

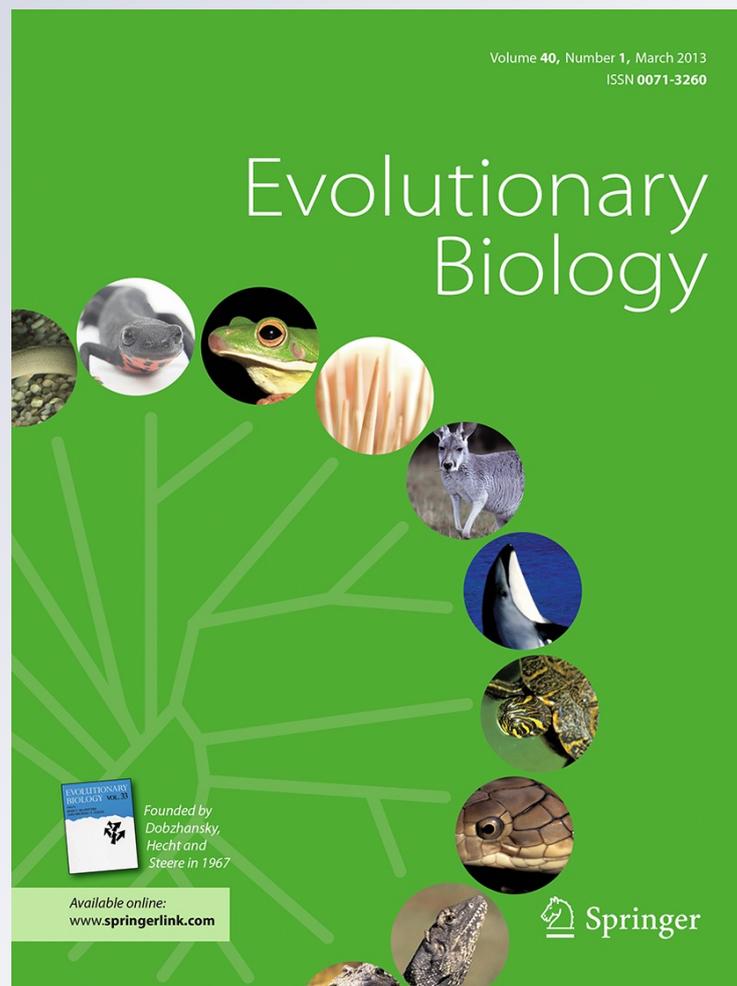
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Two- Versus Three-Dimensional Morphometric Approaches in Macroevolution: Insight from the Mandible of Caviomorph Rodents

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Abstract Morphological variation is one of the most studied dimensions of evolutionary biology, given its close relationship with the ecological aspects of biological diversification. In this work we examine the differences between the use of two- and three-dimensional morphometric techniques for the analysis of macroevolutionary patterns of morphological variation in the mandible of the South American caviomorph rodents, which displays varying degrees of hystricognathy. The variation patterns were similar for 2D and 3D coordinate datasets. However, phylogenetic comparative statistical analyses showed widely different results for mandible variation. Both the phylogenetic signal values and the results of phylogenetic regressions were markedly different between the two macroevolutionary mandible datasets. This suggests that the inclusion of the third coordinate to characterize a hystricognathous mandible may lead to completely different interpretations concerning the processes that could be regulating the morphological diversification of the caviomorph mandible. We also compare these results with the 2D and 3D macroevolutionary patterns of variation in

cranial shape and the intra-specific mandible variation. The variation patterns were similar for 2D and 3D coordinate for all these comparative datasets. The differing results obtained at different evolutionary scales, give strength to the statement about careful selection of data not only in studies of morphological variation in caviomorphs but also for other groups of organisms.

