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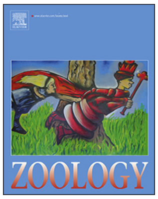

An allometric analysis of sexual dimorphism in *Ctenomys australis*: integrating classic morphometry and functional performance in vivo

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ABSTRACT

The South American rodents of the genus *Ctenomys* (Rodentia, Hystricognathi), which use both forelimbs and incisors to dig, show strong, specialized morphological adaptations to living in the underground niche. In these rodents, the effectiveness of a bite − in this case the potential to inflict physical damage − mostly depends on the strength of the incisors (e.g. bending and torsion stresses) and the power of the masseteric muscle of the jaw. *Ctenomys australis* (the sand dune tuco-tuco) is a highly territorial subterranean rodent that builds large and exclusive burrow systems in coastal sand dunes found continuously along the Atlantic coast of the Buenos Aires province, Argentina. Incisors in this species are used both in agonistic encounters among congeners (i.e. reproductive or territorial intentions) as well as to dig and increase the size of their burrows daily (e.g. acquisition of plant material as food resources). We first characterized sexual dimorphism in cranial traits involved in aggressive interactions between males, analyzing ontogenetic trajectories of both sexes. We also analyzed some remarkable attributes of sexual dimorphism in subadult and adult individuals, the bending and torsion stresses and the bite performance in vivo, hypothesizing that males are able to apply comparatively stronger bite forces at their incisor tips than females, concordant with a broader mandible for the insertion of a powerful adductor musculature. Bite forces were measured in vivo in wild animals of both sexes using a strain gauge load cell force transducer. These individuals were also used to estimate the incisor area (*CA*), the 2nd moment of inertia of the incisors (*I*, or the bending strength to external forces), and the torsional strength of the incisor (*J*), all traits involved in the production of the force that can be withstood at the incisor tips. Ontogeny explained some sexual differences in the cranial traits of adults, expressed as (i) morphological changes related to body size variation, and (ii) morphological changes associated with differences in the slope of ontogenetic trajectories (regardless of body size variation). Recordings of in vivo bite forces were significantly higher in adult males than in females. These differences in bite forces were not associated with the sex itself, but the sexual dimorphism in body size. Although males did not show significantly higher allometric coefficients for *I*, *J* and *CA* than females, males still showed a strong sexual dimorphism in these traits due to body size variation. On the other hand, measurements of mandibular width – an estimator of the relative size of masseteric muscles – in vivo showed strong differences between sexes, suggesting higher force performance at the incisor tips for males. Overall, we observed that many attributes related to bite performance might have been molded by sexual selection, which implies differences in allometric coefficients from some morphological traits throughout ontogeny.

1. Introduction

Sexual selection, including mate preference, is a significant force in the evolution of reproductive isolation, mate recognition, and ultimately in speciation (Lindenfors et al., 2007; Safran et al., 2013). In mammals, mate choice can be predicted by a series of ecological and biological attributes (Safran et al., 2013), and polygynous mating systems are commonly characterized by male-biased sexual dimorphism in both size and/or body weight (Fairbairn, 1997; Cutrera et al., 2010a; Becerra et al., 2012a). As demonstrated by Lindenfors et al. (2007),

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male-biased size dimorphism has been reported in several comparative studies that have shown associations between different estimates of dimorphism and polygyny. Such correlations have been found in many mammalian taxa such as primates (Clutton-Brock and Harvey, 1977; Lindenfors and Tullberg, 1998), ungulates (Jarman, 1983; Pérez-Barbería et al., 2002; Geist and Bayer, 2009), and pinnipeds (Bartholomew, 1970; Cassini, 1999) and several other orders. Sexual dimorphism is less frequent in other mammalian orders like Lagomorpha (Davis and Roth, 2008).

For many mammals, sexual dimorphism is commonly associated with the presence of structures used in aggressive interactions between males. The structures employed in this type of encounters have the potential to produce lethal injuries (e.g. sheep horns, elephant tusks, rhino horns, deer antlers) though these rarely occur (Vassallo, 1998; Becerra et al., 2012a). In some cases, however, the aggression among males is less ritualized, and the weapons involved may cause, for instance, the opponent's death.

Subterranean rodents are normally very territorial and aggressive, being able to exert strong and dangerous forces at their incisor tips (Vassallo, 1998; Van Daele et al., 2009; Becerra et al., 2011). In these species, encounters among individuals of different sex usually depend on the territory monopolized by each male and the male–male competition (Vassallo, 1998; Zenuto et al., 1999). Becerra et al. (2011, 2012a) have argued that inter-male aggressive encounters in these species can be more or less ritualized; usually they are social interactions rather than aiming at the opponent's death or at severe physical damage. It should be noted, however, that little attention has so far been paid to the evolution and maintenance of sexual dimorphism in subterranean rodents (Becerra et al., 2012a). Although some authors have reported both male- and female-biased sexual dimorphism in some characters correlated with body size variation in rodents (Bondrup-Nielsen and Ims, 1990; Yoccoz and Mesnager, 1998; Zenuto and Busch, 1998; Zenuto et al., 1999), only a few studies have considered traits directly involved in aggressive interactions among males (see also Schulte-Hostedde, 2007).

South American rodents of the genus *Ctenomys* (tuco-tucos), which is the most speciose subterranean rodent genus (Reig et al., 1990; Lessa and Cook, 1998; Lessa, 2000; Parada et al., 2011), are characterized by limited dispersal capabilities (Reig et al., 1990; Mora et al., 2006), high territoriality (Vassallo, 1998), small local effective population size (Mora et al., 2006, 2010, 2013a), considerable variation in body size (with species ranging from 90 g in *Ctenomys pundti* to 700–900 g in *C. conoveri*; Mora et al., 2003), and in some cases socially structured mating systems (Lacey, 2000). The distribution and abundance of these subterranean rodents depend, essentially, upon both intrinsic features of the patches such as soil hardness, granulometry, permeability, vegetal cover, terrain elevation, and the dispersal capabilities of the species (Luna and Antinuchi, 2007; Mora et al., 2010, 2013a). These rodents, which use claws and teeth for digging (Vassallo, 1998), show some notable morphological adaptations to life underground (Lessa and Patton, 1989; Lessa, 2000; Mora et al. 2003; Lessa et al., 2008). Thus, *Ctenomys* has been characterized as a 'scratch' (claw) and 'chisel-tooth' digger (Dubost, 1968; Vassallo, 1998). Among other traits, *Ctenomys* has evolved a powerful jaw adductor musculature and procumbent incisors that are used as 'digging tools' mainly for the excavation of its galleries (Vassallo, 1998; Mora et al., 2003; Lessa et al., 2008; Becerra et al., 2011).

Ctenomys australis (the sand dune tuco-tuco) is a highly territorial and herbivorous subterranean rodent that inhabits a relatively secure, permanently sealed burrow system in sand dune habitats with poor primary productivity on the coastal landscape in the south-east of Buenos Aires province, Argentina (Mora et al., 2006, 2010). This species is considered solitary (Busch, 1989; Vassallo and Busch, 1992) and lives underground at very low densities (Cutrera et al., 2010a; Mora et al., 2010). Its high trophic and habitat specialization limits its distribution to the first strip of coastal dunes (Vassallo, 1998; Mora et al., 2006, 2010). This species is one of the largest species within the genus (350–600 g) and the extreme energetic costs associated with digging limit the availability of suitable habitat for this species (Luna and Antinuchi, 2007). The capacity of this species to remove soil decreases dramatically in harder substrates. Vassallo (1998) showed that *C. australis* uses its forelimbs and, less frequently, its incisors when confronted with harder and clayey soils.

As in other species of tuco-tucos, aggressive behavior among adult males in *C. australis* is viewed as an important correlate of their polygynous mating system, leading to a marked sexual dimorphism in body size (Vassallo, 1998; Zenuto and Busch, 1998; Cutrera et al., 2010a). In fact, serious injuries such as profound cuts in the face have been commonly observed in the field as a result of these agonistic encounters between males during the reproductive season (Busch, 1989; Vassallo and Busch, 1992). Thus, sexual selection is likely one of the main forces driving the observed differences in some biological features (e.g. home range size, sex-biased dispersal, robustness of the incisors, mandibular width, etc.) in *C. australis* (Cutrera et al., 2010a; Mora et al., 2010), through the effects exerted by body size dimorphism and differential mating behaviors between sexes, as has been suggested for other solitary mammals (Lindenfors et al., 2007).

Sexual size dimorphism occurs in all *Ctenomys* species, although size differences between males and females are highly variable (Mora et al., 2003). Cutrera et al. (2010a) reported a 1.57 and 3.5 ratio for body mass dimorphism and home range sizes, respectively, in adults of the species *C. australis*. Becerra et al. (2011, 2012a) argued that the aggressiveness is another important determinant of the outcome of intraspecific encounters in *Ctenomys*. These authors reported sexual differences in the absolute measure of bite force in the field (in vivo) for *C. talarum*, and such measures were directly related to the hazardous nature of a bite to conspecifics. Also, these latter authors suggested that the allometric scaling between body mass and bite force ensures that small mammals, in general, can exert bite forces proportionately greater than larger species (see also Wroe et al., 2005; Van Daele et al., 2009).

