Postnatal Ontogeny of Limb Proportions and Functional Indices in the Subterranean Rodent *Ctenomys talarum* (Rodentia: Ctenomyidae)

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ABSTRACT Burrow construction in the subterranean *Ctenomys talarum* (Rodentia: Ctenomyidae) primarily occurs by scratch-digging. In this study, we compared the limbs of an ontogenetic series of *C. talarum* to identify variation in bony elements related to fossorial habits using a morphometrical and biomechanical approach. Diameters and functional lengths of long bones were measured and 10 functional indices were constructed. We found that limb proportions of *C. talarum* undergo significant changes throughout postnatal ontogeny, and no significant differences between sexes were observed. Five of six forelimb indices and two of four hindlimb indices showed differences between ages. According to discriminant analysis, the indices that contributed most to discrimination among age groups were robustness of the humerus and ulna, relative epicondylar width, crural and brachial indices, and index of fossorial ability (IFA). Particularly, pups could be differentiated from juveniles and adults by more robust humeri and ulnae, wider epicondyles, longer middle limb elements, and a proportionally shorter olecranon. Greater robustness indicated a possible compensation for lower bone stiffness while wider epicondyles may be associated to improved effective forces in those muscles that originate onto them, compensating the lower muscular development. The gradual increase in the IFA suggested a gradual enhancement in the scratch-digging performance due to an improvement in the mechanical advantage of forearm extensors. Middle limb indices were higher in pups than in juveniles–adults, reflecting relatively more gracile limbs in their middle segments, which is in accordance with their incipient fossorial ability. In sum, our results show that in *C. talarum* some scratch-digging adaptations are already present during early postnatal ontogeny, which suggests that they are prenatally shaped, and other traits develop progressively. The role of early digging behavior as a factor influencing on morphology development is discussed. J. Morphol. 000:000–000, 2014. © 2014 Wiley Periodicals, Inc.

KEY WORDS: ecomorphology; functional morphology; limb's development; scratch-digging; fossorial adaptations; tuco-tucos

INTRODUCTION

Caviomorph rodents (Rodentia: Hystricognathi) are a characteristic group of South American mammals that evolved in isolation during most of the Cenozoic, reaching taxonomical diversity greater than any equivalent rodent taxon (Vucetich and Verzi, 1999). They represent one of the most diverse clades of rodents in terms of ecology, life history traits, body size, and locomotor habits (Mares and Ojeda, 1982). For example, Octodontoidea includes aquatic, arboreal, terrestrial, fossorial, and completely subterranean species with wide-ranging climbing and digging specializations (Morgan, 2009; and references therein). Particularly, echimyids (spiny rats) and ctenomyids (tuco-tucos) are the two most diverse families of South American caviomorphs (Parada et al., 2011; Fabre et al., 2013). *Ctenomys*, the sole living genus of the family Ctenomyidae, is characterized by a high species richness (∼60 recognized living species; Parada et al., 2011, and reference therein). These species occur over a wide geographical and environmental range, nevertheless they all share adaptations to terrestrial and underground activity (Camín et al. 1995), with specific tuning to

particular ecological conditions (Antinuchi and Busch, 1992; Vassallo, 1998; Lacey et al., 2000; Luna et al., 2002; Mora et al., 2003). Like most other caviomorphs, tuco-tucos are proficient scratch-diggers that dig primarily by means of vigorous scraping movements (Hildebrand, 1985b) which comprise rapid alternating strokes of their forefeet (e.g., Vassallo, 1998) and the use of predominantly parasagittal movements (Kley and Kearney, 2007). Secondarily, they use their large and procumbent incisors to assist in loosening soils (chisel-tooth digging), according to substrate requirements (see De Santis et al., 1998 and references therein; Ubilla and Altuna, 1990; Vassallo, 1998; Stein, 2000). Mammalian scratch-diggers show musculoskeletal modifications, mainly in their forelimbs, that provide increased strength in flexing their large digits and the wrist, extending the elbow, flexing the humerus on the scapula, and stabilizing the shoulder (Hildebrand, 1985a). Limb morphology reflects limb function and, as in many musculoskeletal systems, a trade-off exists between force and speed (Hildebrand, 1985b). Hence, limbs adapted for speed are elongated in their distal segments and their muscles insert relatively close to the joints, while limbs adapted for force have relatively short distal segments and their muscles tend to insert far from the joints they turn (Hildebrand, 1985b). Casinos et al. (1993) showed that, in ctenomyines, the digging activity can exert selective pressure on long bone design, producing shorter and thicker structures. For instance, when compared to nondigging rodents, the humerus of scratch-diggers shows a greater robustness, which provides higher resistance to increased muscle loading; greater articular surface area for elbow stabilization; and a more-distal deltoid process and wider epicondyles, which give greater mechanical advantage to forelimb muscles (Morgan and Verzi, 2006; and references therein). A study of limb morphology and function in caviomorph rodents not including *Ctenomys* (Elissamburu and Vizcaíno, 2004) showed that, in general terms, caviomorph diggers are characterized by morphofunctional indices (ratios) that represent higher humeral and ulnar robustness, higher deltoid and epicondylar development, and increased mechanical advantage of elbow extensor muscles. The scaling and allometry of these indices, which are constructed by means of different linear and functional variables of long bones, have received considerable attention across the major clades of Tetrapoda; particularly, previous studies have been focused on interspecific comparisons among mammals (Kilbourne and Makovicky, 2012). However, few studies have investigated the allometry of limb bone proportions during ontogeny (e.g., Carrier, 1983; Lammers and German, 2002; Young, 2005; Kilbourne and Makovicky, 2012). Furthermore, animal morphology and

behavior are closely related, which suggests that specializations for a particular locomotor behavior (e.g., digging) may affect limb bone morphology throughout ontogeny (Lammers and German, 2002). Most studies that examined morphological specialization for a particular behavior have solely focused on adults. This is of particular concern because natural selection acts, not only at the adult stage, but on the entire life history of animals and adaptations that are advantageous to juveniles may also have considerable influences on adult morphology and behavior (Carrier, 1996). For example, some allometric changes have been shown or suggested to enhance the performance in juveniles (Carrier, 1996; Herrel and Gibb, 2006), which include relatively longer limbs, greater muscular forces and contractile velocities, and higher muscular mechanical advantages (Carrier, 1983, 1995; Heinrich et al., 1999; Young, 2005).

