valkenburgh, 2008). Similarly, many of their craniodental specializations, such as relatively large skull, strong rostrum, robust and generally flaring zygomatic arches, well developed nuchal crest, relatively short and deep mandible, and strong, procumbent incisors with deep implantation also occur in other tooth-digging rodents (Dubost, 1968; Gomes Rodrigues et al., 2016; Hildebrand, 1985; Lessa, 1990, 1993; McIntosh and Cox, 2016; Nevo, 1979, 1999; Stein, 2000; Vieytes et al., 2007). Additionally, *Ctenomys* species have a specialized postglenoid joint morphology that stabilizes the jaw joint (Álvarez et al., 2015; Verzi and Olivares, 2006).

Beyond verifying the existence of these shared features, we explored the simultaneous distribution of craniodental and forelimb variation related to digging among *Ctenomys* species, as estimated by key traits (Table 1). Although variation of these traits was previously studied within the genus (see, for example, Borges et al., 2016; Mora et al., 2003; Morgan and Álvarez, 2013; Verzi and Olivares, 2006; Vassallo, 1998; Vieytes et al., 2007), it had not been examined in a concurrent analysis. Our results showed that specializations for tooth-digging on one hand, and scratch-digging on the other, formed two groups of variables that were distinctly uncor-

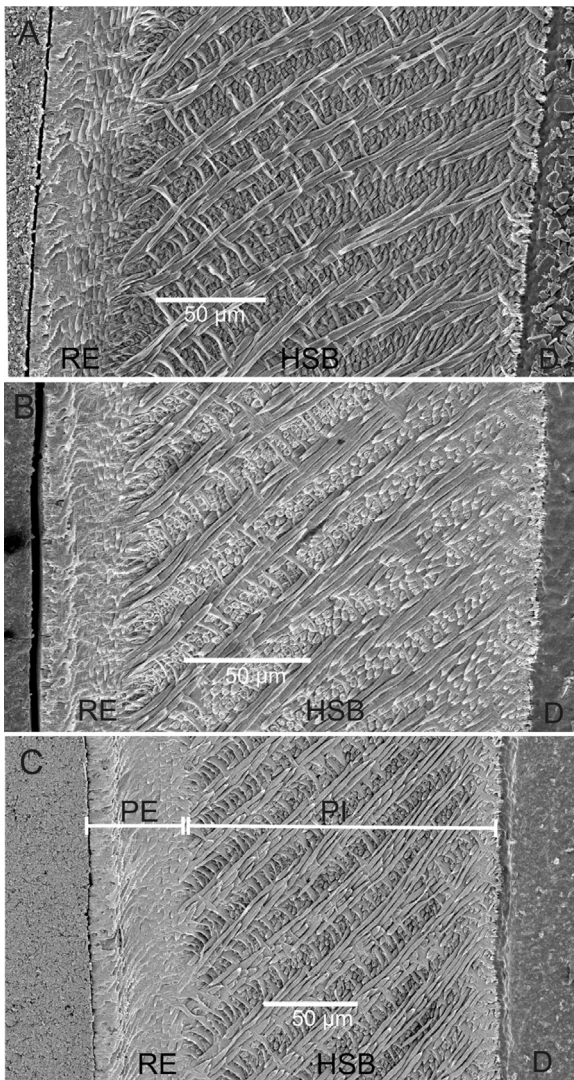


Fig. 3. SEM photographs of enamel microstructure of upper incisors (longitudinal sections, outer tooth surface at left, enamel-dentine junction at right, and occlusal tip down): A. *Ctenomys australis*; B. *Ctenomys talarum*; C. *Ctenomys* sp. 'perucho'. Abbreviations: D, dentine; HSB, Hunter-Schreger Bands; PE, portio externa; PI, portio interna; RE, radial enamel.