In this study we characterized the sexual dimorphism in the subterranean rodent *C. australis*, considering cranial attributes directly involved in aggressive interactions between males (e.g. bending and torsional strength of incisor teeth) and relating them to the ontogenetic trajectories of both sexes. Given that there are energy constraints on body size in subterranean rodents, we analyzed the degree of sexual dimorphism in some attributes of the fighting apparatus of *C. australis* linked to male dominance. We hypothesized that males are able to exert comparatively stronger bite forces at the incisor tips than females, and possess a broader mandible for the insertion of a powerful adductor musculature. Furthermore, in order to improve their robustness and fighting capacity, male incisors must be able to resist greater bending and torsional stresses and have an appropriate angle for attacking the opponent (Becerra et al., 2011). Procumbent incisors, projected forward from the skull, increase the possibility of injuring the opponent during encounters (Mora et al., 2003; Becerra et al., 2011). Thus, we hypothesized that incisors of males are characterized by both increased 2nd and polar moments of inertia (geometrical parameters proportional to the bending and torsional strength, respectively) and a greater procumbency angle than those of females. We also hypothesized that sexual dimorphism in these traits may be explained by allometric changes (or differential growth rates) during ontogeny.

2. Materials and methods

Sexual dimorphism in *C. australis* was explored both through a study on the ontogenetic trajectories of some morphological characters and an exhaustive morphological approach performed on cranial traits of adults involved in aggressive interactions between males (e.g. bending and torsional strength of the incisors and the jaw adductor musculature).

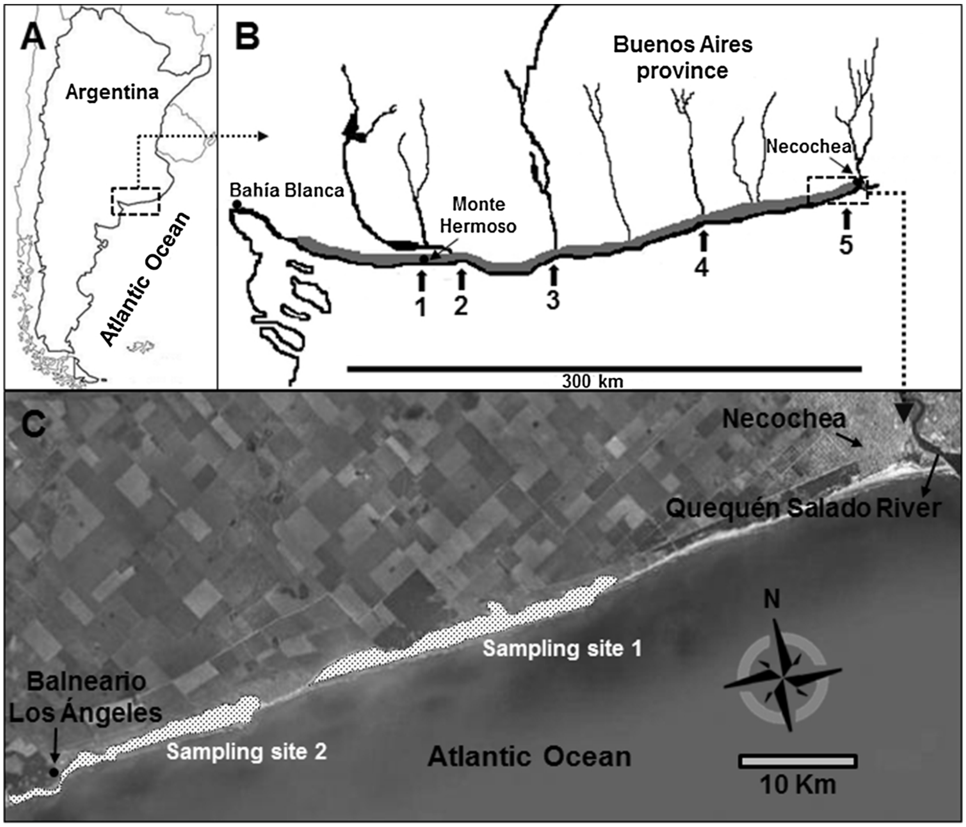

Fig. 1. (A and B) Sampling area of *Ctenomys australis* along the coast of southeastern Buenos Aires province, Argentina, with five locations and their corresponding coordinates where the material for the ontogeny analysis was collected: 1, Monte Hermoso (38°59′S, 61°18′W); 2, Sauce Grande (38°59′S, 61°06′W); 3, Oriente (38°54′S, 60°31′W); 4, Claromecó (38°51′S, 59°59′W); 5, Necochea (38°37′S, 58°50′W) (see Table S1, supplementary data).The grey area represents the entire distribution range of *C. australis*. (C) The image shows the area where the in vivo studies were performed. Dashed polygons in the map show the sand dune area where the sampled specimens were captured (sampling sites 1 and 2), between Balneario Los Ángeles and Necochea.

2.1. Ontogenetic analysis

Specimens for the ontogeny analysis were collected in five different locations along the entire distribution of *C. australis*, from Monte Hermoso to Necochea (see Fig. 1A and B), between May and October in several field trips (from 1987 to 2004). In this analysis, we used an ontogenetic series of skulls in which body masses ranged from 100 to 430 g in females ($n = 61$), and from 90 to 625 g in males ($n = 29$). These masses represent body sizes from pups (post partum offspring) to adults of both sexes. Thus, age classes were divided into three groups: pups or juveniles (100 – 150 g for ♀, 90 – 160 g for ♂), subadults (150 – 260 g for ♀, 160 – 350 g for ♂), and adult individuals (260 – 430 g for ♀, 350 – 625 g for ♂).

Analyses of ontogenetic growth patterns were primarily based on skull traits related to the exertion of strong biting at the tip of the incisors, and to the structural strength of the skull: incisor width (IW); incisor thickness (IT); mandibular width (MW) and zygomatic width (CW) (wide jaws and zygomas indicate well developed masseters; Vassallo and Mora, 2007; Lessa et al., 2008); rostral width (RW); rostral length (RL); diastema length (DL) (shorter facial lengths and wider skulls have been associated with larger bite forces in subterranean rodents; Vassallo and Mora, 2007; Van Daele et al., 2009; McIntosh and Cox, 2016); body length (BL) (variable that reflects very well the overall body size); and basicranial axis length (Ba, length of basioccipital plus basisphenoid), a conservative measure of size that changes little when other skull characters change (Radinsky, 1985; Fig. 2A and C).

We also focused on the variation in the angle of upper incisor procumbency (AIP), which denotes variation in the degree of the anterior projection of the incisors from the skull − a character largely viewed as an adaptation to tooth-digging in several subterranean rodent species (Hildebrand, 1985; Van der Merwe and Botha, 1998; Lessa, 2000; Stein, 2000; McIntosh and Cox, 2016; Fig. 2B) or to achieve a better performance during agonistic inter-male encounters or aggressive biting (Vassallo, 1998; Mora et al., 2003; Becerra et al., 2011, 2013), which is in line with the high territoriality exhibited by most species of tuco-tucos. We measured AIP with a camera lucida (Leica MS5 stereomicroscope) and the values were later transformed into radians for the analyses (see Mora et al., 2003).

In order to study the mandibular adductor muscles in specimens of both sexes, we extracted the superficial masseter muscle (SMM) and lateral masseter muscle (LMM) (Fig. S1 in the supplementary online Appendix). Masses of these muscles were obtained from 14 males and 11 females, considering the age classes described above. The muscles were dissected under an Olympus SZ6 stereomicroscope, weighed to the nearest 0.01 g, and stored at −16 °C for further analysis, following Becerra et al. (2013). The origins and insertions of the muscles were assessed and mapped onto skull photographs based on previous studies such as those by Woods (1972) and Vassallo (1998). The rest of the skull was cleaned and used in the subsequent ontogenetic analyses.

Skull measurements were taken using a digital caliper (0.01 mm). For the purpose of allometric and functional analyses, the data were $\log_{10}$ transformed, adjusted to the allometric growth equation $y = ax^b$, using Ba as standard of skull size. Differences in allometric coefficients for each regression were tested by a slope analysis, using Ba (for all morphological variables) and body length (for SMM and LMM) as independent variables. Body length, a variable directly related to overall body size, was used to assess the possible allometric changes of the masseter muscles during ontogeny. Bivariate equations were calculated as reduced major axis (Model II) regressions because neither variable is considered independent. There is an error associated with the measurements of x and y, and it is the structural relationship between the two variables that is being investigated (see Mora et al., 2003).