The aim of the present study was focused on how limb proportions and functional indices change throughout postnatal ontogeny in the subterranean rodent *Ctenomys talarum* Thomas, 1898 (Rodentia: Ctenomyidae; Los Talas' tuco-tuco). Eilam (1997) studied five rodent species differing in their forms of progression [voles (*Microtus socialis*), gerbils (*Gerbillus dasyurus*), jirds (*Meriones tristrami*), dormice (*Eliomys malnurus*), and jerboas (*Jaculus orientalis*)], and showed that species with a more specialized locomotion undergo greater morphological changes during ontogeny than those with a more generalized locomotion. Thus, considering that the subterranean mode of life in *C. talarum* is a highly specialized (Hildebrand 1985a) and derived behavior when compared to other related caviomorphs (Lessa et al., 2008), we hypothesized important differences between pups, juveniles, and adults in those morphofunctional traits related to digging.

MATERIAL AND METHODS
Specimens

We collected data from an ontogenetic series of 32 specimens of *Ctenomys talarum*, Thomas, 1898 (Appendix I), comprising 12 pups (seven females, five males), six juveniles (three females, one male, and two unsexed) and 14 adults (eight females, six males). The material is housed in the Museo de Ciencias Naturales de La Plata, Buenos Aires, Argentina, and Laboratorio de Morfología Funcional y Comportamiento, FCEyN, Universidad Nacional de Mar del Plata, Buenos Aires, Argentina.

Measurements

Fifteen linear measurements were recorded from the long bones (forelimb: humerus and ulna; hindlimb: femur and tibia) of each specimens (Fig. 1). These measurements, corresponding to diameters and functional lengths (lengths among articular surfaces) of the bones, and muscular insertion sites, were taken using a digital caliper to the nearest 0.01 mm. In pups, because their long bones were too small and fragile to be measured
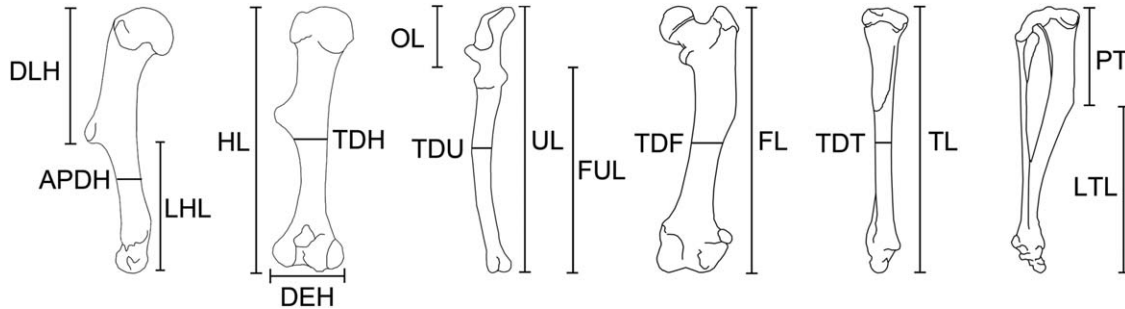
Fig. 1. Linear measurements of the long bones used in this study. Humerus: HL, functional humeral length; DLH, humeral head–distal origin of deltoid process length; TDH, transverse width of the humerus at the diaphyseal midpoint; APDH, anteroposterior diameter of the humerus at diaphysis (taken at a distance of 35% of the humeral length from the distal articular surface); LHL, lower humeral length (difference between total humeral length and distal origin of deltoid process length); DEH, width at epicondyles. Ulna: UL, total ulna length; OL, tip of the olecranon process-center of the trochlear notch length; FUL, difference between total ulna length (UL) and olecranon length (OL); TDU, transverse width of the ulna at the diaphysis. Femur: FL, total femur length; TDF, transverse diameter of the femur at diaphysis. Tibia: TL, distance between the proximal and distal articular surfaces of the tibia; PT, proximal articular surface of the tibiadistal point of the tibial tuberosity length; LTL, lower tibia length (difference between total tibia length and tibial tuberosity length); TDT, width of the tibia at the diaphyseal midpoint.

using the digital caliper, linear measurements were taken by means of digital images. For this purpose, we used an Olympus E620 digital camera attached to a trinocular stereomicroscope (Olympus SZ61,). Digital images were processed using the software NIH ImageJ v. 1.41 (Rasband, 2008).

Morphofunctional Indices

We computed a set of 10 morphofunctional indices (ratios) from linear measurements, which represent attributes of bones, degree of development and mechanical advantage of forelimb muscles related to digging. Ratios are commonly used in biological studies not only because of their functional significance, but also because they represent easy to understand features of organisms (see e.g., Samuels and Van Valkenburgh, 2008). The indices used in this study were chosen because they are good indicators of digging capacity and were based on previous works (Table 1).

We assessed the degree of development of i) the teres major process (ventral prolongation of the vertebral border of the scapula, also called scapular fossa; see Kley and Kearney, 2007), origin site of one of the shoulder flexors (*M. teres major*); ii) the deltoid process, and iii) the olecranon process, by means of the visual examination of bone material and digital photographs. The teres major process is the most prominent trait of the scapulae of fossorial rodents, and it is absent in nonfossorial animals (Lehmann, 1963). In some species, it may even be continued as a shelf along the axillary border of the scapula to the infraglenoid tuberosity, named postscapular fossa (Lehmann, 1963). The importance of these bony modifications lies in their correlation with the mass of the shoulder musculature (Lehmann 1963). To assess the presence and development of the teres major, deltoid and olecranon processes during the prenatal period, we observed two cleared and stained fetuses (~2 months of gestation; total gestation period in *C. talarum* ~3 months), whose cartilages and bones were differentially stained using alcian blue and alizarin red S simultaneously (McLeod 1980). Digital photographs of the scapulae, humeri, and ulnae of eight adults of the semifossorial *Octodon degus* Molina 1782 (Rodentia, Octodontidae; the common degus) were examined and included in some figures in order to highlight the status of some characters in the studied ontogenetic sequence of *C. talarum*. Regarding anatomical adaptations for digging, *O. degus* is considered as a generalized form (Lessa et al., 2008). This species lives in social groups, in which individuals share underground complex burrow systems during night time and emerge to forage above ground during the daytime (Ebensperger et al., 2004).

Statistics

Changes during postnatal ontogeny in the functional indices were analyzed using a two-way multivariate analysis of variance (MANOVA), and differences in the individual indices were assessed by univariate ANOVAs with Scheffe's F and Tamhane's T2 procedures for comparison of individual groups. Factors (independent variables) used in the MANOVA were: age category (pups, juveniles and adults), sex (female, male), and the age category by sex interaction. We included the sex as a factor in order to determine whether sexual dimorphism needs to be accounted for in the analyses. We used an alpha level of 0.05 for all statistical tests.

