

Bite it forward ... bite it better? Incisor procumbency and mechanical advantage in the chisel-tooth and scratch-digger genus *Ctenomys* (Caviomorpha, Rodentia)



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ABSTRACT

The subterranean genus *Ctenomys* (~60 species, ~100–1000 g) constructs its burrows by using both forefeet and teeth throughout a wide range of habitats in South America. They show a high variation in the incisors' angle of attack (procumbency) and a mostly conserved skull morphology, not only amongst their congeners, but within the caviomorph rodents. Traditionally, procumbency has been largely related to tooth-digging. Looking for the possible influence of incisor procumbency on the mechanical advantage (MA) of each of the seven jaw adductor muscles in the genus, we examined 165 skulls representing 24 species. We also evaluated the role of two other relevant traits – i.e. mandibular width and diastema length – in jaw biomechanics and the existence of a relationship between procumbency angle and soil hardness. The in- and out-lever arms (L_i and L_o) of the involve muscles were determined based on their insertion's 3D-coordinates and integrated to calculate their MA. Interspecific scaling relationships for skull and muscle measurements were analyzed through reduced major axis regression performed with phylogenetically independent standardized contrasts. Although the procumbency angle ranged between ~92.5° (*C. mendocinus*) and ~107.2° (*C. occultus*), we found that it was not significantly correlated with the MA of any jaw adductor muscle. This study also showed that the incisor procumbency variation was not associated with the relative rostral length or soil hardness. This result contradicts previous generalizations about a correlation between habitat conditions and the procumbency of the incisors in subterranean rodents. In sum, our results suggest that, within *Ctenomys*, possessing more procumbent incisors may not represent a biomechanical advantage, but might be beneficial in other aspects related to chisel-tooth digging or food processing behaviors.

1. Introduction

Within rodents, caviomorphs have undergone a huge radiation in terms of body size, occupied habitats, locomotor habits and geographical distribution (e.g., Mares and Ojeda, 1982). Particularly, *Ctenomys* species (tuco-tucos; Rodentia, Caviomorpha, Ctenomyidae) are characterized by a subterranean mode of life implying a series of morphological, physiological and behavioral adaptations to live primarily underground (Reig et al., 1990; Lacey et al., 2000; Verzi, 2002; Mora et al., 2003; Begall et al., 2007; Medina et al., 2007; Lessa et al., 2008; Parada et al., 2011). Like other caviomorphs, tuco-tucos are proficient scratch-diggers that primarily dig by means of vigorous scraping movements (Hildebrand, 1985). Secondarily, they use their large and procumbent incisors (i.e.,

incisors with a protrusion angle greater than 90°) according to substrate requirements to assist in loosening and breaking obstacles such as rocks, nodules of CaCO₃ (“tosca”) or hard soil, and fibrous roots (see Ubilla and Altuna, 1990; De Santis et al., 1998; Vassallo, 1998; Stein, 2000). Although they all share the subterranean niche, the wide geographical distribution they have as a genus (Mares and Ojeda, 1982) involves a remarkable range of underlying environmental physical properties. For example, within Argentina, they are found from the coastal sand-dunes (occupied by *C. australis* Rusconi, 1934; Luna and Antinuchi, 2007; Cutrera et al., 2010) to the compact soil in highland grasslands near “El Infiernillo”, Tucumán Province (*C. tuconax* Thomas, 1925; Becerra et al., 2013) and the desert and rocky soil of the north-western mountains (*C. knighti* Thomas, 1919).

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All species of *Ctenomys* share morphological specializations for digging that set them apart from most of the extinct and generalized taxa within Ctenomyidae – with the exception of the extinct *Eucelophorus* – as well as from genera belonging to the sister family of Ctenomyidae, Octodontidae (with the exception of *Spalacopus*), which have different degrees of fossorial behaviors determined by both above- and below-ground habitats (Lessa et al., 2008; Verzi, 2008; Verzi et al., 2010). However, ctenomyids show some interspecific morphological differences in their cranio-dental apparatus, especially in their masticatory biomechanics and functional efficiency (Becerra et al., 2012; Becerra, 2015), which might be related to the wide range of environmental and soil conditions. For instance, their upper incisor procumbency is highly variable, showing significant differences between species (e.g., see Mora et al., 2003). Several authors analyzed the possible ecological effect of incisor procumbency in rodents, and the general conclusion was that the degree of tooth-digging displayed by a species is associated with this trait, beyond the allometric effects (see Landry, 1957; Agrawal, 1967; Hildebrand, 1985; Lessa and Patton, 1989; Lessa, 1990; Lessa and Stein, 1992; Neveu and Gasc, 1999; Bacigalupe et al., 2002; Lessa et al., 2008; Becerra et al., 2013). On the other hand, it was suggested that procumbency angle is related to soil hardness. For example, Mora et al. (2003) reviewed the evidence concerning habitat and procumbency angle for ten *Ctenomys* species and they observed a correspondence of both low procumbency with friable sandy soils, and high procumbency with compact soils composed of humus. Moreover, Vassallo (1998) studied the digging behavior in two sympatric *Ctenomys* species (*C. talarum* and *C. australis*) which differed in size, habitat and procumbency angle. He showed that even though both species behaved as scratch-diggers when confronted with soft, sandy and friable soils, only the most proodont one (i.e. *C. talarum*) used effectively both foreclaws and incisors when digging in extremely hard soils.

One functional explanation for the association between procumbent incisors and tooth-digging behavior is that procumbency isolates the incisor tips from the soft tissues of the mouth, protecting the latter from injury through distance (Van der Merwe and Botha, 1998), and also preventing the ingestion of a high amount of grit during digging. Furthermore, procumbent incisors might also be advantageous since they provide a more favorable angle of attack for digging, through a better anchoring in the flat or concave walls of the burrow (Korth and Rybczynski, 2003). Although procumbency has been largely related to tooth-digging, and several authors have emphasized its benefits for subterranean rodents (Landry, 1957; Lessa, 1990; Vassallo, 1998; Korth & Rybczynski, 2003; Meachen-Samuels & Van Valkenburgh, 2009), its impact on jaw biomechanics has not yet been accurately studied within *Ctenomys*. For example, Vassallo's (1998), Mora's et al. (2003), and Lessa's et al. (2008) analyses on procumbency angle were constrained in the number of species or tested against overall cranio-dental – not biomechanical – metrics, while Vassallo (2000) based his results on non-realistic mathematical simplifications.

The genus *Ctenomys* represents an excellent study model to investigate the role of incisor procumbency in jaw biomechanics because of its relatively conserved skull morphology (i.e., the conserved *bauplan*, or basic structure, of the skull; Vassallo and Mora, 2007), the great variation in upper incisor procumbency (Mora et al., 2003), and the wide range of mechanical demands of the habitats occupied (Mares and Ojeda, 1982). The main purpose of our study was to investigate, in a phylogenetic context, the relationship between incisor procumbency and the mechanical advantage of each of the seven jaw adductor muscles in a sample of 24 *Ctenomys* species. Since even slight cranio-dental morphometric differences may determine shifts in the feeding ecology of closely related species, Cleuren et al. (1995) suggested that a rigorous determination of the orientation of the muscle force vectors appears to be of crucial importance. For that reason, the different linear variables used in this study were obtained from their 3D coordinates on each individual skull.

We hypothesized that procumbency angle affects mechanical

advantage. We predicted that an increase in procumbency will increase the out-lever arm (L_o) and, in turn, will result in a lower mechanical advantage of a specific muscle. We also assessed the role of two other relevant morphological traits in this genus: the mandibular width – a proxy for masseter muscle development (see Becerra et al., 2013, 2014) – and the diastema length (a proxy for rostral size and a good indicator for incisor root insertion in *Ctenomys*; Mora et al., 2003) in jaw biomechanics. We hypothesized that the relative mandibular width across the masseteric crests is associated with the in-lever arm of the deep masseter (PM), and hence, influences its mechanical advantage. We predicted that the larger the mandibular width, the longer the in-lever arm for a muscle and, in turn, the higher the mechanical advantage. On the other hand, we hypothesized that the relative diastema length is associated with the procumbency angle and, consequently, the out-lever arm and all the mechanical advantages. We predicted that an enlargement in relative diastema length will protrude incisors at a higher angle, resulting in the above-mentioned lower mechanical advantage. Finally, we included the comparison between procumbency angle versus soil hardness to test if these variables are associated as it was suggested in previous works (e.g., Vassallo, 1998; Mora et al., 2003).

2. Materials and methods

2.1. Specimens

For the present study, we examined 143 skulls (crania + mandibles) belonging to 24 *Ctenomys* species (Datasheet S1 in the supplementary online Appendix). The ecological data of the different species are summarized in Table 1. We included specimens belonging to the family Octodontidae as an “outgroup” in the analyses of phylogenetic relationships among *Ctenomys* species. The resultant tree was then used to phylogenetically correct our data in the different analyses. Only adults were taken into account, defined by the presence of a functional third molar. Although previous studies showed a significant sexual dimorphism in the overall size of the skull, no such differences were found after standardizing morphological traits by size (e.g., *Ctenomys talarum*, Malizia et al., 1991; García Esponda et al., 2009). Thus, taking into account that this study focused on interspecific differences, and that we used skull traits that were standardized against size, sexes were pooled together.