All of these analyses were performed using Statistica version 7.0 (Statsoft, Tulsa, OK, USA), and SMATR version 2.0 (1,000 iterations; Falster et al., 2006). Osteological materials from the ontogenetic analysis were prepared and deposited in the collections of the Municipal Museum of Natural Sciences “Lorenzo Scaglia” (MMLS), Mar del Plata, Argentina, and in the “Functional Morphology and Behavior Laboratory

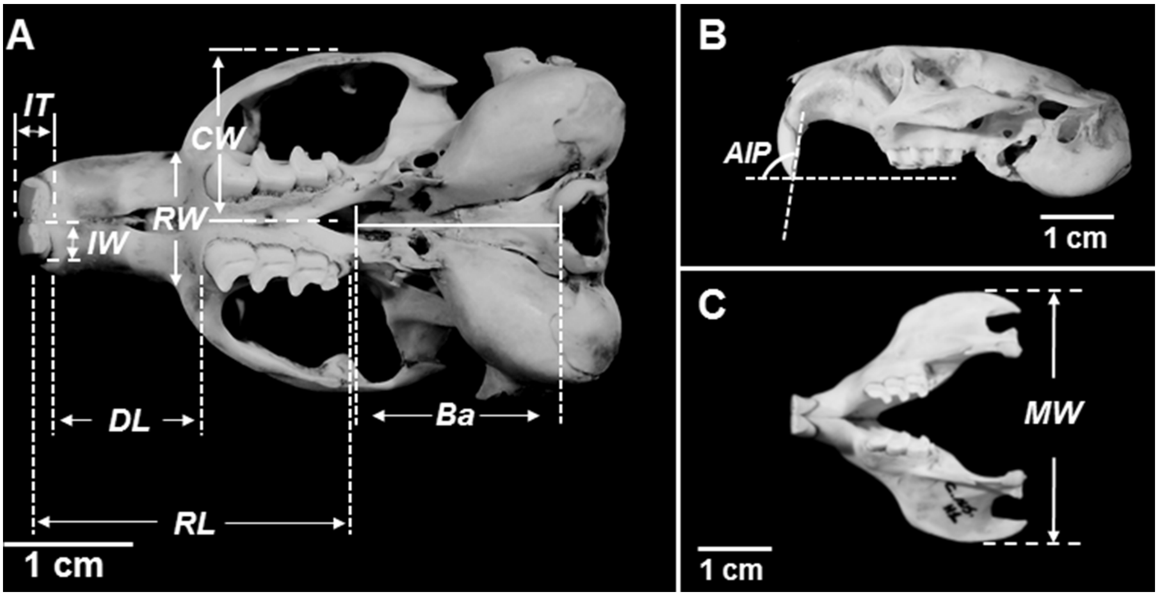

Fig. 2. (A–B) Skull characters related to the force performance at the tip of the incisors used in this study in ventral (A) and lateral (B) view. Dorsal mandibular view (C) is also shown. Abbreviations: AIP, upper incisor procumbency; Ba, length of basioccipital plus basisphenoid; CW, zygomatic width; DL, diastema length; IT, incisor thickness; IW, incisor width; MW, mandibular width; RL, rostral length; RW, rostral width. Scale bars = 1 cm.

(FMBL)", Universidad Nacional de Mar del Plata, Argentina (see Table S1 in the supplementary online Appendix).

2.2. *Analyses of live specimens*

2.2.1. *Sampling area and study design*

Sampling was conducted in a sand dune habitat along the southeastern Atlantic coast, one of the sites localized at 15 km to the southwest of Necochea (38°37′S; 58°50′W, sampling site 1), and the other one at the coastal village of Balneario Los Ángeles (38°40′S, 59°00′W, sampling site 2), Buenos Aires province, Argentina (Fig. 1C). These sampling sites partially overlapped with the study area where the ontogenetic analyses were performed (Fig. 1B). The sand dune habitat in this area reaches altitudes ranging from 30 to 50 m above sea level. The vegetation over dunes is scarce; between 5 and 25% of the typical *C. australis* habitat is covered by natural grassland (Zenuto and Busch, 1995, 1998; Mora et al., 2006) (Fig. 1C and Fig. S2 in the supplementary online Appendix).

For the present study, individuals of *C. australis* were captured from these natural populations in July of 2012 (avoiding the reproductive season between November and February; Zenuto and Busch, 1998). All specimens were collected using snap-traps (Victor no. 0; Oneida Community Ltd., Sherill, NY, USA) with a rubber cover to avoid injuring animals.

The bite force studies and mandibular width measurements with subadults and adults of both sexes (Section 2.2.2) were conducted entirely in the field to avoid high levels of stress in the study animals. In the same way, bending and torsional strength of upper incisors were taken in the field using adult individuals (Section 2.2.3). Animals were weighed after taking the incisor and bite force measurements to avoid manipulation effects that would affect biting performance. Weighing was performed with an electronic scale (0.01 g; see Becerra et al. 2012a). Mean body mass was 326 g (females) and 421.1 g (males).

Different age classes (subadults and adults) were determined by the reproductive status and body size of the collected individuals. An open or plugged vagina and signs of a present or past pregnancy were indicative of sexual maturity in females (following Zenuto and Busch, 1998). The age class of males was inferred from a size (mass) distribution curve obtained from all the individuals that were captured in the sampling area during a period of three years (see Mora et al., 2010).

All procedures followed the recommendations of the American Society of Mammalogists for the use of wild mammals in research (Sikes et al., 2016). At the end of the experiments the animals were released in good physical condition at the site of their capture.

2.2.2. *In vivo bite force and mandibular width measurements*

Individual bite force measurements were taken in the field in 32 females (27 adults and 5 subadults) and 21 males (13 adults and 8 subadults) with a strain gauge load cell force transducer (range 0 – 20,000 g, error 1 g; Necco Technologies, Mar del Plata, Argentina; for a more detailed description see Becerra et al., 2011; Becerra et al., 2012a) (Fig. S2). Bite force measurements were recorded on a PC using the software Hypeterminal (Windows XP application; Microsoft, Redmond, WA, USA) and registered during experimental sessions in the field at the time of capture, when the animals were induced to defensively bite the transducer plates. The bite plates were covered with a thin protective coating made of leather, so the animals did not damage their incisors (this procedure is explained in detail in Becerra et al., 2011, 2012a). The temporal sequence of the experiments in the field was: (i) animals were induced to bite defensively by taking them out of their capture cages; (ii) each session lasted ~1 min and consisted of biting trials that comprised several bites; (iii) trials ended when the animals refused to bite the transducer, which in some instances occurred after 30 – 40 s of recording; (iv) each session was repeated 2 – 4 times per individual. According to Becerra et al. (2011), the strongest bite record from all sessions was assumed to represent maximal bite performance for each individual. In this way, in vivo absolute bite forces were recorded in adult and subadult individuals of both sexes.

A two-way ANOVA was performed to assess the effect of both sex (male/female) and age (adults/subadults) on the in vivo bite force measurements. A Tukey HSD post-hoc test was performed on these variables (age and sex as categorical variables) using bite force as dependent variable. An ANCOVA on bite force was also performed to test for sexual differences (with body weight as covariate).

After taking the bite force measurements, mandibular width (MW) was measured at the maximum width of both masseteric crests, which are extremely well developed in tuco-tucos, using a digital caliper (0.01 mm). MW has been used to estimate the size of the jaw adductor musculature (Olivares et al., 2004; Vassallo and Mora, 2007) and for the calculation of bite forces (Becerra et al., 2011, 2014). An increase in mandibular width in *Ctenomys* species directly incurs a mechanical advantage in tooth-digging and in the ability to break roots or harder substrates (Vassallo, 1998; Mora et al., 2003).

We performed ANOVAs on the MW measurements. In order to control for the effect of body size variation, we also performed an ANCOVA on MW with body weight as covariate.

2.2.3. *Bending and torsional strength of upper incisors*

To avoid any damage to the teeth of the live specimens, we measured the width and thickness of a single upper incisor in 28 female and 20 male adult individuals (the same as those mentioned in Section 2.2.2 plus one additional adult female and seven adult males) and used those metrics to extrapolate its cross-sectional area. Since the shape is not a regular geometric form, the most accurate estimation of the incisor's cross-section should be based on the actual incisor of an average individual within the same species (pictures previously gathered by Becerra et al., 2012b). We virtually scaled that shape according to every incisor's metrics (width and thickness) in order to assess the respective cross-sectional area (CA), the 2nd moment of inertia (I), and the torsional strength of the incisor (J). I represents a geometrical parameter that indicates how resistant a particular structure is to bending stress (Alexander, 1983). Thus, mechanical resistance of the incisors (I, or the bending strength against external forces) was calculated from those pictures (which represent the most genuine shape of the incisor, see Becerra et al., 2012b) by means of the moment calculation macro of Image J (National Institutes of Health, Bethesda, USA), while the torsional strength of the incisors (J) was calculated as $I\text{max} + I\text{min}$ (see Biknevicius et al., 1996; Irgens, 2008). These variables were measured at the incisor tips just proximal to the end of the wear facet (see Verzi et al., 2010a; Becerra et al., 2011, 2012a, 2012b; Vassallo et al., 2016).

In order to assess sexual differences in *C. australis*, ANOVAS and ANCOVAs on CA, I and J (with body weight as covariate) were performed. ANCOVAs provide information on the potential effect of body size variation on these morphological traits, testing for differences in the regression slopes between sexes.

2.3. *Angle of upper incisor procumbency*

The angle of the upper incisor procumbency (AIP, Fig. 2) was measured in adult specimens from the museum material (see Table S1 in the supplementary online Appendix). In order to control for the effect of body size variation, we also performed an ANCOVA on AIP with body weight as covariate.

