

Peramorphic males and extreme sexual dimorphism in *Monodelphis dimidiata* (Didelphidae)

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Abstract The southern short-tailed opossum, *Monodelphis dimidiata*, is a species known not only for its semelparous life cycle, but also for the extreme sexual dimorphism of adults, where males are not only larger, but also have distinctive morphological characters in their skull. Using geometric morphometrics and a suite of statistical tests, I analyzed the postweaning ontogenetic development of this species in order to evaluate the age-class where sexual dimorphism becomes significant and the amount of change exhibited by both sexes. My results showed that *M. dimidiata* partly follows the ontogenetic pattern described for didelphids by previous authors. The character that escapes the general pattern is rostral length, which becomes shorter instead of lengthening throughout the development. This change could be related to an increment in the bite force in the anterior part of the dentition. The amount of sexual dimorphism found for this species is larger than the reported previously for other American marsupials, and I also found a higher rate of growth in males at the attaining of sexual maturity. Based on my results and the information available for other didelphids, I can suggest that *M. dimidiata* males undergo through a process of hypermorphosis, resulting in a

peramorphic condition. It is possible that the extreme sexual dimorphism present in this species is related to reproductive success, specially taking into account their semelparous life cycle.

Keywords Mouse opossum · Ontogeny · Allometry · Heterochrony · Sexual dimorphism · Geometric morphometrics

Introduction

The southern short-tailed opossum, *Monodelphis dimidiata* (Wagner 1847), is a small didelphid marsupial from the Pampas and Atlantic Forest domains of Argentina, Brazil, Uruguay and Paraguay (as *M. sorex* (Hensel 1872), see Vilela et al. 2010) (Pine & Handley 2007; Smith 2008). This species has a peculiar life cycle, which makes it interesting for studying ontogenetic processes. *Monodelphis dimidiata* is a true semelparous breeder, with males that die in summer after the reproductive period, and females that die in autumn after rearing their litters (Reig 1964, 1965; Pine et al. 1985; Baladrón et al. 2012). Associated with the breeding season, in early spring, the growth rate of both sexes strikingly increases, with the males exceeding the females (Pine et al. 1985). As an outcome of this excessive growth rate of males, there is a remarkable dimorphism between both sexes, where males show larger skulls with proportionately larger crests, an enlarged postorbital process of the frontals, widened zygomatic arches, a strongly developed postorbital constriction, deeper lower jaws and saber-like canines (Pine et al. 1985). Also, associated with these body changes, there is a change in feeding preferences, where sexually matured individuals consume more vertebrates (mainly

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rodents) than juvenile individuals (Busch and Kravetz 1991; Goin et al. 1992).

Several ontogenetic studies have been performed in didelphid marsupials (e.g., Maunz and German 1996; Abdala et al. 2001; Flores et al. 2003, 2010; Sebastião and Marroig 2013), but only the first and the last discriminated the ontogenetic pattern of males and females. Maunz and German (1996) analyzed the ontogenetic trajectory of males and females of *Monodelphis domestica* (Wagner 1842), using skull measurements, and discriminating between splanchnocranium and neurocranium. The authors found an heterochronic pattern between sexes, where females are smaller than males, but with the same final shape. On the other hand, Sebastião and Marroig (2013) compared the ontogenetic trajectory of *Didelphis virginiana* Kerr 1792, and *Philander opossum* (Linnaeus 1758) and found a common pattern between both species, which they extended to Didelphimorphia using data available from previous ontogenetic analyses. They also found significant sexual dimorphism, for both species, arising at age 4 in the case of *D. virginiana*, and at age 5 in *P. opossum*; however, growth trajectories were essentially the same for both sexes (Sebastião and Marroig 2013). The ontogenetic pattern described by Sebastião and Marroig (2013) for the order is characterized by a reduction in the braincase, narrowing of the palate, lengthening of the rostrum and enlargement of the temporal space. The authors mentioned, however, that this trend might be present only in large species, making it relevant to analyze whether mouse opossums, such as *M. dimidiata*, follow the same ontogenetic trajectory.

Flores et al. (2013, 2014) optimized the ontogenetic information obtained from 15 cranial variables for several marsupials (didelphids, caenolestids and peramelids) in the phylogenetic hypotheses considered for the group. In their first contribution, they mapped the ontogenetic trend (isometric, positive allometry and negative allometry) and found as plesiomorphic states for marsupials isometry of the condyle-incisive length, isometry of the length of the palate and positive allometry of the length of the mandible, while positive allometry of the zygomatic breadth is synapomorphic for didelphid marsupials (being plesiomorphic for Theria) (Flores et al. 2013). In the second contribution (Flores et al. 2014), the authors mapped the confidence intervals from the multivariate analysis of allometry and discussed changes in growth rates of the different variables, showing increments and decrements in growth rates as synapomorphies or symplesiomorphies of different clades.

The development of geometric morphometric techniques allowed a better representation of shape changes, analyzing not only a few measurements, but the entire shape of the structures (Adams et al. 2004). Moreover,

several statistical methods have been developed to characterize the developmental mechanisms involved in the ontogenetic trajectory of organisms (see a review of those methods in Sheets and Zelditch 2013). Within these methods, Piras et al. (2011) developed a series of multivariate tests for modeling allometric trajectories that allow testing growth rate changes, ontogenetic divergence, convergence or parallelism and heterochronic processes, using the Procrustes distances between groups. Although most of the methods for comparing ontogenetic trajectories were conceived for multiple-species analyses, the extreme sexual dimorphism observed in adults of *M. dimidiata* suggests that an heterochronic process might be involved in the achievement of adult size, making Piras et al.'s tests suitable for testing this hypothesis.

The aims of the present work are: (1) to analyze the postweaning ontogenetic development of *Monodelphis dimidiata*, evaluating the age-class when the sexual dimorphism becomes apparent, and the heterochronic processes (if any) involved in the development of both sexes; (2) to compare the developmental changes found in *M. dimidiata* with previous information provided for didelphids, to see whether they fit in the developmental pattern proposed for the group. For attaining those objectives, I used two-dimensional geometric morphometric analyses of the cranium (in lateral, ventral and dorsal view) and mandible of a large sample of specimens of *M. dimidiata*. Shape changes were evaluated using a regression of shape (Procrustes coordinates) onto size, and the Procrustes distances between the different categories. The age-class where size and shape sexual dimorphism becomes significant was also evaluated. Finally, the tests proposed by Piras et al. (2011) were used to test the heterochronic processes involved in the ontogenetic development.

Materials and methods

Materials and landmarks

I analyzed 175 specimens of *M. dimidiata* (Online Resource 1), with an almost balanced percentage of males and females. Individuals were classified into five age-classes (2–6) according to tooth eruption and wear, based on the model proposed by Gardner (1973) for *Didelphis*: 2—M2, m2 and m3 in position; 3—M3 in position and permanent premolars erupting; 4—permanent premolars and m4 in position; 5—all teeth erupted but with little wear; 6—all teeth moderately to excessively worn. Due to the one-year life cycle of *M. dimidiata*, dental ages become a good proxy for age, since litters are born in a 2-month period (December and January for Buenos Aires Province,

Argentina) and adult males die a year later, while females live a couple of months more (Reig 1964; Pine et al. 1985; Baladrón et al. 2012). It is important to mention that 81 % of the specimens included in the study were collected in Buenos Aires Province, and consequently, they followed the reproductive cycle described above.

