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Size structural changes in the skull of *Didelphis marsupialis* Linnaeus, 1758 (Mammalia: Didelphidae)

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Abstract

A set of 82 skulls *Didelphis marsupialis* (32 males, 40 females and 10 on unknown sex) were investigated to study changes in allometry by bivariate and multivariate analyses using geometric morphometrics. It was found that developmental patterns display a high morphological change during growth, so variation in "aged" skull morphology was rather different to those in "juvenile" morphology. Isometric coordinates were located mainly on zygoma arch. The visceral skeleton continues to change throughout adulthood because of remodelling on *D. marsupialis*, probably due that it participates in the overall metabolism of the organism in progressive and regressive processes, probably as a results of the complex compensation of growth among interrelated structures. Our findings emphasize that developmental processes must be considered beyond teeth eruption for a more comprehensive understanding of ontogenetic process.

Keywords: allometry, Didelphimorphia, isometry, marsupials

INTRODUCTION

Allometry can be viewed as an statistical association between size and shape (Mosimann 1970). Geometric morphometric (GM) study of allometry requires a methodological approach that differs in several aspects from the classic allometry studies (Mitteroecker et al. 2013). Thus, in GM, allometry is expressed as a regression function of the Procrustes shape xy coordinates –i.e., the linear measure of shape differences between landmark configurations- on centroid size (CS) or its logarithm (Mitteroecker et al. 2013) –i.e., the square root of the summed squared distances of each landmark from the centroid of the form- (Zelditch et al. 2004).

The genus *Didelphis* includes the largest living species of American marsupials (Astúa 2015). Species of this genus range in head and body length and weight from *ca.* 300 mm and 500 g in the smallest adults of *Didelphis albiventris* Lund, 1840 to 500 mm

and over 6,000 g in the largest specimens of *Didelphis virginiana* Kerr, 1792 (Astúa 2015). *Didelphis* has been the object of several taxonomic and morphometric analyses in the last decades, some of these focusing in the morphometric variation and diversification within species groups (Reig et al. 1987) (Cerqueira & Lemos 2000) (Lemos & Cerqueira 2002) (Ventura et al. 2002) (Astúa 2015), but none of these allometric studies have been done specifically on *D. marsupialis*.

By definition, changes in rate and timing of ontogenetic processes can only be determined based on absolute time, meaning when the age of compared individuals is known (Sebastião & Marroig 2013). This was actually a general problem when dealing with museum collections, as in our case (studied specimens were housed in two accessed natural history collections and were captured in the wild,

so consequently age information was unavailable). However, the wide range of both skull sizes and dentulous states (from partially teeth erupted to complete dentulous ones) considered here allows to consider the specimens as representing a wide age range, from very young to very old specimens, and thus, this study can be viewed as an ontogenic one.

The purpose of this study is to assess the allometric trajectory of skull in *Didelphis marsupialis* Linnaeus, 1758 and ultimately to assess whether skull shape changes are attributable to a mere change in size (e.g. it is an allometric change), or is influenced by other sources of variation. Our examination of the ontogeny of this species may provide not only results “per se”, but a comparative frame for the ontogeny in all marsupials studying a non-specialized forms as the conservative didelphid as *D. marsupialis* is (Abdala et al. 2001).

MATERIAL AND METHODS

The geometric morphometrics (see (Rohlf 2005) was used to analyze 82 skulls (32 males, 40 females and 10 of unknown sex) belonging to *Didelphis marsupialis* from Colombia. Images of ventral views of skulls were taken using a Nikon D1500 digital camera equipped with an 18-105 mm Nikon DX telephoto lens and saved in JPEG format. A scale (10 mm) was used in this process. From pictures, 14 landmarks were digitized using TpsDig v. 2.26 (Rohlf 2016). These homologous and topologically equivalent landmarks were plotted on ventral aspect (left hemiskull). They were considered to capture variation in skull form (size + shape separately) (Figure 1). For the estimation of shape, each specimen was superimposed by optimally translating, rotating and uniformly scaling them, establishing the measure by means of Procrustes coordinates (Webster & Sheets 2010). For the estimation of size, centroid size (CS) was used. CS and Procrustes coordinates were obtained with the CoordGen8 program (Sheets 1998).