2.2. Data collection

According to Cleuren's et al. (1995) recommendation, we used a 3D model in which the orientation and location of the force vectors of each jaw adductor muscle were accurately determined. To calculate the mechanical advantages of jaw adductor muscles, the cranium and mandible of each specimen were articulated at a gape angle of 10° – which is similar to the gape angle used for *in vivo* measurements of bite force (e.g., Becerra et al., 2014). One-hundred landmarks were collected ($N_{\text{cranium}} = 49$ and $N_{\text{mandible}} = 51$; Fig. 1 and Datasheet S2 in the supplementary online Appendix) on each skull using a MicroScribe G2 X 3D digitizer (Solution Technologies Inc., Oella, MD, USA). Based on Druzinsky et al. (2011) and previous dissections done by the authors (F.B.), we located the origin and insertion areas of each muscle [superficial masseter (S.M.), syn. *Musculus masseter superficialis*; deep masseter (PM), syn. *M. masseter lateralis-pars superficialis*; masseter extension-pars posterior (MXTp), syn. *M. masseter lateralis-pars posterior*; infraorbital zygomaticomandibularis (ZMIO), syn. *M. masseter medialis-pars anterior*; zygomaticomandibularis (ZM), syn. *M. masseter medialis-pars posterior*; temporalis (T), syn. *M. temporalis*; pterygoid (Pg), *M. pterygoides*]. Once these areas were located, six landmarks were set at their most anterior, posterior, lateral, medial, dorsal and ventral margins, and centroids were calculated as the arithmetic mean of these points (see Datasheet S3 in the supplementary online Appendix for the

Table 1
Available ecological data for the *Ctenomys* species included in this study. PA, upper incisor procumbency angle; NA, not available.

Taxon	PA (mean)	Habits	Soil	Soil bulk density (kg m ⁻³)	Habitats	Diet	Distribution	References
<i>C. argentinus</i>	104.4°	NA	Found in regions with sandy soils.	1285.250	Dry areas with xeric vegetation at about 600 m.	NA	This species occurs in north-central Argentina in Formosa and Chaco.	Bidau and Lessa (2008)
<i>C. australis</i>	95.0°	Nocturnal Diurnal Subterranean	Very friable and soft soils.	1270.175	Scrub. Sand dune habitats with grasses (<i>Panicum</i> ; <i>Poa</i>).	Herbivorous (above and below ground)	This species occurs in south-eastern Buenos Aires Province, Argentina, in a very narrow distribution along the coast.	Mares and Ojeda (1982) Bidau (2013)
<i>C. boliviensis</i>	102.2°	Nocturnal Diurnal Subterranean	Colonies are found in areas with friable, not often flooded soils.	1409.188	Open savanna areas that have been disturbed by different agricultural and cattle grazing activities (http://www.iucnredlist.org).	Herbivorous (above and below ground)	Around the city of Santa Cruz, central Bolivia; Mato Grosso in south-western Brazil; western Paraguay; Formosa Province in Argentina. In Bolivia it is found at 400–500 m asl.	Mares and Ojeda (1982) Dunnung and Bernal (2016)
<i>C. dorbigyi</i>	101.5°	NA	NA	1245.063	NA	NA	North eastern Argentina, in Corrientes Province. Two small outlier localities included in southern portion of the distribution (Bidau and Lessa, pers. comm.).	Bidau et al. (2008a)
<i>C. flammarioni</i>	95.9°	NA	Sandy soils.	1251.688	Seashore dunes, in an area of high salinity and poor vegetation.	Herbivorous (above and below ground)	Endemic to Brazil. Occurs along the south-eastern coast.	Catzeffis et al. (2008)
<i>C. haigi</i>	99.0°	NA	NA	1001.375	Arid, steppe grassland punctuated at irregular intervals by wet meadows known as “mallines”. Its primary habitat is Patagonian Steppe, and it is also found in low Monte and Valdivian rain forest.	NA	Neuquén, Río Negro, and Chubut provinces, Argentina; in the Monte de Llanuras and Mesetas ecoregions.	Bidau (2016a)
<i>C. lami</i>	96.00°	NA	Inhabits a region with sandy soil named ‘Coxilha das Lombas’ in Brazil.	1273.875	Found in the vicinity of sand dunes.	Herbivorous (above and below ground)	Endemic to southern Brazil, in the state of Rio Grande do Sul.	Bidau et al. (2008b)
<i>C. latro</i>	102.0°	NA	Soft and sandy soils.	1269.625	Dry areas with xeric vegetation at about 600 m.	NA	North-western Argentina in Tucumán and Salta Provinces.	Bidau et al. (2008c)
<i>C. leucodon</i>	120.1°	Nocturnal Diurnal Subterranean	Colonies are found in areas with friable soils.	1206.438	Altiplano open grasslands, in both primary habitat and areas disturbed by grazing.	Herbivorous (above and below ground)	Altiplano highlands surrounding Lake Titicaca, extending from La Paz, Bolivia, to southern Puno, Peru, between 3,800–4,500 m asl.	Mares and Ojeda (1982) Zeballos and Vivar (2016)
<i>C. magellanicus</i>	101.3°	Nocturnal Diurnal Subterranean	Sandy, compact soil.	1162.875	Steppes in Patagonia (Lessa, pers. comm.).	Herbivorous (above and below ground)	Endemic to extreme southern South America including southern Chile and southern Argentina.	Mares and Ojeda (1982) Bidau et al. (2008d)
<i>C. mendocinus</i>	92.5°	Nocturnal Diurnal Subterranean	Aridisols. Poorly developed sandy and gravelly soils.	1286.813	Semixeritic to xeric habitats with grasses and shrub communities (<i>Larrea</i> sp.).	Herbivorous (above and below ground)	Occurs in Argentina on eastern slopes of the Andes from Santa Cruz north to Mendoza provinces, 460–3,400 m asl.	Mares and Ojeda (1982) Rosi et al. (1992, 2002) Camín et al. (1995) Bidau and Ojeda (2016)

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Table 1 (continued)

Taxon	PA (mean)	Habits	Soil	Soil bulk density (kg m ⁻³)	Habitats	Diet	Distribution	References
<i>C. occultus</i>	107.2°	NA	NA	1267.313	Xeric vegetation.	NA	This species is known for only a few localities in Tucumán Province (Alberdi, Lamadrid, Monteagudo and Simoca), in the Chaco Seco region, Argentina.	Patton et al. (2015)
<i>C. opimus</i>	96.2°	Nocturnal Diurnal Subterranean	Sandy and gravelly soils, usually on slopes.	1335.563	Habitats with sparse vegetation and loose soil.	Herbivorous (above and below ground)	Northwest Argentina, south-western Bolivia, southern Peru, northern Chile, 2,500–5,000 m asl on the Andean steppe.	Mares and Ojeda (1982) Dunnum et al. (2016)
<i>C. perrensi</i>	101.2°	Nocturnal Diurnal Subterranean	Sandy soils.	1219.438	Savanna, marshes.	Herbivorous (above and below ground)	North-eastern Argentina, in the central area of western Corrientes Province.	Mares and Ojeda (1982) Bidau (2016b)
<i>C. portousi</i>	100.8°	Nocturnal Diurnal Subterranean	Fine sandy soils to slightly gravelly sandy soil (Echeverría, unpubl. data).	1215.750	The original habitat was Pampas grasslands. Due to changes in land use, it is now mostly found at the edges of fields.	Herbivorous (above and below ground)	Eastern Argentina in Buenos Aires and La Pampa Provinces.	Mares and Ojeda (1982) Woods and Kilpatrick (2005) Bidau et al. (2008e)
<i>C. pundi</i>	98.0°	NA	NA	1259.963	Historically, its natural habitat was Pampas.	NA	Southern Córdoba and southern San Luis Provinces, Argentina.	Bidau et al. (2008f)
<i>C. rionegrensis</i>	98.6°	NA	Sandy soils.	1442.125	It is restricted to sand dunes (Lessa, pers. comm.).	NA	South-eastern-most part of Río Negro (Uruguay) between the Río Negro and Río Uruguay. Coast of Río Uruguay in Entre Ríos Province, Argentina.	Bidau et al. (2008g) Patton et al. (2015)
<i>C. roigi</i>	102.8°	NA	Sandy soils.	1347.938	Sand dunes and edges of rivers.	NA	Argentina, near the Paraná River in Corrientes Province. It can be found over an area smaller than 100 km ² (Bidau, pers. comm.)	Bidau et al. (2008h)
<i>C. seaglitai</i>	100.7°	NA	NA	1280.125	NA	NA	Only known from the vicinity of the type locality Los Cardones, Tucumán Province, Argentina.	Patton et al. (2015)
<i>C. sociabilis</i>	96.5°	NA	Black soil but extremely light and easy to work with.	1110.938	Arid, steppe grassland punctuated at irregular intervals by wet meadows known as “mallines”.	The primary dietary item is the grass <i>Poa pratensis</i> .	Neuquén Province, Argentina, within the area of Reserva Nacional del Parque Nacional Nahuel Huapi.	Pearson and Christie (1985)
<i>C. talarum</i>	100.9°	Nocturnal Diurnal Subterranean	Sandy and friable to hard, clayey soils. Some populations inhabit hard soils rich in humus and with low percentage of silt or clay.	1280.271	Lives in well vegetated grassy habitats. Inhabits highly variable habitat from sand dunes to inland grasslands.	Herbivorous (above and below ground)	Eastern Argentina along the coast in Buenos Aires Province, possibly extending to Santa Fe Province.	Mares and Ojeda (1982) Bidau (2016c)
<i>C. torquatus</i>	94.6°	Nocturnal Diurnal Subterranean	Sandy, rock-free soils.	1297.604	Scrub, grassland. Wide range of habitats (Lessa, pers. comm.). Prefers sandy habitats, and in Uruguay it does not occur in cultivated areas.	Herbivorous (above and below ground)	From central Uruguay to southern Brazil, in Rio Grande do Sul State.	Mares and Ojeda (1982) Redford and Eisenberg (1992) Bidau (2016d)

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Table 1 (continued)

Taxon	PA (mean)	Habits	Soil	Habitats	Soil bulk density (kg m ⁻³)	Habitats	Diet	Distribution	References
<i>C. tuconax</i>	105.5°	Nocturnal Diurnal Subterranean	Well-compacted, humus-rich or loamy soils.	Scrub, grassland. Humid plains to 3000 m.	1331.938		Herbivorous (above and below ground)	North-western Argentina, in Tucumán Province.	Mares and Ojeda (1982) Reig et al. (1990) Bidau et al. (2008)
<i>C. tucumanus</i>	105.2°	NA	Deep humid soils in the piedmont and forest borders of Tucuman city (Contreras, pers. comm.).	Humid areas of plains.	1331.375		NA	Central Tucumán Province, Argentina.	Bidau et al. (2008)

detailed mathematical derivation from landmarks to centroids). Since the zygomaticomandibularis infraorbitalis (ZMIO), superficial masseter (SM) and temporalis (T) are not straightforwardly orientated for jaw adduction, we took into account their tendons' (for SM and T) or muscle's main line of action (for ZMIO) for the biomechanical analysis.