3. Results

3.1. *Ontogenetic trajectories*

Male ontogeny presented significant positive allometries ($b > 1$) for IW, IT, MW and DL, whereas negative allometric coefficients were observed for CW ($b < 1$) (Table 1; Figs. 2 and 3). Other variables (RW, RL, SMM and LMM) scaled according to expectation under isometry ($b = 1$; Table 1). Females showed an isometric condition for IW, RL, MW, and SMM, significant positive allometries for DL and IT, and significant negative allometries for RW, CW, and LMM (Table 1).

The regression slopes of the variables IW, IT, RW and LMM showed significant differences between the sexes (see slope analysis in Table 1; case *c* in Fig. 4), denoting that the greater proportions of these variables observed in males seem to be explained not only by differences in body size but also by the growth rates themselves. The allometric growth of these morphological traits denotes a strong sexual dimorphism in adults of this species.

Even though no significant differences between the sexes were observed in the allometric coefficient for MW after controlling for body size (ANCOVA, differences in slope: $P = 0.17$), significant differences between the sexes were observed in the elevation (or lateral transposition; Klingenberg, 1998; Klingenberg and Spence, 1993) of this morphological trait (ANCOVAs, shift in elevation: y-intercept, $P < 0.001$; case *b* in Fig. 4). This pattern suggests a clear intersexual difference in this variable due to lateral transposition. Neither differences in the regression slope (changes in the allometric coefficient) nor in the overall position at the regression line (changes only due to body size variation) were observed.

Whereas the allometric growth equations ($y = ax^b$) for SMM and LMM were regressed against body length (BL, n♀ = 11, n♂ = 14), the other morphological traits were regressed against basicranial axis length (Ba; n♀ = 61, n♂ = 29; see Section 2.1 for details).

3.2. *Bite force performance, bending strength and torsional strength in vivo*

In vivo absolute bite forces recorded in the subterranean rodent *C. australis* were significantly higher in adult males (360 – 550 g) than females (270 – 450 g; mean bite force 82.2 vs. 57.9 N; ANOVA: $F_{[1,38]} = 42.09$, $P < 0.001$; Table 2). Bite forces in subadult males (310 – 360 g) were not significantly higher than in subadult females (250 – 270 g; mean bite force 52.43 vs. 46.85 N; ANOVA: $F_{[1,12]} = 1.01$,

Table 1
Allometric coefficients (b) and the corresponding 95% confidence intervals (CI, standardized major axis) from the ontogeny comparisons between sexes. The probability of fit to an isometric condition (due to a positive or negative allometry) is shown for each sex. Values of F and P from slope test (or from differences in allometric coefficients; ANCOVA) are also shown. Asterisks indicate statistically significant ($^{*}P < 0.05$, $^{**}P < 0.01$) differences between allometric coefficients and H_0 (isometry) or between each other. Abbreviations: Ba, length of basioccipital plus basisphenoid; BL, body length; CW, zygomatic width; DL, diastema length; IT, incisor thickness; IW, incisor width; LMM, lateral masseter muscle; MW, mandibular width; RL, rostral length; RW, rostral width; SMM, superficial masseter muscle; ♀, females; ♂, males.

	♀	$F_{♀\text{-H0}}$	Probability$_{♀\text{-H0}}$	♂	$F_{♂\text{-H0}}$	Probability$_{♂\text{-H0}}$	$F_{♂\text{-}♀}$	Probability$_{♂\text{-}♀}$
Log$_{10}$ Ba/Log$_{10}$ IW	$b = 1.01$ (0.89 – 1.15)	0.03	$P = 0.86$	$b = 1.29$ (1.12 – 1.49)	13.11	**	9.28	**
Log$_{10}$ Ba/Log$_{10}$ IT	$b = 1.25$ (1.11 – 1.41)	13.47	**	$b = 1.98$ (1.60 – 2.51)	39.55	**	4.97	*
Log$_{10}$ Ba/Log$_{10}$ RW	$b = 0.73$ (0.65 – 0.82)	28.33	**	$b = 0.92$ (0.80 – 1.05)	1.69	$P = 0.20$	9.10	**
Log$_{10}$ Ba/Log$_{10}$ RL	$b = 0.95$ (0.85 – 1.07)	0.76	$P = 0.94$	$b = 1.03$ (0.91 – 1.16)	0.22	$P = 0.64$	2.10	$P = 0.36$
Log$_{10}$ Ba/Log$_{10}$ MW	$b = 1.00$ (0.88 – 1.13)	0.01	$P = 0.94$	$b = 1.12$ (1.00 – 1.25)	4.54	*	5.70	$P = 0.17$
Log$_{10}$ Ba/Log$_{10}$ DL	$b = 1.12$ (1.00 – 1.26)	4.18	*	$b = 1.21$ (1.08 – 1.35)	11.43	**	2.50	$P = 0.12$
Log$_{10}$ Ba/Log$_{10}$ CW	$b = 0.75$ (0.66 – 0.84)	26.48	**	$b = 0.85$ (0.74 – 0.97)	5.96	*	3.91	$P = 0.05$
Log$_{10}$ BL/Log$_{10}$ SMM$^{1/3}$	$b = 0.94$ (0.76 – 1.16)	0.45	$P = 0.52$	$b = 1.13$ (0.90 – 1.42)	1.31	$P = 0.27$	1.82	$P = 0.18$
Log$_{10}$ BL/Log$_{10}$ LMM$^{1/3}$	$b = 0.71$ (0.55 – 0.92)	8.76	*	$b = 1.28$ (0.99 – 1.67)	4.27	$P = 0.06$	9.27	**

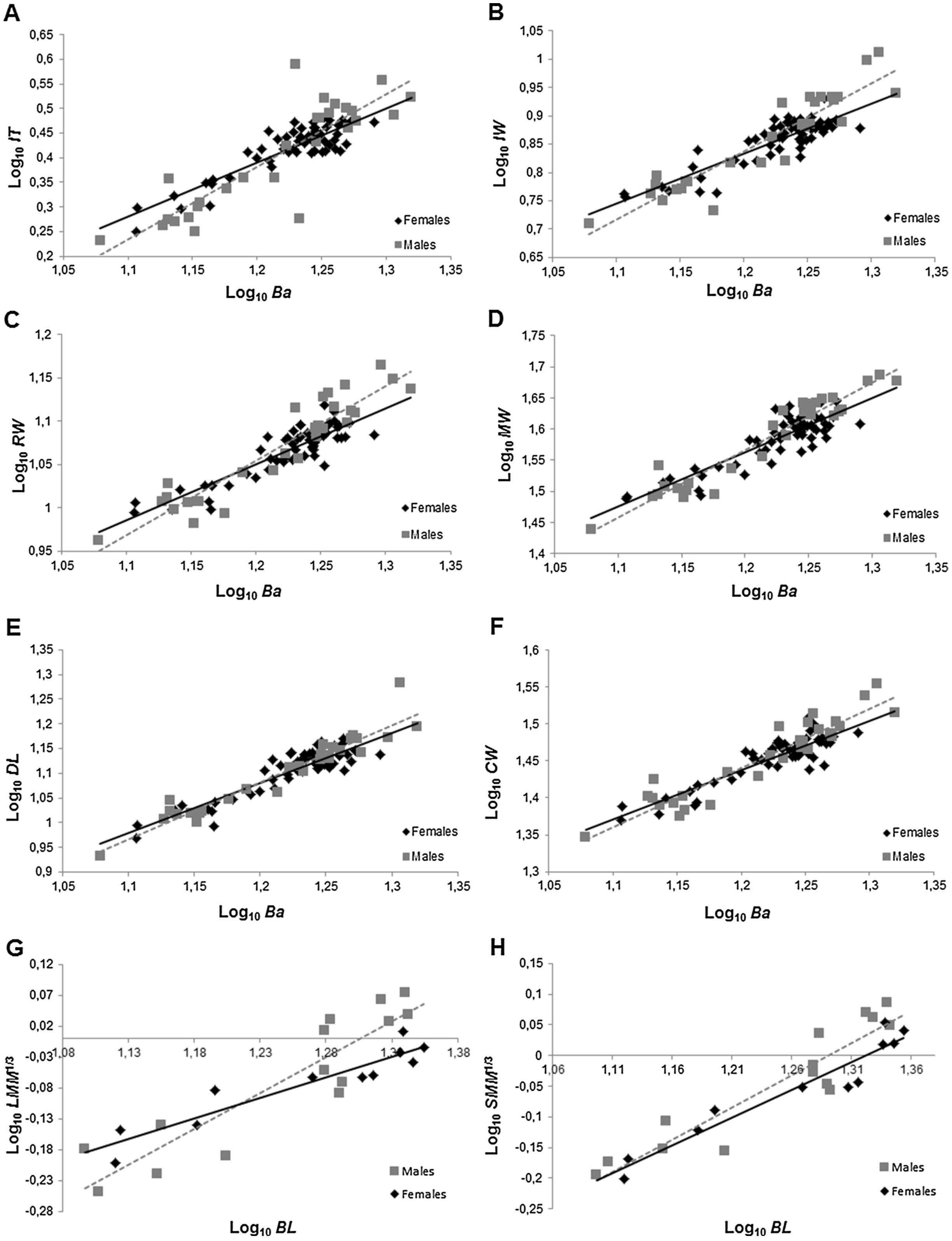

Fig. 3. Scatter-plots of ontogenetic scaling for different skull variables measured on both sexes of *C. australis*. Variables in plots (A–F) are regressed against basicranial axis length (Ba), while variables in plots (G) and (H) are regressed against body length (BL). The data are $\log_{10}$-transformed (all variables were measured in millimeters, except for the superficial (SMM) and lateral masseter muscle (LMM) which were measured in grams). Females are represented by black diamonds, while males are denoted with gray squares. Gray dashed lines and black solid lines show the calculated allometric equations' slope for males and females, respectively. Abbreviations as in Fig. 2.