Keywords Caviomorph rodents · Evolutionary diversification · Geometric morphometrics · Mandible morphology · Shape dimensionality

Introduction

Achieving an understanding of the processes involved in the biological diversification of the various groups of organisms is one of the main goals of evolutionary biology (Schluter 2000; Gavrilets and Losos 2009). In this context, morphology is one of the most studied dimensions, given its close relationship with ecological dimensions in diversification processes (Wainwright 2007; Gavrilets and Losos 2009; Perez et al. 2009). In this sense, the study of patterns of morphological variation provides an approach toward the understanding of the factors responsible for organismic diversification, be it under a structural, adaptive or historical perspective. One of the morphological structures that has been most analyzed in such studies is the mandible of mammal groups (e.g., Cardini 2003; Klingenberg et al. 2003; Cheverud 2004; Perez et al. 2009; Álvarez et al. 2011). Because of the characteristics of the mammalian mandible and its strong link with diverse ecological roles, such as food prehension and processing, as well as digging in species that have evolved fossorial habits, it provides an

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excellent model to study evolutionary diversification processes.

The great majority of recent studies on mandibular morphology and the factors affecting or originating its variation at macroevolutionary level have been performed using geometric morphometric techniques (Cardini 2003; Cardini and O'Higgins 2005; Barrow and MacLeod 2008; Meloro et al. 2008; Mancina and Balseiro 2010; Milenković et al. 2010; Álvarez et al. 2011; Hautier et al. 2011; Meloro and O'Higgins 2011). These works employed mostly two-dimensional data, i.e., x , y landmark coordinates. Such procedures may be used confidently when the mandible is relatively flat, as in most mammalian groups. However, in a few groups of mammals including the South American members of the rodent infraorder Hystricognathi (caviomorphs), the mandible displays a hystricognathous condition, more or less marked in the different lineages of the clade (Vassallo and Verzi 2001; Hautier et al. 2011).

The existence of a mammal group with variable degree of hystricognathy allows an evaluation of the differences in the application of two- and three-dimensional geometric morphometric techniques. In particular, it makes it possible to assess if a considerable amount of morpho-functional and/or phylogenetic information might be lost when analyses fail to incorporate the third dimension of morphological change in the mandible of caviomorph rodents. In this sense, the goal of this work is to analyze and compare the amount of information contributed by both bi- and three-dimensional geometric datasets of caviomorphs. The sample included several species with diverse degree of hystricognathy and bi- and three-dimensional geometric morphometric techniques were applied to represent mandibular morphology. In this context, and with comparative purposes, we also analyzed 2D and 3D datasets for the cranium, and studied the shape variation within samples of two morphologically divergent caviomorph genera, *Cavia* and *Ctenomys* (Vassallo and Verzi 2001; Hautier et al. 2011). On the basis of previous studies (Cardini and Thorington 2006; Álvarez 2012; Álvarez et al. 2011) the expected results are: (a) the pattern of morphological differentiation among phylogenetic clades will be similar whether two- or three-dimensional data are employed and (b) similar associations between these data and certain variables relevant for the evolution of the group. In order to test these expectations we estimated the phylogenetic relationships between caviomorph species based on mitochondrial and nuclear DNA sequences (Lemey et al. 2009), quantified mandible size and shape variation using geometric morphometric techniques (Adams et al. 2004), and measured the associations of mandible shape with phylogenetic relationships and size using ordinary and phylogenetic comparative techniques (Rohlf 2001; Blomberg et al. 2003; Klingenberg and Gidaszewski 2010).

Materials and Methods

Sample

We analyzed 200 mandibles representing 24 genera and seven families (Table 1; Online Resource 1). The specimens included in this study were adults defined by the presence of a functional third molar. Males and females were pooled for the analyses. Specimens are housed in the mammalogical collections of Museo de La Plata (La Plata, Argentina), Museo Argentino de Ciencias Naturales “Bernardino Rivadavia” (Buenos Aires, Argentina), Museo Municipal de Ciencias Naturales de Mar del Plata “Lorenzo Scaglia” (Mar del Plata, Argentina), and Instituto Argentino de Investigaciones de las Zonas Áridas (Mendoza, Argentina).

