
Article

Orbit orientation in didelphid marsupials (Didelphimorphia: Didelphidae)

Patricia PILATTI and Diego ASTÚA*

Laboratório de Mastozoologia, Departamento de Zoologia, Universidade Federal de Pernambuco. Av. Prof. Moraes Rego S/N, Cidade Universitária, Recife, PE 50670-901, Brazil

*Address correspondence to Diego Astúa. E-mail: diegoastua@ufpe.br; d.a.moraes@gmail.com.

Received on 9 January 2016; accepted on 25 May 2016

Abstract

Usually considered a morphologically conservative group, didelphid marsupials present considerable variation in ecology and body size, some of which were shown to relate to morphological structures. Thus, changes on orbit morphology are likely and could be related to that variation. We calculated orbit orientation in 873 specimens of 16 Didelphidae genera yielding estimates of orbits convergence (their position relative to midsagittal line) and verticality (their position relative to frontal plane). We then compared similarities in these variables across taxa to ecological, morphological and phylogenetic data to evaluate the influencing factors on orbit orientation in didelphids. We found an inverse relation between convergence and verticality. Didelphids orbits have low verticality but are highly convergent, yet orbit orientation differs significantly between taxa, and that variation is related to morphological aspects of the cranium. Rostral variables are the only morphological features correlated with orbit orientation: increasing snout length yields more convergent orbits, whereas increase on snout breadth imply in more vertical orbits. Size and encephalization quotients are uncorrelated with orbit orientation. Among ecological data, diet showed significant correlation whereas locomotion is the factor that less affects the position of orbits. Phylogeny is uncorrelated to any orbital parameters measured. Ecological factors seemingly play a more important role on orbit orientation than previously expected, and differentiation on orbit orientation seems to be more functional than inherited. Thus, despite the apparent homogeneity on didelphid morphology, there is subtle morphological variability that may be directly related to feeding behavior.

Key words: convergence, Didelphidae, frontation, orbit orientation, verticality.

The orientation of the orbits can be estimated by 2 main angular measurements: convergence and frontation (or verticality). Convergence refers to more or less convergent or divergent orbit orientation. Highly convergent orbits point to the same direction, converging to the midsagittal plane of the cranium, whereas divergent orbits are more laterally directed. Frontation or verticality refers to more or less dorsal orientations of orbits relative to the cranium. Its formal designation is based on measurement methodology, using nasion–inion chord for frontation or the cranium frontal plane for verticality to compare with orbital plane. Orbits with high frontation/verticality levels are oriented perpendicular to the frontal

plane, so facing the snout and are related to a vertical cranial posture. Low levels of frontation/verticality mean more horizontal orbits that are positioned more dorsally in the cranium (Cartmill 1972; Noble et al. 2000; Heesy 2004, 2005, 2008).

Orbital convergence results in the overlap of the visual fields of each eye. High orbital convergence leads to the superposition of large areas of each visual field, thus yielding a wider area with stereoscopic vision (Heesy 2004). This in turn increases the visual acuity, because it improves eye light sensibility, definition of contrasts and brightness, quality of the image projected on retina, as well as the perception of depth or tridimensionality (Pettigrew et al.

1988; Ross et al. 2007). In mammals with low orbital convergence (i.e., high orbital divergence), the visual field overlap is smaller and the final visual field is wide to panoramic (Heesy 2004).

The earliest studies on orbit orientation focused on Primates. Their cranial morphological features associated with the visual system are conspicuous due to highly specialized conditions (Ross and Kirk 2007). The eyes face forward in almost all primate species, a distinct condition between mammals. These studies focused on peculiar primate features, such as the visual apparatus, to help understanding their origins and evolutionary history (Jones 1916; Smith 1924). Thus, the visual system morphology was initially related to arboreal habits.

The orbit orientation in Primates was seen as an adaptation for stereoscopic vision, an essential feature to locomotion on the arboreal environment as a response to accuracy on visual estimates of distance and direction needs (the arboreal theory; Cartmill 1997). On the other hand, ongoing comparative studies suggest that visual adaptations relating to primate origins, including orbit orientation, are a consequence of nocturnal predation issues, rather than an arboreal hypothesis. It is because these adaptations associated with a better image quality and visual acuity would be important features in low light environments to distinguish camouflaged preys as much as to pursuing and catching moving prey (the nocturnal visual predation hypothesis [NVPH]; Cartmill 1972; Ross et al. 2007). Both hypotheses are still under debate nowadays (Noble et al. 2000; Ross and Kirk 2007; Heesy 2008).

