

Cranial ontogeny of *Lutreolina crassicaudata* (Didelphidae): a comparison with *Didelphis albiventris*

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Metatherians experience the greatest developmental changes during extrauterine life. Following previous studies on *Didelphis albiventris* Lund, 1840, we examined the postweaning cranial allometry of size of the Neotropical marsupial *Lutreolina crassicaudata* (Desmarest, 1804). Our aim was to compare growth patterns of both species to identify traits particular to each species and traits common to both species. This may contribute toward identifying a common developmental plan for didelphids. We measured 15 cranial variables in 32–43 specimens from just-weaned young to old adult. Total length of the skull was the estimator of overall size in least squares and reduced major axis regressions. The skull of *Lutreolina crassicaudata* grows at a rate slower than the overall change in size in its neurocranial components, palate, and postcanine rows, and it grows relatively faster in the rest of the splanchnocranium. This pattern closely resembles that of *Didelphis albiventris*, from which it differs mainly in the allometry of the muzzle. In both species, allometry explains most postweaning changes of the trophic apparatus on functional grounds, in relation to interspecific differences in diet. We hypothesize that most local allometric departures from a generalized didelphid plan would relate to main dietary trends.

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Introduction

The reproductive strategy of marsupials involves a short gestation period followed by a long lactation, in which the young are attached to the mother's nipple (Lillegraven 1976). After weaning, when young have been released from their physical bond, a critical period of growth begins. The young are no longer nursed on a permanent basis and initiate dietary components typical of adults.

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Abdala *et al.* (2001) studied the ontogeny of the skull of *Didelphis abiventris* Lund, 1840 during the postweaning period. By a combination of allometry and qualitative observations, the analysis of cranial growth of this species of *Didelphis* revealed that many osteological modifications were clearly related to the change from lactation to adult trophic function. The morphological transformation continued during the whole life at a constant logarithmic rate in most cranial areas. *Didelphis* was an important choice for Abdala *et al.* (2001) since the genus is regarded as a conservative form extensively used as a model in studies of the function of mammalian skull complexes (see Crompton and Hiiemae 1970, Crompton and Hylander 1986, Crompton 1989).

In this work, we analyze the postweaning cranial growth of *Lutreolina crassicaudata* (Desmerest, 1804) within the comparative frame provided by the study of *Didelphis albiventris* (Abdala *et al.* 2001). *Lutreolina* is a distinct genus of Neotropical marsupials with a single extant species closely related to *Didelphis* (Jansa and Voss 2000). By comparing the allometry of the skull in both species, it is possible to discriminate the growth traits particular to each species from the traits that may conform a common ground to the developmental plan of Neotropical marsupials. Identification of that basic plan is relevant because specialization in the rest of marsupials can be seen as evolutionary departures from generalized didelphid-like forms. Thus, with our comparison between two species, we provide a hypothesis of postweaning growth that can be tested in the presence of new data from other didelphids.

Material and methods

We studied 43 skulls of *Lutreolina crassicaudata*. Specimens are stored in Argentina at Colección Mamíferos Lillo (CML), Museo Argentino de Ciencias Naturales (MACN), and Instituto Argentino de Investigaciones en Zonas Áridas (IADIZA). The list of specimens examined follows: IADIZA 3299, 3302, 3304, 3428, 3429, 3297, 3296, 3427, 3298, plus 1 uncataloged specimen; CML 802, 803, 805, 806, 807, 809, 810, 811, 918, 919, 1353; MACN 43.21, 43.37, 43.73, 52.76, 13069, 13265, 13285, 13713, 14904, 15365, 17267, 17268, 17923, 19190, 19191, 20809, 30248, 32233, 34547, 39466, 45101, 49242. Specimens ranged from 36.7 mm (a just-weaned juvenile with M² erupting) to 83.6 mm (an adult with heavily worn dentition) in total length of skull. Although males tend to be larger than females (Graipel *et al.* 1996), both sexes were pooled together in a single sample because overlaid regression lines of each sex were virtually identical by visual inspection in all variables.