$P = 0.334$; Table 2). However, a two-way ANOVA (with age and sex as categorical variables and bite force as dependent variable) shows a significant effect of both sex (male/female) and age (adults/subadults) on the bite force measurements (age: $F_{[1,49]} = 34.27$, $P < 0.001$; sex: $F_{[1,49]} = 17.9$, $P < 0.001$; interaction between age and sex: $F_{[1,49]} = 6.9$, $P < 0.05$). The interaction between the categorical

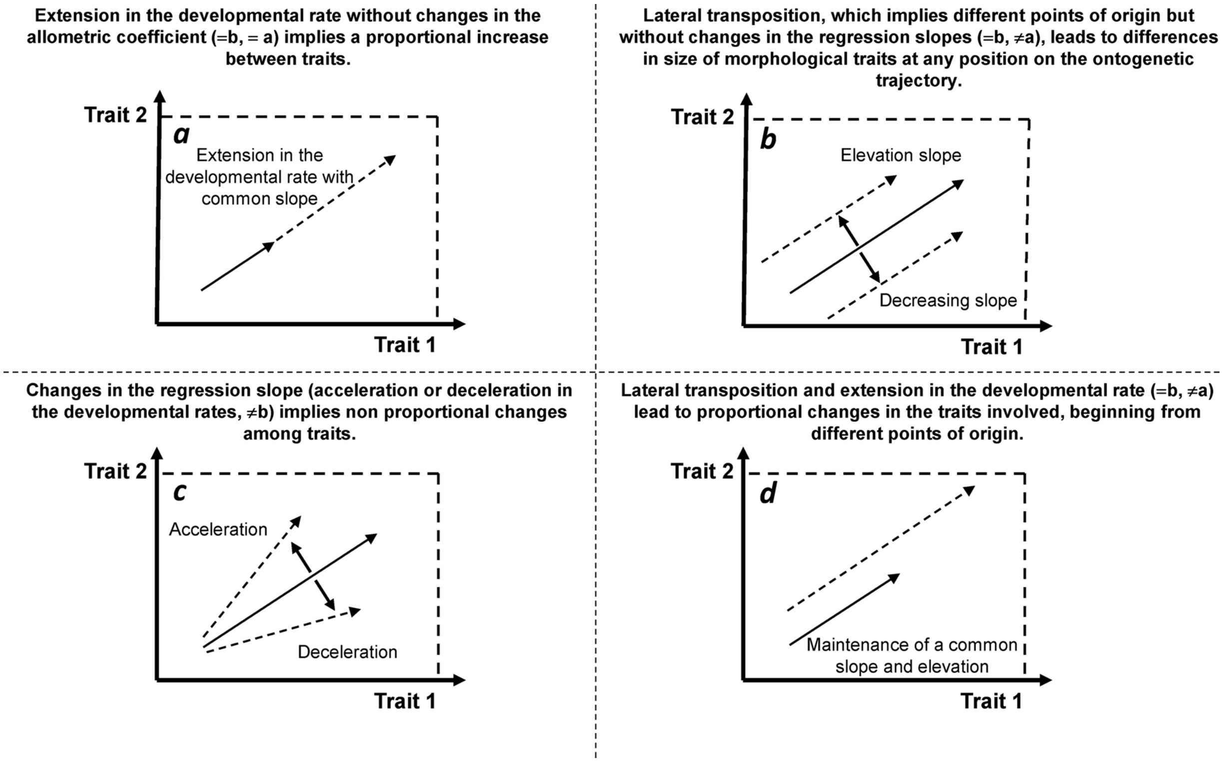

Fig. 4. Bivariate plots represent the possible evolutionary changes in morphological attributes of the skull resulting in sexual differences during ontogeny. The allometric equations were linearized by applying the logarithm of the data ($\log_{10} y = \log_{10} a + b \log_{10} x$). Some variables seem to fit case ***a*** (e.g., CA, *I* and *J*), case ***b*** (e.g., MW) or case ***c*** (e.g., LMM, IT, IW and RW). In this study no particular examples were observed for case ***d***. Abbreviations as in Fig. 2.

variables suggests that males not only have higher bite forces than females, but these differences are enhanced with the age of the individuals. A Tukey HSD post-hoc test performed on these variables (age and sex as categorical variables and bite force as dependent variable) showed highly significant differences between adult males and females, and between subadult and adult males ($P < 0.001$), but not between subadult males and females nor between subadult and adult females.

In order to assess the differences in the allometric coefficients for bite force, we performed an ANCOVA with body mass as covariate. This showed that bite force differences in adult/subadult individuals were not associated with the sex itself, but with the dimorphic body size (ANCOVA, allometric coefficients: $P = 0.53$; intercept: $P = 0.067$; Fig. 5A). In conjunction, these results suggest that differences in body size between sexes seem to explain the differences in bite performance in adult individuals.

Maximum mandibular width (MW, an estimator of the relative size of the masseteric muscles) estimated in vivo was significantly higher in adult males than in adult females (mean mandibular width 48.25 vs. 42.59 mm; ANOVA: $F_{[1,32]} = 49.62$, $P < 0.001$; Table 2). In addition, a two-way ANOVA (with age and sex as categorical variables and MW as dependent variable) showed a significant effect of both sex (male/female: $F_{[1,49]} = 41.71$, $P < 0.001$) and age (adults/subadults: $F_{[1,49]} = 65.79$, $P < 0.001$), and their interaction ($F_{[1,49]} = 5.5$, $P < 0.05$). The interaction between the categorical variables suggests that males not only have higher MW than females, but these differences are enhanced with the age of the individuals. Interestingly, maximum mandibular width and bite force showed the same pattern of variation among sexes and age classes (Fig. 6 and Table 2).

After controlling for body size, MW of subadult/adult individuals showed highly significant differences (ANCOVA, $F_{[1,46]} = 0.37$, $P < 0.001$) between sexes. Thus, greater relative size of the adductor muscles leads to higher bite forces at the tips of the incisors for males. These results were slightly different to those observed in the ontogenetic analysis, where the between-sex differences relative to the regression slopes were not significant. It should be noted, however, that there is some variation in the estimations of MW between the collection material (see Section 2.1) and live individuals (in vivo measurements). Measurements of live specimens include the thickness of the integument (epidermis and dermis) and possibly lack the accuracy of the direct measurements performed on the osteological material. Likewise, in vivo measurements only considered subadult and adult individuals in the analyses.

Both *I* and *J* were significantly higher in males than in females (ANOVAs: ♂ $F_{[1,46]} = 11.6$, $P < 0.001$; ♀ $F_{[1,46]} = 8.99$, $P < 0.05$;

Table 2
In vivo bite force and body measurements (mean ± SD) in the subterranean rodent *C. australis*.

	Bite force (N)	Body mass (g)	Body length (mm)	Head length (mm)	Head height (mm)	Mandibular width (mm)
Males						
Adults ($n = 13$; > 350 g)	82.20 ± 12.50	467.00 ± 62.80	222.12 ± 13.10	58.90 ± 2.16	37.28 ± 1.50	48.25 ± 2.98
Subadults ($n = 8$; 160 – 350 g)	52.43 ± 11.75	311.25 ± 30.90	118.19 ± 12.60	53.00 ± 2.40	32.84 ± 1.50	40.75 ± 1.72
Females						
Adults ($n = 27$; $>$ 260 g)	57.90 ± 10.54	341.30 ± 38.80	201.65 ± 10.80	54.80 ± 1.40	33.62 ± 0.92	42.59 ± 1.67
Subadults ($n = 5$; 150 – 260 g)	45.58 ± 7.56	230 ± 18.70	174.00 ± 3.95	50.84 ± 1.60	31.00 ± 1.33	37.46 ± 1.60
All specimens	61.88 ± 16.24	357.8 ± 83.71	202.40 ± 18.70	55.24 ± 3.10	34.25 ± 2.40	43.34 ± 4.06