Phylogenetic Inference

Phylogenetic relationships among caviomorph genera were studied through Bayesian Inference methods. Sequences from the growth hormone receptor (GHR; 823 bp), transthyretin hormone (TTH; 987 bp) and mitochondrial subunit 12S (12S; 949 bp) genes were obtained from Genbank (for accession numbers, see Online Resource 2). When a sequence was not available for the species used to obtain morphometric data, the sequence for a cogenetic species was used. jModelTest 0.1 (Posada 2008) was employed to determine the most appropriate model of sequence

Table 1 Studied caviomorph taxa and number of specimens examined for each genera (N)

Taxa	N	Taxa	N
Cavioidea		Octodontoidea	
Caviidae		Abrocomidae	
<i>Cavia</i>	9	<i>Abrocoma</i>	6
<i>Galea</i>	9	Echimyidae	
<i>Microcavia</i>	11	<i>Myocastor</i>	9
<i>Dolichotis</i>	12	<i>Proechimys</i>	3
<i>Pediolagus</i>	4	<i>Thrichomys</i>	6
<i>Hydrochoerus</i>	9	Octodontidae	
<i>Kerodon</i>	4	<i>Aconaemys</i>	6
Dasyproctidae		<i>Ctenomys</i>	10
<i>Dasyprocta</i>	16	<i>Octodon</i>	8
Cuniculidae		<i>Octodontomys</i>	11
<i>Cuniculus</i>	8	<i>Octomys</i>	7
Chinchilloidea		<i>Pipanacoctomys</i>	11
Chinchillidae		<i>Spalacopus</i>	4
<i>Chinchilla</i>	5	<i>Tympanoctomys</i>	12
<i>Lagidium</i>	10		
<i>Lagostomus</i>	10		

evolution for each analyzed gene. The best fit model for the genes GHR, TTH and 12S was GTR + G. The Bayesian Inference method was implemented using MrBayes 3.1.2. (Ronquist and Huelsenbeck 2003). Two simultaneous analyses were performed using the algorithm MCMC (*Markov chain Monte Carlo*) with 1,000,000 generations. Sampling frequency was 100 and burnin was set at 25 %.

Mandible Two- and Three-Dimensional Coordinates

Mandible shape (Fig. 1) was captured as three-dimensional coordinates. The three-dimensional coordinates (3D) of thirteen landmarks were obtained with a Microscribe G2X (Immersion Corp., San Jose, California, USA). We also represented mandible morphology through two-dimensional coordinates (2D) of the same landmarks. These were recorded on digital images of mandibles in lateral view using the software tpsDig 2.12 (Rohlf 2008).

To estimate the precision of landmark registering, intra-observer error associated with the placement of 2D and 3D landmarks coordinates was evaluated using a sample of the studied mandibles. The results obtained using a Procrustes analysis (see below) showed low levels of error in the placement of landmarks in 2D and 3D (PROTEST analysis: $m_{12} = 0.923$, $P = 0.001$ and $m_{12} = 0.907$, $P = 0.001$, respectively).

Morphometric Analyses

For both 3D and 2D datasets, raw coordinates were aligned using Generalized Procrustes analyses (Rohlf and Slice 1990; Mitteroecker and Gunz 2009). The mean shape for each caviomorph genus was computed by averaging the aligned coordinates (Procrustes shape coordinates). We

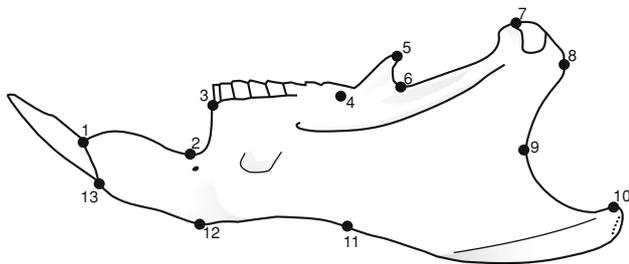


Fig. 1 Landmarks used in this study to represent mandible configuration. 1 antero-dorsal border of incisor alveolus, 2 extreme of diastema invagination, 3 anterior end of mandibular toothrow, 4 anterior end of base of coronoid process, 5 tip of coronoid process, 6 maximum curvature of incisura mandibulae, 7 anterior edge of condylar process, 8 posterior-most edge of postcondyloid process, 9 maximum curvature of curve between postcondyloid process and angular process, 10 tip of angular process, 11 dorsal-most point on ventral border of mandibular corpus, 12 posterior extremity of mandibular symphysis, 13 antero-ventral border of incisor alveolus