We observed 20 qualitative features of the skull of *Lutreolina crassicaudata* and contrasted the aspect of each trait between a set of the youngest and a set of the oldest specimens. For comparative purposes, we observed the same traits as Abdala *et al.* (2001) in *Didelphis albiventris*, including selected areas of both the neurocranium and splanchnocranium.

Fourteen variables representing longitudinal and transversal dimensions of the skull itself and its main components (Fig. 1) were submitted to an analysis of allometry of size. We defined the total length of the skull as a linear estimator of overall size (Simpson *et al.* 1960, Emerson and Bramble 1993). The equation used was the power function with error term assumed multiplicative. The function was linearized via a log (base 10) transformation of both members (Alexander 1985). This yields an expression of the form:

$$\log y = \log b_0 + b_1 \log x + \log e$$

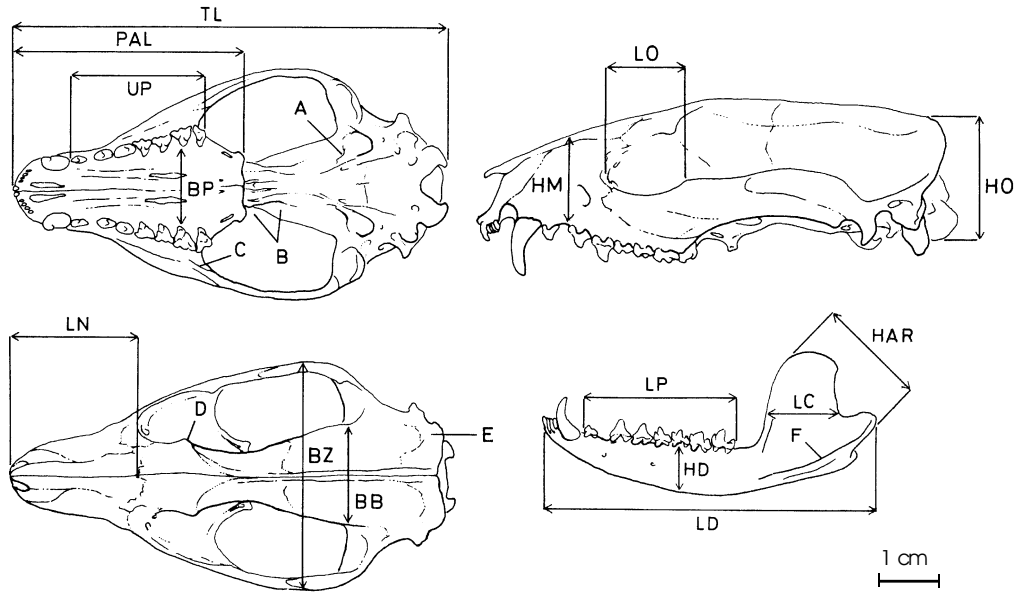


Fig. 1. Cranial measurements of *Lutreolina crassicaudata*. TL – total length of the skull, PAL – length of palate, UP – length of upper postcanine row, BP – breadth of palate, BZ – breadth of zygoma, BB – breadth of braincase, LN – length of nasals, HO – height of occipital plate, LO – length of orbit, HM – height of muzzle, HAR – height of ascending ramus of the dentary, LC – length of coronoid process of the dentary, LD – length of dentary, HD – height of the dentary at the level of second lower molar, LP – length of lower postcanine row. These measurements were used in our study of allometry. Some of the qualitative traits analyzed: A – site of the secondary foramen ovale, covered by alisphenoid wall; B – sphenorbital region; C – maxillary boss for the origin of the superficial masseter muscle; D – supraorbital process; E – lambdoidal crest; F – inferior masseteric line (this line, in dorsal view, is recurved outwardly).

where y is any of the measured skull variables, b_0 is the y -intercept, b_1 is the slope of the line or coefficient of allometry, x is the total length of the skull, and e is the error term. Model estimation was done by using both least squares (LS) and reduced major axis (RMA) methods (Niklas 1994, Abdala *et al.* 2001). The latter is likely more appropriate since the total length of the skull is indeed estimated with error. However, the change of all variables can be thought of as dependent on overall size, which is represented by the total length of the skull. This means that a least squares model may be suitable as well. Therefore, we report results using both methods, which are arithmetically related (Niklas 1994). Alpha level was set at 0.003 (Bonferroni correction of the nominal 5% alpha divided by 14, ie the number of regressions performed using the same sampling units; see Hair *et al.* 1995).