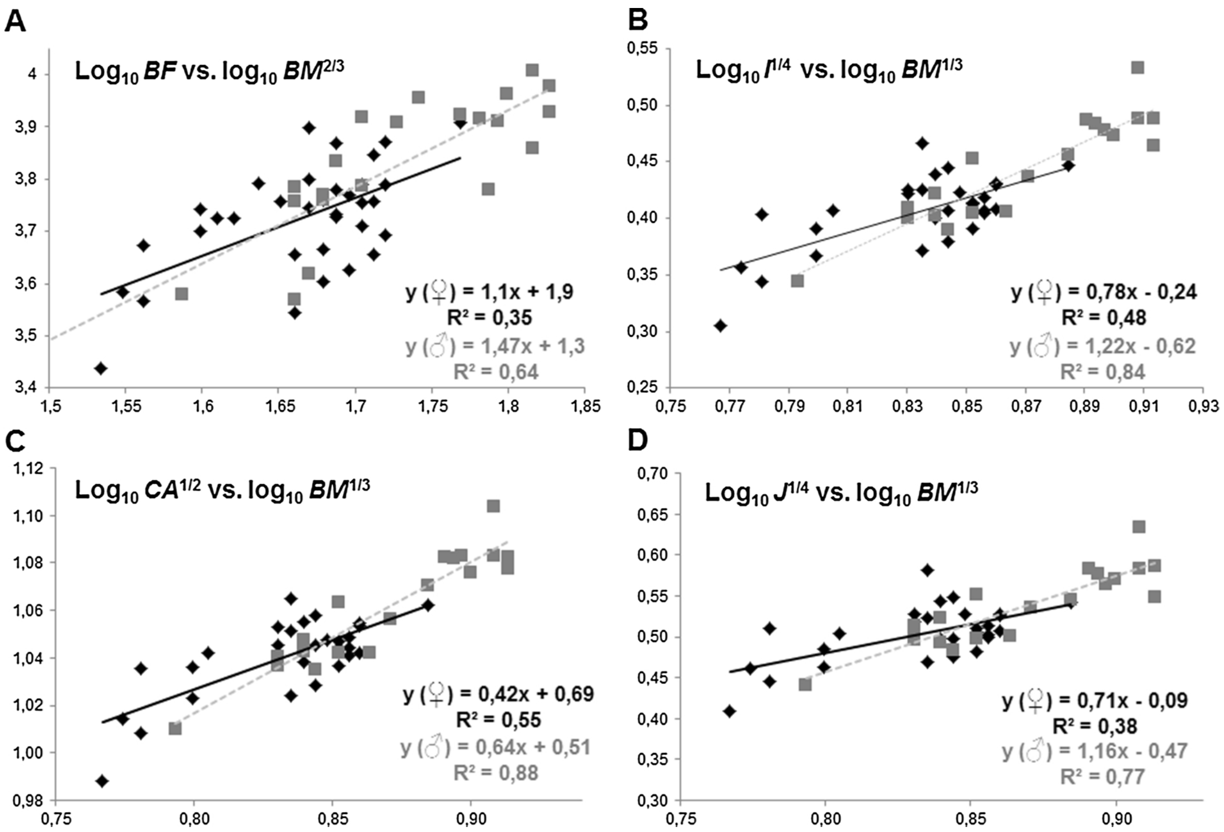

Fig. 5. Scatter-plots of in vivo (A) bite force (BF), (B) bending strength (*I*), (C) cross-sectional area of incisors (CA), and (D) torsional strength (*J*) in both sexes of *C. australis*. The data are $\log_{10}$-transformed (bite forces were measured in N) and linearized, and the allometric equations and adjustments are shown. All variables are regressed against body mass (BM).

Fig. 7), however, CA did not show any significant differences between the sexes (ANOVA: $F_{[1,46]} = 0.75, P = 0.4$; Fig. 7). Similarly as found in the in vivo absolute bite forces measured, there were no statistical differences between the sexes in the allometric coefficients for CA, *I* or *J* (ANCOVAs, allometric coefficients: $P = 0.48$, $P = 0.32$, $P = 0.52$, respectively; Fig. 5B–D). However, males still showed a strong sexual dimorphism in the characters that confer higher bending and torsional strength by their overall position at the slope (ANCOVAs, changes along the common slope: $P < 0.001$ for all comparisons; case *a* in Figs. 4 and 5B–D; Table 3). Consequently, sexual dimorphism in these morphological traits seems to be associated with body size variation, but not with sex. Thus, sexual dimorphism of these traits (involved in bending and torsional strength of the incisors) was not independent of body size.

3.3. Variation in upper incisor procumbency

The angle of the upper incisor procumbency (AIP) was not significantly different between adults of both sexes (mean AIP of 92.2° for ♂ vs. 92.1° for ♀; ANOVA: $F_{[1,56]} = 0.08, P = 0.97$). After controlling for body size, no significant differences between the sexes were observed for AIP (ANCOVA: $F_{[1,36]} = 0.95, P = 0.34$).

4. Discussion

In this study we aimed to characterize sexual dimorphism in the subterranean rodent *C. australis*, focusing on cranial traits directly involved in the aggressive interactions between males (e.g. bite force; robustness of jaw adductor muscles; bending and torsional strength of the incisors). To this aim the ontogenetic trajectories of these traits were assessed for both sexes. Sexual dimorphism may be the outcome of morphological changes related solely to body size differences between the sexes, and/or it also may respond to differential growth rates (allometric changes) in morphological traits associated with male–male interactions. Therefore, the question here is whether body size variation between the sexes is accompanied by changes in cranial proportions, and if so, how the ontogenetic trajectories of both sexes differ to produce this variation.

4.1. Ontogenetic trajectories

The robustness of the *Ctenomys* mandibular apparatus has been viewed as the outcome of both selection pressures related to the occupation of the underground niche (Vassallo, 1998; Mora et al., 2003; Lessa et al., 2008; Vassallo et al., 2016), as well as selection linked to its social system and high territoriality (i.e. male– male competition for mates, Becerra et al., 2012a). Incisors in this species are actively used during inter-male aggressive encounters (Vassallo and Busch, 1992), which most probably characterize the polygynous mating system of this species (Zenuto and Busch, 1998; Cutrera et al., 2010a; Mora et al., 2010). We suggest that, in *C. australis*, attributes associated with biting performance might have been molded by sexual selection, involving gender differences in ontogenetic trajectories for some cranial traits.

In this regard, Fig. 4 shows four possible types of morphological change affecting a particular trait during the ontogeny of an organism. In case *a*, morphological change is only associated with an increase in body size (i.e. sexual dimorphism is almost exclusively related to body size increase during ontogeny). In case *b*, the morphological change during ontogeny is related to early changes in the intercept, but not in the slope (lateral transposition; Klingenberg and Spence, 1993; Klingenberg, 1998). In cases *a* and *b*, the ratio between the growth rates of traits does not change during ontogeny; nevertheless, lateral transposition (*b*) implies that a dissociation between the growth rates of these traits has occurred in earlier stages of ontogeny (Klingenberg, 1998; Verzi et al., 2010a). In case *c*, morphological change in a trait is related to different growth rates (changes in the regression slope) between sexes during a particular stage of ontogeny. This situation does

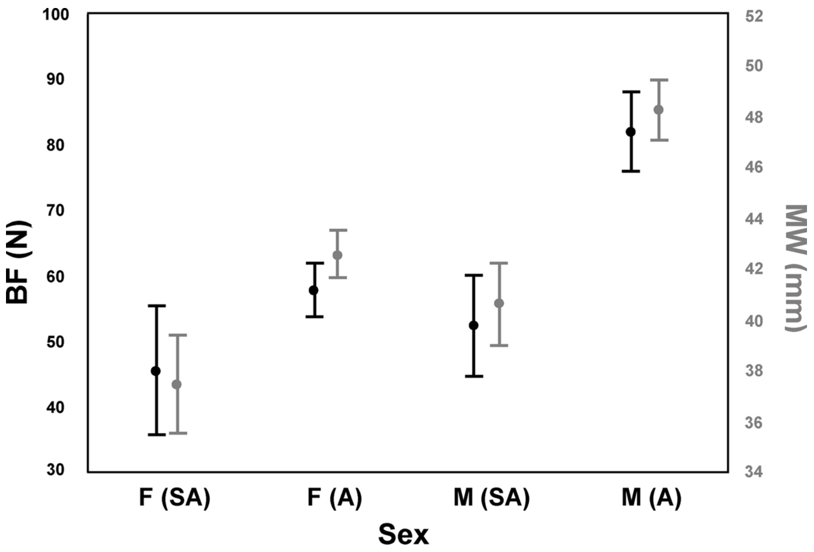

Fig. 6. Relationships among means and 95% confidence intervals for bite force (BF, in black) and mandibular width (MW, in gray) between the sexes for different age classes: F (SA) = subadult females; F (A) = adult females; M (SA) = subadult males; M (A) = adult males.