Because the use of ratios can pose problems in statistical analyses, as they tend to violate assumptions of normality and homoscedasticity included in parametric tests (Sokal and Rohlf, 1981), ratios were log_{10} transformed prior to MANOVA. Normality was tested with the Shapiro–Wilk test and homogeneity of variance with the Levene's test (Zar, 1999). Despite the fact that distributions were normal except for URI and BI (both $P < 0.05$), and variances were homogeneous except for IFA, CI, and BI (all $P < 0.05$), we used ANOVA because it is a robust test and operates well even with considerable deviations from normality and homoscedasticity (Zar 1999). All tests were performed with STATISTICA v8.0 (StatSoft, Milton Keynes, UK) and PASW Statistics v18.0 (SPSS, Hong Kong, China).

Differences among the three age group vectors of the morphofunctional indices' means (i.e., the three age group centroids) were described with stepwise discriminant analysis, which determines linear combinations of variables that maximize separation among individuals belonging to each age group. This technique reviews and selects variables for inclusion in the model that contribute most to discrimination among groups. In discriminant analysis used in a descriptive way, the grouping variable (age group) has an explanatory role and the response variables (morphofunctional indices) correspond to the outcomes (Huberty and Hussein, 2003).

To examine scale effects on long bone allometry, naso-occipital length was taken as a proxy of body size (Schleich and Vassallo, 2003). This variable was recorded from each skull using a digital caliper to the nearest 0.01 mm. Linear measurements (Fig. 1) were log_{10} transformed prior to performing bivariate reduced major axis (RMA, model II) regressions, a method that is chosen because of its appropriateness for examining allometric relationships (Warton et al., 2006). This analysis assumes that neither variable is independent in the strict sense, with both variables containing error in some extent. In this case, symmetrical line-fitting techniques (model II), are more suitable than standard ordinary least squares regression

TABLE 1. Morphological indices used in the analyses, their abbreviation, calculations, and their inferred functional significance

Index	Calculation	Functional significance
Relative position of the deltoid process (RDP)	Humeral head-distal origin of deltoid process length divided by humeral length (DLH/HL)	This index is an estimator of in-lever arm of the deltoid and pectoral muscles.
Humerus robustness index (HRI)	Anteroposterior diameter at diaphysis divided by humerus length (APDH/HL)	Indicates robustness of the humerus and its ability to resist bending stresses.
Relative width at epicondyles (EI)	Epicondylar width of the humerus divided by humeral length (DEH/HL)	Indicates the relative area available for the origins of the forearm flexors, pronators, and supinators, and hence their degree of development. It is considered as a good indicator of fossoriality.
Index of fossorial ability (IFA)	Olecranal length divided by the functional ulnar length (OL/FUL)	This index reflects the mechanical advantage of the triceps and dorsoepitrochlearis muscles during elbow extension, and it is considered as a good indicator of fossoriality.
Robustness of the forearm (URI)	Transverse diameter of the ulna at the diaphyseal midpoint divided by functional ulnar length (TDU/FUL)	Reflects the resistance of the ulna to bending, and also indicates the relative surface available for the insertion of muscles involved in pronation and supination of the forearm, and flexion of the manus and digits.
Brachial index (BI)	Functional ulnar length divided by humerus length (FUL/HL)	Indicates the relative proportions of proximal (humerus) and middle (ulna) elements of forelimbs (i.e., forearm), and gives an indication of the extent to which the forelimb is apt for fast movement.
Femur robustness (FRI)	Transverse diameter of the femur at the diaphyseal midpoint divided by the functional femoral length (TDF/FL)	Indicates robustness of the femur and its ability to resist bending and shearing stresses.
Tibial spine index (TSI)	Proximal tibia length, which measures the distance from the proximal articular surface of the tibia to the distal point of the tibial tuberosity, divided by the tibial length (PT/TL)	Reflects the strength of the leg and the relative width available for the insertion of the gracilis, semitendinosus and semimembranosus muscles and the foot flexors.
Robustness of the tibia (TRI)	Transverse diameter of the tibia divided by the tibial length (TDT/TL)	Indicates robustness of the tibia and its ability to resist bending and shearing stresses.
Crural index (CI)	Tibia length divided by femur length (TL/FL)	Indicates the relative proportions of proximal (femur) and middle (tibia) elements of the hindlimb (forelegs). As for BI, this index has been considered as indicative of how well the hindlimbs are apt for speed.

Measurements indicated above are illustrated in Figure 1.
Indices follow Hildebrand, 1985b; Biewener and Taylor, 1986; Lessa and Stein, 1992; Biknevicius, 1993; Casinos et al., 1993; Vassallo, 1998; Vizcaíno et al., 1999; Fernández et al., 2000; Stein, 2000; Vizcaíno and Milne, 2002; Elissamburu and Vizcaíno, 2004; Morgan and Verzi, 2006; Samuels and Van Valkenburgh, 2008; Samuels et al., 2013.

(see Sokal and Rohlf, 1981), and simulation investigations have indicated that these methods provide more stable estimates, particularly if sample sizes are small (Riggs et al., 1978). RMA analyses were run using the software RMA (v1.17; Bohonak, 1998) with equations in the form of:

$$\log y = \log a + b(\log x) \tag{1}$$

where y is the raw measurement, a is the y-intercept, x is the proxy for body size, and b is the slope. By taking the antilog of both sides, it is revealed that the equation for the regression line becomes the general allometric equation,

$$y = ax^b, \tag{2}$$

where a is the allometric constant and b is the allometric exponent (Schmidt-Nielsen, 1984).

Confidence intervals for regression slopes were generated using a bootstrapping routine (10,000 replicates). If the confidence interval excluded a value of 1.0 (the slope value expected for isometric growth for linear measurements vs. the naso-occipital length), then allometry (i.e., a departure from isometry) was considered significant. Thus, negative allometry was indicated by slopes significantly <1.0, positive allometry by slopes significantly >1.0, and isometry by slopes not significantly different from 1.0. In the power Eq. (2), the allometric exponent b (regression slope) details the relative magnitude of y versus x change, thus indicating ontogenetic change. Based on this fact, we compared regression slopes using parallelism (homogeneity of slopes) test to describe possible differences in the relative magnitude of change between morphological variables used for constructing the morphofunctional indices.