The centroid corresponding to the pivot (P), i.e. the temporo-mandibular joint, was also calculated. The length of each adductor muscle's in-lever arm (L_i) was trigonometrically determined based upon the three-dimensional (3D) coordinates of the corresponding attachments' and pivot's centroids (Fig. 2A). The out-lever arm for incisor biting was determined as the linear distance from the incisors' tip to the pivot (L_o , Fig. 2B), and the mechanical advantage ($MA = L_i/L_o$) of each muscle was calculated (see Datasheet S3 in the supplementary online Appendix for a detailed mathematical derivation from landmarks to lever arms; for improvements by 3D lever modelling see Davis et al., 2010; Becerra et al., 2013). Some of the recorded landmarks were used to calculate the skull linear metrics (e.g., basilar length, diastema length, Fig. 2B; mandibular width, Fig. 2C).

In rodents, the shape of the upper and lower incisors has evolved differently, with the upper incisors being more variable than the lower ones, in terms of their degree of procumbency (Landry, 1957). For this reason, we focused our study on the upper incisors, which may shift their position because their roots are enclosed inside incisor capsules that protrude on either side of the rostrum (Lessa, 1990). Procumbency of the upper incisors (i.e., angle of Thomas or angle of attack; see Reig et al., 1965; Fig. 2B) was measured using the ruler tool in Adobe Photoshop CS5 from digital photographs of specimens' skulls in lateral view.

To evaluate the relationship between procumbency and soil hardness, we included the bulk density (<http://soilgrids.org>) as an indicator of soil compaction because it has a direct relationship with soil hardness, as more compact soils have greater density (Borges et al., 2016; and references therein). This variable (kg m⁻³) was constructed by Hengl et al. (2014) by compiling previously published soil profile data and environmental layers at different depths, at a 1-km resolution, and using regression-kriging interpolation at non-sampled locations. To obtain the bulk density data (soil compactness) for each species we used the geographical coordinates of previously studied populations from the literature (Datasheet S4 in the supplementary online Appendix). We recorded values corresponding to the four nearest pixels to the geographical location of the sampled population, and at four depths (0 cm, 5 cm, 15 cm and 30 cm). Then, we calculated the mean density of soil inhabited by the various species and used the data in subsequent analyses.

2.3. Phylogenetic tree construction

To phylogenetically correct our data, first we constructed a phylogenetic tree using the mitochondrial cytochrome-b sequences of the 24 ctenomyid species included in this study and 5 octodontid genera, as an outgroup. Sequences were obtained from GenBank in FASTA format (accession numbers are listed in Datasheet S5 in the supplementary online Appendix) and were aligned with MEGA 6 software (Tamura et al., 2013) using the ClustalW algorithm (Larkin et al., 2007). Tamura-Nei + I + G substitution model was selected using ModelGenerator 0.85 software (Keane et al., 2006), based on the AIC, AIC2 and BIC criteria. The final phylogenetic tree was built using the MEGA 6 software (Tamura et al., 2013) under a maximum likelihood criterion using the selected substitution model and 1,000 bootstrap replications.

The resulting tree for *Ctenomys* and a character matrix – including basilar length (BL), out- (L_o) and in-lever arms (L_i s), procumbency angle (PA), diastema length (DL) and bulk density (BD) – were imported to Mesquite (Maddison and Maddison, 2015) to obtain the raw phylogenetically independent contrasts (PICs) using the PDAP package. Finally, PICs were standardized by dividing the raw contrasts by their standard deviations, and these standardized PICs were used to compute the reduced major axis regression analyses.

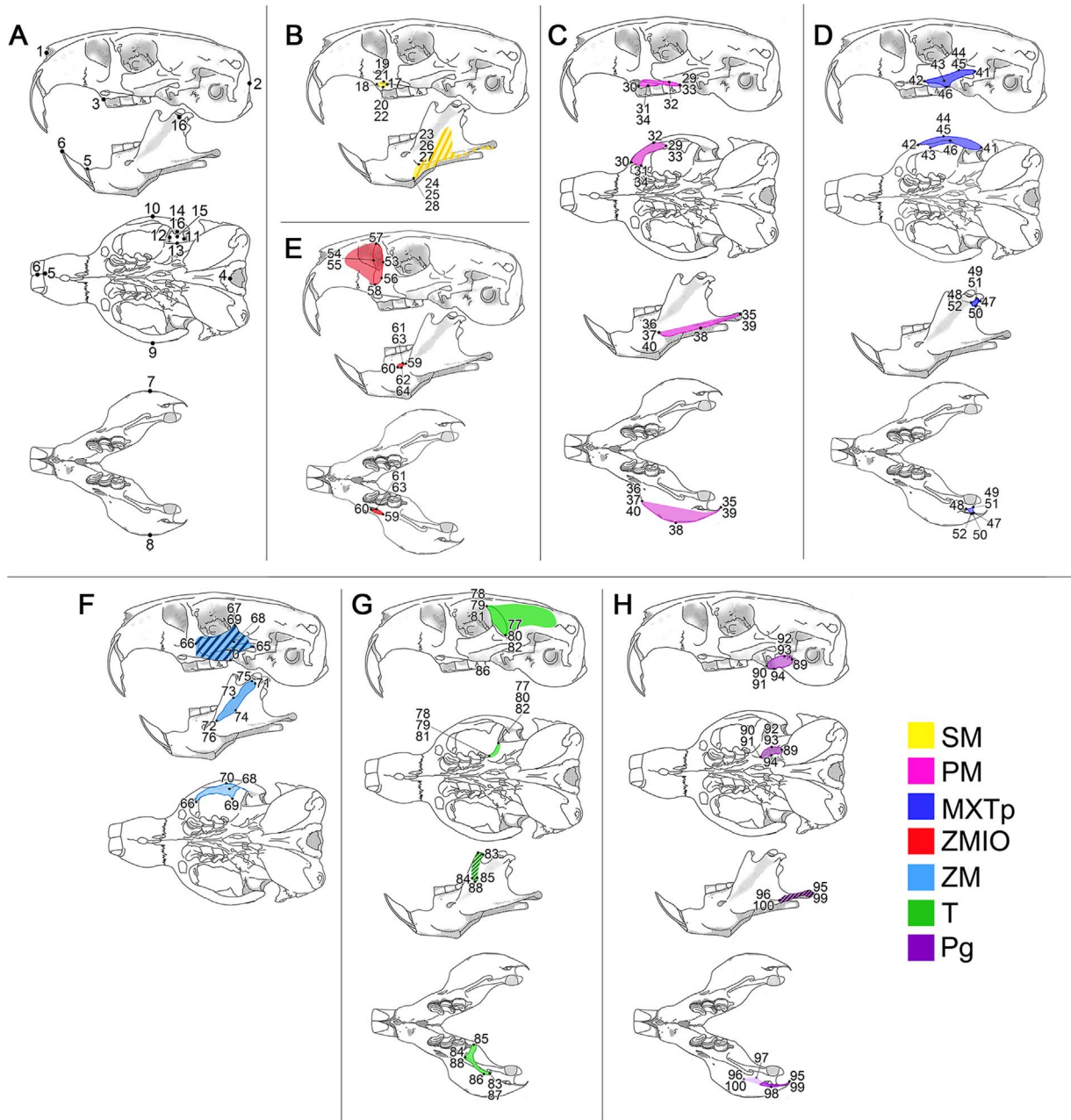


Fig. 1. Attachment areas of cranial and mandibular muscles and landmarks (black dots with numbers) considered in the present study. Colored areas represent the attachment sites of the different muscles. Full color areas: external attachment site; hatched areas: muscle insertions on the medial – internal – side of the bone; colored borders (lines) represent margins of the muscle attachment areas considered for biomechanical analyses. (A) Landmarks recorded to calculate reference lengths (1–10) and the location of the mandibular condyle (11–16); (B–H) landmarks recorded at the most external margins of the jaw adductor muscles’ attachment sites according to the longitudinal, sagittal and transverse planes; (B) superficial masseter (SM); (C) deep masseter (PM); (D) posterior extension of the masseter (MXTp); (E) infraorbital zygomaticomandibularis (ZMIO); (F) zygomaticomandibularis (ZM); (G) temporalis (T); (H) pterygoid (Pg).

2.4. RMA and allometry

We performed bivariate reduced major axis regressions (RMA, model II) to examine the potential relationships between different morpho-functional variables and indices (see all combinations in [Tables 2 and 3](#)). All linear measurements (in mm) and procumbency (PA, in radians) were log₁₀-transformed. RMA regressions were run using phylogenetically independent standardized contrasts ([Felsenstein, 1985](#)) to account for non-independence of related taxa ([Harvey and Pagel, 1991](#)). For this purpose, we used the PDTREE module of the phylogenetic diversity analysis program (PDAP version 1.16; [Midford et al., 2010](#)) of the program Mesquite ([Maddison and Maddison, 2015](#)). Due to the body size differences within this genus, we tested for

allometries of our interest variables (procumbency, out- and in-lever arms, diastema length) against basilar length (BL). All in-lever arms (L_is) were regressed against the out-lever arm (L_o) in order to test whether the mechanical advantages (L_i/L_o) were as conservative throughout these taxa as [Becerra et al. \(2014\)](#) suggested for higher taxonomical levels.