performed principal component analyses (i.e., relative warp analyses, RWA) in order to describe the major trends of morphological variation in a reduced dimensional space. Shape changes were visualized by means of differences in outline diagrams. Morphometric analyses were performed using MorphoJ (Klingenberg 2010).

Statistical Analyses

The resulting morphometric ordinations obtained for each dataset were compared using Procrustes analysis (PROTEST; Gower 1971; Peres-Neto and Jackson 2001). This procedure allows analysis of the degree of similarity between the arrangements of genera in the respective morphospaces obtained from different data types (RWs that explained 90 % of total variation). Significance was assessed via permutation tests with 10,000 permutations. This analysis was carried out using the vegan package (Oksanen et al. 2010) for R (ver. R 2.11.1.; R Development Core Team 2011).

The phylogenetic signal of mandibular shape variation was assessed through the univariate K statistic proposed by Blomberg et al. (2003), and the multivariate *tree length* measure proposed by Laurin (2004) and extended for geometric morphometric techniques by Klingenberg and Gidaszewski (2010). The K statistic was calculated for the first RW which accounted for a large amount of shape variation. *Tree length* was used to measure the phylogenetic signal of the entire shape variation. The presence of phylogenetic structure was evaluated using a permutation test that randomizes the observed mean shapes (for each genus in this case) among the tips of a phylogeny describing intergeneric relationships. Squared changes along tree branches (their sum represents the *tree length*) are computed for observed data and for each permutation step. The P value for this test represents the proportion of permutations that results in a tree length equal or less than the observed with the original data (Figueirido et al. 2010; Klingenberg and Gidaszewski 2010). Statistical analyses were performed using the picante package for R (Kembel et al. 2010) and MorphoJ (Klingenberg 2010).

Relationships between mandible shape variation and size were assessed through regression analyses. Size is considered to have important influence on caviomorph mandible shape variation (Álvarez 2012). To taking into account the phylogenetic structure of data in a comparative analysis, we fitted the variables into a Phylogenetic Generalized Least Squares regression model (Martins and Hansen 1997; Rohlf 2001). Shape variation was represented by the first ten relative warps (approximately 90 % of total variation). Size was represented by the ln centroid size of mandible configurations (Online Resource 3). Aside standard multivariate statistics for regression, we reported

the statistic η^2 that represents the percentage of shape variation explained by size

Additional Morphometric Datasets

With comparative purposes, we applied the same approach to other 3D and 2D shape datasets, such as the cranium of caviomorphs. We selected 27 landmarks that were recorded both three- and two-dimensionally. The treatment of data and morphometric and phylogenetic comparative analyses were the same as for the mandible. At the same time, we analyzed 3D vs 2D mandible shape variation within two caviomorph genera, *Ctenomys* and *Cavia*, that represent opposing morphologies regarding mandible flaring and several other features (Vassallo and Verzi 2001; Hautier et al. 2011). We ran morphometric analyses in order to assess the similarities between 3D and 2D ordination of specimens for both genera and the influence of size over shape variation using ordinary regression analysis.

Results

The phylogeny obtained from Bayesian analysis of the three-gene matrix (Fig. 2) was in agreement with partial previous hypotheses (Rowe and Honeycutt 2002;

Honeycutt et al. 2003; Spotorno et al. 2004; Blanga-Kanfi et al. 2009; Upham and Patterson 2012). Likewise, the relationships between the studied genera correspond to previous proposals. The traditionally recognized familial clades within each superfamily were recovered: Octodontidae and Echimyidae within the Octodontoidea, Caviidae, Dasyproctidae and Cuniculidae within Caviioidea, and Chinchillidae (=Chinchilloidea in this case). Posterior probabilities were above 0.9.