RESULTS
Multivariate Analysis

MANOVA found significant differences in the studied morphofunctional indices among age
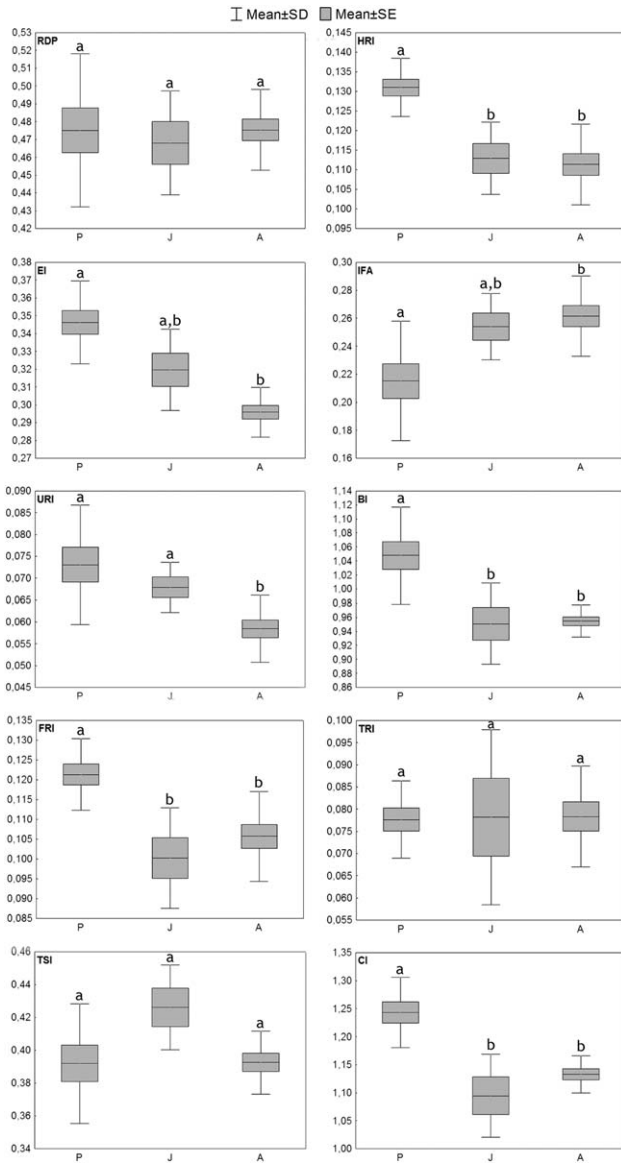
Fig. 2. Box and whisker plots (mean $\pm$ SE and $\pm$ SD) of each functional index by age (P = pups; J = juveniles; and A = adults). The same letters above the box and whisker plots represent similar means after the use of post hoc Scheffe's F and Tamhane's T2 tests.

categories (Wilks' $\lambda = 0.062$; df = 20; $P < 0.001$), but a nonsignificant multivariate effect of sex (Wilks' $\lambda = 0.586$; df = 10, $P > 0.05$). According to this analysis, seven of the ten indices evaluated were informative of the variation between groups. ANOVAs found that indices that showed significant univariate differences between ages were (Fig. 2): robustness of the humerus (HRI; $F_{2,29} = 16.520$, $P < 0.001$); epicondylar index (EI; $F_{2,29} = 21.619$, $P < 0.001$); index of fossorial ability (IFA; $F_{2,29} = 6.847$, $P < 0.01$); ulna robustness index (URI; $F_{2,29} = 8.134$, $P < 0.01$); brachial index (BI; $F_{2,29} = 12.706$, $P < 0.001$); femur robustness

(FRI; $F_{2,29} = 10.600$, $P < 0.001$); and crural index (CI; $F_{2,25} = 17.500$, $P < 0.001$). Conversely, the relative position of the deltoid process (RDP; $F_{2,29} = 0.114$, $P > 0.05$), the robustness of the tibia (TRI; $F_{2,25} = 0.020$, $P > 0.05$), and the tibial spine index (TSI; $F_{2,25} = 2.800$, $P > 0.05$) showed nonsignificant univariate differences between ages. Post hoc tests revealed that *C. talarum* pups, when compared to adults, were characterized by relatively short and massive humeri, ulnae and femora (high HRI, URI, and FRI), relatively enlarged humeral epicondyles (high EI), a reduced olecranon process (low IFA), and longer middle limb elements (higher CI and BI). Juveniles showed intermediate values of morphofunctional indices (EI, IFA, and URI) or nonsignificant differences when compared to adults (HRI, FRI, CI, and BI). Post hoc tests' results, mean values and standard deviations of morphofunctional indices for age groups are summarized in Figure 2.

Stepwise discriminant analysis performed with those indices for which the MANOVA revealed a significant relationship with age categories (i.e., HRI, EI, IFA, URI, BI, FRI, and CI), showed a highly significant separation of the groups (Wilks' $\lambda = 0.097$, $F_{12,40} = 7.347$, $P < 0.0001$), while the variables that contributed most to discrimination among groups (i.e., variables included in the model) were EI, CI, BI, URI, IFA, and HRI. Standardized coefficients of the canonical discriminant function, eigenvalues, proportion of variance explained by each function, and Wilks' lambda are summarized in Table 2. The analysis yielded two discriminant functions, which accounted for 100% of the total variance in the data (Table 2; Fig. 3). The first discriminant function (DF1) accounted for 88.84% of the total variance and was negatively correlated with fossorial ability index (IFA) and positively correlated with brachial index (BI), epicondylar index (EI), robustness of the ulna (URI), and robustness of the humerus (HRI). Pups had positive scores for DF1 (Fig. 3) reflecting relatively longer forelimb middle elements (higher BI), wider humeral epicondyles (higher EI), shorter and more massive ulna and humerus (higher URI and HRI). Juveniles and adults had mainly negative DF1 scores (Fig. 3) associated with relatively longer olecranon processes, which represent a greater fossorial ability (higher IFA). The second discriminant function (DF2) accounted for 11.16% of variance and was positively correlated with ulnar robustness (URI) and epicondylar index (EI) and negatively correlated with crural index (CI). Juveniles showed positive DF2 scores; however, only two individuals showed strongly positive DF2 scores and fell outside the range of variation seen in adults (Fig. 3). These individuals were characterized by unusually low values for CI and, particularly, one of them showed also very low values for URI and EI. Conversely, pups and adults were

TABLE 2. *Standardized canonical discriminant function coefficients, eigenvalues, proportion of variance explained by each function and Wilks' lamda*

Functional index	DF1	DF2
EI	0.556	0.307
CI	0.018	−0.989
BI	0.645	0.140
URI	0.490	0.714
IFA	−0.458	−0.278
HRI	0.389	−0.206
Eigenvalue	5.206	0.654
% Variance	88.84	11.16
Wilks' λ	0.097	0.604

Fig. 4. Detail of the forearm transparency of a ~2 months of gestation fetus of Los Talas' tuco-tuco *C. talarum*. Notice that the teres major process is still absent.

scattered throughout, which suggest that some pup and adult individuals display extreme values for URI, EI, and CI (Fig. 3).