Specimens were photographed with the palate parallel to the photographic plane for dorsal and ventral view of the skull, the sagittal plane parallel to the photographic plane for lateral view, and the plane including coronoid process and horizontal ramus parallel to the photographic plane for the mandible. All images included a ruler for scale.

Landmark placement was based on previous analyses on didelphid marsupials (Astúa de Moraes et al. 2000; Astúa 2010). For the dorsal view of the skull, I digitalized 23 landmarks and 44 semilandmarks, covering both sides (Fig. 1a); for the lateral view of the skull (right side), I digitalized 11 landmarks and 31 semilandmarks (Fig. 1b); for the ventral view of the skull, I digitalized 23 landmarks covering both sides (Fig. 1c); and for the mandible (right mandible), I digitalized 7 landmarks and 12 semilandmarks (Fig. 1d). I used the application MakeFan6 (Sheets 2003) to ensure consistent placement of the semilandmark coordinates, using an equidistant placement or an equiangular placement depending on the structure being analyzed. I digitized both landmarks and semilandmarks using the software tpsDIG 2.12 (Rohlf 2008a).

Geometric morphometrics

I aligned the landmark coordinates using the generalized Procrustes analysis (GPA) which superimposes specimens eliminating the effect of position, orientation and scale (Bookstein 1991). For the alignment, semilandmarks were slid using the minimum bending energy method (Bookstein 1997). I used the software TPSrelw 1.46 (Rohlf 2008b) for the GPA and sliding of semilandmarks. The aligned specimens were then exported to MorphoJ 1.05b (Klingenberg 2011) to perform the regression of shape onto size. For the analyses of the ventral and dorsal view of the skull, only the symmetric component of shape variation was used for the analyses (see Klingenberg et al. 2002).

As another way of visualizing the shape differences between sexes and age categories, I estimated the differences in shape (using Procrustes distance) and size (using centroid size) between each age category and the average shape or size of the youngest age category. Differences were visualized using boxplots, and estimations were performed for each sex separately. The Wilcoxon rank test was used to test the statistical significance between different age and sex classes. The statistical significance of the *p* value was determined using a *p*-plot and a sharpened Bonferroni method (García 2004). Distance calculations,

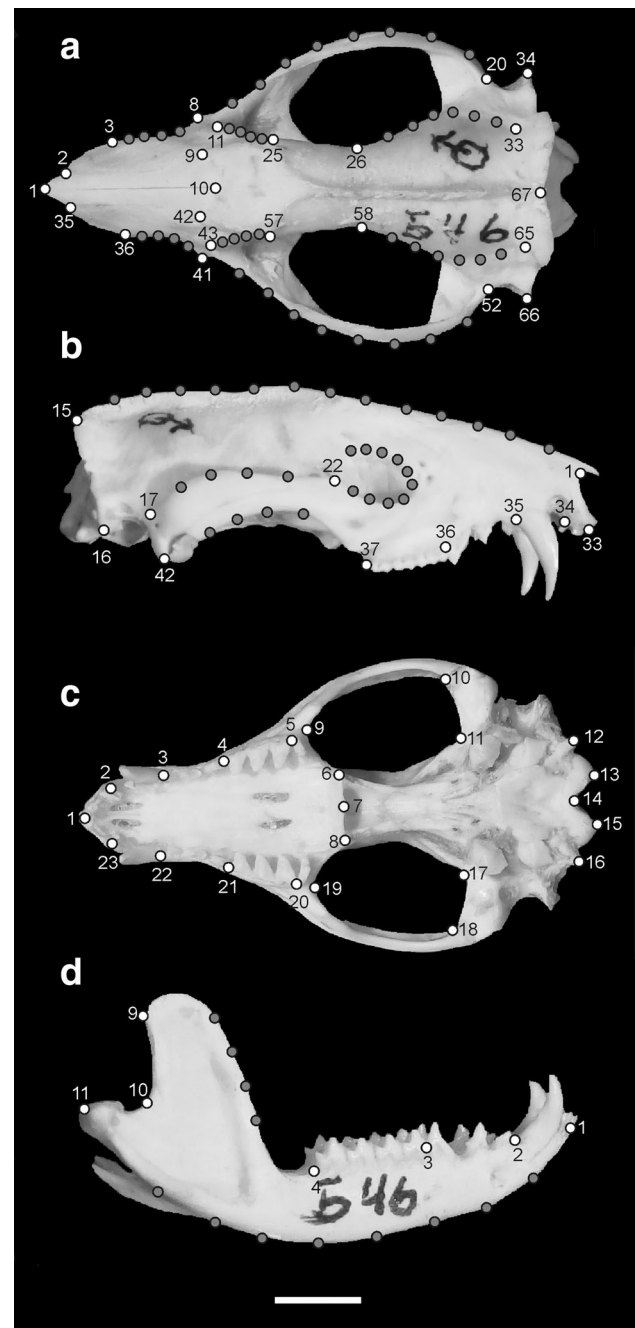


Fig. 1 Landmarks (white circles) and semilandmarks (gray circles) used in the analyses. **a** Dorsal view of the skull. **b** Lateral view of the skull. **c** Ventral view of the skull. **d** Mandible. Bar 1 cm

boxplots and the Wilcoxon rank tests were performed using R 2.15.0 (R Development Core Team 2014).

Comparing ontogenetic trajectories

To test whether both sexes differ in their allometric trajectories, I used the statistical procedure proposed by Piras et al. (2011) which uses regression coefficients, predicted

values and residuals from a multivariate regression between shape and size (regressing each sex separately). All the analyses were performed in R, using the scripts provided by the authors, also distributed with the online workbook by Zelditch et al. (2012).

Using the Procrustes distances between predicted shapes at small and large sizes, I tested whether developmental trajectories were divergent, parallel or convergent. If distance remains the same at both sizes, developmental trajectories are parallel; otherwise, they are divergent or convergent if the largest distance is at larger or smaller sizes, respectively. A multipermutation procedure was used to test the null hypothesis of parallelism against convergence or divergence, randomly reassigning sex affiliation (1000 permutations).

Under parallel trajectories, I tested whether the groups showed predisplacement, postdisplacement or did not differ in the elevation of the ontogenetic trajectory using a multivariate intercept test, performing a multivariate regression between shape and size and calculating the Euclidean distance between vectors of intercept coefficients. Significance was tested using the permutation procedure to generate random distribution of distances and comparing them with the observed distances (1000 permutations).

Under overlapping trajectories, I tested for the dispersion along allometric trajectories using a permutation test (randomly reassigning sex affiliation) upon separate per-group multivariate regressions between shape and size, estimating the mean-squared error for each regression (MSE).

Finally, to check whether one of the groups is peramorphic (or paedomorphic), I used a multipermutation procedure on distances between the shapes predicted at maximum size values recorded for any group. After assessing the significance for shape distances, the multivariate distances between predicted shapes at maximum per-group size value and origin was quantified in order to determine which trajectory lies below the other.

Results

Shape changes throughout ontogeny

Dorsal view of the skull

The most dominant feature of shape change throughout ontogeny is the relative narrowing of the braincase (Fig. 2a). Smaller changes also occur in other structures: The postorbital process (landmarks 25 and 57) is anteriorly displaced; the zygomatic arches are laterally expanded, enlarging the temporal fossa; and the rostrum is relatively

shorter in older categories, but since its breath does not change, it gives the aspect of being more robust than the rostrum of juveniles (Fig. 2a).