The patterns of shape variation observed were similar for the 3D and 2D datasets (Fig. 3). The percentage of variation explained by the first two RWs of each dataset was approximately 60 %. Most of the cavioids were placed on negative values of the RW1, while octodontoids occupied positive values. Chinchilloids were located in intermediate positions. In the case of the cavioids, *Cuniculus* and especially *Dasyprocta* were placed towards more central values of RW1, showing mandibular morphologies similar to those displayed by octodontoids (deeper and shorter corpus; higher coronoid and condylar processes; greater degree of histricognathy). Among the octodontoids, the position of the genus *Myocastor* was drastically altered depending on the dimensionality of the shape data: in the 3D space it was segregated from the other representatives of the superfamily and even from the rest of caviomorphs, and located towards negative values of RW2 because of its

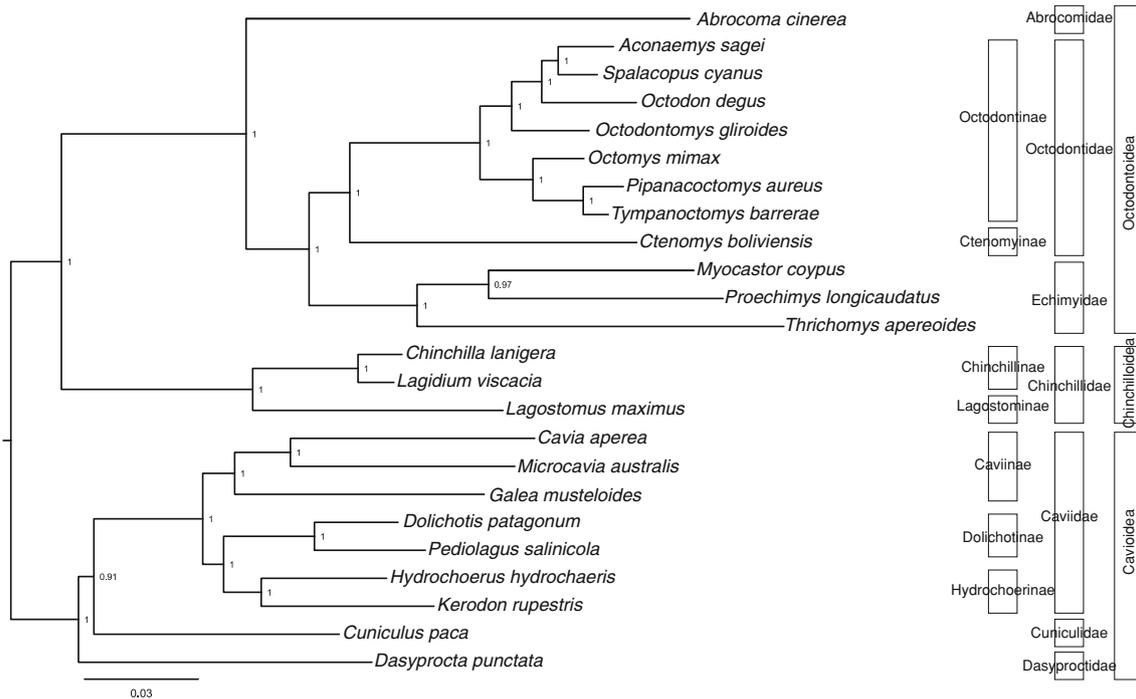


Fig. 2 Phylogenetic tree topology obtained for South American hystricognath (caviomorph) rodents, estimated through Bayesian Inference. Sequences for growth hormone receptor (GHR), transthyretin hormone (TTH) and mitochondrial subunit 12S (12S) genes were

combined. Numbers in nodes represent posterior probabilities support. Suprageneric clades are indicated (subfamilies, families, and superfamilies)