Specimens studied are deposited in the mammal collection of the *Departamento de Biología* of the *Universidad del Valle* in Cali (Colombia) and *Instituto de Ciencias Naturales* of the *Universidad Nacional de Colombia* in Bogotá (Colombia). Every specimen had been taxonomically identified to the species level, and as each one was initially collected for other studies, no ethical permission was considered to be obtained. None presented malformations. A complete list of specimens may be obtained upon request. A 2k-means clustering was used to assign each specimen to 3 size group according to CS, which were interpreted as “juveniles” (n=25, range 62.2-114.3), “subadults” (n=25, range 119.8-151.4) and “adults” (n=32, range 154.1-196.4) (names in brackets are just a way to nominate size groups as age groups).

A two-way NPMANOVA test using correlation as distance measure and 9,999 permutations was applied to know if differences between sexes and age groups existed for form (size + shape). As no sexual differences appeared between sexed specimens (n=72) (Table 1 and Figure 2), all sexes were pooled.

CS was then transformed to its natural logarithm to increase the fit of the model (percentage of shape variance explained by size) and a multivariate regression analysis was performed using this log-transformed CS as independent variable. Since the isometric value (i.e., no shape change according to age) can be affected by the number of variables considered, a bivariate regression on Principal Components (PC) 1 and 2 was also performed, calculating reduced major axis coefficients.

All standard statistical procedures were performed using the

PAST® software package (Hammer et al. 2001).

RESULTS AND DISCUSSION

Allometry, e.g. the dependence of some (all) shape vector(s) e , that is, dependence of shape and size (Mosimann 1970), was detected for *D. marsupialis* in the multivariate regression ($p \lll 0.001$, $R^2=0.290$, Wilk's $\lambda=0.092$, $F_{28,53}=18.5$). Bivariate regressions of PC1 and PC2 (which explained respectively a 33.16 and 12.79 % of the total observed variance) showed that allometry plays a clear role in shape variation and differentiation in PC1 ($r^2=0.856$, $p \lll 0.001$) but not in PC2 ($r^2 \lll 0.0001$, $p=0.948$) (Figure 3). Although sexual dimorphism was statistically non-significant in our sample, I deemed necessary to statistically test for a change in slope or position between curves for males and females given. ANCOVA (ANalysis of COVariance) test reflected no statistical difference between sexes ($p=0.949$, Figure 4) so I assumed that *D. marsupialis* exhibits parallel growth trajectories for both sexes and with similar slopes homogeneity ($F=2.396$, $p=0.126$). So, both genders grow equally.

Analysing separately Procrustes coordinates in multivariate regression (Table 2), it was clear that some of skull components produce an isometric morphological arrangement, most of which were located on lateral part of neurocranium (y4, y5, y6, x7 and y9), mainly along the zygomatic arch. Observed modifications signalled by the rest of allometric landmarks can be associated directly to functional changes in visceral skeleton (splanchnocranium complex) (Figure 5). The visceral skeleton continues to change throughout adulthood because of remodelling on *D. marsupialis*, probably due that it participates in the overall metabolism of the organism in progressive and regressive processes, probably as a results of the complex compensation of growth among interrelated structures (more than in response to variation in diet). Similar bony compensations have been reported by Maunz and German (Maunz & German 1996) for the palate and the mandible of *M. domestica*, and Abdala et al. for *Didelphis albiventris* (Abdala et al. 2001). I relate this allometry trend with an overall strengthening of the skull and the development of a skull shape that favours a strong bite. As I am dealing with weaned individuals, those changes can be associated with the shift from milk suckling to the active feeding.

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Figure 1. Landmarks used to the study of skull form (ventral side of left hemicranium). The 14 chosen landmarks were considered to sufficiently capture the morphology of the ventral size and shape. These landmarks were present on all 82 studied specimens of *Didelphis marsupialis*.