The scatterplot of the regression scores versus log centroid size (69 % explained variance) showed substantial overlap between categories 3, 4 and many specimens of category 5 (Fig. 2b). Regarding the sex, there is a large overlap between both sexes in the smaller sizes, but males have larger centroid sizes than females and, consequently, exceed females in shape (Fig. 2b). The analysis of Procrustes distances is consistent with the regression, where there are no statistically significant differences in shape between age-classes 3 and 4, and 4 and 5 in males (Fig. 2c; Table 1). Sexual shape dimorphism starts at age-class 5, where females are more different from the smallest individuals than males (i.e., have a larger Procrustes distance; Fig. 2c; Table 1). At age-class 6, males exceed female shape (Fig. 2c; Table 1). Sexual size dimorphism starts at age-class 6, where males are significantly larger than females (Fig. 2d; Table 1). At age-class 5, the median size of females is larger than the median size of males, although that difference is not statistically significant (Fig. 2d; Table 1).

Ventral view of the skull

There are few shape changes in this view throughout ontogeny, being the most important changes the relative lengthening of the zygomatic arches (anteroposterior), and the relative narrowing of the basicranium and the braincase (Fig. 3a). There are almost no shape changes in the palate, except for a small posterior displacement of landmark 1 (placed between the incisors 1), causing a shortening of the snout (Fig. 3a).

The scatterplot of the regression scores versus log centroid size (81 % explained variance) showed a large overlap between categories 3 and 4, while the remaining categories form a continuum (Fig. 3b). Regarding the position of the specimens according to their sex, there are no differences at smaller sizes, but males reach much larger sizes than females (Fig. 3b). The analysis of the Procrustes distances was consistent with the distribution of the age-classes on the regression plot, with almost no differences between age-classes 3–5 (Fig. 3c). Statistically significant differences in shape are evident between age-classes 5 and 6 in males; none of the remaining comparisons were significant (Table 1). Sexual dimorphism becomes evident at age-class 6, and both for shape and size were males became statistically different from females (Fig. 3c, d; Table 1).

Lateral view of the skull

The most important changes in this view were located in the braincase, which became much less bulbous in the

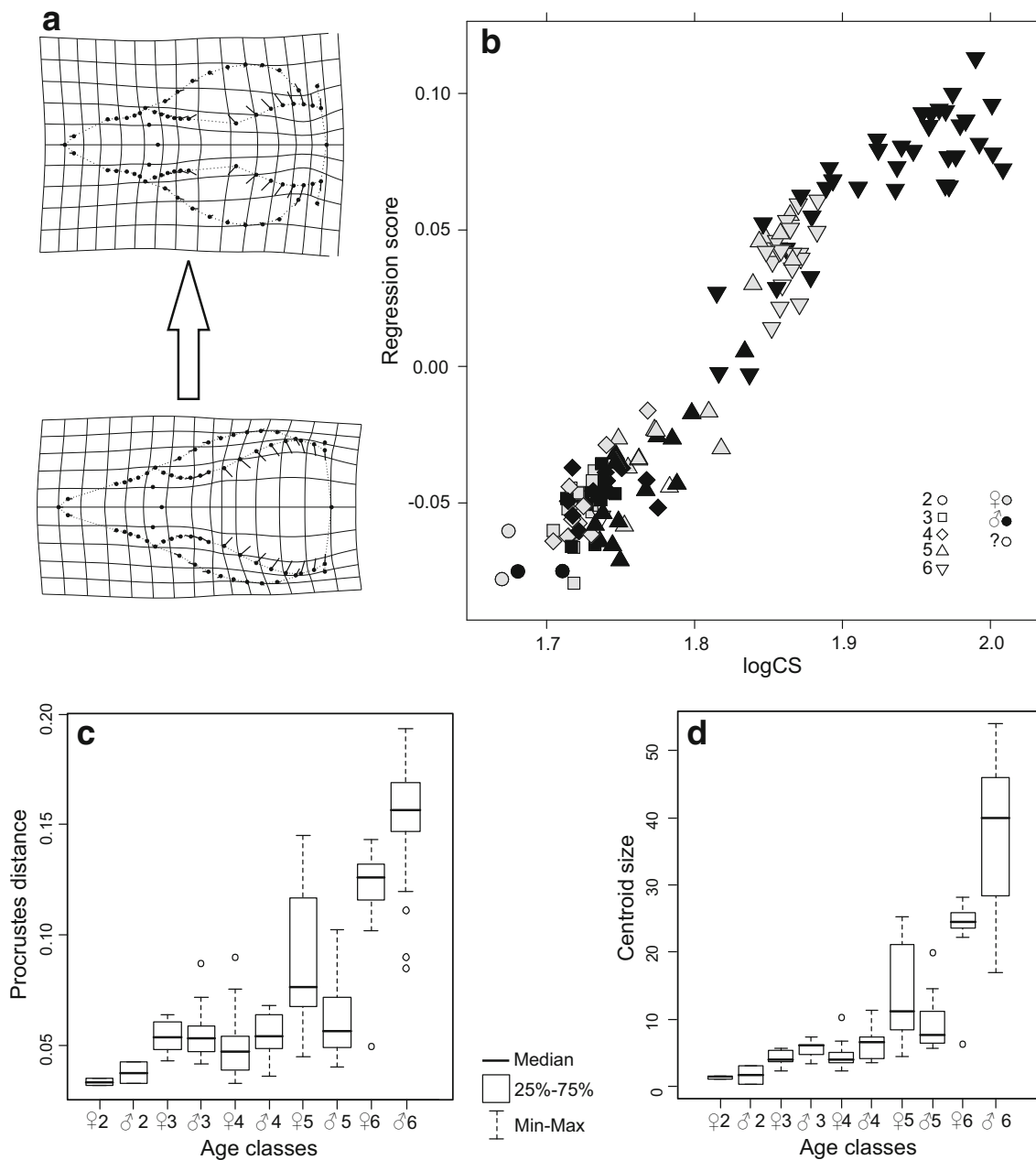


Fig. 2 Results from the morphometric analysis of the dorsal view of the skull. **a** Shape changes from age-class 2 (*bottom*) to age-class 6 (*top*) with respect to the consensus shape. **b** Multivariate regression of the Procrustes coordinates against the log-transformed centroid size.

c Boxplots of Procrustes distance of each specimen of all age-classes respect to the mean of age-class 3. **d** Boxplots of centroid size versus age

adults, and the zygomatic arches, that became more robust throughout the ontogeny, being longer (anteroposterior) and higher (dorsoventral) in adults (Fig. 4a). Orbits were also relatively smaller in older specimens (Fig. 4a). Finally, there was a relative reduction in the rostrum length in adults, which together with its increase in height gives adults the aspect of having a more robust rostrum (Fig. 4a). The shortening of the rostrum is also accompanied by an anterior displacement of the end of the tooth row (landmark

37; Fig. 4a), and consequently, adults present a relatively shorter tooth row.