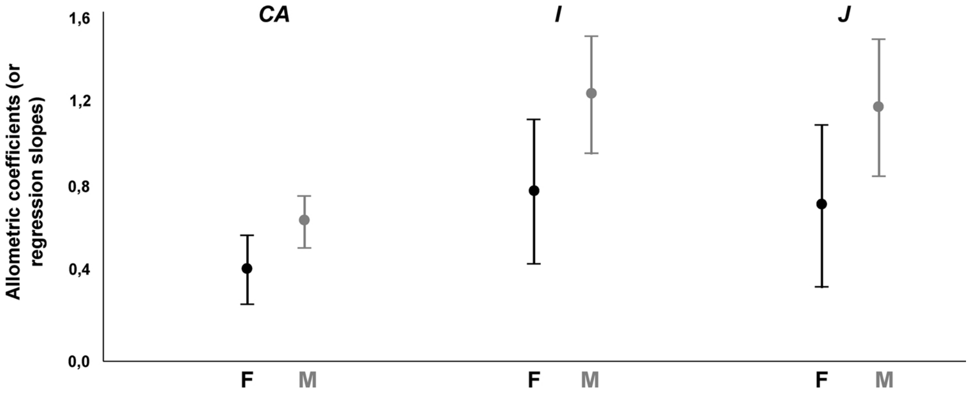

Fig. 7. Allometric coefficients (or regression slopes) for area of incisors (CA), bending strength (*I*).

not necessarily imply a constant acceleration (or deceleration) in growth rates of a particular trait related to the sex, but may respond to any change either at the beginning or at the end of the ontogenetic trajectory. The fourth modality (case *d*), which involves a combination of lateral transposition and extension from the developmental rate, was not observed for any trait in this study. Based on the conceptual scheme depicted in Fig. 4 it might be said that a complex pattern of morphological change accounts for the dimorphism observed in *C. australis*. Some variables in our study appear to show sexual dimorphism only as a response to growth extension during ontogeny (case *a* in Fig. 4). Because males achieve greater body sizes than females, their incisors would reach greater structural strength as revealed by indicators measured in this study (i.e. higher values of CA, *I* and *J*). This happens without changes in the regression slope of the ontogenetic trajectory. We are confident that since these variables have been estimated, albeit indirectly, in the same way for both sexes this result would not be affected by the method used. On the other hand, other traits seem to suggest that there are allometric changes throughout ontogeny. Such is the case with variables related to the proportions of the incisors (IW and IT) and of the masseter lateralis muscle (LMM, case *c* in Fig. 4). Sexual dimorphism may also result from lateral transposition (MW, case *b* in Fig. 4).

Finally, environmental selective pressures during ontogeny (e.g. digging and cutting roots) and agonistic encounters between adult individuals (e.g. male–male interactions) could have molded the evolution of robust incisors and strong masseteric muscles in *C. australis* (and in other *Ctenomys* species, see also Vassallo et al., 2016). With respect to bite force, the ANCOVA analysis indicates that sex differences in biting capability were not associated with the sex itself, but with overall dimorphism in body size. In this manner, this functional attribute of the mandibular apparatus seems to follow the same pattern observed for the incisor strength indicators CA, *I* and *J*, i.e. sex differences arising without significant changes in the allometric coefficient. Here, we have globally focused on changes throughout postnatal ontogeny, without focusing our attention on each particular age class.

4.2. Dimorphism and the mating system of C. australis

Ecological (e.g. spatial location of resources), social (e.g. tolerance among individuals) and sexual features (e.g. body size, secondary characters associated with the immune status) are currently recognized as having a significant impact on mate choice in mammals (Cutrera et al., 2010b). Mate choice is usually focused on environment-dependent aspects of the male phenotype, such as the quality of resources that a male might monopolize (Safran et al., 2013). Although in many mammalian species male spatial organization is influenced by the distribution of the females (because a male's reproductive success depends principally on the number of mates he can find and defend; Clutton-Brock, 1989; Komers and Brotherton, 1997), the mating system in *C. australis* is most probably a resource-defense polygyny −as was proposed by Zenuto et al. (1999) for *C. talarum* − in which males monopolize resources (foraging areas and territory) that will then be used by

Table 3

Allometric coefficients (*b*) and the corresponding 95% confidence intervals (CI, standardized major axis) from the comparisons between subadult/adult sexes and from all data. *P*-values (probability) and R^2 (regression coefficients, with their own probability *P*-values) from the slope test (or from differences in allometric coefficients; ANCOVA) between the sexes are also shown. Asterisks indicate statistically significant ($^*P < 0.05$, $^{**}P < 0.01$) differences between allometric coefficients. The possibilities of allometric changes observed in this study are explained in Fig. 4 (cases ***a***, ***b*** and ***c***). Abbreviations: CA, cross-sectional area of the incisors; *I*, bending strength of the incisors; *J*, torsional strength of the incisors; BM, body mass.

	♀	♂	Along common slope shift	Elevation shift	Slope shift
Log_{10} $\text{CA}^{1/2}$ vs. $\log_{10}$ $\text{BM}^{1/3}$	*b* = 1.147 (0.842 – 1.560) $R^2 = 0.40^{**}$	*b* = 1.319 (1.05 – 1.66)* $R^2 = 0.78^{**}$	$\Theta = 10.321$ $P < 0.001^{**}$	$\Theta = 1.49$ $P = 0.22$	$\Theta = 0.54$ $P = 0.48$
Log_{10} $\text{I}^{1/4}$ vs. $\log_{10}$ $\text{BM}^{1/3}$	*b* = 1.12 (0.84 - 1.5) $R^2 = 0.48^{**}$	*b* = 1,33 (1.09 – 1.62)** $R^2 = 0.84^{**}$	$\Theta = 10.86$ $P < 0.001^{**}$	$\Theta = 1.12$ $P = 0.29$	$\Theta = 0.97$ $P = 0.32$
Log_{10} $\text{J}^{1/4}$ vs. $\log_{10}$ $\text{BM}^{1/3}$	*b* = 1.16 (0.85 – 1.58) $R^2 = 0.38^{**}$	*b* = 1.32 (1.04 – 1.67) $R^2 = 0.77^{*}$	$\Theta = 10.22$ $P < 0.001^{**}$	$\Theta = 1.58$ $P = 0.21$	$\Theta = 0.47$ $P = 0.52$

reproductive females (Zenuto and Busch, 1998; Cutrera et al., 2010a). The main outcomes of the present study are that *C. australis* has a robust excavatory/masticatory apparatus and is capable of exerting great bite forces at the tip of the incisors, comparable to other species of tuco-tucos of the same body size, such as *Ctenomys tuconax* (Becerra et al., 2013). Since males of *C. australis* (similar to other species of *Ctenomys*) also use the incisors during aggressive encounters (which is evident from the scars in the face of males in natural populations; Busch, 1989; Vassallo and Busch, 1992), both their greater bite force and strong incisors appear especially apt for that type of mating system.

It is interesting to note that there is evidence that other biological attributes such as home range size and dispersal rate also show sexual dimorphism in *C. australis* (Cutrera et al., 2010a; Mora et al., 2010). It has been shown that these attributes strongly depend on body mass. Mora et al. (2010) and Cutrera et al. (2010a) have described important sexual differences in some attributes of *C. australis* with home range sizes and depth of the galleries being larger and deeper for males than females. Considering data on dispersal rates, some sexual differences seem to be present in different species of *Ctenomys*; these studies are in agreement, in general, with a male-biased dispersal, at least for some landscape scale (Cutrera et al., 2005, 2010b). In *C. australis*, male-biased dispersal was found at lower spatial scales (< 4 km) in continuous habitats, suggesting that males might move longer distances than females. Therefore, dispersal rates may also be considered an important component of sexual dimorphism both in this species as well as in other mammals, influencing significantly the genetic and morphological differentiation among populations.

In addition to these latter ecological and life history traits, sexual dimorphism also exists in those characters that constitute the fighting apparatus of *C. australis* (Cutrera et al., 2010a; Mora et al., 2010). In the present study, differences in bite force − the ability to exert external forces at the tips of the incisors − were not primarily associated with the sex itself, but with the sexual dimorphism in body size. Concomitantly, although males and females did not show statistical differences in the allometric coefficients either for *CA*, *I* or *J* − variables which denote the higher bending and torsional stress resistance at the tip of the incisors −, they showed strong sexual dimorphism in these characters due to the increase in body size (Table 3 and Fig. 4). However, mandibular width − an estimator of the relative size of the masseter muscles and, ultimately, bite force − was related not exclusively to body size, but also to the sex.

Remarkably, maximum mandibular width and bite force showed the same pattern of variation among sexes and age classes (Fig. 6). Thus, greater relative size of the masseteric muscles leads to higher bite forces at the tip of the incisors, in agreement with the conclusions of Becerra et al. (2014) regarding caviomorph rodent species. Overall, gender seems to play a minor role in morphological changes linked to the strengthening of the incisors; changes in these traits seem to be mainly associated with body size variation.

Therefore, sexual selection acting on male body size can account for behavioral and morphological traits on which reproductive success strongly relies. Nevertheless, as we showed, other cranial traits involve ontogenetic changes beyond size (e.g. mandibular width). Similar results were described by Becerra et al. (2013) in *C. tuconax*, which has a body mass comparable to that of *C. australis*. Interestingly, sexual dimorphism in *C. tuconax* was explained by sexual selection acting on overall body size, which leads to important differences in the mandibular apparatus between sexes (Becerra et al., 2013).