An RMA regression assumes that neither variable is independent in the strict sense, with both variables containing error to some extent. In this case, symmetrical line-fitting techniques (model II) are more suitable than the standard ordinary least squares regression (see [Sokal and Rohlf, 1981](#)), and previous simulation research has indicated that these methods provide more robust estimates, particularly if sample sizes are small ([Riggs et al., 1978](#)). For allometric analyses we used the following

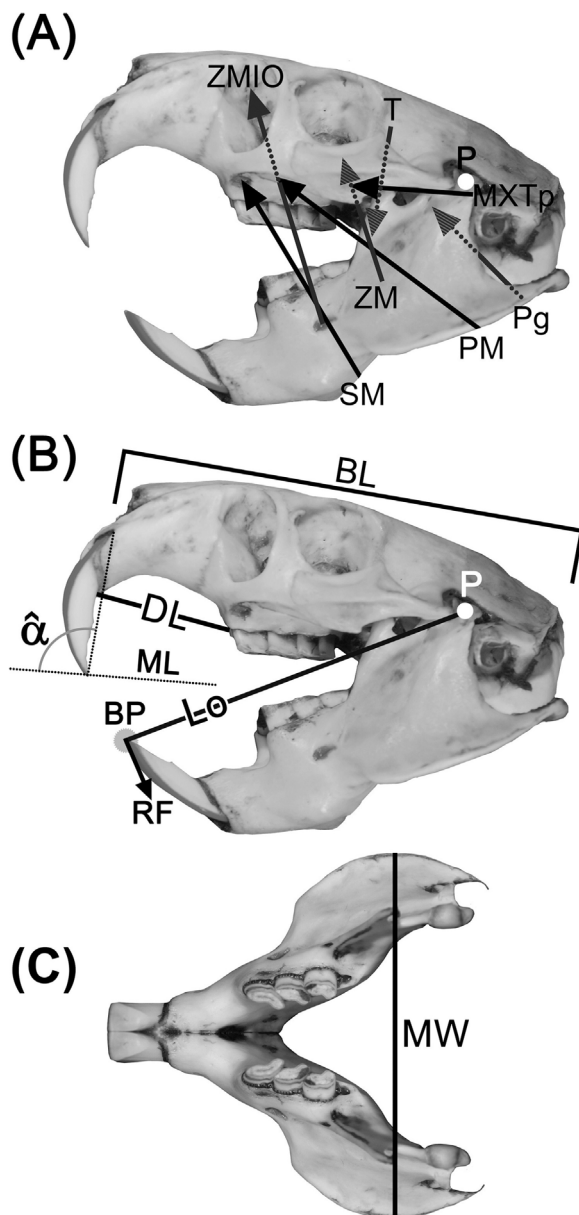


Fig. 2. Biomechanical model and morphological variables considered in the present study. (A) Lines of action of the different jaw adductor muscles considered for bite force estimation based on the computation of the static force equilibrium. (B) Upper incisor procumbency or $\hat{\alpha}$ = angle of Thomas. (C) Mandibular width measurement. Muscle abbreviations as in Fig. 1. Abbreviations: BL, basilar length; BP, biting point; DL, diastema length; L_o , out-lever arm; ML, molar row line; MW, mandibular width; P, pivot (temporo-mandibular joint, site in which the mandibular condyle articulates); RF, food or soil reaction force.

equation:

$$y = ax^b, \quad (1)$$

where y is the raw measurement, x is the proxy for body size, and a and b are the scaling constant and exponent, respectively (Schmidt-Nielsen, 1984). It was transformed to:

$$\log(y) = \log(a) + b * \log(x) \quad (2)$$

where a becomes the y -intercept and b becomes the slope in the RMA analyses run with the software package PAST (v3.06; Hammer et al., 2001). Confidence intervals for regression slopes were generated using a bootstrapping routine (10,000 replicates). If the confidence interval excluded the value 1.0—the slope value expected for isometric scaling

for a linear measurement (e.g., L_o , L_i or diastema length) vs. the basilar length – a departure from isometry (i.e., allometry) was considered significant. Negative allometry was indicated by slopes significantly (i.e., considering the confidence interval) < 1.0 ; positive allometry, by slopes significantly > 1.0 ; and isometry, by slopes not significantly different from 1.0. Conversely, since procumbency angle (radians) is a dimensionless parameter, the isometric expectation when it is regressed against basilar length is a lack of change across body size variation; i.e., confidence intervals should include the null slope (zero).

2.5. Comparative methods

2.5.1. Phylogenetic signal analysis

To determine how trait variation is correlated with the phylogenetic relatedness of species, we quantified the phylogenetic signal in incisor procumbency angle (PA), basilar length (BL), mandibular width (MW), diastema length (DL), out-lever arm (L_o), in-lever arms (L_i s) and mechanical advantages (L_i/L_o) of each jaw adductor muscle. For this purpose, we used Blomberg's K method, which measures the phylogenetic signal by quantifying the amount of observed trait variance relative to the trait variance expected under Brownian motion (Blomberg et al., 2003). Thus, K is a scaled ratio of the variance among species over the contrasts' variance (the latter of which will be low if phylogenetic signal is high), and varies continuously from zero (i.e., the null expectation) to infinity. The null hypothesis for this statistic is the lack of signal (i.e., the trait has evolved independently of phylogeny and close relatives are not more similar on average than distant ones; Kamilar and Cooper, 2013). Values close to 1 are expected if phylogenetic signal is strong (i.e., Brownian motion model), while K -values > 1 indicate that close relatives are more similar than expected under that model. These analyses were carried out using the 'phylosig' function in the phylogenetics package phytools v.0.5-20 (Revell, 2012) within R v.3.1.3 (R Core Team, 2014).

2.5.2. Character color maps

The observed and ancient reconstructed values of the morphological variables used in this study (continuous characters) were mapped – using a color scale – onto the branches of the resulting phylogenetic tree for *Ctenomys*. These maps show the evolution of characters – and their concomitant diversification – and were constructed using the *contMap* function in the phylogenetics package *phytools* v.0.5-20 (Revell, 2012) within R v.3.1.3. We included a tree of bulk density mapped on the phylogeny to illustrate the different soil hardnesses at which *Ctenomys* species are found.

3. Results

3.1. Phylogenetic tree construction

The obtained phylogenetic tree for the 24 *Ctenomys* species included in this study (see Fig. S1 in the online Appendix) showed slight differences with previously published trees (see, for example, Parada et al., 2011).

3.2. RMA and allometry

The overall RMA regression analyses performed on standardized phylogenetically independent contrasts indicated that mechanical advantages were not significantly related to the upper incisor procumbency angle or the relative mandibular width (Table 2). On the other hand, we found a significant relationship between the mechanical advantage of the superficial masseter (SM) and infraorbital zygomatic-mandibularis (ZMIO) with the rostral size (DL/BL) (Table 2).

The allometric analyses showed that the SM, PM, ZMIO, ZM and Pg in-lever arms scaled isometrically with the basilar length (Table 3; Fig. 3B, C, E, F, H). The out-lever arm (L_o) and the remaining in-lever

Table 2

Results of reduced major axis regressions (RMA, type II) performed upon phylogenetically independent standardized contrasts of different morpho-functional variables and indices (x variables: PA, upper incisors' procumbency, in radians; MW/BL, relative mandibular width; DL/BL, relative diastema length; y variables: MA, mechanical advantage) of each jaw adductor muscle of the skull of 24 species of the subterranean genus *Ctenomys*. Numbers in parentheses represent the 95% confidence interval. BL, basilar length; DL, diastema length; MW, mandibular width; MXTp, posterior extension of the masseter muscle; Pg, pterygoid; PM, deep masseter; SM, superficial masseter; T, temporalis; ZM, zygomaticomandibularis; ZMIO, infraorbital zygomaticomandibularis. Bold script indicates significance at $P < 0.05$.

Standardized contrasts	Slope <i>a</i>	Intercept <i>b</i>	<i>R</i> ²	<i>t</i>	<i>P</i> (uncorr.)	Permutation <i>P</i>
SM MA vs. PA	1.377 (0.962, 4.325)	0.109 (0.044, 0.286)	0.043	0.967	0.345	0.342
PM MA vs. PA	-0.980 (-3.273, -0.570)	-0.041 (-0.189, 0.013)	0.002	-0.190	0.851	0.848
MXTp MA vs. PA	1.343 (0.518, 4.185)	0.122 (0.026, 0.240)	0.064	1.200	0.243	0.242
ZMIO MA vs. PA	1.713 (1.220, 5.200)	0.130 (0.042, 0.347)	0.050	1.055	0.304	0.308
ZM MA vs. PA	1.276 (0.898, 3.648)	0.095 (0.039, 0.221)	0.145	1.888	0.073	0.072
T MA vs. PA	-1.106 (-3.578, -0.722)	-0.035 (-0.193, 0.028)	0.008	-0.416	0.681	0.682
Pg MA vs. PA	2.033 (1.289, 6.742)	0.129 (0.014, 0.417)	0.033	0.852	0.404	0.403
SM MA vs. MW/BL	-0.439 (-1.448, -0.111)	0.083 (-0.005, 0.202)	0.012	-0.515	0.612	0.608
PM MA vs. MW/BL	-0.316 (-1.080, -0.098)	0.053 (-0.002, 0.166)	0.002	-0.219	0.829	0.829
MXTp MA vs. MW/BL	0.446 (0.094, 0.701)	-0.006 (-0.066, 0.063)	0.103	1.556	0.135	0.132
ZMIO MA vs. MW/BL	-0.600 (-1.829, -0.1426)	0.106 (-0.011, 0.201)	0.083	-1.377	0.183	0.178
ZM MA vs. MW/BL	-0.419 (-0.645, -0.092)	0.078 (0.006, 0.135)	0.086	-1.404	0.175	0.171
T MA vs. MW/BL	-0.387 (-1.213, -0.087)	0.075 (0.007, 0.185)	0.014	-0.541	0.594	0.595
Pg MA vs. MW/BL	-0.723 (-1.006, -0.218)	0.112 (0.009, 0.213)	0.159	-1.994	0.059	0.056
SM MA vs. DL/BL	-1.071 (-1.446, -0.584)	-0.032 (-0.079, 0.018)	0.339	-3.280	0.004	0.004
PM MA vs. DL/BL	-0.771 (-2.660, -0.387)	-0.030 (-0.187, 0.013)	0.008	-0.409	0.686	0.683
MXTp MA vs. DL/BL	1.090 (0.619, 3.716)	0.111 (0.012, 0.268)	0.035	0.874	0.392	0.392
ZMIO MA vs. DL/BL	-1.465 (-2.089, -0.771)	-0.051 (-0.103, 0.007)	0.467	-4.289	0.000	0.000
ZM MA vs. DL/BL	-1.023 (-2.986, -0.545)	-0.032 (-0.184, 0.022)	0.149	-1.915	0.069	0.069
T MA vs. DL/BL	-0.944 (-2.918, -0.606)	-0.027 (-0.137, 0.042)	0.096	-1.497	0.149	0.151
Pg MA vs. DL/BL	-1.766 (-5.631, -0.943)	-0.077 (-0.382, 0.031)	0.076	-1.310	0.204	0.206

Table 3

Results of reduced major axis regressions (RMA, type II) performed upon phylogenetically independent standardized contrasts of different morphological variables of the skull of 24 species of the subterranean genus *Ctenomys*. Numbers in parentheses represent the 95% confidence interval. BD, bulk density; DL, diastema length; L_o, out-lever arm; L_i, in-lever arm; PA, procumbency angle. Muscle abbreviations as in Fig. 1. Statistics: NS, non-significant; (+), positive allometry; (-) negative allometry and (=), isometry. Bold script indicates significance at $P < 0.05$.