Subsequent studies on orbit orientation (Noble et al. 2000; Ravosa et al. 2000; Heesy 2004, 2005, 2008; Finarelli and Goswami 2009) included not only representatives of Primates, but also species of varied marsupial (Didelphimorphia, Dasyuromorphia, and Diprotodontia) and eutherian orders (Artiodactyla, Carnivora, Chiroptera, Cingulata, Dermoptera, Erinaceomorpha, Hyracoidea, Macroscelidea, Perissodactyla, Pilosa, Rodentia, and Scandentia), as well as birds (Iwaniuk et al. 2008). Because they addressed a wider group of taxa, studies on orbital orientation that included didelphids presented taxonomically limited sample (relative to the diversity of living didelphid species). The most taxon-rich study up to date used 15 species, representing 8 genera, with 63 specimens sampled (Heesy 2005; 2008).

Didelphidae, the single family of the order Didelphimorphia, are New World marsupials forming a monophyletic group with 18 genera and more than 100 species, and represent currently the greater taxonomic radiation of marsupials out of Australasian region, ranging from southern Canada to Patagonia occupying most vegetation types in that range (Voss and Jansa 2009; Astúa 2015a).

In marsupials, orbits are highly convergent, but with little verticality: they are convergent but much more dorsal than frontal (Heesy 2008). Compared with other mammals, the braincase portion of didelphid cranium is pronouncedly small (Hiemae and Jenkins 1969) and in some mammals differences on encephalization quotients are known to be associated to orbit orientation variation (Noble et al. 2000). Cartmill's (1972) hypothesis states that an increase on endocranial volume leads the orbital margins to a more anterior position on the cranium. Thus, enlarged cranial frontal regions in taxa with high encephalization levels can lead orbits to a more vertical configuration, for example.

Just as the neurocranium, the splanchnocranium is also likely to affect orbit orientation. Influences of rostrum shape on orbit orientation have been reported in Eutheria (Cox 2008). Short and wide snouts imply in high convergent orbits, like in carnivorans, whereas long and narrow snouts restrict orbit convergence to lower degrees,

such as large herbivorans (i.e., Artiodactyla and Perissodactyla). Didelphids are all omnivores in a broad sense, with a diet constituted of insects and invertebrates, small vertebrates, fruits and nectar, and the relative use of these resources indicates a continuum from predominantly frugivorous to predominantly insectivorous and carnivorous species (Astúa de Moraes et al. 2003, Astúa 2015a). As these interspecific diet differences might be related to morphological variation on rostrum shape, we expect that they could also affect orbit orientation, with larger snouts related to low convergence. In addition, if didelphids fit in the NVPH, we expect that animalivorous species show more convergent orbits.

Didelphids that include higher proportions of fruit in their diets search for food mainly above the ground and some species are strictly arboreal (e.g., *Caluromys philander*). Those with high insect contents may be strictly terrestrial (e.g., *Metachirus nudicaudatus*), whereas others are more generalist and scansorial in substrate use (e.g., *Didelphis marsupialis*). Didelphids also include the sole semi-aquatic marsupial known, the water opossum *Chironectes minimus* (Vieira 2006a). Therefore, to assess if different locomotion habits affect orbit orientation as predicted by the Arboreal Theory for Primates, we test if arboreal didelphids have more convergent orbits than the non-arboreal species.

Although usually considered a morphologically conservative group, didelphid marsupials present a considerable amount of variation in ecology, as mentioned for diet and locomotion, and that variation were shown to be associated with morphological structures and/or body size. Didelphids represent the most basal lineage within living marsupials, a position confirmed by morphological and molecular phylogenetic studies (Amrine-Madsen et al. 2003; Horovitz and Sanchez-Villagra 2003; Asher et al. 2004; Cardillo et al. 2004). Still, although Didelphidae are now understood as a derived group in Metatheria (Oliveira and Goin 2006), the physiological and morphological features of members of this family are usually interpreted as similar to basal features of early mammals (Retief et al. 1995). Characters such as lower encephalization, nocturnal habits, convergent orbits, non-specialized omnivore dentition and a generalized morphological aspect of the body are examples of these basal features (Pirlot 1981). As a result, they are frequently used as models for marsupial ancestors or as living models of a putative primitive mammal (Lemelin 1999; Schmitt and Lemelin 2002; Lemelin et al. 2003; Rasmussen 2005; Hokoç et al. 2006).