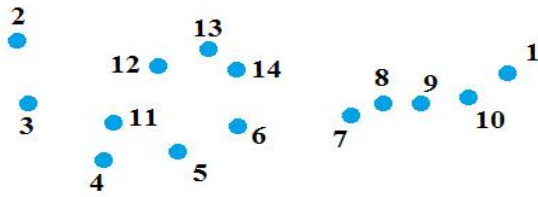


Table 1. Two-way NPMANOVA test using correlation distance for Procrustes coordinates in *Didelphis marsupialis* form (size + shape) skulls of sexed specimens (32 males and 40 females). No sexual differences appeared.

Source	Sum of squares	Degreed of freedom	Mean square	F	<i>p</i>
Sex	-3.77E-11	2	-1.89E-11	-1.4856	0.949
Age	3.78E-09	2	1.89E-09	148.8	0.0001
Interaction	-6.95E-11	4	-1.74E-11	-1.3672	0.2341
Residual	9.27E-10	73	1.27E-11		
Total	4.60E-09	81			

Table 2. Slopes and intercepts for each Procrustes coordinates. Most of Procrustes coordinates presented significative values in regression multivariate fit. Isometric values appear in bold.

Variable	Slope	Error	Intercept	Error	R	<i>P</i>
x1	-0.07383	0.005463	0.60938	0.011622	-0.83392	2.36E-22
y1	-0.00857	0.004382	0.06815	0.009322	-0.21363	0.053964
x2	0.06961	0.006608	-0.54380	0.014059	0.76229	8.88E-17
y2	-0.07157	0.00591	0.27053	0.012574	-0.80437	8.94E-20
x3	-0.02169	0.006353	-0.30555	0.013517	-0.3566	0.001008
y3	0.01181	0.005691	-0.04480	0.012108	0.22604	0.041154
x4	-0.08329	0.008832	-0.04561	0.018789	-0.72557	1.25E-14
y4	-0.00044	0.006606	-0.10806	0.014055	-0.00736	0.94766
x5	-0.06519	0.008091	0.02507	0.017214	-0.66931	6.24E-12
y5	0.00356	0.008721	-0.11182	0.018554	0.04565	0.68377
x6	0.04501	0.012734	-0.09935	0.027091	0.36754	0.000682
y6	0.00115	0.008954	-0.06113	0.01905	0.01438	0.89797
x7	0.00491	0.012446	0.16505	0.026478	0.04407	0.69417
y7	0.02801	0.005628	-0.09363	0.011974	0.48630	3.62E-06
x8	0.04339	0.005608	0.14498	0.011931	0.65432	2.62E-11
y8	0.02358	0.003231	-0.06519	0.006874	0.63228	1.88E-10
x9	0.02540	0.005623	0.23716	0.011963	0.45089	2.13E-05
y9	0.00353	0.003192	-0.01998	0.006790	0.12277	0.27183
x10	0.04470	0.006662	0.28103	0.014173	0.60017	2.53E-09
y10	0.01395	0.002929	-0.02985	0.006232	0.47001	8.38E-06

x11	-0.07143	0.004744	-0.06932	0.010092	-0.85976	4.63E-25
y11	0.06410	0.009179	-0.18318	0.019527	0.61545	7.61E-10
x12	0.02090	0.010637	-0.18939	0.022631	0.21461	0.052848
y12	-0.01399	0.007702	0.09917	0.016386	-0.19904	0.073024
x13	0.03071	0.006118	-0.12762	0.013015	0.48943	3.07E-06
y13	-0.02696	0.004562	0.15143	0.009706	-0.55124	8.02E-08
x14	0.03075	0.009562	-0.08205	0.020343	0.33842	0.001872
y14	-0.02819	0.005604	0.12835	0.011922	-0.49027	2.93E-06

Figure 2. Comparison of sizes (expressed as Centroid Size, CS) between males (n=11) and females (n=18) “adults” of *Didelphis marsupialis* skulls. The median is shown with a horizontal line inside the box. The short horizontal lines were drawn from the top of the box up to the largest data point less than 1.5 times the box height from the box (the “upper inner fence”), and similarly below the box. The means of CS were 187.87 and 186.89 for males and females respectively. Mann-Whitney test reflected no statistical differences between genders (U=636, $p=0.794$). No outliers appeared.