The scatterplot of the regression scores versus log centroid size (39 % explained variance) showed an overlap between age categories 3 and 4 and between age categories 5 and 6; some specimens of category 5 were also overlapped with categories 3 and 4 (Fig. 4b). Specimens of age-class 2 were placed apart from the remaining specimens in the x axis (centroid size), but their values on the y axis

Table 1 Results of the Wilcoxon rank tests for comparisons of Procrustes distance and centroid size between sexes and age-classes

	Dorsal		Ventral		Lateral		Mandible	
	w	p	w	p	w	p	w	p
<i>Procrustes distance</i>								
♀2 versus ♂2	1	0.667			0	0.667	1	0.999
♀3 versus ♂3	64	0.976	53	0.2109	42	0.897	70	0.999
♀4 versus ♂4	85	0.605	29	0.5414	83	0.827	67	0.683
♀5 versus ♂5	247	0.006	91	0.9812	115	0.869	178	0.616
♀6 versus ♂6	88	0.00008	111	6.8 E⁻⁶	58	4.4 E⁻⁸	191	0.00023
2 versus 3	0	0.00068			2	0.0060	23	0.352
3 versus 4	27	0.684	118	0.2584	122	0.0044	306	0.913
4 versus 5			208	0.4938	307	0.120	168	1.6 E⁻⁵
♀4 versus ♀5	31	0.00098						
♂4 versus ♂5	75	0.4035						
♀5 versus ♀6	79	0.00248	131	0.3389	141	0.889	46	4.6 E⁻⁶
♂5 versus ♂6	15	1.4 E⁻¹¹	50	7.7 E⁻⁵	63	3.5 E⁻⁶	4	2.4 E⁻¹³
<i>Centroid size</i>								
♀2 versus ♂2	2	0.999			0.5	0.999	0	0.667
♀3 versus ♂3	23	0.01699	11	0.0113	22.5	0.130	47	0.192
♀4 versus ♂4	34	0.08795	29	0.5414	37	0.0194	61	0.461
♀5 versus ♂5	231	0.0275	92	0.3669	118.5	0.0653	188	0.415
♀6 versus ♂6	117	9.3 E⁻⁶	102	1.3 E⁻⁷	120.5	0.00012	95	5.4 E⁻⁸

Significant *p* values (after Bonferroni correction) are given in bold. Corrected *p* values for the Procrustes distance analysis and centroid size analysis are as follows: dorsal view of the skull *p* 0.0107/*p* 0.0214; ventral view of the skull *p* 0.00793/*p* 0.0123; lateral view of the skull *p* 0.0093/*p* 0.0133; mandible *p* 0.0098/*p* 0.00954. ♀ females; ♂ males

(regression score) were similar to those of age-classes 3, 4 and 5 (Fig. 4b). Sex distribution followed the same pattern as in other views. The boxplot of the Procrustes distances was consistent with the scatterplot of the regression. Age categories 2–3 and 3–4 were statistically significantly different, as well as categories 5–6 for males (Fig. 4c; Table 1). Sexual dimorphism becomes evident at age-class 6, and both for shape and size were males become statistically different from females and larger (Fig. 4c, d; Table 1).

Mandible

Throughout ontogeny, the mandible becomes more robust, mainly due to a relative shortening of the mandibular body (with little change in its height) in adults (Fig. 5a). Other changes in adults include a posterior displacement of the condylar process, and the expansion, both anteroposterior and dorsoventral, of the coronoid process (Fig. 5a).

The scatterplot of the regression score versus the centroid size (68.6 % of explained variance) showed a substantial overlap between age categories 3, 4 and 5, while only some specimens of age-class 5 overlapped with specimens of age-class 6 (Fig. 5b). Specimens of age-class 2 were separated only by centroid size as in the lateral

view of the skull (Fig. 5b). The analysis of the Procrustes distances between age categories was consistent with the results of the regression, where the only age-classes that showed statistically significant differences were 5 and 6 for both sexes (Fig. 5c; Table 1). Sexual dimorphism starts at age-class 6, both for shape and size (Fig. 5c, d; Table 1).

Ontogenetic trajectories in males and females

The three views of the skull and the mandible showed similar results. The ontogenetic convergence test, using common small and large sizes for both sexes, resulted in a constant distance between predicted shapes at both ends of the distribution, implying parallel ontogenetic trajectories (*p* > 0.05; Table 2). The test for shift in elevation (multivariate intercept test) was not significant for all the analyses, meaning that both sexes do not have differences in the elevation of the ontogenetic trajectory and have the same intercept (Table 2).

The dispersion along the ontogenetic trajectories (mean-squared error test) was equal for both sexes in three views (lateral, ventral and mandible), while for the dorsal view of the skull, the test was statistically significant, although at the limit of the significance level (*p* = 0.048; Table 2).

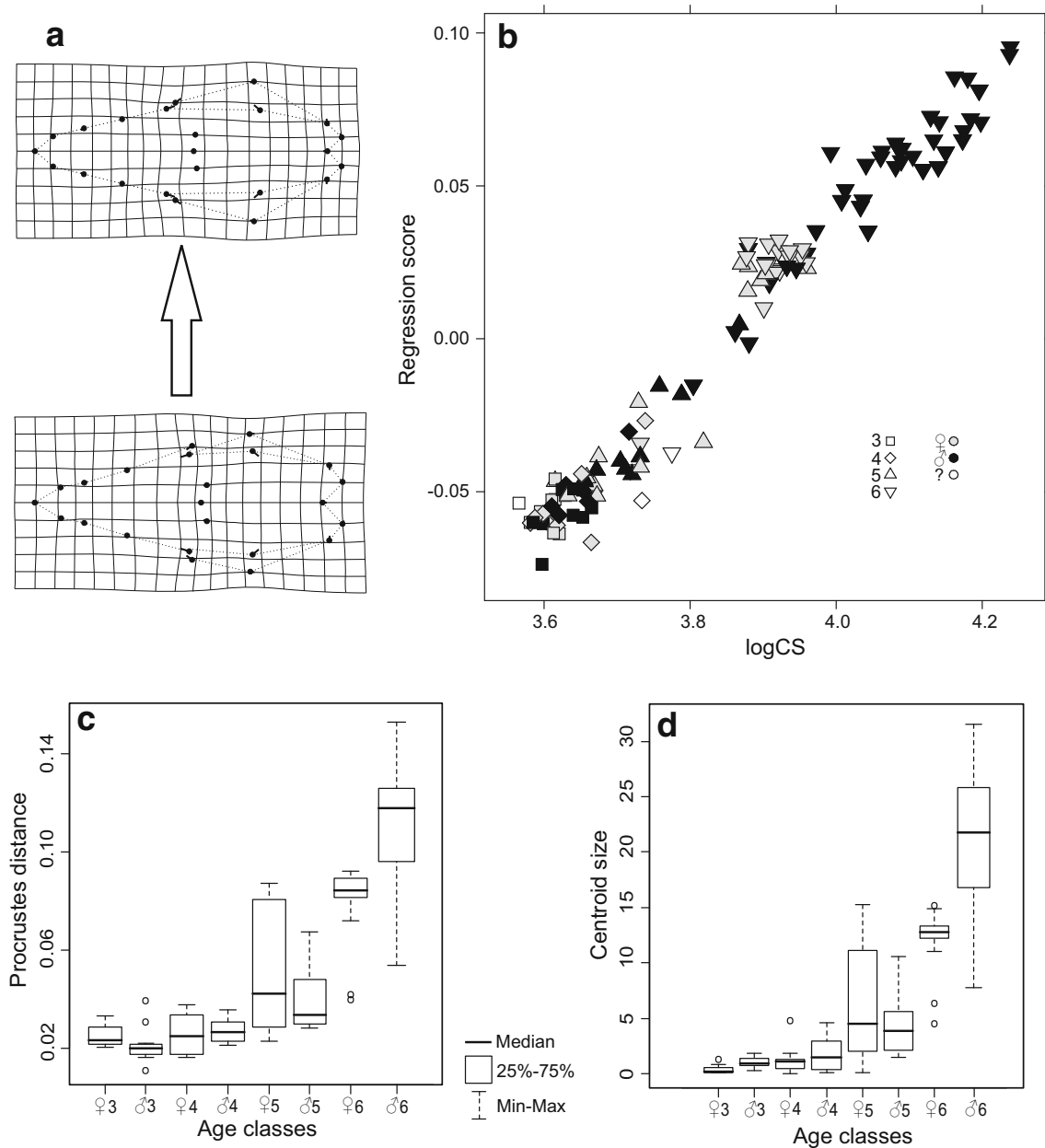


Fig. 3 Results from the morphometric analysis of the ventral view of the skull. **a** Shape changes from age-class 3 (*bottom*) to age-class 6 (*top*) with respect to the consensus shape. **b** Multivariate regression of the Procrustes coordinates against the log-transformed centroid size.