In this study, we present a detailed analysis of orbit orientation parameters focusing exclusively in didelphids, calculating and describing orbital orientation (convergence and verticality) in a taxon-rich sample that covers most of the existing morphologic diversity, to estimate if there is any variation on orbit orientation between the species. Then we discuss which variables may influence orbital parameters in didelphids such as: (1) morphological (encephalization and rostrum shape), and (2) ecological (diet and locomotion), as well as (3) historical factors (phylogenetic data), to test if the variability of orbital orientation, if there is, is due to phylogenetic inertia, with close-related species showing similar orbital morphology.

Materials and Methods

Taxa sampled and data collection

We obtained the data from examined specimens housed in the following mammal collections: American Museum of Natural History (AMNH), British Museum Natural History (BMNH), The Field Museum (FMNH), Museum of Natural History, University of

Kansas (KU), Louisiana State University, Museum of Natural Science (LSUMZ), Museu Nacional do Rio de Janeiro (MN), Museum of Southwestern Biology, University of New Mexico (MSB), Museo de Historia Natural de la Universidad Nacional Mayor de San Marcos (MUSM), Museum of Vertebrate Zoology, University of California, Berkeley (MVZ), Museu de Zoologia da Universidade de São Paulo (MZUSP), Sam Noble Oklahoma Museum of Natural History, University of Oklahoma (OMNH), Coleção do Departamento de Zoologia, Universidade Federal de Minas Gerais (UFMG), Coleção de Mamíferos, Universidade Federal de Pernambuco (UFPE), Coleção da Universidade Federal de Santa Catarina (UFSC), National Museum of Natural History (USNM), and University of Wisconsin Zoological Museum (UWZM; see [Online Appendix](#)).

We used 873 specimens from 16 didelphid species in this analysis, representing each one genus ([Table 1](#)). Species are referred to only by genus name, when appropriate. These represents didelphid genera known at the time of data collection, except rare ones such as *Chacodelphys* and *Cryptonanus*, that could not be included in this work due to the lack of available specimens. We included 2 species of *Marmosa* to represent the morphotypes or *Marmosa* (*Marmosa*) and *Marmosa* (*Micoureus*). Taxa with sample lower than 50–60 specimens represent all available and suitable specimens in all the examined collections, and thus represent close to all of the existing specimens, particularly for rare taxa such as *Caluromysiops*, *Lestodelphys*, and *Glironia*.

We follow the taxonomic arrangement by [Gardner \(2008\)](#) with modifications according to [Voss et al. \(2014\)](#). We used only adult individuals in the study, to avoid influence of ontogenetic variation. Specimens were defined as adults when all molars were fully erupted and third premolars replaced and fully erupted ([Tyndale-Biscoe and Mackenzie 1976](#); [Tribe 1990](#); [Astúa and Leiner 2008](#)). Although some of the taxa analyzed here are knowingly dimorphic in cranial size or shape ([Astúa 2010](#)), we pooled males and females in order to assess the overall variance in each species. This also allowed for the inclusion of specimens with unknown sex, particularly important for rare taxa such as *Caluromysiops*, *Glironia*, *Hyladelphys*, and *Lestodelphys*.

Table 1. Orbit orientation parameters of convergence and verticality calculated for each species (SD; n = sample size)

Species	n	Convergence (°)		Verticality (°)	
		Mean	SD	Mean	SD
<i>Caluromys lanatus</i>	65	53.87	4.69	48.02	4.33
<i>Caluromysiops irrupta</i>	06	48.05	1.65	57.31	2.82
<i>Chironectes minimus</i>	88	60.00	4.58	41.01	3.84
<i>Didelphis albiventris</i>	58	54.18	4.28	43.34	4.16
<i>Glironia venusta</i>	02	61.05	1.30	36.14	1.58
<i>Gracilinanus agilis</i>	67	58.23	4.58	41.46	4.09
<i>Lestodelphys halli</i>	04	57.92	4.62	43.53	3.91
<i>Lutreolina crassicaudata</i>	71	50.33	3.717	47.57	3.69
<i>Marmosa (Micoureus) demerarae</i>	80	57.69	3.63	41.69	2.85
<i>Marmosa (Marmosa) murina</i>	66	57.37	4.16	43.0	3.54
<i>Marmosops incanus</i>	64	57.05	4.04	39.67	3.67
<i>Metachirus nudicaudatus</i>	63	52.55	4.39	41.13	4.46
<i>Monodelphis brevicaudata</i>	60	52.34	3.69	42.31	3.68
<i>Philander opossum</i>	58	53.83	5.01	43.09	5.91
<i>Thylamys elegans</i>	70	55.38	4.32	42.91	3.91
<i>Tlacuatzin canescens</i>	51	55.29	3.51	44.54	3.27
TOTAL	873				