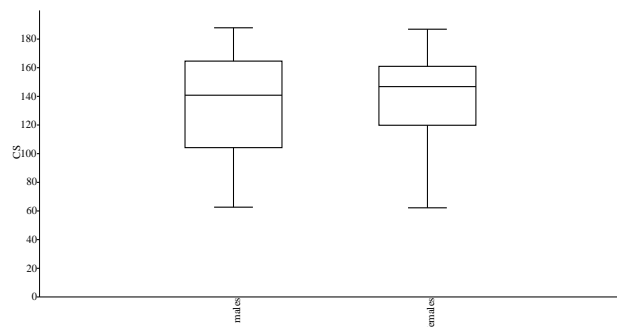


Figure 3. Bivariate scatterplot of the scores of the first Principal Component (PC1) with centroid sizes (CS, log transformed) of *Didelphis marsupialis* skulls. Regression was performed with the whole sample. Allometry plays a clear role in shape variation and differentiation in PC1 ($r^2=0.856$, $p<<0.001$). Each symbol refers to an age group: “juveniles” (n=25, filled squares), “subadults” (n=25, empty squares) and “adults” (n=32, crosses).

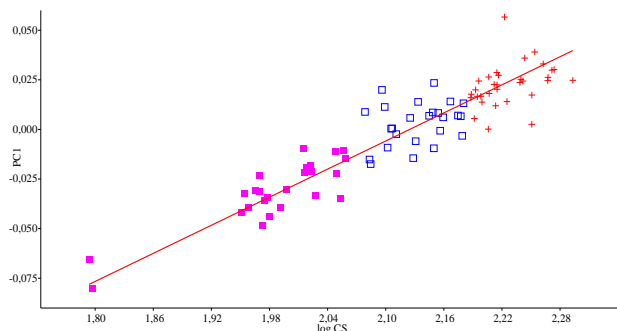


Figure 4. Bivariate regression of PC1 on log CS for both sexes between males (n=32) and females (n=40) of *Didelphis marsupialis* skulls. ANCOVA test reflected no statistical difference between sexes ($p=0.949$). Scatterplots presented similar slopes homogeneity (F=2.396, $p=0.126$).

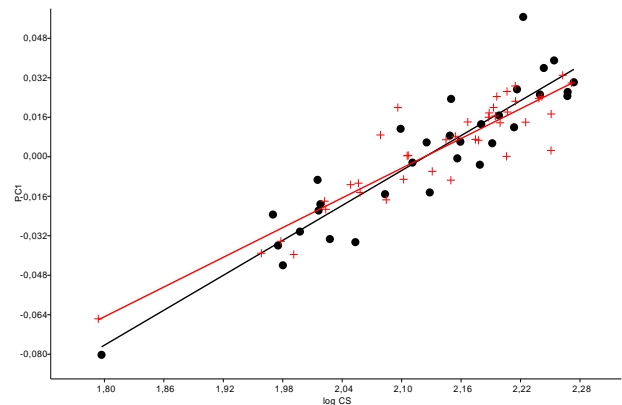
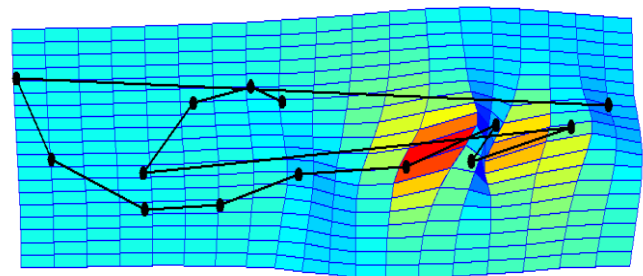


Figure 5. ‘Expansion factors’ which display the area expansion (or contraction) factor around each landmark, indicating the degree of local growth on the skull (left hemiskull in ventral view) on *Didelphis marsupialis*, for males and females jointly, average values. The expansions are color-coded for all grid elements, with green for expansion and purple for contraction. See figure 1 for the detailed situation of landmarks.