We also estimated departures from isometry by setting the null allometry coefficient to 1.0 – the expected slope for equal rate of change in 2 related linear variables. A coefficient significantly < 1.0 is said to be ‘negatively’ allometric, whereas the converse holds for ‘positive’ departures (Emerson and Bramble 1993). Values of p in the range 0.01–0.003 were considered to support a marginally significant trend, likely requiring a greater sample size.

Results and discussion

Qualitative comparisons

Lutreolina crassicaudata shares most developmental traits with *Didelphis albiventris* in the neurocranium. In juveniles of both species the postorbital constriction and a secondary foramen ovale are lacking, the petrosal is relatively large, the dorsal margin of the foramen magnum is formed by the interparietal (= supraoccipital) instead of the exoccipitals, and the exoccipitals are only partially fused to the basioccipital.

Some differences were apparent as well. First, in *L. crassicaudata*, the supra-orbital border of the frontal develops earlier so that the juvenile (eg specimens with definitive upper third premolar and upper fourth molar not yet fully erupted) shows an even greater process as compared to an adult specimen of *Didelphis albiventris*. Second, in adults of *L. crassicaudata*, the sphenorbital fissure is close to both the foramen rotundum and the ethmoidal foramen. In adults of *D. albiventris*, the sphenorbital fissure is separated from the foramen rotundum by an elongated wall of the alisphenoid, and is separated from the ethmoidal foramen by a wall of the orbitosphenoid (Abdala *et al.* 2001). Third, an important difference between *L. crassicaudata* and *D. albiventris* relates to the completion of the secondary foramen ovale in adults. It is fully closed by the alisphenoid, with no trace of the primary foramen ovale, in *L. crassicaudata*. It is not entirely closed in *Didelphis marsupialis*, and it is variable in *D. albiventris* and *D. virginiana*, in which the primary or secondary foramina can be present. Probably, the secondary foramen ovale is completed by membranaceous tissue in the latter two species. This character deserves a more detailed examination, since Wroe (1997) assigned a considerable phylogenetic significance to its variation in dasyuromorphians. Fourth, the portion of the occipital plate surrounding the foramen magnum is more protruded backwardly in *L. crassicaudata* than in any other extant didelphid. Fifth, the anterior edge of the orbit is located at the level of (either deciduous or definitive) upper third premolar in both the young and adult *L. crassicaudata* (a similar condition to the young *D. albiventris*), whereas it is displaced backwards to the level of the upper first molar in the adult *D. albiventris*.

In the splanchnocranium, there were also a majority of similarities and a few differences. The sagittal crest, the paraoccipital process, the maxillary boss for the origin of the superficial masseter, the condyloid process, the curvature of the inferior masseteric line, and the palatal vacuities are poorly developed in young of both *L. crassicaudata* and *D. albiventris*. By contrast, the lambdoidal crest in *L. crassicaudata* starts growing earlier than that of *D. albiventris* (juveniles with incomplete definitive dentition show a crest of a size comparable to that of an adult *D. albiventris*), and achieves a final size comparatively greater in adults. In *L. crassicaudata*, the highly developed lambdoidal crest, which provides the origin for the posterior fibers of the temporal muscle, only compares to some fossil didelphids such as *Hyperdidelphis* (Goin and Pardiñas 1996). The nasals remain

wide in adults of *L. crassicaudata*. In contrast, in *D. albiventris* the nasals become slender and narrow in adulthood. The maxillary boss for the origin of the superficial masseter is relatively weaker in adults of *L. crassicaudata* in comparison to *D. albiventris*. The inferior masseteric line becomes recurved outwards in adult *L. crassicaudata*, likely increasing the insertion area of the superficial masseter muscle, whereas it remains straight in *D. albiventris*.