Morphology: orbit orientation

We used digital photographs of the cranium to obtain estimates of orbit orientation. Although most studies measure orbit orientation directly from the crania using a Microscribe 3D digitizer ([Heesy 2008](#); [Finarelli and Goswami 2009](#)) or a dihedral goniometer ([Noble et al. 2000](#); [Ravosa et al. 2000](#)), we used cranium photographs pre-existent in a database of one of us (DA) to recover the landmarks. These digital images allowed us incorporate a taxon-rich set of specimens of Didelphidae not evaluated until nowadays, housed in many collections worldwide, which would not be possible otherwise.

All images were taken in dorsal and lateral views, oriented at 90° from each other. The camera was set and leveled on the photocopy stand. Cranium position was standardized under camera lenses to avoid position distortion at pictures. For the dorsal view, they were positioned with the frontal plane parallel to the camera lenses in a way that landmarks III and IV ([Figure 1](#)) are at the same distance from the worktable surface and with both occipital condyles aligned at the same distance from the worktable surface, to avoid any lateral tilting. Crania on lateral view have the midsagittal plane parallel to camera lenses by aligning landmarks I and II ([Figure 1](#)) at equal distances from the worktable surface. We avoid lateral tilting of the cranium by aligning right and left moral series relative to the photograph. We used only crania with at least one intact orbit. Only intact crania were useful in lateral view.

To recover the convergence and verticality of the orbits, we established 8 landmarks in dorsal view and 7 in lateral view (numbered landmarks in [Figure 1](#)). All landmarks were chosen only in structures visible and homologous in all specimens, and were digitized using tpsDig software ([Rohlf 2016a](#)). In total, 3 landmarks on orbital margins define the orbital plane. The first landmark marks the postorbital process of the frontal bone, which indicates the

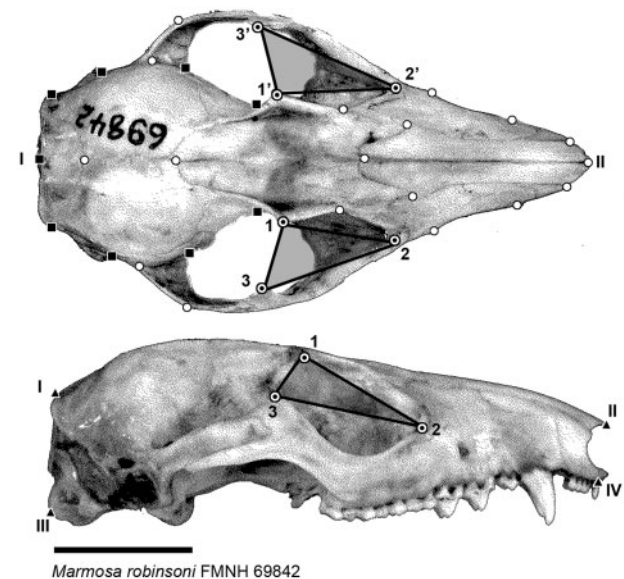


Figure 1. Landmarks used on dorsal (above) and lateral view (below). Target symbols on orbital margins define the orbital plane (represented by the shaded triangle). Triangle symbols indicate 4 landmarks used to match the dorsal and lateral views for 3D reconstruction (see text for further details). Square symbols indicate 9 landmarks used to calculate braincase centroid size. Landmarks used to determine cranial centroid size include round white symbols, square symbols from the braincase landmarks, and the orbit landmarks. See text for further details and landmark definitions.

posterior limit of the supraorbital margin (landmark 1 of the lateral view, landmarks 1 and 1' of the dorsal view). The second is between the 2 lacrimal foramina of the lacrimal bone (landmark 2 of the lateral view, landmarks 2 and 2' of the dorsal view). The third landmark is the tip of the frontal process of the jugal, a projection that provides support to postorbital ligament and indicates the posterior limit of infraorbital margin (landmark 3 of the lateral view, landmarks 3 and 3' of the dorsal view).

We defined 4 additional landmarks to match the dorsal and lateral views for use in steps necessary for the 3D reconstruction, defined as follows in the lateral view (Figure 1): (I) posterior edge of sagittal crest on the midline of interparietal bone, (II) anterior extremity of internasal suture, (III) maximum curvature of the posterolateral surface of the occipital condyle, and (IV) margin of alveolar process of first upper incisor, in premaxilla. Landmarks I and II are the same in the dorsal and lateral views.