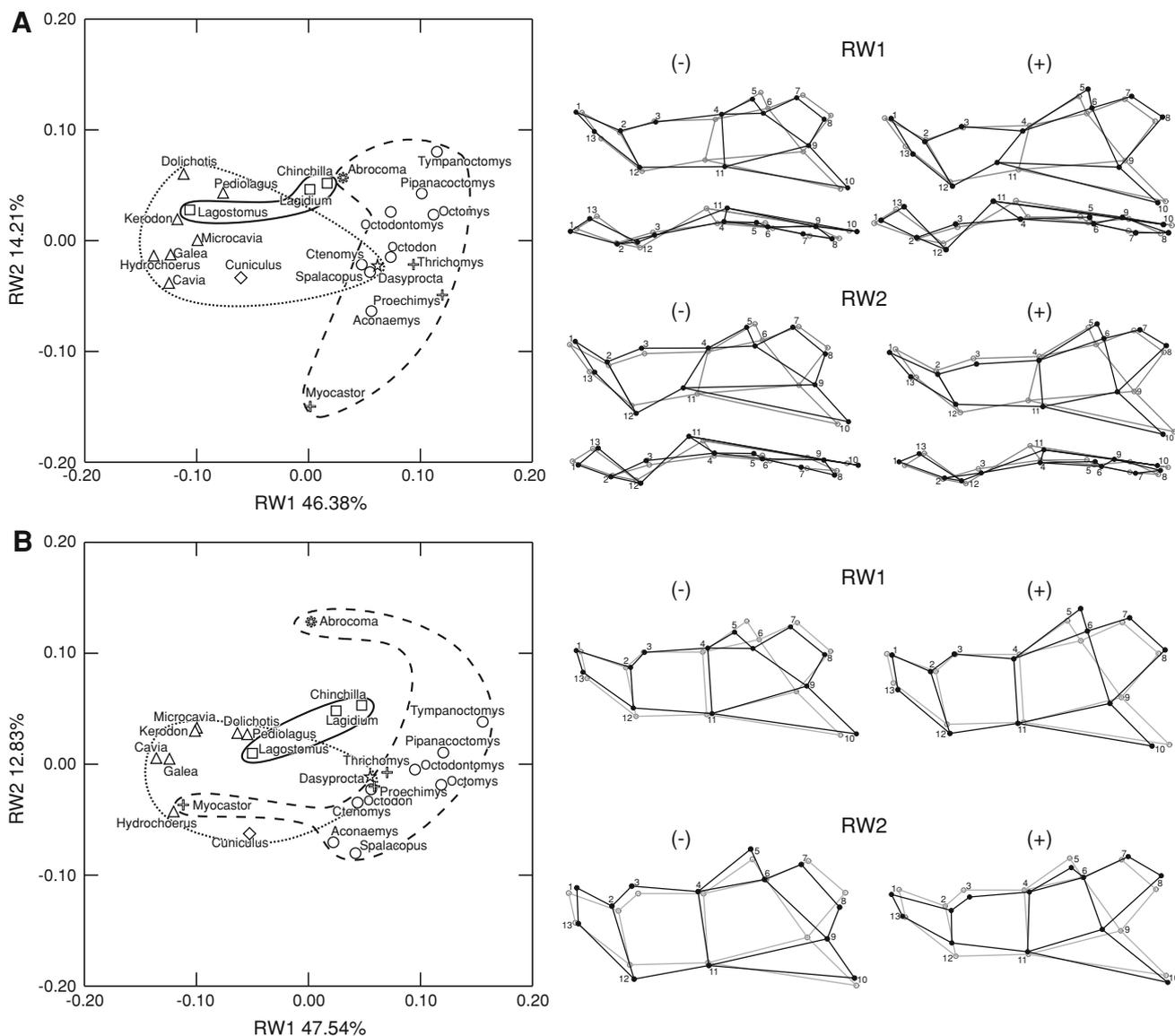


Fig. 3 Ordination of 24 caviomorph genera in the morphospace defined by the first two relative warps (RWs), based on 3D (a) and 2D (b) coordinate datasets. Symbols represent caviomorph families: triangles caviids, diamonds cuniculids, stars dasyproctids, circles octodontids, crosses echimyids, asterisks abrocomids, squares