Allometry and feeding habits

Size, as estimated by the total length of the skull, significantly explained the variation of all cranial measurements (Table 1). The range of adjusted R^2 values (0.54–0.98, most > 0.80) was virtually identical to that reported for *D. albiventris*. Residuals did not show systematic trends, and no outliers were detected. Both regression methods yielded compatible results except for the length of the lower postcanine row. LS results suggest a (marginally significant) ‘negative’ allometric trend, whereas the trend is ‘positive’ after RMA results. This is expectable because precisely this measurement is the one with the greatest variability, and hence, the one with the lowest R^2 . Since RMA coefficient is obtained by dividing least squares coefficient by the corresponding Pearson’s correlation coefficient (Niklas 1994), lower R^2 mean lower Pearson’s r , and consequently, greater difference between least squares and RMA coefficients. In another two cases, RMA failed to detect the ‘negative’ allometric trend suggested by least squares (length of nasals and height of occipital plate; Table 1). In all the other variables both methods arrived at the same results. This suggests that increasing of the sample size would be advisable for the 3 variables that show differences.

The most striking differences in cranial allometry between *L. crassicaudata* and *D. albiventris* are in the muzzle. The length of nasals is either isometric (RMA) or ‘negative’ (LS) in *L. crassicaudata*, whereas it is ‘positive’ in *D. albiventris*; and the height of muzzle is isometric in *L. crassicaudata*, and ‘negative’ in *D. albiventris*. Altogether, this yields a short and high muzzle in *L. crassicaudata*. This is a species-specific configuration that is produced by the retarded growth of the nasal area, which becomes short in length, and the continuous growth in height of the maxillary region. By contrast, in *D. albiventris*, the slender muzzle results from a ‘positive’ growth in length (as expressed by the nasals) and a deceleration of the growth in height of the maxillary region (‘negative’ allometry of the height of muzzle).

Another difference is the height of the ascending ramus of the mandible, which is isometric in *L. crassicaudata* and strongly ‘positive’ in *D. albiventris*. This means that adults of *D. albiventris* achieve a coronoid higher than expected by its size. It may imply differential performance in occlusal muscles and trophic function. This idea is reinforced by the differences in the maxillary boss for the origin of the superficial masseter, the development of the lambdoidal crest, and the shape of the inferior masseteric line.

Table 1. Regression models and summary statistics of the logarithm (base 10) of each cranial variable of *Lutreolina crassicaudata*, calculated by least squares (LS) method or via reduced major axis (RMA) method. Independent variable was total length of the skull. Abbreviations: n – sample size, R^2 – adjusted coefficient of determination, $\log b_0$ – y -intercept, b_1 – coefficient of allometry or slope of the regression line, t_{iso} – one-tailed Student’s t -value under the null hypothesis of isometry (expected null coefficient is 1; $\text{df} = n - 2$), P_{iso} – P -value for t_{iso} . Last three columns present allometric trends in *L. crassicaudata* (results from LS and RMA) and *Didelphis albiventris* (results from LS, Abdala *et al.* 2001). Sign “+” indicates ‘positive’ allometry or rate of growth of corresponding variable greater than 1 (unity is the value expected under isometry), sign “-” indicates ‘negative’ allometry or rate of growth of the variable lesser than 1, sign “=” stands for isometry of the variable, ie rate of growth statistically not different from 1, sign in brackets indicates marginally significant. ^a All Student’s t -values ≥ 6.1 ; associated $p < 0.001$, with degrees of freedom $n - 2$.