Finally, these results show that these morphological attributes of the skull may be used during aggressive encounters between males, characterizing a typical polygynous mating system also present in other species of this genus, like *C. talarum* (Zenuto et al., 1999; Becerra et al., 2012a). The establishment of dominance hierarchies among males and the occurrence of ecological differences such as different home range sizes observed in the field for *C. australis* (Cutrera et al., 2010a; Mora et al., 2010) agree with the strong sexual dimorphism observed here in some morphological traits of the skull.

4.3. Underground activity, use of incisors in soft and hard substrates and their relationship with sexual dimorphism

The incisors of subterranean rodents are able to perform functions in both foraging and digging (e.g. several species of Bathyergidae, Geomyidae, Octodontidae, Spalacidae, Cricetidae, Echimyidae, Aplodontiidae and Ctenomiydae; Van der Merwe and Botha, 1998; Lessa, 2000; Stein, 2000; Mora et al., 2003; Vassallo and Mora, 2007; Lessa et al., 2008; Van Daele et al., 2009), activities which typically require the exertion of strong forces for breaking the soil (Mora et al., 2003). Although *Ctenomys* has been considered a 'scratch' (claw) and 'chisel-tooth' digger by several authors (Dubost, 1968; Lessa et al., 2008), the use of each specific component for digging is highly variable and most probably depends on the soil hardness (Vassallo, 1998; Mora et al., 2003; Luna and Antinuchi, 2007; Becerra et al., 2013). Noticeably, most of the *Ctenomys* species have evolved a powerful jaw musculature and procumbent incisors that are used as 'digging tools' mainly for the excavation of galleries (Vassallo, 1998; Lessa, 2000; Lessa et al., 2008; Becerra et al., 2011), which provides important insights about specific adaptations of ctenomyids to the subterranean mode of life (Mora et al., 2003).

Some authors have associated the body size differences in *Ctenomys* with the hardness of the soil they inhabit, with strong selection for energy efficiency in digging (in conjunction with local environmental conditions) severely limiting these species to particular habitats (Mora et al., 2003; Luna and Antinuchi, 2007). Accordingly, many authors support the idea that the capacity of tuco-tucos to dig the soil decreases dramatically in harder substrates (see also Luna and Antinuchi, 2007 and references therein). The positive allometry of incisor width and thickness present in most *Ctenomys* species (Mora et al., 2003; Vassallo and Mora, 2007; Verzi et al., 2010b) shows that, in larger species, relatively more powerful incisors might be able to resist greater bending forces. These results seem to contradict some observations in which several species of tuco-tucos of large body sizes inhabit friable soils.

Mora et al. (2003) also detected a correspondence between species with low angles of upper incisor procumbency (AIP, a character largely viewed as an adaptation to digging with teeth) and friable sandy soils, and between species with high AIP and compact soils. Higher upper incisor procumbency was also observed by Vassallo (1998) in species that inhabit relatively compact soils, allowing a more effective angle of attack. Our results show that AIP is highly variable in *C. australis*, with no clear differences between the sexes. However, this species has one of the lowest AIP within the genus (Vassallo, 1998; Mora et al., 2003).

Although *C. australis* uses the incisors for breaking down hard substrates (e.g. roots and bulbs), it is essentially a 'scratch' digging rodent in a typical sand dune habitat (Vassallo, 1998), while the smaller species *C. talarum* combines both 'scratch' and 'chisel-tooth' digging to break down the soil. From an adaptative perspective, the more frequent use of the incisors on hard substrates may explain the higher values of procumbency in *C. talarum* than in *C. australis*. Some larger species, like *C. tuconax*, are also capable of digging in harder substrates (Becerra et al., 2013).

According to Verzi (1999) and Vieytes et al. (2007), enamel thickness seems to be a good predictor of the bending stress to which the incisors are subjected in *Ctenomys* and other caviomorph rodents. These authors reported greater enamel thickness for *C. talarum* compared to *C. australis*, suggesting that the occupancy of harder soils in the former species requires more frequent chisel-tooth digging (see also Vassallo, 1998). This relationship between tooth digging and habitation in hard soils was also reported in the ctenomyid fossil *Eucelophorus* sp. (Vieytes et al., 2007).

Bite forces measured in *C. australis* in the present study were higher than those previously reported for *C. talarum* (males: 150 – 210 g; females: 130 – 180 g; Becerra et al., 2012a), and similar to values reported for *C. tuconax* (males: 310 – 550 g; females: 250 – 450 g; Becerra et al., 2013). *C. tuconax* has a body size similar to *C. australis*, and adult males also show significantly higher bite forces (74.90 N) than adult females (53.80 N) (Becerra et al., 2013). In addition, bite force recordings in *C. talarum* were significantly higher in adult males than in females (32 vs. 27 N), but in both cases lower than those in *C. australis* and *C. tuconax*. It should be noted, however, that *C. tuconax* uses its incisors to disaggregate soils that are significantly more compact than those occupied by other similar-sized species such as *C. australis* (Becerra et al., 2013). Since several species of *Ctenomys* occupy diverse habitats and soil types regardless of their body size (Mora et al., 2003), the hypertrophy of the masseteric musculature in some large *Ctenomys* species (e.g. *C. australis*) may result from other factors rather than soil hardness, for example the density of roots in the soil through which the galleries must pass.

Sexual selection also seems to have influenced the evolution of some morphological traits, such as the increased bending and torsional strength of the incisors and the hypertrophy of the masseteric crests. Ultimately, these attributes provide an advantage in the production of forces involved in aggressive encounters between males during mating and territory defense (Vassallo and Busch, 1992; Vassallo, 1998; Becerra et al., 2011).

4.4. Linking the climate changes during the Late Cenozoic and the evolution of skull traits involved in specializations for digging in ctenomyids

Ctenomys is the only living genus of the family Ctenomyidae, whose extinct and living taxa show different commitments to life underground (Fernández et al., 2000; Lessa et al., 2008; Becerra et al., 2013). Tracing the evolution of this behavior in ctenomyid rodents from the Pliocene to the extant taxa reveals the acquisition of several morphological adaptations for digging, which include the forelimbs, the mandibular apparatus, the incisors (Verzi, 1999; Vucetich et al., 1999; Mora et al., 2003; Vieytes et al., 2007; Lessa et al., 2008), and other physiological and behavioral adaptations (Luna and Antinuchi, 2007). In addition, some authors argue that the relatively drier and open biomes characterizing the southern portion of South America have promoted, in ctenomyids and other related caviomorph rodents, the strengthening of the mandibular apparatus and the acquisition of dental attributes which allow the processing of abrasive diets (Verzi et al., 2010b).

In this regard, one of the most significant transformations in the evolution of ctenomyids has been the progressive enlargement of the masseteric crest, where the masseteric muscles insert, which was accompanied by an overall strengthening of the skull (Mora et al., 2003, 2013b; Verzi 2008). According to Vassallo and Mora (2007), the evolution of the masseteric crest in ctenomyids was strongly correlated with an increase in the volume of masseteric muscles. Thus, an increase in mandibular width in *Ctenomys* species has directly involved a mechanical advantage in chisel-tooth digging and the ability to break roots or harder substrates (Vassallo, 1998; Mora et al., 2003). Clearly, the acquisition of a robust mandibular apparatus with strong incisors and the ability to exert powerful bites proved compatible with two functions typical of open, relatively arid environments: digging, and processing abrasive diets.

5. Conclusions

For the moment, it is not possible to know at what point in the evolutionary history of the genus *Ctenomys* resource-defense polygyny began to constitute the predominant mating system (see, however, Woodruff et al., 2013). Whereas sexual size dimorphism is widespread among extant *Ctenomys* species (Bidau and Medina, 2012), there are no data on this issue in extinct species of the Ctenomyidae. However, it seems clear that at some point sexual selection began to operate as a significant force within *Ctenomys*, intertwined with selection pressures coming from southern South American environments where it originated and diversified.

We analyzed the sexual dimorphism in cranial characters implicated in aggressive interactions between males, analyzing (i) cranial ontogenetic trajectories of both sexes, and (ii) in vivo incisor strength and bite performance in adult individuals, hypothesizing that adult males are able to exert stronger bite forces at their incisor tips than adult females. We found that cranial traits involved in aggressive interactions of *C. australis* undergo significant changes throughout postnatal ontogeny. Traits such as incisor width and thickness showed sexual dimorphism regardless of body size increase (i.e. change in proportions due to allometry). Contrarily, differences in bite force were not associated with the sex, but with sexual dimorphism in body size itself. Mandibular width, a morphological proxy of bite force, was highly dimorphic and has resulted from a lateral transposition of the ontogenetic trajectory.

Although some cranial attributes studied in *C. australis* are probably the outcome of selection pressures related to abrasive diets and dento-excavation, sexual selection acting on morphological traits (particularly those attributes directly involved in aggressive interactions between males) should not be ruled out. The fact that some of the studied traits were significantly correlated with body mass suggests that sexual selection acting on male body size can account for the dimorphism in several jaw and incisor traits in this species. It remains to be seen whether other factors may underlie the morphological and functional differences between the sexes reported here. For example, although both sexes have very good digging capabilities, it is not known whether males perform this activity more frequently because they have larger home range areas.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.zool.2018.02.005.

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