c Boxplots of Procrustes distance of each specimen of all age-classes respect to the mean of age-class 3. **d** Boxplots of centroid size versus age

Finally, the peramorphosis test was highly significant in all the cases (Table 2).

Discussion

The southern short-tailed opossum, *M. dimidiata*, is a species known not only for its semelparous life cycle, but also for the extreme sexual dimorphism of adults, where males are not only larger, but also have distinctive

morphological characters in their skull (Pine et al. 1985). Those distinctive characters, which include large canines, even led some authors to suggest that *M. dimidiata* could be treated as a pigmy sabertooth predator (Blanco et al. 2013); however, their analyses had some flaws, and the hypothesis was recently rejected by Chemisquy and Prevosti (2014).

Previous authors have analyzed the ontogenetic pattern of larger species of didelphids such as *Didelphis albiventris* Lund 1840, *D. marsupialis*, *Philander opossum*, *Lutreolina*

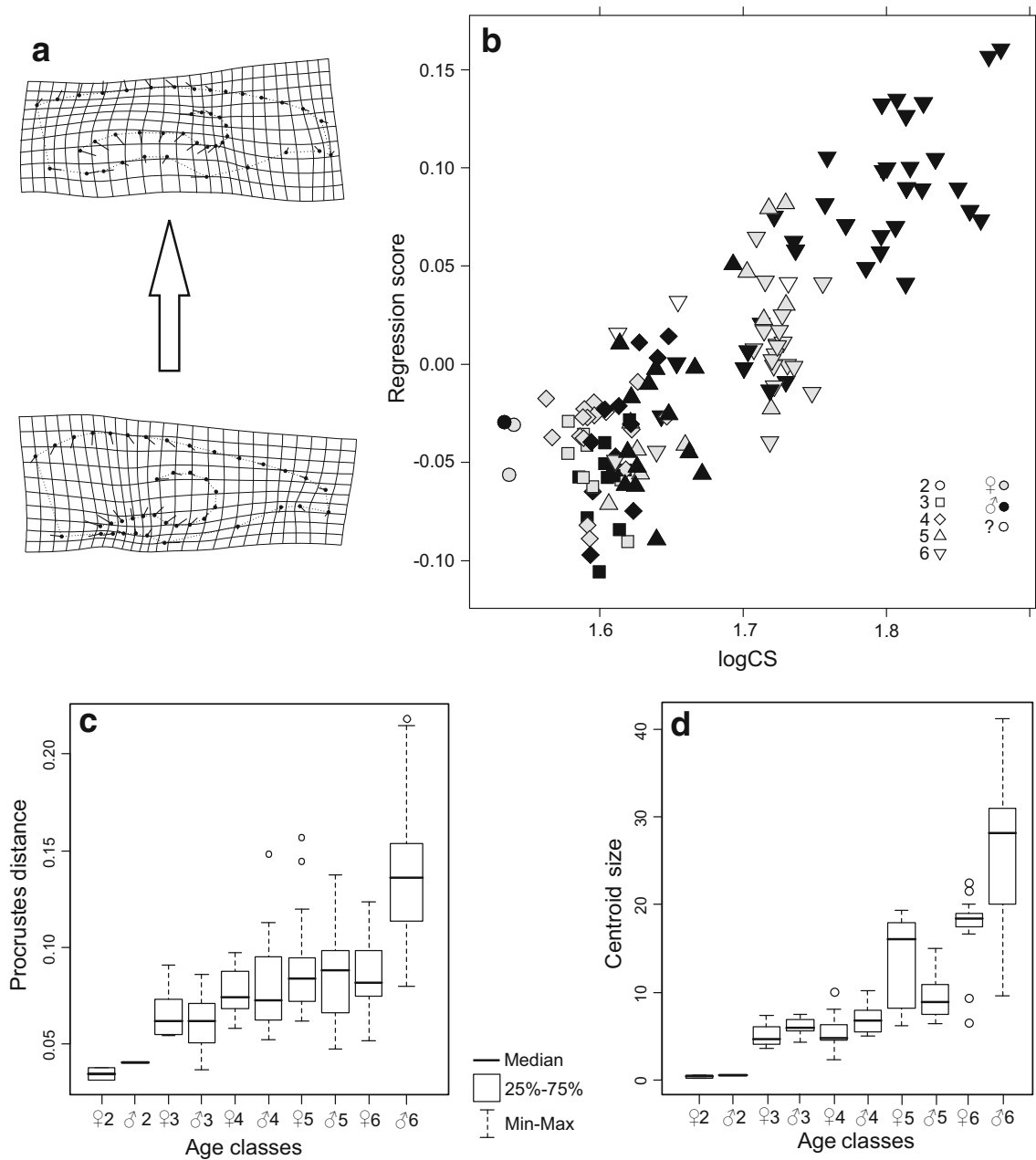


Fig. 4 Results from the morphometric analysis of the lateral view of the skull. **a** Shape changes from age-class 2 (bottom) to age-class 6 (top) with respect to the consensus shape. **b** Multivariate regression of the Procrustes coordinates against the log-transformed centroid size.

c Boxplots of Procrustes distance of each specimen of all age-classes with respect to the mean of age-class 2. **d** Boxplots of centroid size versus age

crassicaudata (Desmarest 1804) and *Caluromys philander* (Linnaeus 1758) (Abdala et al. 2001; Flores et al. 2003, 2010; Sebastião and Marroig 2013); based on those results, a general change pattern for the order was proposed (Flores et al. 2003; Sebastião and Marroig 2013); and also, the allometric tendencies of those didelphids and other marsupials were mapped on a phylogeny (Flores et al. 2013, 2014). In this context, the analysis of a mouse opossum is interesting, in order to see whether it follows the general

pattern reported for the larger species. Keeping in mind that the analyses performed previously are methodologically different from the analyses of this contribution, the ontogenetic trend of *M. dimidiata* can be compared with the findings of those authors.