chinchillids. Superfamilies are indicated by line type: dashed (Octodontoidea), dotted (Caviioidea) and full (Chinchilloidea). Mandible shape changes along the first two relative warps (RW1, RW2), from negative (-) to positive (+) values, are shown as outline diagrams for both 3D and 2D datasets

deeper mandible, with a laterally expanded angular process and highly reduced coronoid process. In contrast, in the 2D space it was close to genus *Hydrochoerus*.

The three-dimensional landmark coordinates allowed us to assess differences in mandible shape that involve flaring of the angular process, a morphological difference that cannot be evaluated in a lateral view of the mandible. The caviomorph genera that occupied positive values of RW1 and negative values of RW2, such as the echimyids, octodontids, and some cavioids, showed more lateralized angular processes. Toward negative values of RW1, where most cavioids occurred, angular processes are less flared laterally and

expanded posteriorly. The use of 3D landmark coordinates resulted in better separation of mandibular morphologies, mostly among the members of Octodontoidea. Despite this, the correlation between both ordinations was high and significant (PROTEST analysis: $m_{12} = 0.908$, $P = 0.001$). Thus, it may be advanced that, together with the shape changes observed in the lateral view of mandible, there exist important latero-medial variations linked to the configuration of the angular process (Fig. 3).

The clear phylogenetic structure observed in both ordinations was supported by the statistical analyses. However, the phylogenetic signal was much higher for the 3D data

(2D data: $K = 1.319$, $P = 0.0001$, *tree length* = 0.149, $P < 0.0001$; 3D data: $K = 2.129$, $P = 0.0001$, *tree length* = 0.164, $P < 0.0001$). 3D mandible shape was highly associated with size variation ($\lambda_{20,26} = 0.125$, $\eta^2 = 0.875$, $F = 2.374$, $P = 0.020$), but the relationship between 2D morphological variation and size was lower and not statistically significant ($\lambda_{20,26} = 0.491$, $\eta^2 = 0.509$, $F = 0.555$, $P = 0.910$).

Analyses within both genera *Cavia* and *Ctenomys* yielded somewhat different results with respect to what was observed for the analyses of mandible shape among the whole sample of caviomorph genera. Firstly, PROTEST analyses resulted in significant, but lower, correlation between 2D and 3D ordinations of specimens of each genus (*Cavia*: $m_{12} = 0.752$, $P < 0.001$; *Ctenomys*: $m_{12} = 0.599$, $P = 0.003$). Ordinary Least Squares regression analyses carried out for *Cavia* 2D and 3D shape datasets (90 % of shape variation) against size resulted moderate and non-significant (2D data: $\lambda_{8,12} = 0.373$, $\eta^2 = 0.627$, $F = 2.516$, $P = 0.073$; 3D data: $\lambda_{8,12} = 0.600$, $\eta^2 = 0.400$, $F = 1.000$, $P = 0.483$). For *Ctenomys*, 2D shape is related moderately and significantly with size while 3D shape also showed a moderate relationship but non-significant (2D data: $\lambda_{8,16} = 0.380$, $\eta^2 = 0.620$, $F = 3.260$, $P = 0.021$; 3D data: $\lambda_{8,12} = 0.523$, $\eta^2 = 0.477$, $F = 1.821$, $P = 0.147$).

Shape analyses of caviomorph cranium showed similar genera ordination for both 3D and 2D morphospaces (PROTEST analysis: $m_{12} = 0.938$, $P < 0.001$). It could be observed similar phylogenetic structure to that observed for the mandible. Estimations of phylogenetic signal resulted significant for both 2D and 3D shape datasets (2D data: $K = 1.498$, $P < 0.001$, *tree length* = 0.143, $P < 0.001$; 3D data: $K = 1.045$, $P < 0.001$, *tree length* = 0.151, $P < 0.001$). Contrary to what we observed for the mandible, cranium shape is highly associated with size variation for both datasets (2D data: $\lambda_{16,30} = 0.055$, $\eta^2 = 0.945$, $F = 6.109$, $P < 0.001$; 3D data: $\lambda_{16,30} = 0.097$, $\eta^2 = 0.903$, $F = 4.153$, $P < 0.001$).