Standardized contrasts	Allometric coefficient <i>b</i>	Intercept	<i>R</i> ²	<i>t</i>	<i>P</i> (uncorr.)	Permutation <i>P</i>	Scaling
L _o vs. BL	1.118 (1.003, 1.194)	0.010 (-0.021, 0.039)	0.959	22.179	4.6987E-16	0.0001	(+)
L _i SM vs. BL	0.947 (0.740, 1.088)	0.038149 (-0.008, 0.083)	0.866	11.654	1.25E-10	0.0001	(=)
L _i PM vs. BL	1.180 (0.831, 1.401)	0.031 (-0.048, 0.102)	0.774	8.490	3.13E-08	0.0001	(=)
L _i MXTp vs. BL	2.521 (1.328, 3.485)	0.211 (-0.087, 0.446)	0.420	3.903	8.18E-04	0.0008	(+)
L _i ZMIO vs. BL	0.910 (0.642, 1.139)	0.049 (-0.023, 0.108)	0.703	7.048	5.90E-07	0.0001	(=)
L _i ZM vs. BL	1.060 (0.752, 1.293)	0.048 (-0.058, 0.139)	0.572	5.300	2.96E-05	0.0002	(=)
L _i T vs. BL	1.988 (1.535, 2.548)	0.044 (-0.151, 0.269)	0.447	4.123	4.84E-04	0.0009	(+)
L _i Pg vs. BL	1.039 (0.599, 1.411)	-0.006 (-0.136, 0.125)	0.244	2.602	1.66E-02	0.0158	(=)
PA vs. BL	-0.299 (-1.022, -0.223)	-0.049 (-0.097, 0.006)	4.14E-05	-0.029	9.77E-01	0.9770	N.S.
DL vs. BL	1.681 (1.209, 2.023)	-0.117 (-0.252, -0.010)	0.717	7.291	3.53E-07	0.0001	(+)
L _o vs. DL	1.5038 (1.142, 1.794)	-0.133 (-0.246, -0.022)	0.735	7.628	1.75E-07	0.0001	(+)
PA vs. L _o	0.834 (0.483, 2.725)	16.908 (7.549, 57.972)	0.025	0.741	4.66E-01	0.4688	N.S.
PA vs. DL	0.178 (0.120, 0.600)	-0.036 (-0.078, 0.051)	0.003	0.265	7.93E-01	0.7960	N.S.
L _i SM vs. L _o	0.847 (0.713, 0.954)	0.030 (-0.010, 0.067)	0.911	14.653	1.69E-12	0.0001	(=)
L _i PM vs. L _o	1.056 (0.830, 1.222)	0.021 (-0.044, 0.083)	0.837	10.392	9.84E-10	0.0001	(=)
L _i MXTp vs. L _o	2.255 (1.075, 3.15)	0.188 (-0.128, 0.450)	0.378	3.574	1.79E-03	0.0018	(+)
L _i ZMIO vs. L _o	0.814 (0.629, 1.005)	0.040 (-0.024, 0.091)	0.765	8.275	4.77E-08	0.0001	(=)
L _i ZM vs. L _o	0.948 (0.729, 1.144)	0.039 (-0.048, 0.118)	0.647	6.207	3.71E-06	0.0001	(=)
L _i T vs. L _o	1.779 (1.348, 2.265)	0.0266 (-0.164, 0.252)	0.498	4.570	1.67E-04	0.0001	(+)
L _i Pg vs. L _o	0.929 (0.560, 1.272)	-0.015 (-0.155, 0.117)	0.250	2.650	1.50E-02	0.0145	(=)
BD vs. PA	-0.046 (-0.152, -0.020)	-13.422 (-26.868, -0.652)	0.000	-0.10	9.22E-01	0.9200	N.S.

arms (MXTp and T) scaled with positive allometry with the basilar length (Table 3; Fig. 3A, D, G). Procumbency angle ranged from 92.5° (*C. mendocinus*, $N = 1$) to 107.2 ± 4.55° (*C. occultus*, $N = 6$), and *C. leucodon* was the most proodont species (120.1 ± 2.55°; $N = 2$). This variable was not significantly related to body size (Table 3; Fig. 3I) and its variation across the genus is illustrated in Fig. 4.

An isometric relationship was found when the out-lever arm (L_o) was regressed against the in-lever arm (L_i) of five of the seven jaw adductor muscles (SM, PM, ZMIO, ZM and Pg; Table 3; Fig. 5A, B, D, E, G), while the in-lever arm of the two remaining muscles (MXTp and T)

scaled with positive allometry (Table 3; Fig. 5C, F).

A non-significant relationship was found when procumbency was regressed against diastema length (Table 3; Fig. 6A). Furthermore, when diastema length was regressed against basilar length or the out-lever arm, the data showed an overall positive allometry (Table 3; Fig. 6B, C).

The relationship between procumbency angle and bulk density was found to be non-significant relationship, i.e. *Ctenomys* species can be found in soft or harder soils, regardless of whether they do or do not have procumbent upper incisors (Table 3).

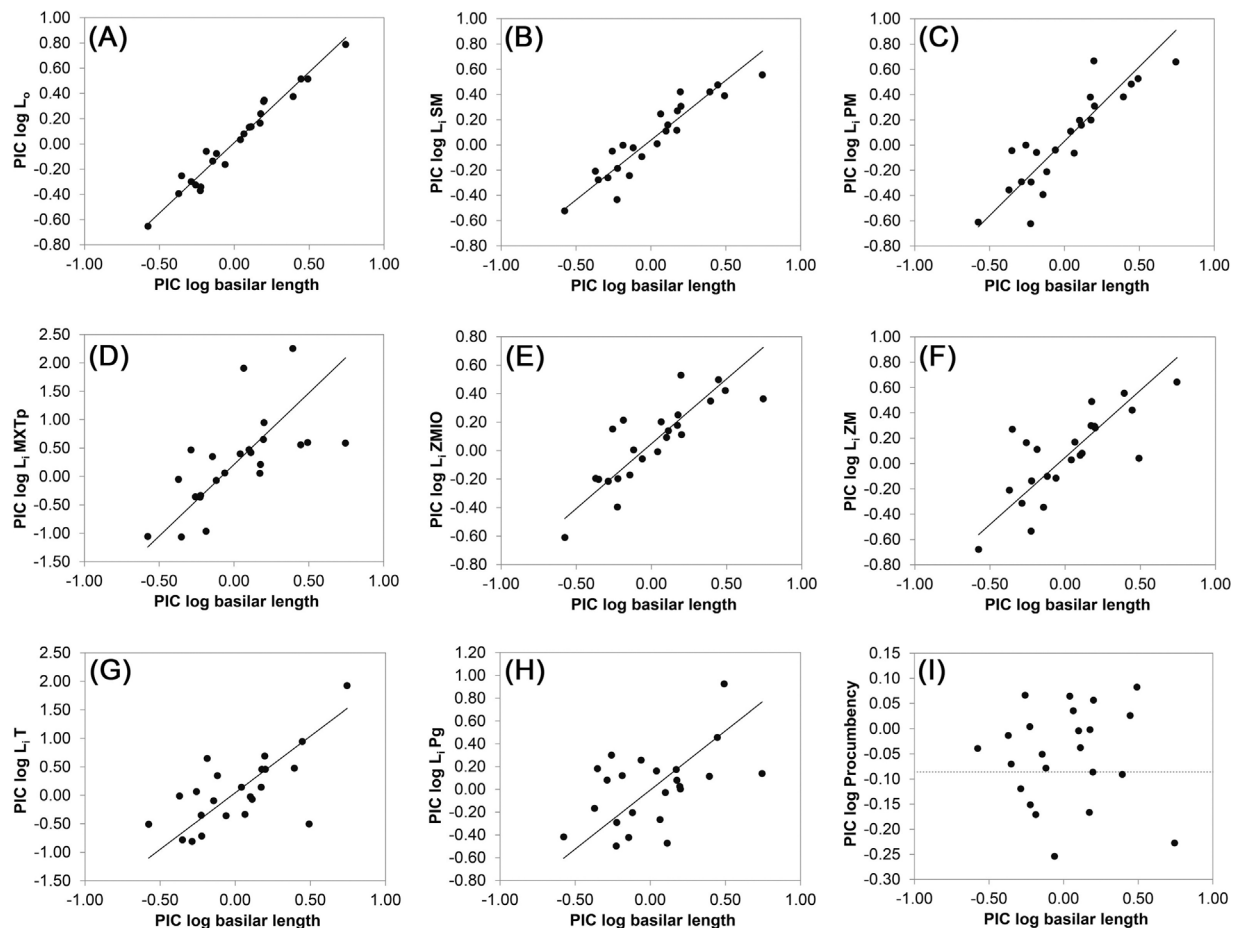


Fig. 3. Scatter-plots of RMA regression analyses performed on phylogenetically independent standardized contrasts (PICs) of (A) the out-lever arm (L_0); (B–H) the in-lever arms (L_i) of the different jaw adductor muscles; (I) the procumbency angle against the basilar length (a skull proxy for body size) in adult individuals of *Ctenomys* species. Black dots represent the mean value for each species. Muscle abbreviations as in Fig. 1.