The ability of the discriminant model to separate individuals into age categories was assessed using the classification phase. Age categories of individuals were correctly classified in 96.4% of the cases, with 78.6% correct classification when cross-validated (where each case is classified using the discriminant functions calculated from all the remaining cases). Classification success was high, being 100% for pups and adults, and 80% for juveniles. Of 28 specimens included in the analysis, only one juvenile was misclassified as a pup.

Teres Major, Deltoid, and Olecranon Processes Development

It was observed that the teres major process appears postnatally: it was absent in 2 months of gestation fetuses ($N = 2$) as well as in all pups
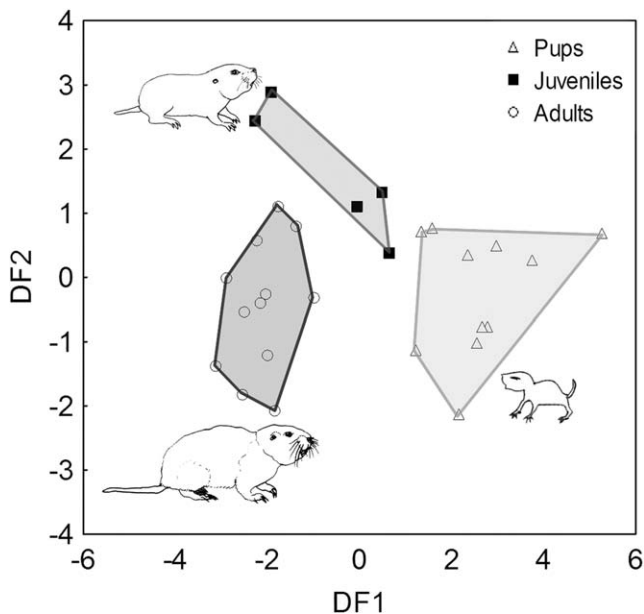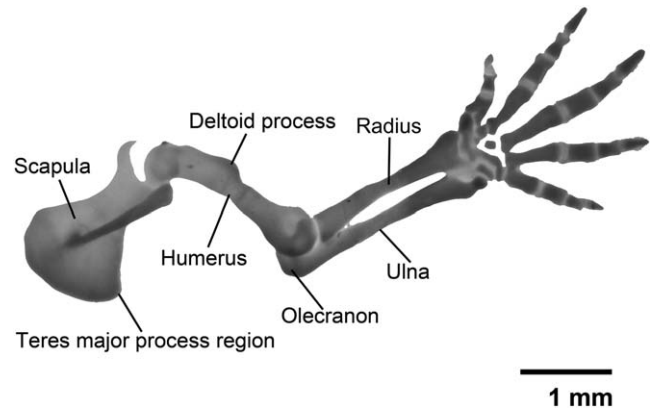
sampled ($N = 12$; Figs. 4 and 5A). The process begins to develop during the juvenile stage (incipient for three of six of the observed juveniles—Figure 5B—and conspicuous for the other 50%). Finally, 12 of 14 adults showed a conspicuous process (Fig. 5C); while in the other 2 of 14 it was incipient. Regarding the development of the teres major process, the scapula of *O. degus* (Fig. 5D) showed similar development to that observed during the juvenile stage of *C. talarum* (i.e., incipient). Conversely, we observed that the deltoid process was already present in 2 months of gestation fetuses, although it was relatively poorly developed (Fig. 4). We found that all the studied pups presented an incipient deltoid process (Fig. 6A), while in juveniles and adults it was

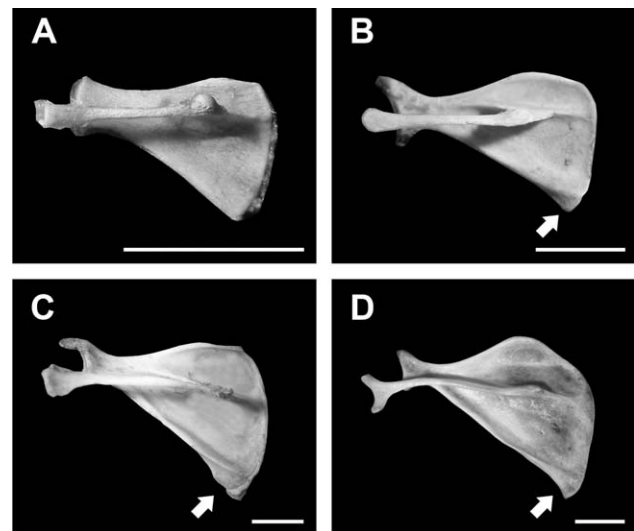Fig. 3. Biplot of DF1 and DF2 values for *C. talarum* at different ages.

Fig. 5. Right scapulae in lateral view of Los Talas' tuco-tuco *C. talarum* (**A, B, C**) and degu *O. degus* (**D**) showing the relative development of the teres major process (white arrow). References: (A) absent (pup); (B) incipient (juvenil); (C) conspicuous (adult); (D) incipient (adult). Scale = 5 mm.
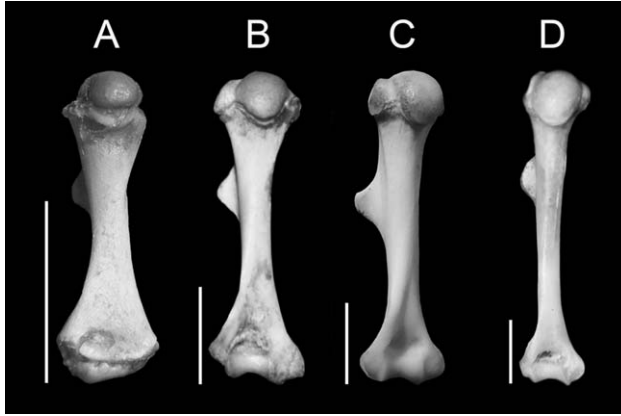
Fig. 6. Left humeri in posterior view showing the relative development and position of the deltoid process in the subterranean *C. talarum* at different age categories (**A**: pups; **B**: juveniles; and **C**: adults), and in the semifossorial *O. degus* (**D**: adult). Note that *O. degus* has a relatively more slender humerus. Scale = 5 mm.