BIBLIOGRAPHY

1. Abdala, F., Flores, D.A. & Giannini, N.P., 2001. Postweaning ontogeny of the skull of *Didelphis albiventris*. *Journal of Mammalogy*, 82, pp.190–200.
2. Astúa, D., 2015. Morphometrics of the largest new world marsupials, opossums of the genus *Didelphis* (Didelphimorphia, didelphidae). *Oecologia Australis*, 19(1), pp.117–142.
3. Cerqueira, R. & Lemos, B., 2000. Morphometric differentiation between Neotropical black-eared opossums, *Didelphis marsupialis* and *D. aurita* (Didelphimorphia, Didelphidae). *Mammalia*, 64, pp.319–327.
4. Gardner, A.L. & Jr. Handley, C.O., 2007. Marsupials, Xenarthrans, Shrews, and Bats. In U. of Chicago, ed. *Mammals of South America*. Chicago.
5. Hammer, Ø., Harper, D.A.T. & Ryan, P.D., 2001. PAST, v. 2.17c. *Palaeontologia Electronica*, 4(1), pp.1–229.
6. Lemos, B. & Cerqueira, R., 2002. Morphological differentiation in the White-Eared Opossum Group (Didelphidae: Didelphis). *Journal of Mammalogy*, 83(2),

- pp.354–369.
7. Maunz, M. & German, R.Z., 1996. Craniofacial heterochrony and sexual dimorphism in the short-tailed opossum (*Monodelphis domestica*). *Journal of Mammalogy*, 77(4), pp.992–1005.
 8. Mitteroecker, P. et al., 2013. A brief review of shape, form, and allometry in geometric morphometrics, with applications to human facial morphology. *Hystrix*, 24(1), pp.59–66.
 9. Mosimann, J.E., 1970. Size allometry: size and shape variables with characterizations of the lognormal and generalized gamma distribution. *Journal of the American Statistical Association*, 65(330), pp.930–945.
 10. Reig, O.A., Kirsch, J.A.W. & Marshall, L.G., 1987. Systematic relationships of the living and Cenozoic american “opossum-like” marsupials (Suborder Didelphimorphia), with comments on the classification of these and of the Cretaceous and Paleogene New World and European metatherians. In *Possums and opossums: studies in evolution*. Sydney: Surrey Beatty and Sons, pp. 1–89.
 11. Rohlf, F.J., 2005. Geometric morphometrics simplified. *Trends in Ecology & Evolution*, 20(1), pp.13–14.
 12. Rohlf, F.J., 2016. tpsDIG2, v. 2.26. Available at: <http://life.bio.sunysb.edu/morph/>.
 13. Sebastião, H. & Marroig, G., 2013. Size and shape in cranial evolution of 2 marsupial genera: *Didelphis* and *Philander* (Didelphimorphia, Didelphidae). *Journal of Mammalogy*, 94(6), pp.1424–1437. Available at: <http://www.bioone.org/doi/abs/10.1644/11-MAMM-A-349.1>.
 14. Sheets, H.D., 1998. IMP: CoordGen8- Coordinate Generation Utility. Available at: <http://www.canisius.edu/~sheets/morphsoft.html>.
 15. Vaughan, T.A., Ryan, J.M. & Zapplewski, N.J.C., 2000. *Mammalogy* S. C. Publishing, ed., Fort Worth, Texas.
 16. Ventura, J. et al., 2002. Morphometrics of the genus *Didelphis* (Didelphimorphia: Didelphidae) in Venezuela. *Journal of Mammalogy*, 83(4), pp.1087–1096.
 17. Webster, M. & Sheets, H.D., 2010. A Practical Introduction to Landmark-based Geometric Morphometrics. *The Palaeontological Society Papers*, 16, pp.163–188.
 18. Zelditch, M.L., Swiderski, D.L. & Sheets, H.D., 2004. *Geometric morphometrics for biologists: a primer*, Boston, MA: Elsevier Academic Press. Available at: http://cataleg.udl.cat/record=b1279303~S11*cat [Accessed May 4, 2016].