Discussion

Both 3D and 2D macroevolutionary datasets of the mandible shape led to broadly similar ordination results. The percentages of explained variation in the RWs were similar, as well as the overall arrangement of caviomorph genera in the respective morphospaces. The lack of total correlation between the two ordinations could be due to the fact that some genera, such as the octodontoid *Myocastor*, changed their relative position in each of the morphospaces. In contrast with this overall similarity between ordinations, some noteworthy differences were observed

between the macroevolutionary statistical analyses applied to each dataset. While phylogenetic signal values that are close to 1, such as observed for 2D data, suggest that the analyzed character has evolved following a Brownian movement model, values higher than 2 as obtained for 3D shape would indicate stasis in character change (Losos 2008). Likewise, the phylogenetic regressions results suggest that the choice of 2D or 3D data could strongly influence the inferences about the importance of size for the morphological diversification of the mandible in caviomorph rodents. Cranial shape analyses showed similar ordination to that observed for 3D mandible dataset. However, both 3D and 2D cranial shape variation associated highly and significantly with size and displayed significant phylogenetic signal. These similarity results differ from what we would expect for a marked three-dimensional structure such as the cranium, probably because of the great importance of size to structure the cranial shape variation that we observed in our results. Therefore, the decision whether or not to include the third coordinate in the analyses can lead to fundamentally different interpretations of the processes that could have guided the morphological diversification only of the caviomorph mandible.

The macroevolutionary results for the mandible differed from previous works (e.g., Cardini and Thorington 2006) at intra-specific scale in which no differences were recorded between 3D and 2D data in an analysis of ontogenetic shape changes of the sciurid rodent genus *Marmota*. We also assessed intra-specific shape variation but on datasets including adults of two morphologically disparate caviomorph genera such as *Ctenomys* and *Cavia*, which represent opposite trends regarding major differences traits such as mandible width (Vassallo and Verzi 2001; Hautier et al. 2011). The intra-specific results are partially concordant with those of Cardini and Thorington (2006). 3D and 2D ordinations for each genus correlated significantly but weakly. Regarding regression analyses, mandible shape variation resulted moderately associated with size changes for both *Ctenomys* and *Cavia*. These results are somewhat expected since the sample analyzed included only adult specimens and because the degree of shape variation that can be added if the third dimension is to be included probably is not much higher than the one observed for two-dimensional shape when dealing with intra-specific datasets. The marked difference in the results with the macroevolutionary analyses of mandible variation suggests that a great extent of the effect of size is probably in the third dimension (i.e., lateral flaring) of the mandible, which is a highly variable trait mainly among caviomorph species, as we could see and as noted already by Hautier et al. (2011), but with lower variation at intra-specific level. Disparate results obtained for the several datasets analyzed in this

study reveal some difficulty in generalizing the results obtained at high macroevolutionary scales to a lower scale, or vice versa.

Until now, morphological and phylogenetic patterns of caviomorph mandible had been analyzed mostly on the basis of bidimensional variation, following the tradition of 2D studies of the rodent mandible (Perez et al. 2009; Fornel et al. 2010; Álvarez et al. 2011). Caviomorph mandible shows important tridimensional variation linked to its hystricognath condition. Hautier et al. (2011) showed that hystricognathy is a highly homoplastic feature among rodents, especially variable within Cavimorpha. Including in shape analyses all the dimensions in which an anatomical structure can vary could be significant to understand the evolution of the diverse lineages and the diversification of the group. The differing results obtained at different evolutionary scales, give strength to our statement about careful selection of data not only in studies of morphological variation in caviomorphs but even likely for other groups of organisms.

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