Variables	n	Least squares						RMA			Summary of allometry		
		R^2	$\log b_0$	b_1^a	t_{iso}	P_{iso}	b_1^a	t_{iso}	P_{iso}	LS	RMA	<i>Didelphis albiventris</i>	
Length of nasals	43	0.89	-0.21	0.86	-2.91	0.0029	0.95	-1.04	0.1519	-	=	+	
Height of muzzle	32	0.91	-0.59	0.94	-1.06	0.1494	1.01	0.18	0.4294	=	=	-	
Length of palate	39	0.98	-0.16	0.95	-2.27	0.0147	0.96	-1.64	0.0551	=	=	=	
Breadth of palate	40	0.77	0.47	0.46	-13.17	0.0001	0.58	-10.24	0.0001	-	-	-	
Length of upper postcanine row	43	0.92	-0.04	0.80	-5.40	0.0001	0.87	-3.67	0.0003	-	-	-	
Length of lower postcanine row	32	0.54	0.13	0.73	-2.33	0.0135	1.32	2.69	0.0057	(-)	+	-	
Length of dentary	42	0.97	-0.16	1.03	1.08	0.1440	1.03	1.28	0.1045	=	=	+	
Height of dentary	42	0.82	-1.45	1.27	2.92	0.0029	1.37	3.95	0.0002	+	+	+	
Height of coronoid	35	0.92	-0.59	1.04	0.76	0.2273	1.05	1.06	0.1480	=	=	+	
Length of coronoid	42	0.86	-1.36	1.32	3.80	0.0002	1.37	4.40	0.0001	+	+	+	
Breadth of zygoma	39	0.90	-0.21	0.96	-0.88	0.1934	0.99	-0.28	0.3921	=	=	=	
Breadth of braincase	43	0.71	0.52	0.40	-15.55	0.0000	0.50	-12.76	0.0001	-	-	-	
Height of occipital plate	36	0.80	-0.28	0.81	-2.72	0.0052	0.90	-1.49	0.0729	(-)	=	-	
Length of orbit	42	0.74	-0.13	0.66	-5.51	0.0001	0.88	-1.97	0.0279	-	-	-	

The assessment of the actual functional differences must await a biomechanical analysis. However, lines of evidence support a trend towards increased carnivory in *L. crassicaudata*. Specifically, the paracristids and metacristids are long and oriented more in parallel to the toothrow (Goin *et al.* 1992), a disposition appropriate for slicing meat (Van Valen 1969). In contrast, *D. albiventris* shows an intermediate condition between more carnivorous species (*L. crassicaudata*) and more insectivorous species (eg *Thylamys*; Goin *et al.* 1992). Additionally, Emerson and Radinsky (1980) hypothesized that the backward protrusion of the occipital plate could be related to a greater potential for dorsiflexion of the head on the neck, perhaps related to maximizing gape. Observations both in the field and in captivity assign mainly predatory habits to *L. crassicaudata* (Walker 1975, Eisenberg 1989, Mares *et al.* 1989, Barquez *et al.* 1991, Redford and Eisenberg 1992).

The basic didelphid plan

Lutreolina crassicaudata shares most postweaning growth patterns with *Didelphis albiventris*. By integrating the results and functional interpretations from the present paper for *L. crassicaudata* with those from Abdala *et al.* (2001) for *D. albiventris*, we attempt to outline a common developmental ground for the didelphids, as follows.

(1) The neurocranium is negatively allometric. The condition of the foramina and the anterior edge of the orbit in adult *L. crassicaudata* resemble that of the young *D. albiventris*. This suggests that completion of some growth processes may depend on final adult size.

(2) Changes in splanchnocranial components are heterogeneous. While the animal grows, crests expand as to provide origin or attachment for masticatory muscles. The mandible is thickened, and the lateral development of the condyloid process enhances the mandibular joint and reduces the lateral rotation. Mandible construction proceeds in a complex way, with the length of dentary isometric, height of dentary positively allometric, and the length of the lower postcanine series tending to be negatively allometric. The pattern of tooth emergence, which is particular to didelphids, affects mandibular growth in such a way that the lower postcanine series grows at a slower rate than the upper series, until the same length is achieved in both series at adulthood (see Abdala *et al.* 2001 for a detailed account of the process).

The palate grows with exactly the same pattern in both *L. crassicaudata* and *D. albiventris*. The width of the posterior palate is negatively allometric but the total palate length is isometric. This means that young have a short and wide palate that supports the tongue during suckling (Abdala *et al.* 2001). This condition changes as lactation is replaced by mastication (adults have a long and narrow palate).

(3) The neurocranium and the splanchnocranium interact to provide space for the large temporal muscles, principally via the isometry of the breadth of the zygoma and the negative allometry of the braincase.

Here we provide the common growth patterns of two different genera. This may conform the basic plan of didelphids, which is verifiable against new data from other didelphids. We hypothesize that major departures from this basic plan will correlate mainly with dietary trends, as we have seen in *L. crassicaudata*. Therefore, species may show particular patterns of postweaning growth in relation to omnivory, carnivory, and insectivory – the main dietary trends in didelphids.

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