One of the most important changes described previously is the relative reduction in the braincase (i.e., scales with a negative allometry with body size), which is considered as a general trend in vertebrates (Emerson and Bramble 1993)

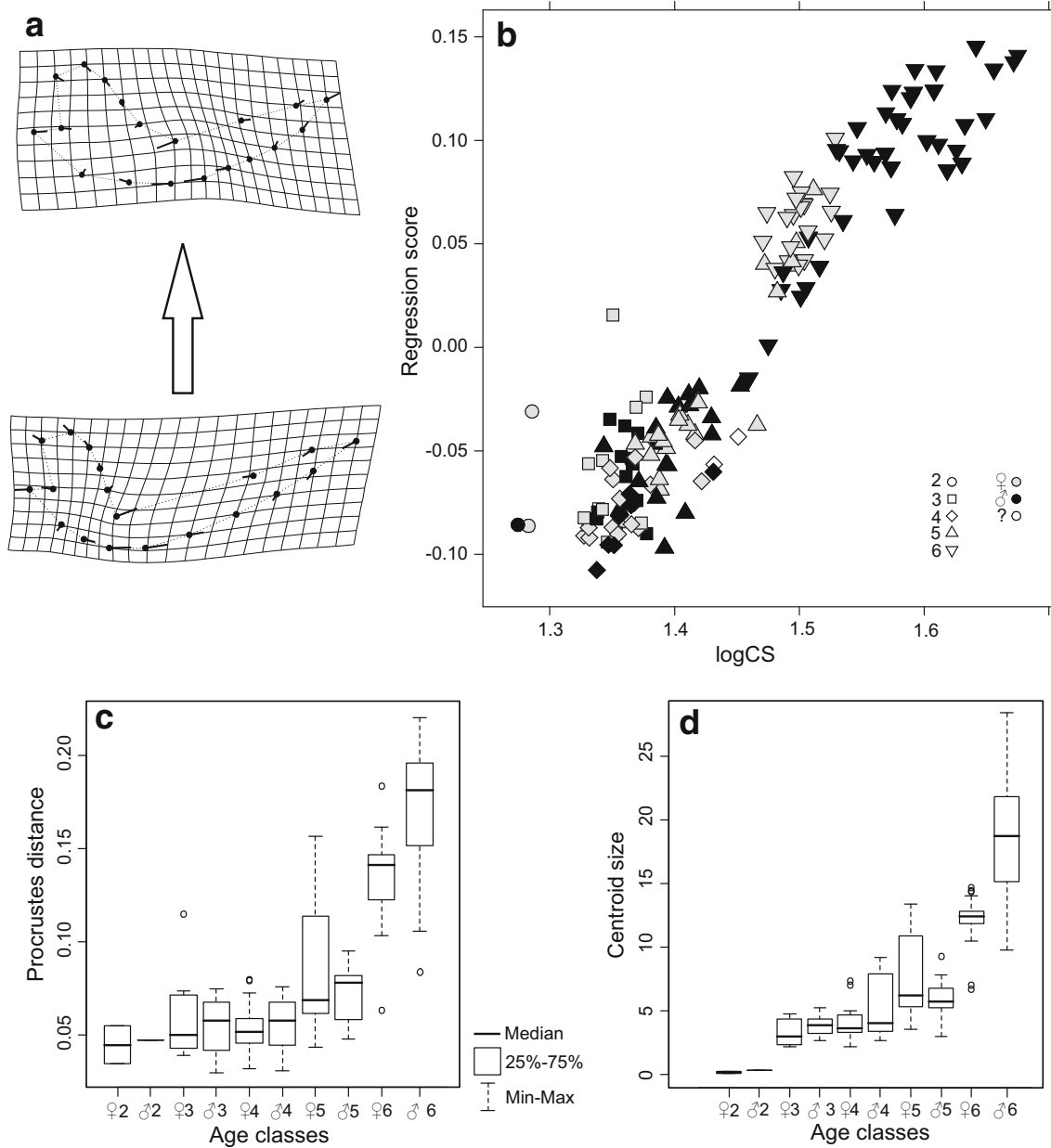


Fig. 5 Results from the morphometric analysis of the mandible. **a** Shape changes from age-class 2 (bottom) to age-class 6 (top) with respect to the consensus shape. **b** Multivariate regression of the Procrustes coordinates against the log-transformed centroid size.

c Boxplots of Procrustes distance of each specimen of all age-classes with respect to the mean of age-class 2. **d** Boxplots of centroid size versus age

Table 2 Results of male–female comparison of ontogenetic trajectories

	Dorsal	Lateral	Ventral	Mandible
Multivariate ontogenetic convergence test	0.129	0.056	0.229	0.077
Multivariate intercept test	0.182	0.072	0.283	0.264
Mean-squared error test	0.048	0.249	0.174	0.438
Peramorphosis test	0.001	0.001	0.001	0.001

Significant *p* values are given in bold

and is also present in *M. dimidiata*. If we add to the relative reduction in the braincase, the positive allometry of the zygomatic breadth (although the change is not as striking as other modifications), the enlargement of the temporal space described by Sebastião and Marroig (2013) also occurs during the ontogenetic development of *M. dimidiata*. The positive allometry of the zygomatic breadth was also described as a synapomorphy for didelphid marsupials (reversing in *Caluromys*) by Flores et al. (2013).

Mandible development also follows the pattern previously described for didelphids (Abdala et al. 2001; Flores et al. 2003, 2010, 2013) with a relative shortening of the postcanine tooth row, a relative heightening of the mandibular body, and a relative heightening and lengthening of the coronoid process, making the process more robust in adults. One interesting feature present in the ontogenetic development of *M. dimidiata* is the relative shortening of the anterior part of the mandible (i.e., landmarks 1, 2, 3 and semilandmarks 19, 18 and 17 are posteriorly displaced; Figs. 1d, 5a); however, the posterior displacement of the coronoid process makes the total length of the mandible isometric. Since none of the previous analyses which describe mandible ontogeny have a measurement which describes the anterior part of the mandibular body (Abdala et al. 2001; Flores et al. 2003, 2010), it is impossible to evaluate whether this is a unique feature of *M. dimidiata* or it is present in other marsupials. This shortening of the anterior part of the mandible throughout ontogeny has been previously described in short-faced carnivorans such as felids and hyenas (Tanner et al. 2010; Segura 2014). The biomechanical implications of this feature are discussed below.

The slenderization of the palate described previously for other didelphids (Abdala et al. 2001; Flores et al. 2003, 2010; Sebastião and Marroig 2013) appears to be absent in *M. dimidiata*, where the palate tends to become relatively more robust throughout ontogeny (although the changes are small). This is caused by the ontogenetic isometry of the palate breadth and the negative allometry of its length. Also, the rostrum in *M. dimidiata* becomes shorter throughout ontogeny instead of lengthening, as described for other didelphids (Sebastião and Marroig 2013; Flores et al. 2013), and the height of the muzzle becomes relatively larger instead of being relatively constant or shorter as reported for other marsupials (Flores et al. 2010). All these features combine to make the rostrum of an adult specimen of *M. dimidiata* more robust than the rostrum of a young one. The shortening of the rostrum, more extreme anteriorly, is accompanied by the relative shortening of the anterior part of the mandible described above.

Rostral length, as well as palate width and length, might have an effect on bite force and the biomechanical functioning of the masticatory apparatus (Thompson et al.

2003). *Monodelphis dimidiata* has an isometric growth of the palate and interglenoid widths (Fig. 3), so it is expected that the region of maximal bite force values will be located in the same area of the jaw in adults and juveniles (Thompson et al. 2003). It is possible that the negative allometries of the palate and the rostrum lengths are related to an increment of the bite strength in the anterior part of the dentition, rostral to the point of maximal bite force. Taking into account that adults of *M. dimidiata* develop large canines (Chemisquy and Prevosti 2014), it is possible that this increment in bite force is related to the use of those hypertrophied teeth. The increment on the height of the coronoid process can also contribute to a stronger bite by enlarging the temporalis movement arm (Radinsky 1981). The increment in bite strength could be related to diet changes in adults and is consistent with the stomach content analyses published by Goin et al. (1992) who reported a shift in the diet from mainly insectivores in juveniles and small adults (<60 g) to carnivores in large adults (more than 60 g).