conspicuous and well developed (Fig. 6B,C). We observed that differences between ages were related to an expansion of the proximal border of the deltoid process; however, a distal positioning from the shoulder joint was already observed in very young pups (Fig. 6A), which implies an enhanced mechanical advantage of the *M. deltoideus* and *M. pectoralis major* which insert in the deltoid process and act across the shoulder joint. Figure 6D exemplifies the moderately distal position of the deltoid process in the semifossorial *O. degus*. Finally, as it was observed in the deltoid process, the olecranon process was also relatively poorly developed in 2 months of gestation fetuses (Fig. 4), incipient in all the examined pups, and more developed (i.e., conspicuous) in juveniles–adults than in pups (Fig. 7). We observed that
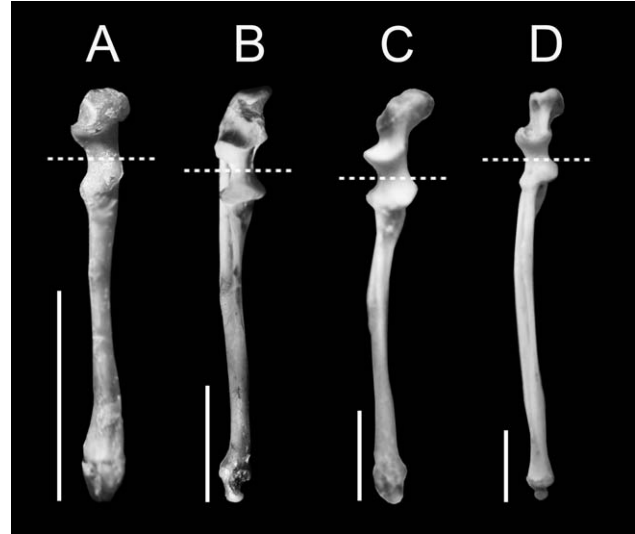

Fig. 7. Left ulna in dorsal view showing olecranon process development in the subterranean *C. talarum* at different age categories (**A**: pups; **B**: juveniles; and **C**: adults), and in the semifossorial *O. degus* (**D**: adult). Olecranon length was established as the distance from the head of the olecranon to the midpoint of the semilunar—or trochlear—notch of the ulna (white dotted lines). Scale = 5 mm.

this process was mediolaterally narrow and nearly straight in pup–juveniles and much broader and more curved in adults. Strikingly, in pups and juveniles of *C. talarum* the status of this character was highly similar to the one observed in adults of *O. degus* (see Lessa et al., 2008).

Allometric Analysis

The allometric pattern in bone development was assessed using regressions of $\log_{10}$ transformed

TABLE 3. *Results of regressions describing ontogenetic growth of long bones*
(forelimbs: humerus and ulna; hindlimbs: femur and tibia)

Long bone	Variable	Y-intercept	Slope	Slope C.I. limits	R^2
Humerus	HL	−1.179	1.575 (+)	1.519, 1.631	0.991
	TDH	−1.632	1.258 (+)	1.156, 1.360	0.953
	APDH	−1.536	1.189 (+)	1.088, 1.289	0.949
	LHL	−1.475	1.587(+)	1.487, 1.686	0.972
	DLH	−1.557	1.609 (+)	1.499, 1.719	0.967
	DEH	−1.242	1.283 (+)	1.203, 1.364	0.972
Ulna	UL	−0.951	1.480 (+)	1.423, 1.537	0.989
	FUL	−0.956	1.419 (+)	1.349, 1.490	0.982
	TDU	−1.766	1.164 (I)	0.984, 1.344	0.829
	OL	−2.179	1.827 (+)	1.668, 1.985	0.946
Femur	FL	−1.365	1.731 (+)	1.661, 1.800	0.989
	TDF	−1.975	1.495 (+)	1.381, 1.610	0.958
Tibia	TL	−1.059	1.569 (+)	1.503, 1.636	0.989
	PT	−1.480	1.593 (+)	1.466, 1.720	0.954
	LTL	−1.265	1.559 (+)	1.459, 1660	0.974
	TDT	−2.262	1.631 (+)	1.451, 1.810	0.926

See Figure 1 for references.
Note. (+) denotes positive allometry (e.g. increasingly gracile long bones during ontogeny) and (I) denotes isometry (constant proportions during ontogeny).

TABLE 4. Results of parallelism tests describing differences in the magnitude of change between morphological linear variables related to functional indices

Functional index*	Morphometrical variables[†]	F	DFn, DFd	P	Magnitude of change
RDP	DLH vs. LHL	0.061	1, 60	n.s.	DHL = LHL
HRI	APDH vs. HL	52.856	1, 60	***	APDH < HL
EI	DEH vs. HL	39.774	1, 60	***	DEH < HL
IFA	OL vs. FUL	18.559	1, 60	***	OL > FUL
URI	TDU vs. FUL	13.623	1, 60	**	TDU < FUL
BI	FUL vs. HL	13.306	1, 60	**	FUL < HL
FRI	TDF vs. FL	17.520	1, 60	***	TDF < FL
TRI	TDT vs. TL	0.008	1, 52	n.s.	TDT = TL
TSI	PT vs. TL	0.292	1, 52	n.s.	PT = TL
CI	TL vs. FL	14.789	1, 56	**	TL < FL

*Each index is defined in Table 1.
[†]Measurements indicated above are illustrated in Figure 1.
P: ** <0.01 and ***<0.001.

measurements against the logaritmized naso-occipital length (a proxy of body size). All but one (TDU) of the analyzed morphometrical variables deviated significantly from isometry, showing a positive allometry (Table 3). Parallelism tests showed that the magnitudes of change in length of the ulna, the humerus and the femur were greater than the corresponding magnitudes of change in width, that is, these bones elongate faster than their diameter increases, so that throughout postnatal ontogeny they appear to become more slender (Table 4). It was observed that the olecranon lengthens faster than the functional length of the ulna. However, the epicondyles widen at a lesser rate than the elongation of the humerus (Table 4). We found nonsignificant differences in the growing rate of the proximal and distal segments of the humerus (DLH, LHL) and the tibia (PT, LTL) (Fig. 1; Table 4), which is consistent with the fact that the deltoid process and the tibial spine do not shift their relative position along the humerus and tibia during postnatal ontogeny. Moreover, we found that proximal elements of each limb (i.e., humerus and femur) lengthen faster than the middle ones (i.e., ulna and tibia), which results in relatively shorter middle limb elements.