Using the coordinates of each landmark from the 2 images, we created a 3D configuration of landmarks to calculate the 3D orbit orientation. The dorsal images were taken aligning the landmarks III and IV parallel to the table and focal planes at the time of the image capture, and by keeping the same distance from right and left molar rows from the table (by checking their alignment visually at the table level) so that the skull was not tilted to its right or left. The lateral images were taken by aligning the sagittal plane of the skull perpendicular to the table and focal planes. The same criterion (alignment of molar rows) was used to verify that the cranium was not tilted to its right or left when positioned for the lateral images. We then translated and rotated the dorsal and lateral configurations as follows: the lateral landmarks were translated to fit landmark III at 0,0, and rotated so that landmark IV had $y=0$ (thus, III–IV fits the x -axis), whereas the dorsal landmarks were translated to fit landmark I onto 0,0, and rotated so that landmark II had $y=0$ (thus, I–II fits the x -axis, and the sagittal plane is perpendicular to the image plane, passing through I–II). Landmarks III–IV were only used as such for this rotation/translation step, but I and II were further used for distance measurements (see below).

These new aligned coordinates were then used to reconstruct the 3D coordinates: for each orbit landmark, the new x and y coordinates of the dorsal images are the 3D x and y , and their respective y -coordinate in the new lateral view is their z -coordinate. Finally, we used these coordinates to generate the factors A, B, and C of the plane equation for each plane used in subsequent calculations (i.e., the orbit plane, the sagittal plane, and the frontal plane):

$$Ax + By + Cz + D = 0$$

where

$$A = y_1 \cdot (z_2 - z_3) + y_2 \cdot (z_3 - z_1) + y_3 \cdot (z_1 - z_2),$$

$$B = z_1 \cdot (x_2 - x_3) + z_2 \cdot (x_3 - x_1) + z_3 \cdot (x_1 - x_2)$$

$$C = x_1 \cdot (y_2 - y_3) + x_2 \cdot (y_3 - y_1) + x_3 \cdot (y_1 - y_2),$$

and $x_1, y_1, z_1, x_2, y_2, z_2, x_3, y_3, z_3$, are, respectively, the x, y , and z coordinates of any 3 points that define a plane, either the 3 landmarks that define the orbit plane or any 3 landmarks contained in the sagittal or frontal plane, after the 3D reconstruction.

Convergence was calculated as the dihedral angle between the orbital and sagittal planes (Figure 2), and verticality as the dihedral

angle between the orbital plane and the frontal plane (Figure 2). The dihedral angle was calculated as:

$$\cos \alpha = \frac{A_1 A_2 + B_1 B_2 + C_1 C_2}{\sqrt{A_1^2 + B_1^2 + C_1^2} \times \sqrt{A_2^2 + B_2^2 + C_2^2}},$$

where $A_1, B_1, C_1, A_2, B_2, C_2$ are the A, B, and C factors from any 2 pair of planes, as defined in the previous equation.

Morphology: encephalization

We used the same images of the cranium in dorsal view to estimate encephalization. Encephalization was calculated as the quotient between the centroid size of the braincase and the centroid size of the whole cranium. Centroid size is the size variable used in geometric morphometrics (Monteiro and Reis 1999), and has the property of retaining in a single variable the multivariate structure of size. It corresponds to the square root of the sum of squared distances between each landmark and the centroid of the landmark configuration. In total, 33 landmarks were set to obtain centroid size for the cranium, and 9 of these were used to calculate the centroid size of the braincase (Figure 1). Landmarks were digitized using tpsDig software (Rohlf 2016a), and Procrustes alignments and centroid sizes calculation were performed on TpsRegr (Rohlf 2016b).