Summing up, although *M. dimidiata* partly follows the ontogenetic pattern described for Didelphimorphia, one important feature such as rostral length escapes the general pattern, being shorter instead of lengthening throughout the development. More mouse opossums need to be studied in order to see whether this is a feature unique to this species, or whether it is something characteristic of the smallest species of the order.

Apart from the shape changes, the analyses showed significant sexual dimorphism in cranial size and shape in almost all the views sampled. Both for the skull and mandible, sexual dimorphism (size and shape) becomes statistically significant at age-class 6, where males are much larger than females and show a greater difference in shape, being the median of females outside (or almost outside) the 25–75 % percentile of males (Figs. 2c, d, 3c, d, 4c, d, 5c, d). In the dorsal view of the skull, shape sexual dimorphism is statistically significant at age-class 5, but in this case females have larger Procrustes distances from the younger specimens than males and are also bigger (Fig. 2c, d). This tendency (i.e., bigger females with larger Procrustes distances at age-class 5) is also present in the other views (except for the shape differences in the lateral view of the skull), but differences are not statistically significant.

Astúa (2010) analyzed cranial sexual dimorphism in New World marsupials using geometric morphometrics, including in his sample several species of *Monodelphis*. He found sexual size dimorphism in *M. brevicaudata*, *M. glirina* and *M. domestica*, while sexual shape dimorphism was only present in the last one. Astúa (2010) could not confirm sexual dimorphism in *M. dimidiata* since he only sampled one female, but he found it smaller than the males in all the views analyzed. Pine et al. (1985) reported sexual

size dimorphism for *M. dimidiata* based on weights and body measurements of animals captured in Balcarce (Buenos Aires Province, Argentina). The animals that presented sexual dimorphism belonged to the age-classes 6 and 7 used by Pine et al. (here considered as age-class 6). Similar results were reported by Bergallo and Cerqueira (1994) for *M. domestica*, where males were larger than females after age-class 6. It is noteworthy the amount of dimorphism present in *M. dimidiata*, where at age-class 6 the centroid size and Procrustes distances of males and females showed no overlap in their 25–75 % percentile (something similar was already reported for this species by Pine et al. 1985). A similar amount of size sex dimorphism could be inferred for *M. glirina* and *M. brevicaudata* based on the reports of Ventura et al. (1998) and Astúa (2010). However, the data available from those analyses do not allow a proper comparison with my results. None of the remaining species of new world opossums analyzed by Astúa (2010) showed such a disparity in centroid size between males and females.

Pine et al. (1985) also found that males were more variable than females in a set of cranial characters, which they associated with sexual dimorphism and the virtual cessation of growth after attaining sexual maturity in females. My results are consistent with that observation, since age-class 6 males exhibit a broader range of centroid size values and Procrustes distance values than females (Figs. 2c, d, 3c, d, 4c, d, 5c, d), implying that they not only are more variable in size but also in shape.

The analyses of the heterochronic patterns between males and females showed that although both sexes shared the same growth tendency, males exceed females. These results put the observations of the regression plots and the boxplots of centroid size and Procrustes distance in a statistical context, since the observation of those plots insinuates that males could be peramorphic relative to females. According to Gardner (1973) and Bergallo and Cerqueira (1994), during pregnancy and lactation, didelphid females stop growing since energetic resources are allocated in reproduction, while males continue to grow. In species that live for more than one cycle (e.g., *M. domestica*; see Bergallo and Cerqueira 1994), females continue to grow after the reproductive season, but they never reach male size. In the case of *M. dimidiata*, the sexual dimorphism is so strong that although females live longer than males (males die after reproduction, while females die after the weaning of the litter), their growth rate is not sufficient to reach male shape nor size.

As mentioned above, the amount of change in females between age-class 5 and age-class 6 was much smaller than that in males at that same period, being the difference in shape statistically significant for males but not for females (Figs. 2c, 3c, 4c, 5c; Table 1). This large difference in

distances could imply that males have a higher rate of growth than females during the transition between age-classes 5 and 6. This hypothesis of the change in rates is also supported by the fact that males die before females do (Baladrón et al. 2012), and consequently, they attain a larger and more different shape in less time. Since both sexes share the same ontogenetic trajectory (Table 2), it is possible that males undergo through a process of hypermorphosis, resulting in a peramorphic condition for males (or peratypic sensu Reilly et al. 1997). It is important to mention that, as stated above, the one-year life cycle of individuals make dental age a good proxy for the real age, reducing the possible biases in the estimation of the heterochronic processes. Another piece of evidence for the peramorphic condition of males (vs. a paedomorphic condition in females) comes from the results reported by Astúa (2010) and discussed above, where none of the other species that exhibit sexual dimorphism showed such a large difference between sexes, indicating that clearly *M. dimidiata* would be going beyond the ontogenetic pattern of didelphids.

Pine et al. (1985) proposed that the sexual dimorphism could be related to trophic niche segregation between males and females. However, this hypothesis could be discarded for *M. dimidiata* based on the observations of Goin et al. (1992) and González and Claramunt (2000), where both sexes were found to eat vertebrates in similar proportions. Instead, the existence of larger males could be related to reproductive success as the result of winning agonistic encounters with other males (Astúa 2010). In Australian semelparous species of *Antechinus*, it was reported that larger males fertilized more females (Kraaijeveld-Smit et al. 2003), and this was done not by female selection of larger males, but by dominance of larger males and their ability to survive longer and consequently being able of fertilizing more females (Fischer and Cockburn 2006). Since *M. dimidiata* females are thought to be polygynous (Baladrón et al. 2012), it is possible that the extreme sexual dimorphism present in this species is related to the same factors as in the Australian *Antechinus*, although behavioral analyses are needed to test that hypothesis.