DISCUSSION

This study revealed that limb proportions in *C. talarum* undergo significant changes throughout postnatal ontogeny, and no significant differences between sexes were observed. Several morphological traits associated with scratch-digging are already present during early ontogeny, yet some of these traits undergo postnatal changes that might affect their functional capacity. Differences between ages were related to differential growth rates of long bones' traits, and according to discriminant analysis, main differences were found in the forelimb design. Particularly, pups could be differentiated from juveniles and adults by a set of morphofunctional characteristics as relatively shorter and thicker humeri and ulnae, wider epicondyles, longer middle limb elements, a proportionally shorter olecranon, and incipient deltoid and teres major processes. Because in mammals there is a general elongation of the limbs during ontogeny (see Polly 2007), we observed that bony features and pattern of growth were in accordance with this phenomenon. This result is interesting because, despite the fact that in subterranean rodents the limb bones are relatively short and robust during adulthood (e.g., Elissamburu and De Santis, 2011; Morgan and Álvarez, 2013), the pattern of growth in length and diameter is maintained in *C. talarum*. Pups have shorter bones with greater diameters, which result in greater robustness indices. However, although these indices may reflect the overall strengthening of a long bone when used for interspecific analyses (e.g., Elissamburu and Vizcaíno, 2004), it would be inaccurate to state that pups present stronger bones because the degree of mineralization of the bone cortex is what provides stiffness (see Farnum, 2007). Previous studies, on adult individuals, showed that *Ctenomys* has relatively broader subperiosteal diameters and narrower endosteal diameters, which result in thicker humeral cortices, and these morphological traits of humeral shafts contribute to a greater resistance to bending and torsion during digging activity due to a greater rigidity (Biknevicius, 1993). According to Carrier and Leon (1990) and Carrier (1996), long bones grow following a positive allometry in endothermic taxa, being relatively thicker early in ontogeny to compensate for low density of osteons and stiffness (Kilbourne and Makovicky, 2012). This might be the case of *C. talarum*, but further investigation is required to evaluate the relationship between thickness and density of bones, and robustness in pups and juveniles of this species.

The posteroventral portion of the scapula significantly enlarges during postnatal ontogeny, shaping a distinctive teres major process for the origin of the hypertrophied *M. teres major* (one of the main

retractor muscles of the forelimb; Kley and Kearney, 2007; see also the pioneering study by Lehmann, 1963). As stated by Kley and Kearney (2007), adaptive modifications of the pectoral girdle, in mammals that are highly specialized for scratch-digging, are most evident in the shape and size of the scapula. We found that this process is absent in young pups, incipient in juveniles and becomes conspicuous during adulthood. The examination of scapulae in a semifossorial caviomorph (*O. degus* adults) showed that they were similar to *C. talarum* pups–juveniles. Like other traits, scapular morphology responds to the combined influence of historical (phylogenetic) and functional factors, and particularly, the scapular shape of octodontoids might be advantageous for digging, although not a fossorial specialization (Morgan, 2009; and references therein). In the case of *C. talarum*, our results suggest that differences in the degree of development of the teres major process may be associated to differences in the relevance of digging activity, which start to be more important from the beginning of the juvenile stage.

Another scratch-digging adaptation observed in subterranean rodents is the well developed and more-distally positioned deltoid process of the humerus, site of insertion of the *M. deltoideus* and *M. pectoralis*, two flexor muscles of the forearm. Because distal positioning of the deltoid process enlarges the in-lever arm (L_i) of these muscles (Fernández et al., 2000), it may also increase their external forces ($F_{ext} = F_{int} \times L_i/L_o$; being L_i/L_o the ratio between the in-lever and out-lever arm or mechanical advantage; F_{int} the internal force produced by muscle contraction, and F_{ext} the resultant external force exerted by a muscle; Hildebrand 1988). However, our MANOVA and ANOVA results showed that there were no differences in the relative position of this process during postnatal ontogeny, suggesting that the mechanical advantage of these muscles do not significantly change with aging. This phenomenon occurs because, as it was shown by parallelism tests, both the distal and proximal (LHL and DLH; Fig. 1) segments of the humerus elongate at a similar rate (Table 4). Lessa et al. (2008) showed that a moderately distal position of the deltoid process is inferred to have characterized all living and extinct ctenomyids, and independently acquired by the octodontid *Spalacopus*. However, these authors observed that adult individuals of *C. talarum* present a more-distally positioned deltoid process. Strikingly, our results demonstrated that *C. talarum* pups already present a distally positioned process, suggesting that this condition is prenatally acquired (see Fig. 4). The visual examination of the bony material showed that the proximal border of the deltoid process expands laterally, particularly starting

from the juvenile stage, thus increasing the surface available for muscle insertion.

One particular forelimb adaptation that enables fossoriality is a greater biepicondylar breadth (Lehmann, 1963; Goldstein, 1972; Hildebrand, 1985a), as was observed in adult individuals of *Ctenomys* (Biknevicius, 1993). In this study, we found that the relative width at epicondyles (EI) is higher in pups when compared to juveniles and adults. The medial epicondyle of the humerus is the origin site of digital and carpal flexors and of the pronator, whereas the lateral epicondyle is the origin site of carpal extensors and supinator (Woods, 1972; Lessa et al., 2008). The importance of a greater biepicondylar breadth is that it not only provides a larger surface for muscles' insertion, but might also increase their effective force by altering muscles' line of action (Hildebrand, 1988; Vassallo, 1998; Fernández et al. 2000), and thus compensating for the lower muscular development in young individuals. Conversely, the index of fossorial ability (IFA), which reflects the relative development of the olecranon process, is significantly lower in pups than in adults, and juveniles show intermediate values, that is, the olecranon in pups is relatively the shortest, in contrast to adults, and, therefore, their *M. triceps brachii*, *M. anconeus*, and *M. dorsoepitrochlearis* have the lowest and highest mechanical advantage during forearm extension, respectively. The status of this character in pups and juveniles is similar to what it is observed in the generalized and semifossorial *O. degus* by Lessa et al. (2008). Changes in IFA throughout postnatal ontogeny are related to differences in the elongation rates of the olecranon and the ulna (Table 4).

Some traits (namely, the teres major and olecranon processes, and the epicondyles of the humerus) undergo postnatal changes that might affect digging capacity. This fact is consistent with an increasing commitment to the digging behavior, in which young pups begin to loosen and transport soil early during postnatal ontogeny (after thermoregulatory independence age; ~2 weeks); construct simple burrows after 18–20 days; and start to excavate complex burrow systems (with nest a feeding chambers) near to the weaning age (Echeverría, 2011; Fig. 8).