3.3. Comparative methods

3.3.1. Phylogenetic signal

The result of Blomberg's K -statistic showed, in general, a low but significant phylogenetic signal in most – 14 out of 19 – of the studied morphological variables (Table 4), which implies a substantial variation among species within *Ctenomys* subclades. The mandibular width, the mechanical advantage of the infraorbital zygomaticomandibularis (ZMIO), and the procumbency angle showed the highest K -statistic values (Table 4).

3.3.2. Character color maps

Fig. 7 illustrates the variation in the morphological and soil variables analyzed in the present study. The ancestral state reconstruction of procumbency angle shows that the *Ctenomys*' common ancestor likely had a relatively similar value to that observed in the clade composed by *C. roigi*, *C. perrensi* and *C. dorbignyi*. *C. leucodon* showed an outlier value, being the more procumbent species. In general terms, it can be observed that the sampled species show a relatively narrower mandibular width than their common ancestor, except for *C. boliviensis*. The bulk density color map shows that some species inhabit harder (i.e., more compact) soils than the common ancestor. This is the case in species such as *C. tuconax*, *C. opimus*, *C. occultus*, *C. roigi*, *C. rionegrensis* and *C. flamarioni*. *C. sociabilis* is a species that inhabits less compact soil.

4. Discussion

Previous studies suggested that not only the bite force output –

which varies interspecifically in *Ctenomys* (e.g., Borges et al., 2016) – but also the upper incisor procumbency would be key factors for successful chisel-tooth digging, especially when ctenomyids are confronted with harder soil conditions (Lessa, 1990; Lessa and Stein, 1992; Vassallo, 1998; Fernández et al., 2000; Becerra et al., 2012). As previously observed by Mora et al. (2003), we found that procumbency is a variable trait and is not influenced by size, yet it displays significant phylogenetic signal. Thus, it is possible to observe both small and large species possessing either high or low procumbency angles, a pattern previously detected in other subterranean taxa, such as Geomyidae (Lessa and Patton, 1989).

Furthermore, concerning our main objective, we found that the mechanical advantages of the jaw adductor muscles are not significantly related to upper incisor procumbency. Hence, it is possible to observe species differing significantly in procumbency but showing a similar mechanical advantage for a particular muscle. This result is relevant since it implies that the possession of procumbent incisors would not diminish the external forces applied during the incisal bite. As mentioned in Section 1, procumbency may convey several functional advantages in the context of chisel-tooth diggers. For example, besides favoring the protection of facial soft tissues by distance (Van der Merwe and Botha, 1998) or improving the angle of attack with regard to the substrate (Korth and Rybczynski, 2003), it may contribute to incisor sharpness (see Druzinsky, 2015), which facilitates substrate penetration. Furthermore, procumbency may entail longer lower jaws, and hence, an increase in gape (McIntosh and Cox, 2016; and references therein) which may facilitate biting off bigger lumps of hard soil or fibrous roots.

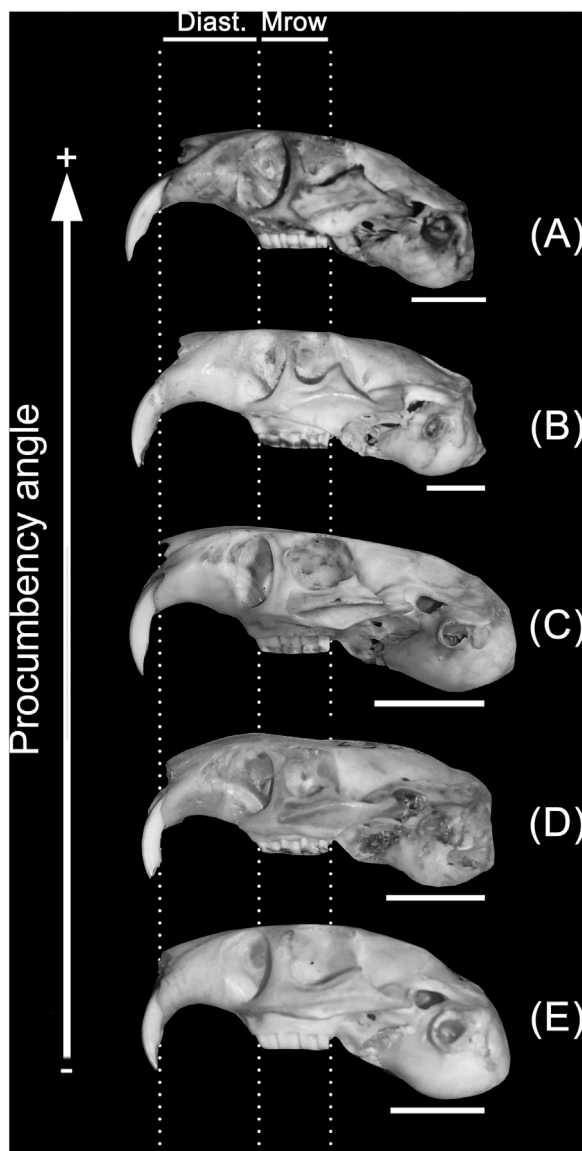


Fig. 4. *Ctenomys* skulls in lateral view illustrating the variability in upper incisor procumbency. (A) *C. leucodon*, (B) *C. tuconax*, (C) *C. talarum*, (D) *C. tucumanus*, (E) *C. opimus*. Abbreviations: Diast., diastema length; Mrow, molariform teeth row length. Scale bars = 1 cm.

As to the role of the mandibular width, we found that it is not related to the mechanical advantage of any jaw adductor muscle. This is understandable since, except for the deep masseter which inserts on the masseteric crest, there is no anatomical or biomechanical relationship between the adductor muscles and this structure. On the other hand, we found that species with shorter rostrum possess higher mechanical advantages for two important muscles (SM, superperforator; ZMIO, infraorbital zygomaticomandibularis). This is in line with Lessa (1990), who discussed that an improvement in the incisor bite force might be achieved by a shortening of the out-lever arm, an elongation of the in-lever arm, an increase of the effective muscle force, or a combination of them all. A shortening of the diastema length – relative to the basilar length – allows a more anterior displacement of the molar series, and consequently, the muscle insertions and their action vectors. This would result in a maximization of the in-lever arms and, therefore, the mechanical advantages.

As stated above, procumbency has been classically related to chisel-tooth digging, especially in harder soil conditions. However, and disagreeing with what was previously suggested in studies such as those by

Vassallo (1998) or Mora et al. (2003), we found that procumbency is not related to bulk density (the proxy for soil hardness used in this study) across the wide range of soil types inhabited by the genus. For instance, the most proodont species (*C. leucodon*, the white-toothed tuco-tuco) inhabits friable soils, while a species that displays a $\sim 20^\circ$ lower procumbency angle (*C. talarum*) inhabits either sandy and friable or hard and clayey soils (see Table 1 for references). The main difference between our results and previous ones may be due to the fact that we worked with a larger sample, recorded the soil compactness in specific localities – and not just a mean value for the whole distribution range in which different populations occur –, and statistically analyzed our data.

Since the upper incisors of rodents are enclosed within the maxillary portion of the rostrum – describing a smooth helicoidal arc as a result of continuous growth – Lessa (1990) advanced the hypothesis that incisor procumbency must increase in relation with rostral allometric growth (a relatively longer rostrum leads to a more flattened incisor, producing a higher procumbency; see Fig. 1D in Lessa, 1990). Accordingly, Mora et al. (2003) observed a positive allometric relationship between diastema length (a proxy for rostral size in *Ctenomys*) and procumbency. However, once the data was standardized by phylogenetic independent contrasts, we found a non-significant relationship between these variables. This suggests that, in *Ctenomys* species, a relatively longer rostrum might not necessarily lead to higher incisor procumbency. For example, within our sample, *C. haigi* and *C. dorbignyi* have relatively long and short rostra, respectively, but they show highly similar procumbency values (PA: 99° and 101.5° , respectively).

Another possible factor for the variation observed in the angle at which incisors protrude is the incisors' root position. For instance, Van der Merwe and Botha (1998) suggested that a posterior displacement of this root – in chisel-tooth digging rodents – allows an increased procumbency. This is the case in the chisel-tooth digging African mole-rats, which are characterized by short rostra, but also very procumbent upper incisors rooted behind the last molars (see McIntosh and Cox, 2016). Within *Ctenomys*, in the most procumbent species (i.e., *C. leucodon*) the incisor root reaches the first molar, unlike in most others in which it solely reaches the premolar (A.I.E., personal observation). This posterior shift of the incisors' root would not have been the only feature enhancing the evolution of procumbency in ctenomyids and octodontids, but the outward displacement of the incisors' bony capsule lateral to the molar row (Lessa et al., 2008) as well.

Becerra et al. (2014) concluded that the SM, PM, ZMIO and ZM, when pulling in concert, account for over 90% of the bite force due to their high mechanical advantage and/or massive development. Interestingly, our results showed that the in-lever arms for those muscles – which are mainly overlapping the molar series region – scaled isometrically with both the basilar length and the out-lever arm, implying that this “middle” region would be highly conserved within the genus. Since in mammals the pivot is located right behind the molar tooth row, the out-lever arm of the jaw adductor musculature would be mainly associated with the length of the most anterior region of the basilar length (i.e., diastema + molar series; Fig. 4). Therefore, it is logical to deduce that the positive allometry exhibited by the out-lever arm in relation to basilar length would be a reflection of the greater variation observed in the rostral region, as is shown in Table 3. These results partially disagree with previous studies which suggested that changes in skull shape (e.g., in the out-lever arm and/or rostral allometry) are mainly associated with incisor procumbency and the chisel-tooth digging model, which, in turn, has classically been related to soil hardness (e.g., Vassallo, 1998; Mora et al. 2003).