related. Thus, PC1 summarized tooth-digging specializations, with species positioned along this axis in agreement with the findings of Verzi and Olivares (2006). Furthermore, the enamel microstructure of one of the species highly specialized for tooth-digging, *Ctenomys* sp. 'perucho', presented high EI and greater HSBi (Table 1; Vieytes et al., 2007), while the primarily scratch-digger *C. australis* (Vassallo, 1998) showed lowest EI and HSBi values, and the occasionally tooth-digging *C. talarum* (Vassallo, 1998) had intermediate values. Because these variables have been shown to be rather homogeneous in ecologically and behaviorally dissimilar octodontoids, we assume that these slight differences are informative (Vieytes et al., 2007). These enamel traits of *Ctenomys* sp. 'perucho' could represent adaptive responses to biomechanical requirements, similar to those detected previously in the specialized fossil ctenomyid *Eucelophorus* (Vieytes et al., 2007) and other tooth-digging rodents (Geomyidae, Buzas-Stephens and Dalquest, 1991). Thus, although it was not possible to analyze the enamel pattern of incisors in all the species studied here, these differences suggest a trend of increasing tooth-digging specialization that agrees with our PC1. On the other hand, the second axis of variation (PC2) summarized scratch-digging specializations, which

were not necessarily coupled with tooth-digging specializations (see Fig. 2).

Additionally, our results showed that morphological specialization patterns in *Ctenomys* were not influenced by size. This differs from previous proposals (Schleich and Vassallo, 2003; Vassallo and Mora, 2007) which indicate that cranial shape variation related to digging is constrained by size among *Ctenomys* species (see Verzi and Olivares, 2006; Verzi et al., 2010b). Regarding postcranial variables, although raw measurements showed moderate correlation with size, this was not the case for the indexes built to capture morphofunctional specialization.

The comparison of *Ctenomys* with other subterranean rodents shows some interesting parallels regarding patterns of specialization. In the subterranean pocket gophers (Geomyidae), which are particularly similar to tuco-tucos in general appearance (Reig et al., 1990), it has been hypothesized that increasing specialization for scratch- or tooth-digging would be alternative, so that a given taxon has stronger specializations at either craniodental or forelimb level, but not both (Lessa, 1993; Lessa and Stein, 1992, Fig. 3; Lessa and Thaler, 1989). Among tuco tucos, some species follow this pattern, combining more specialized craniodental morphology with less specialized forelimbs (Fig. 2, quadrant IV: *C. leucodon*, *Ctenomys* sp. 'bellavista', *Ctenomys* sp. "perucho") and viceversa (Fig. 2, quadrant II: *Ctenomys rionegrensis*, *Ctenomys* sp. 'marmol'). However, at least some *Ctenomys* species exhibit strong specializations at both craniodental and forelimb level (Fig. 2, quadrant I: *C. dorbigny*, *C. lewisi*, *C. steinbachi*) showing that these would have not been mutually exclusive in the evolution of the genus. Similar results were found in a recent analysis of the geomyid *Thomomys* (Marcy et al., 2016). It is not possible at present to assess the influence of phylogeny on this pattern because several of the taxa in our sample are undescribed populations that have not yet been included in phylogenetic analyses. Nevertheless, at least preliminarily, the distribution of taxa in the morphospace shows some degree of phylogenetic structure; when our results are inspected in light of the molecular results obtained by Parada et al. (2011), it becomes apparent that the species belonging to the 'mendocinus' group (*C. flamarioni*, *C. australis*, *C. azarae*) are restricted to the portion of the morphospace that encompasses lesser craniodental specialization (Fig. 2, quadrants II and III); similarly, species belonging to the 'frater' group, i.e. *C. lewisi* and *C. frater*, show strong specialization at both craniodental and forelimb level (Fig. 2, quadrant I).

The fact that the species of our sample occupy all sectors of the morphospace suggests the evolution of diverse strategies for digging in *Ctenomys* from a threshold of specialization that would have already been acquired in the late Pliocene (Verzi et al., 2010a). Furthermore, the apparent lack of constraint in the specialization of tuco-tucos toward either or both digging modes might have played a role in the successful occupation of diverse environments, especially those most challenging, such as hard soils and/or with high proportion of roots and other elements that hinder burrow construction. Additional studies are necessary to explore the influence of phylogeny as well as relevant environmental characteristics on these patterns of morphological variation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.mambio.2017.07.005>.

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