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References

- Abdala F, Flores DA, Giannini NP (2001) Postweaning ontogeny of the skull of *Didelphis albiventris*. *J Mammal* 82:190–200
- Adams DC, Rohlf FJ, Slice DE (2004) Geometric morphometrics: ten years of progress following the ‘revolution’. *Ital J Zool* 71:5–16
- Astúa D (2010) Cranial sexual dimorphism in New World marsupials and a test of Rensch’s rule in Didelphidae. *J Mammal* 91:1011–1024
- Astúa de Moraes D, Hingst-Zaher E, Marcus LF, Cerqueira R (2000) A geometric morphometric analysis of cranial and mandibular shape variation of didelphid marsupials. *Hystrix* 10:115–130
- Baladrón AV, Malizia AI, Bó MS, Liébana MS, Bechard MJ (2012) Population dynamics of the southern short-tailed opossum (*Monodelphis dimidiata*) in the Pampas of Argentina. *Aust J Zool* 60:238–245
- Bergallo HG, Cerqueira R (1994) Reproduction and growth of the opossum *Monodelphis domestica* (Mammalia, Didelphidae) in northeastern Brazil. *J Zool (Lond)* 232:551–563
- Blanco RE, Jones WW, Milne N (2013) Is the extant southern short-tailed opossum a pigmy sabretooth predator? *J Zool* 291:100–110
- Bookstein FL (1991) Morphometric tools for landmark data: geometry and biology. Cambridge University Press, Cambridge
- Bookstein FL (1997) Landmark methods for forms without landmarks: morphometrics of group differences in outline shape. *Med Image Anal* 1:225–243
- Busch M, Kravetz FO (1991) Diet composition of *Monodelphis dimidiata* (Marsupialia, Didelphidae). *Mammalia* 55:619–621
- Chemisquy MA, Prevosti FJ (2014) It takes more than large canines to be a sabretooth predator. *Mastozool Neotrop* 21:27–36
- Emerson SB, Bramble DM (1993) Scaling, allometry and skull design. In: Hanken J, Hall BK (eds) *The skull*, vol 3. The university of Chicago Press, Chicago, pp 384–416
- Fischer DA, Cockburn A (2006) The large-male advantage in brown antechinuses: female choice, male dominance, and delayed male death. *Behav Ecol* 17:164–171
- Flores DA, Giannini NP, Abdala F (2003) Cranial ontogeny of *Lutreolina crassicaudata* (Didelphidae): a comparison with *Didelphis albiventris*. *Acta Theriol* 48:1–9
- Flores DA, Abdala F, Giannini NP (2010) Cranial ontogeny of *Caluromys philander* (Didelphidae: Caluromyinae): a qualitative and quantitative approach. *J Mammal* 91:539–550
- Flores DA, Abdala F, Giannini N (2013) Post-weaning cranial ontogeny in two bandicoots (Mammalia, Peramelomorpha, Peramelidae) and comparison with carnivorous marsupials. *Zoology* 116:372–384
- Flores DA, Abdala F, Martin GM, Giannini NP, Martinez JM (2014) Post-weaning cranial growth in shrew opossums (Caenolestidae): a comparison with bandicoots (Peramelidae) and carnivorous marsupials. *J Mammal Evol*. doi:10.1007/s10914-014-9279-0
- García LV (2004) Escaping the Bonferroni iron claw in ecological studies. *Oikos* 105:657–663
- Gardner AL (1973) The systematics of the genus *Didelphis* (Marsupialia: Didelphidae) in North and Middle America. *Spec Publ Mus Texas Tech Univ* 4:1–81
- Goin FJ, Velázquez C, Scaglia O (1992) Orientación de las crestas cortantes en el molar tribosfénico. Sus implicancias funcionales en didelfoideos (Marsupialia) fósiles y vivientes. *Rev Mus La Plata (Nueva Ser)* 9:183–198
- González EM, Claramunt S (2000) Behaviors of short-tailed opossums, *Monodelphis dimidiata*. *Mammalia* 64:271–285
- Klingenberg CP (2011) MorphoJ: an integrated software package for geometric morphometrics. *Mol Ecol Resour* 11:353–357
- Klingenberg CP, Barluenga M, Meyer A (2002) Shape analysis of symmetric structures: quantifying variation among individuals and asymmetry. *Evolution* 56:1909–1920
- Kraaijeveld-Smit FJL, Ward SJ, Temple-Smith PD (2003) Paternity success and the direction of sexual selection in a field population of a semelparous marsupial, *Antechinus agilis*. *Mol Ecol* 12:475–484
- Maunz M, German RZ (1996) Craniofacial heterochrony and sexual dimorphism in the short tailed opossum (*Monodelphis domestica*). *J Mammal* 77:992–1005
- Pine RH, Handley CO Jr (2007) Genus *Monodelphis*. In: Gardner AL (ed) *Mammals of South America*, vol 1. The University of Chicago Press, Chicago, pp 82–107
- Pine RH, Dalby PL, Matson JO (1985) Ecology, postnatal development, morphometrics, and taxonomic status of the short-tailed opossum, *Monodelphis dimidiata*, an apparently semelparous annual marsupial. *Ann Carnegie Mus* 54:195–231
- Piras P, Salvi D, Ferrara G, Maiorino L, Delfino M, Pedde L, Kotsakis T (2011) The role of post-natal ontogeny in the evolution of phenotypic diversity in *Podarcis* lizards. *J Evol Biol* 24:2705–2720
- R Development Core Team (2014) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. <http://www.R-project.org>. Accessed 8 Mar 2015
- Radinsky LB (1981) Evolution of skull shape in carnivores 1. Representative modern carnivores. *Biol J Linn Soc* 15:369–388
- Reig OA (1964) Roedores y marsupiales del partido de General Pueyrredón y regiones adyacentes (Provincia de Buenos Aires, Argentina). *Publ Mus Mun Cienc Nat, Mar del Plata* 1:203–224
- Reig OA (1965) Datos sobre la comunidad de pequeños mamíferos de la región costera del Partido de General Pueyrredón y los partidos limítrofes (Prov. de Buenos Aires, Argentina). *Physis* 25:205–211
- Reilly SM, Wiley EO, Meinhardt DJ (1997) An integrative approach to heterochrony: the distinction between interspecific and intraspecific phenomena. *Biol J Linn Soc* 60:119–143
- Rohlf FJ (2008a) TpsDig, ver. 2.12. Department of Ecology and Evolution, State University of New York at Stony Brook, Stony Brook
- Rohlf FJ (2008b) TpsRelw, ver. 1.46. Department of Ecology and Evolution, State University of New York at Stony Brook, Stony Brook
- Sebastião H, Marroig G (2013) Size and shape in cranial evolution of 2 marsupial genera: *Didelphis* and *Philander* (Didelphimorphia, Didelphidae). *J Mammal* 94:1424–1437
- Segura VA (2014) Ontogenia craneana postnatal en cánidos y félidos neotropicales: funcionalidad y patrones evolutivos. Dissertation, Universidad Nacional de La Plata
- Sheets HD (2003) IMP-integrated morphometrics package. Department of Physics, Canisius College, Buffalo
- Sheets HD, Zelditch ML (2013) Studying ontogenetic trajectories using resampling methods and landmark data. *Hystrix* 24:67–73
- Smith, P (2008) FAUNA Paraguay Handbook of the Mammals of Paraguay Number 26 *Monodelphis sorex*. www.faanaparaguay.com/monodelphissorex.html. Accessed 15 Dec 2014
- Tanner JB, Zelditch ML, Lundrigan BL, Holekamp KE (2010) Ontogenetic change in skull morphology and mechanical advantage in the spotted hyena (*Crocuta crocuta*). *J Morphol* 271:353–365
- Thompson EN, Biknevicius AR, German RZ (2003) Ontogeny of feeding function in the gray short-tailed opossum *Monodelphis domestica*: empirical support for the constrained model of jaw biomechanics. *J Exp Biol* 206:923–932
- Ventura J, Pérez-Hernández R, López-Fuster MJ (1998) Morphometric assessment of the *Monodelphis brevicaudata* group (Didelphimorphia: Didelphidae) in Venezuela. *J Mamm* 79:104–117

- Vilela JF, De Moraes Russo CA, De Oliveira JA (2010) An assessment of morphometric and molecular variation in *Monodelphis dimidiata* (Wagner, 1847) (Didelphimorphia: Didelphidae). *Zootaxa* 2646:26–42
- Zelditch ML, Swiderski DL, Sheets HD (2012) A practical companion to geometric morphometrics for biologists: running analyses in freely-available software. <http://booksite.elsevier.com/9780123869036/>. Accessed 14 May 2015