Finally, limb middle elements (i.e., ulna and tibia) seemed to be relatively longer in pups than juveniles and adults, which showed nonsignificant differences between them, that is, pups have relatively elongated forearms and forelegs (the highest BI—brachial index— and CI—crural index). Conversely, lower indices in juveniles and adults indicated the opposite condition, that is, relatively shorter forearms and forelegs. Allometric analysis showed that this pattern of growth was due to a faster lengthening of the proximal elements of each limb (i.e., humerus and femur) than the
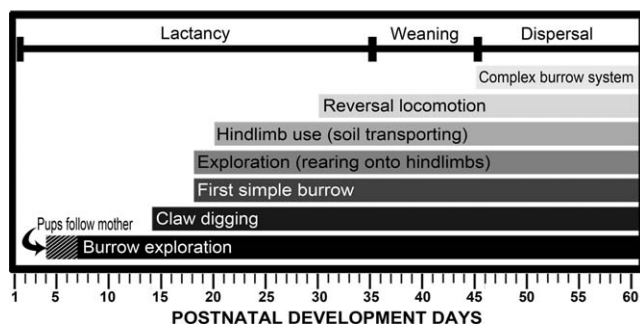
Fig. 8. Postnatal ontogeny of salient behaviors related to digging in *C. talarum* (modified from Echeverría, 2011).

middle ones, and this is important to consider, as it relates to effective out-lever arm and mechanical advantage of many muscles used in digging. For example, the relative shortening of the forelimb middle elements (e.g., the portion of the ulna distal to the olecranon fossa), provides a short out-lever for triceps extension (Hopkins and Davis, 2009). Furthermore, the overall shortening of the forelimb (i.e., reducing the out-lever arm) and the enlargement of muscular attachments (increasing both the in-lever arm and the area of insertion), results in an improved mechanical advantage for the digging muscles (Hildebrand, 1985a). In armadillos (Mammalia, Xenarthra, Dasypodidae) it was demonstrated that the brachial index correlates well with digging habits (Vizcaíno and Milne, 2002), and in fossorial rodents it was shown that they display many significantly different features from other groups, including shortened forearms (low BI) and hands (Samuels and Van Valkenburgh, 2008). Thus, the elongated forearms in *C. talarum* pups, in conjunction with what it was observed in the other indices, might suggest an incipient fossorial ability. Conversely, among caviomorphs, the relative length of the tibia is variable and its relationship with locomotor habits is unclear yet. According to Candela and Picasso (2008), this variability may indicate that low values of crural index correspond to the generalized condition present in arboreal and large terrestrial taxa, while high values correspond to more cursorial, fossorial, or leaping taxa. However, another study has shown that fossorial and semi-fossorial rodent taxa present low crural index values (Samuels and Van Valkenburgh, 2008).

In the case of digging in *C. talarum*, morphological ontogeny and behavioral ontogeny probably interact to each other. For example, in mammals it has been shown that patterns of muscle contraction (e.g., muscle exercise) are related to bone stresses (Herring and Teng, 2000) and this interaction between muscle and bone causes a differential growth in areas closest to peak stresses (Young et al., 2009; and references therein). Epigenetic effects of the early biomechanical environment

have been documented in several groups of mammals, and therefore, it is widely accepted that both genetic and epigenetic factors determine the final shape and strength of the skeleton (Carter et al., 1998; Nowlan and Prendergast, 2005). In *C. talarum* the early digging activity may positively affect, for example, the resistance of the humerus to bending or the diameter of its epicondyles.

CONCLUSIONS

This study has shown that, in *C. talarum*, several digging adaptations are already present during early postnatal ontogeny (e.g., incipient olecranon, relatively wide epicondyles, relatively distal deltoid process, short and massive long bones of the forelimb), particularly during lactation stage (pups, 1–35 days), which suggest that they are shaped peri or prenatally. Moreover, during early stages most of the analyzed functional indices did not reach the level of maturity that confers an adequate mechanical performance during digging. Probably, the pups may have the mechanical ability to disaggregate the substrate although the ability to build functional burrow systems (defined by the presence of a main tunnel, foraging tunnels, and nest) may require a maturation period of approximately 45–60 days, age corresponding to the stages of weaning-dispersion (Fig. 8). Conversely, during early infancy a morphological compensation might exist in some traits related to scratch-digging. For example, relatively broader epicondyles might increase carpal and digital flexors' effective force by altering muscles' line of action; or relatively shorter and thicker long bones (humeri, femora, and ulnae) might compensate their low rigidity. Thus, the postnatal period in *C. talarum* (1–65 to 70 days) might be of great relevance not only for "practicing" the construction of burrow systems, but also to achieve through body exercise the appropriate musculoskeletal development, effectiveness, and/or efficiency for such conduct. Finally, a recent study on palaeocastorine beavers (Rodentia, Castoridae), which are members of the burrowing fauna of the Oligocene of North America, showed that some morphological changes in crania and postcrania throughout ontogeny may be associated with increased burrowing ability in adults, probably as a consequence of greater burrowing needs with increased size (Calede, in press; and references therein). The data presented by Calede (in press) can be compared to those described by Calede and Hopkins (2012) for an early mylagaulid (Rodentia, Aplodontoidea, Mylagaulidae, *Alphagualus pristinus*) supporting greater adaptations to fossoriality in the forearm of the mylagaulid than in the paleocastorine. This description of *A. pristinus* gave insights into the ontogeny of these burrowing mammals, suggesting that the developmental trajectory

mimics evolution in the increase of fossorial modifications of the skeleton with time (Calede and Hopkins, 2012; see also Vassallo and Mora, 2007; Verzi et al., 2010). As Calede (in press) stated, common trends in the ontogeny of these two burrowing groups (i.e., paleocastorines and mylagaulids) relate to convergence in scratch-digging behavior and include changes in the proportion and development of forelimb traits. Accordingly, in *C. talarum*, changes in limbs throughout ontogeny are consistent with increased burrowing ability in adults (Echeverría, 2011) and greater ontogenetic changes are observed in the pectoral girdle, especially in the forelimbs, suggesting a general pattern in burrowing rodents.

APPENDIX I

Specimens examined. Institutional acronyms correspond to LMFCMP—Laboratorio de Morfología Funcional y Comportamiento, Universidad Nacional de Mar del Plata, Argentina; MLP—Museo de La Plata, Argentina.

Ctenomys talarum Argentina, Buenos Aires, Punta Indio MLP 26.VIII.01.4, 26.VIII.01.14, 9.II.99.7, 26.VIII.01.10, 1.XI.95.13, 23.VIII.01.5, 23.VIII.01.3, 23.VIII.01.4, 26.VIII.01.7; Argentina, Buenos Aires, Magdalena MLP 1.XI.95.15, 26.VIII.01.3, 26.VIII.01.1; Argentina, Buenos Aires, Punta Piedras MLP 1.VIII.00.8, 1.VIII.00.12, 1.VIII.00.14, 1.VIII.00.13; Argentina, Buenos Aires, Mar de Cobo LMFCMP H1MZ, H1ABB, M2O, H1S, M4S, M1D, M2S, H2JL, H4O, H2A, H1A, H1F, H3O, M1MY, H2MY, H1ABA.

ACKNOWLEDGMENTS

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