To verify the validity of braincase size from 2D photographs as proxies for a 3D variable (endocranial volume, considered in turn a proxy for brain weight), we ran a regression analysis between endocranial volume and braincase centroid size for a subset of specimens for which we had both crania and photographs at hand (75 specimens from 6 genera). We determined endocranial volumes for this subset using the method described in Eisenberg and Wilson (1981) and Iwaniuk and Nelson (2002), by inserting of lead balls (ca. 1mm diameter) through the foramen magnum until completely filling the

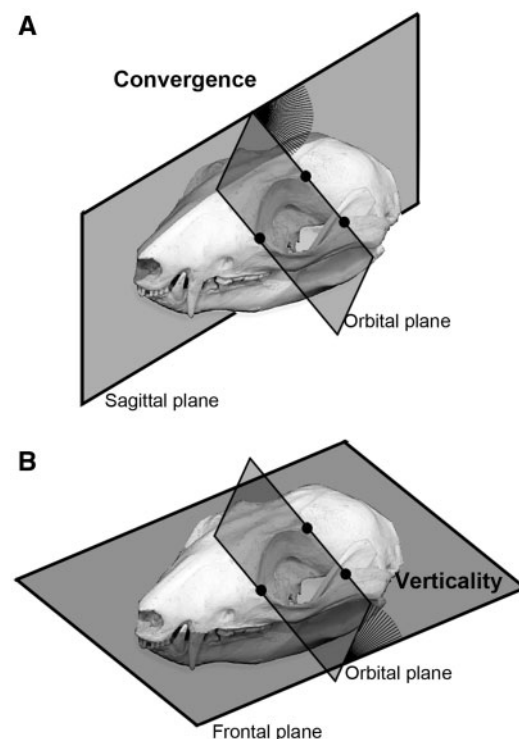


Figure 2. Planes and angles used to define orbit convergence (A) and verticality (B).

endocranial space. Lead balls were then transferred to a 5-mL syringe graduated every 0.1 mL. We measured each volume twice and used the mean of these 2 measurements as the estimated endocranial volume. Endocranial volume and braincase centroid size are highly correlated ($r = 0.97$, $P < 0.001$), and thus centroid size of the braincase was used afterwards as endocranial volume estimator, because centroid size was available for many more specimens and taxa than volume, thus allowing a much more representative sample of didelphids.

Morphology: rostrum measurements

We made 2 measurements of the snout in dorsal view to assess the influence of the snout proportions on the orbit orientation: Rostrum Relative Length (RL) and Rostrum Proportional Width (RPW). RL is the ratio between length of the rostrum anterior to the orbits (taken as the fraction of distance between landmarks I and II that is anterior to the segment 2-2'; Figure 1) and total cranial length (distance between landmarks I and II; Figure 1). It measures how long or short is the snout in relation to the cranium. In turn, the RPW is the ratio between rostral breadth at the anterior end of the orbits (distance between landmarks 2 and 2'; Figure 1) and its own length (as measured above). It captures the widening/narrowing of the snout and, thus, shows whether it is wider or narrower in relation to cranium.

Morphology: size

Skull size has previously been used as a proxy for body size in mammals (Emerson and Bramble 1993). Unpublished data from our research group have shown that cranium centroid size has a high correlation with body weight. Thus, we used cranial centroid size as a proxy for body size, to assess the existence of a potential allometric effect of size on orbital orientation from photographed crania. Cranium centroid size was preferred over body weight because the latter was not available for all specimens. In addition, body weight may vary due to short-term ecological condition, whereas cranial size is more related to the ontogeny and individual life history of each specimen.

Ecology: locomotion

We reviewed locomotion type for each genus based on information derived from the literature. The articles used were mainly about vertical stratification, use of space, substrate preferences, habitat selection, movements, and locomotion of the marsupials (Fish 1993; Passamani 1995; Freitas et al. 1997; Lemelin 1999; Pires and Fernandez 1999; Cunha and Vieira 2002; Grelle 2003; Vieira and Monteiro 2003; Santori et al. 2005; Delciellos and Vieira 2006; Vieira 2006a; Vieira 2006b).

Based on this information, we classified the species according to 4 strata: water, ground, understory, and canopy. For each species, we attributed a value from 0 to 5 corresponding to use frequencies of each stratum. Each value was converted in a proportion of *use of substrate frequency* that ranges from 0 to 1, which 0 means no use of such substrate, 1 means exclusive use of a substrate and intermediate values means rare, occasional or frequent use. Then we calculated a *use of substrate frequency index* for each species as the ratio between the value for each strata and the sum of values of all 4 strata, generating a proportion of substrate use ranging from 0 to 100% of use. Then we calculated the *similarity index* (S) between each pair of species as the sum of the root squares of the multiplication of the proportion values for each strata of each pair of species,

as exposed below. The similarity index ranges from 0 to 1, where 0 means that the species of such pair never use the same substrate, whereas 1 means the same substrate use in the same proportion. We built the distance matrix as a dissimilarity matrix by calculating $1 - S$ for each pair of species, following Astúa (2009) and Marroig and Cheverud (2001):

$$S = \sqrt{X_{1,1} \times X_{1,2}} + \sqrt{X_{2,1} \times X_{2,2}} + \sqrt{X_{3,1} \times X_{3,2}} + \sqrt{X_{4,1} \times X_{4,2}},$$

where S is the similarity index, $X_{1,1}$ is the index of use of first stratum frequency by the first species, $X_{1,2}$ is the index of use of first stratum frequency by the second species, $X_{2,1}$ is the index of use of second stratum frequency by the first species and so on.