Despite body size differences among species, skull proportions are relatively conserved in *Ctenomys*, suggesting that it would have been defined early in the clade's history. In fact, the out-lever arm and the in-lever arms of the three main contributors to bite force showed a weak but significant phylogenetic signal. This can be explained by high rates of trait evolution leading to large differences among close relatives, and

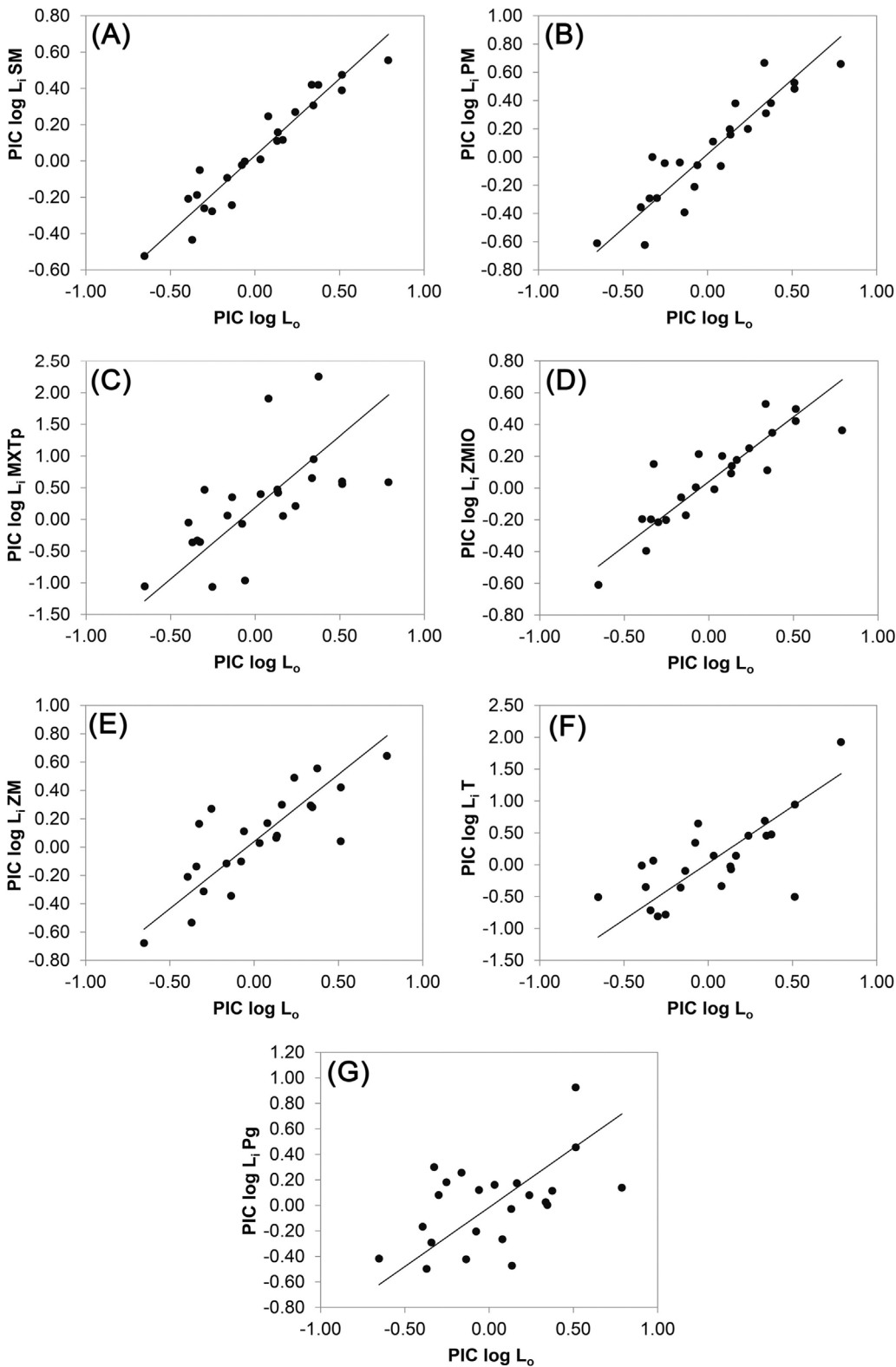


Fig. 5. (A–G) Scatter-plots of RMA regression analyses performed on phylogenetically independent standardized contrasts (PICs) of the different jaw adductor muscles' in-lever arms (L_i) against the out-lever arm (L_o). Black dots represent the mean values for each species. Muscle abbreviations as in Fig. 1.

it may be related to the maintenance of a key function: the exertion of the high forces required to effectively perform both chisel-tooth digging and foraging (Mora et al., 2003). In the genus *Ctenomys*, speciation is considered to have happened rapidly since its appearance in the late Pliocene (Reguero et al., 2007; Verzi et al. 2010), achieving its current diversity during the Pleistocene (~60 recognized living species; Parada et al., 2011 and references therein). Adaptive radiations are expected to

be characterized by a weak phylogenetic signal (Kamilar and Cooper, 2013) such as that observed in the procumbency angle or mandibular width, and this may be related to the fact that closely related species have diversified into different niches (e.g., soils differing in bulk density, see Fig. 7).

In general terms, ctenomyids present a relatively conserved cranial *bauplan*, with both rostral length and incisor procumbency (i.e., the

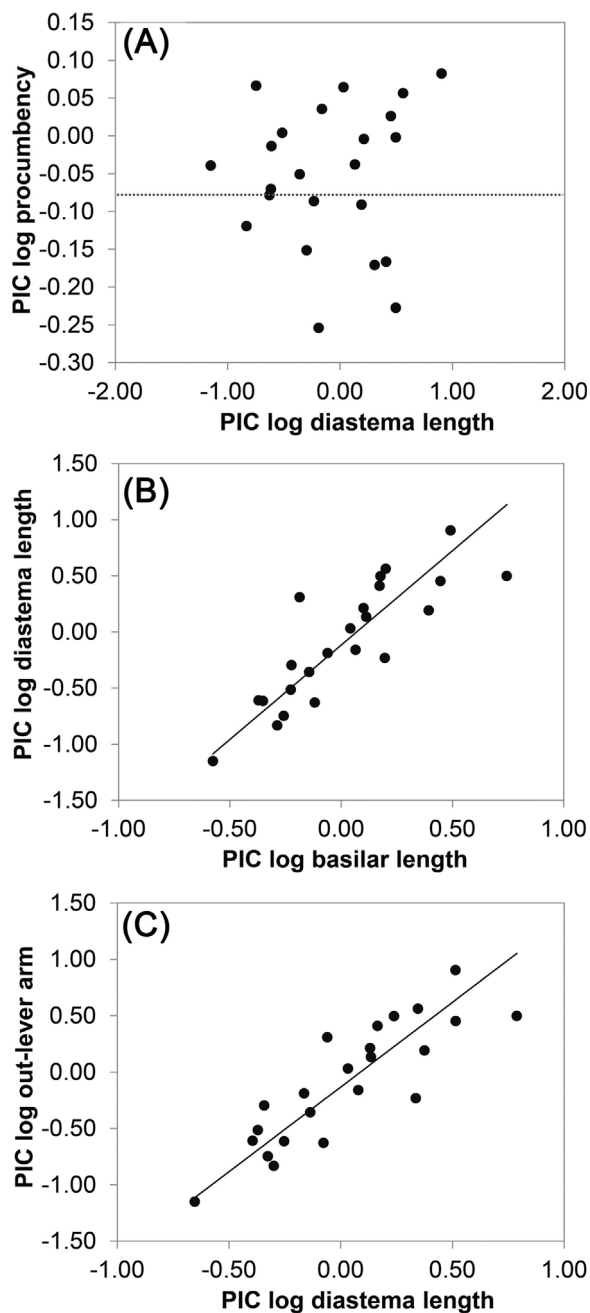


Fig. 6. (A–C) Scatter-plots of RMA regression analyses performed on phylogenetically independent standardized contrasts (PICs) of different biomechanical variables against basilar length (a skull proxy for body size) and diastema length (a skull proxy for rostral size) in *Ctenomys* species. Black dots represent the mean values for each species.

most anterior portion of the skull and classically related to tooth-digging) being the morphologically most variable traits. Changes in this section would imply less interference with other biological systems and functions than modifications in the other sections more backwards in the skull. Thus, the functional independence of gnawing and chewing would have developed into an also independent evolution of the anterior (i.e., where the incisors are embedded) and “middle” (i.e., where the molars are) regions of the skull, in response to different environmental conditions. On the other hand, the low morphological variation in the middle and posterior sections of the cranium can also be interpreted from an evolutionary point of view. The development of subterranean habits associated with more arid and open environments would have constrained these characters early in the evolution of the family Ctenomyidae. Furthermore, the *bauplan* achieved by ctenomyids

Table 4

Phylogenetic signal in *Ctenomys* traits using *K*. BL, basilar length; DL, diastema length; L_o , out-lever arm; L_i , in-lever arm; MA, mechanical advantage; MW, mandibular width; MXTp, posterior extension of the masseter muscle; PA, procumbency angle; Pg, pterygoid; PM, deep masseter; SM, superficial masseter; T, temporalis; ZM, zygomaticomandibularis; ZMIO, infraorbital zygomaticomandibularis. Bold script indicates significance at $P < 0.05$.

Morphological trait	Statistic <i>K</i>	<i>P</i> -value
PA	0.35	0.001
BL	0.21	0.024
MW	0.47	0.001
DL	0.22	0.040
L_o	0.26	0.016
L_i SM	0.26	0.012
L_i PM	0.27	0.005
L_i MXTp	0.25	0.016
L_i ZMIO	0.23	0.023
L_i ZM	0.21	0.054
L_i T	0.22	0.054
L_i Pg	0.19	0.072
MA SM	0.30	0.007
MA PM	0.21	0.053
MA MXTp	0.21	0.036
MA ZMIO	0.40	0.001
MA ZM	0.26	0.018
MA T	0.15	0.267
MA Pg	0.22	0.033

would be so robust and powerful that it would not have required drastic changes to adapt to the different soil conditions, varying only in the length of the diastema and the incisor procumbency. Finally, even though the mechanics and shape-related differences were only significant in the anterior region of the cranium, it should also be taken into account that the body size differences seen within this genus would involve differences in bone thickness and overall skull robustness, which may ultimately affect their resistance to mechanical loadings during burrow digging.