Ecology: diet

We recover feeding data for each genus from information found on the literature. The articles used were mainly about behavior and diet and for those species without feeding data available, we used data from congeneric species (Santori et al. 1995; Emmons and Feer 1997; Freitas et al. 1997; Cáceres et al. 2002; Vieira and Astúa 2003; Santori et al. 2012; Astúa 2015a). Then, we classified each genus according to categories: invertebrates, vertebrates, and fruits. The proportion of *food item frequency* for each species, the *food item frequency index*, the *similarity index* (S) between each pair of species, and the distance matrix as a dissimilarity matrix were all calculated as described above for locomotion, following Marroig and Cheverud (2001) and Astúa (2009).

History: phylogeny

Phylogenetic data were obtained from the Interphotoreceptor Retinoid-Binding Protein (IRBP) nuclear gene sequences obtained from GenBank. We used MEGA2 (Kumar et al. 2001) and the model K2P + G (Voss and Jansa 2003) to calculate the genetic distance between each pair of species. We built a phylogenetic distance matrix between each pair of species using these genetic distances as proxies, as in Astúa (2009).

Data analysis

We used correlation analyses to examine relationships between convergence and verticality for all didelphids and for each genus. We compared the genera for each orbital parameter through analysis of variance (ANOVA), followed by a Tukey *a posteriori* test.

We ran correlations between matrices followed by Mantel tests between each orbital parameter with each likely factor of influence to evaluate whether and how biological variables affect orbit orientation. A distance matrix was calculated to each variable and Mantel tests were run between various combinations of matrices; one of orbital angle (convergence, verticality) and another from the factors (encephalization, cranium size as body size, RL, RPW, locomotion, diet, and phylogeny).

Results

Orbit orientation

Orbit orientation is shown in Table 1 and Figures 3 and 4. Mean convergence for the 16 species was 55.4°, ranging from 39.1° to 70.7°. Mean verticality was 43.1°, ranging 28.7° to 65.0°. *Glironia* and *Chironectes* have the most convergent orbits of the group (means 61.05° and 60.00°, respectively), whereas *Caluromysops* and *Lutreolina* have the lower values of convergence (48.05° and