In sum, although possessing procumbent incisors would represent a functional advantage in many aspects related to chisel-tooth digging or food processing, our results suggest that, within *Ctenomys*, it might not affect the leverage of jaw adductor muscles. Further studies are required on the digging performance of different species and populations in their natural habitats, taking into account not only the cranial and jaw morphology, but also habitat characteristics such as physical properties of the soil (e.g., hardness, bulk density, humidity, texture) and vegetation (e.g., plant biomass, dietary items or hardness and shape of underground plant obstacles) measured *in situ*. These studies would provide essential information to better understand the role of incisor procumbency during chisel-tooth digging or primary food processing in ctenomyids.

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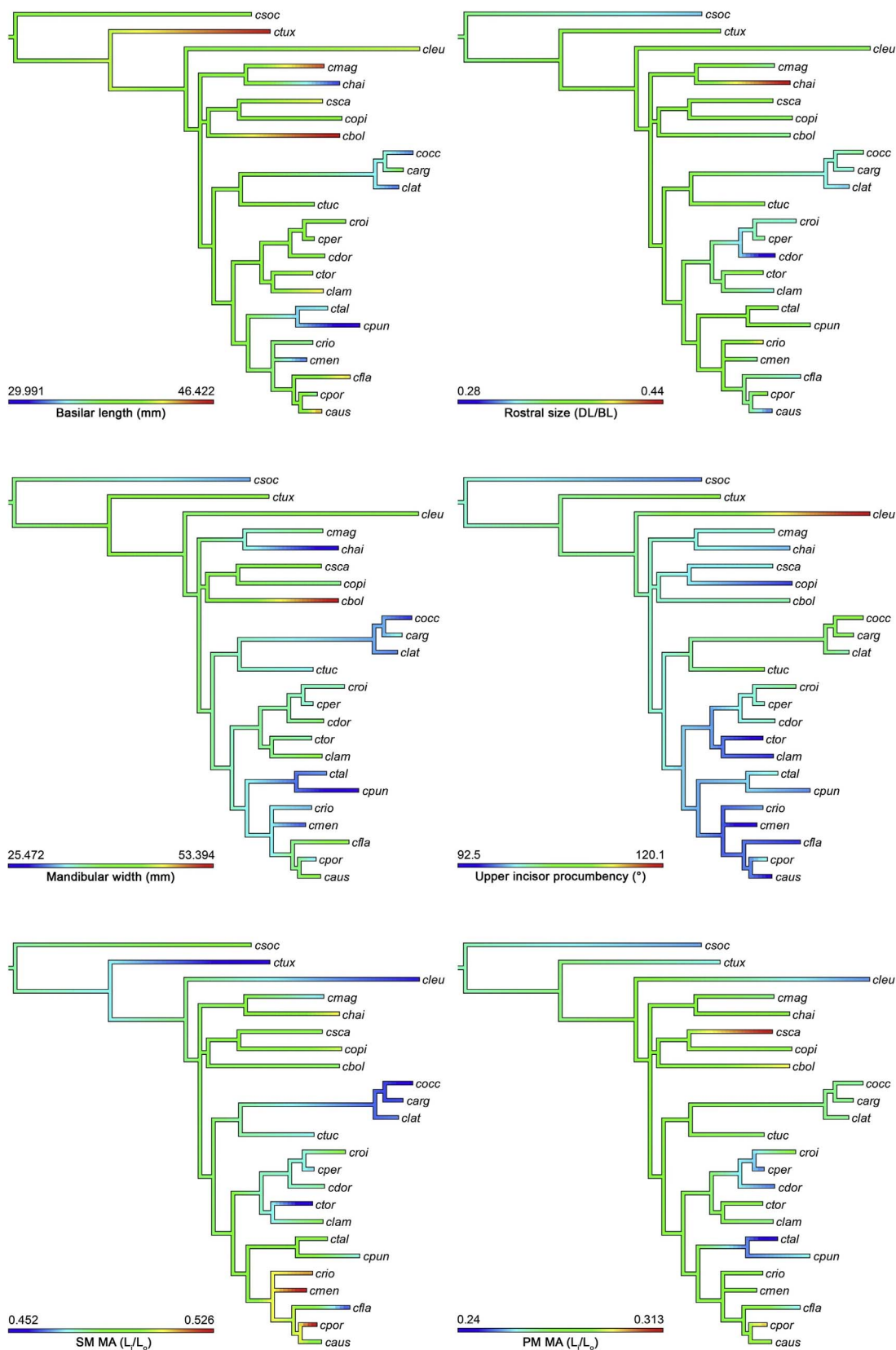


Fig. 7. Evolution and diversification (colored character maps) of different variables analyzed in 24 *Ctenomys* species in this study: carg, *C. argentinus*; caus, *C. australis*; cbol, *C. boliviensis*; cdor, *C. dorbignyi*; cfla, *C. flamarioni*; chai, *C. haigi*; clam, *C. lami*; clat, *C. latro*; cleu, *C. leucodon*; cmag, *C. magellanicus*; cmen, *C. mendocinus*; cocc, *C. occultus*; copi, *C. opimus*; cper, *C. perrensi*; cpor, *C. porteousi*; cpun, *C. pundi*; crio, *C. rionegrensis*; croi, *C. roigi*; csca, *C. scagliai*; csoc, *C. sociabilis*; ctal, *C. talarum*; ctor, *C. torquatus*; ctux, *C. tuconax*; ctuc, *C. tucumanus*. Abbreviations: MA, mechanical advantage; MXTp, posterior extension of the masseter; Pg, pterygoid; PM, deep masseter; SM, superficial masseter; T, temporalis; ZM, zygomaticomandibularis; ZMIO, infraorbital zygomaticomandibularis.

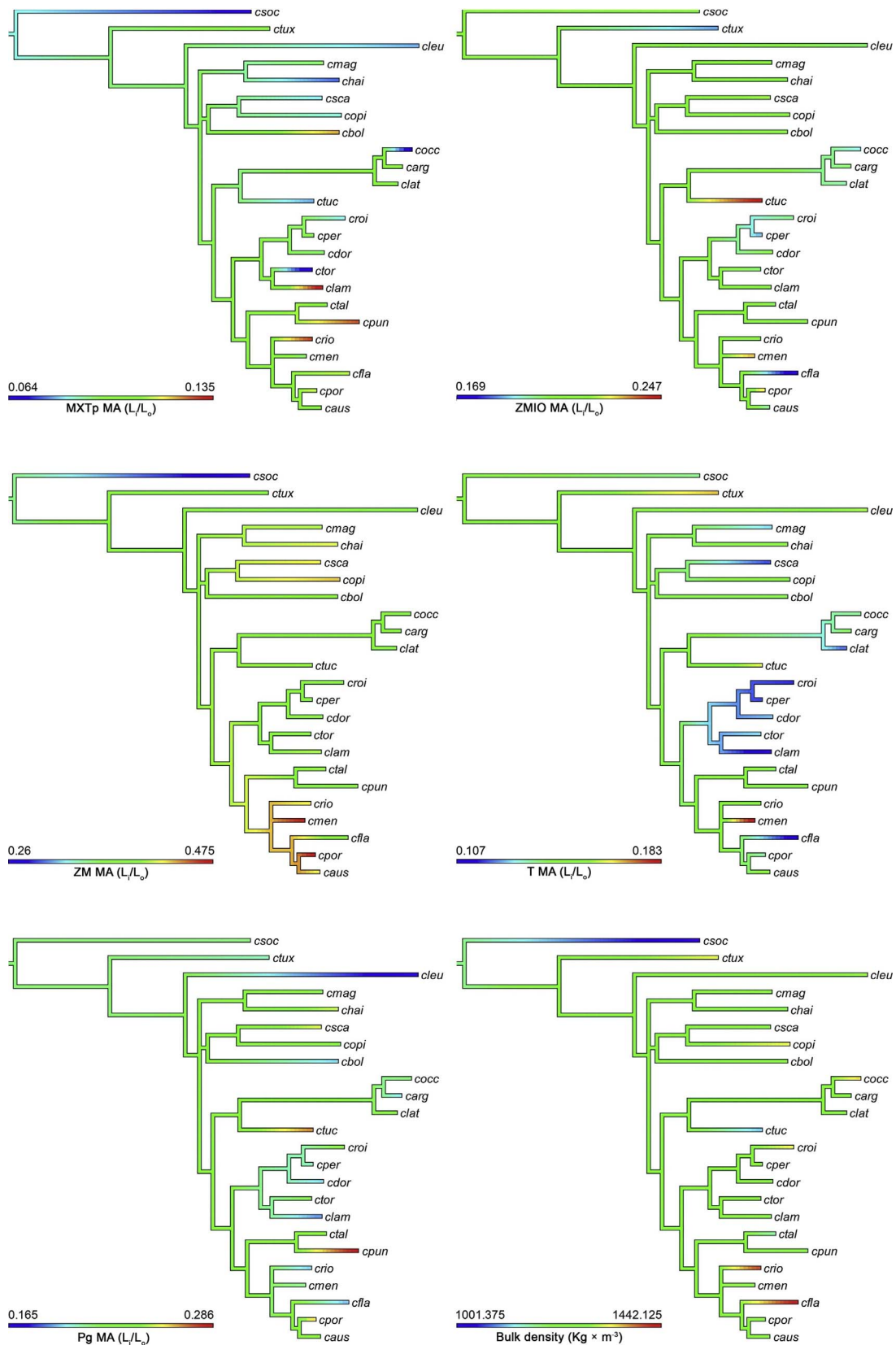


Fig. 7. (continued)

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.zool.2017.08.003>.

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