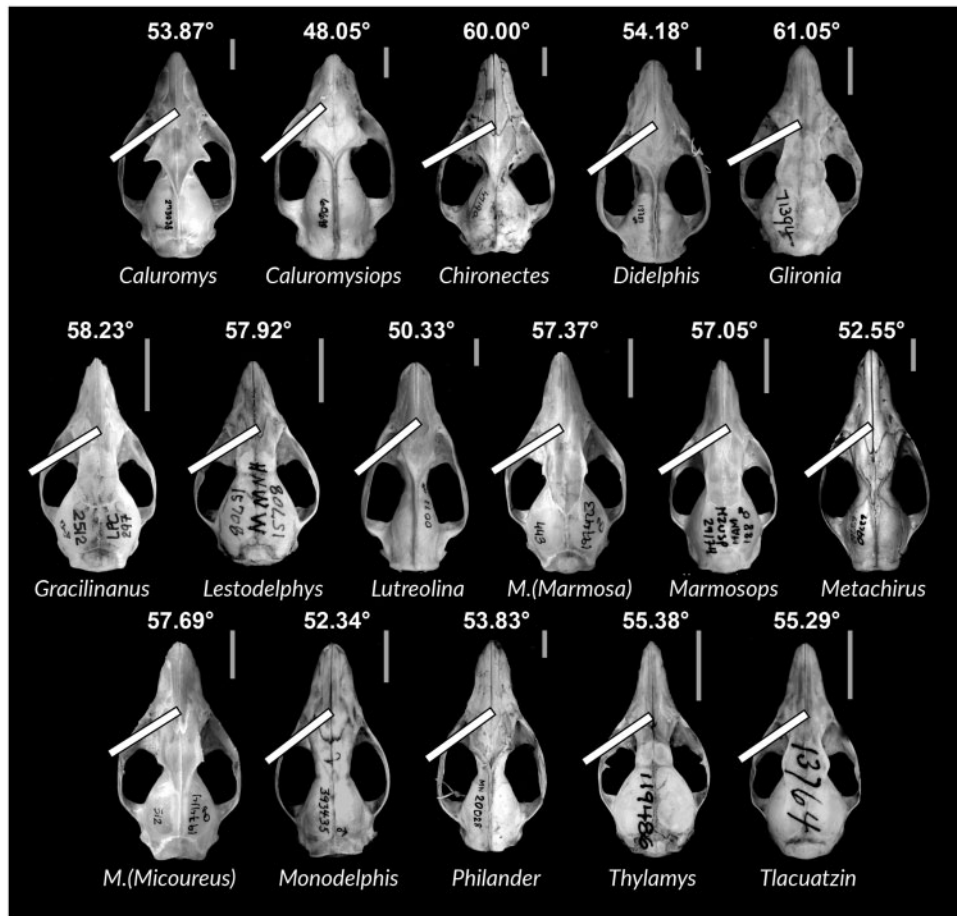


Figure 3. Means of convergence per genus. White bars illustrate the inclination of orbital plane to its referential plane (sagittal plane) for visual comparison only (as convergence angles represent dihedral angles between planes, as defined in text). Gray bar = 1 cm.

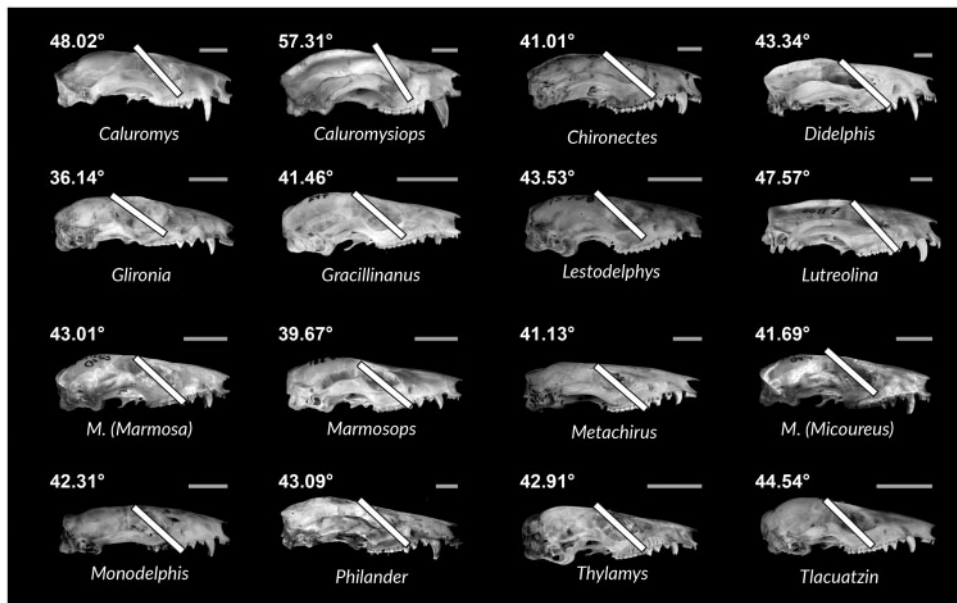


Figure 4. Means of verticality per genus. White bars illustrate the inclination of orbital plane to its referential plane (frontal plane), for visual comparison only (as verticality angles represent dihedral angles between planes, as defined in text). Gray bar = 1 cm.

50.33° orbit convergence means, respectively), showing the most divergent orbits. For verticality, *Caluromysiops* presented the higher value (mean = 57.31°). Its orbits are the most vertical, being oriented to rostrum rather than to braincase. *Caluromys* and *Lutreolina* come next with 48.02° and 47.57° orbit verticality angles. *Glironia* has the less verticals orbits (mean = 36.14°), followed by *Marmosops* (mean = 32.62°).

Across all taxa, convergence angle is negatively correlated with verticality angle ($r = -0.83$; $P < 0.001$). This indicates that in didelphids these angles have antagonist actions on orbit orientation. Highly convergent orbits correspond to less vertical orbits and vice versa. As a group, didelphid orbits are very dorsal and moderately convergent. The results of correlation analyses for each genus are shown in Table 2, and coefficients range from -0.88 to *Chironectes* and *Lutreolina* to -0.96 for *Gracilinanus*, *Lestodelphys*, and *Metachirus*. We could not perform correlation analysis with *Glironia* because the data are from only 2 specimens.

Nevertheless, analyzing taxon per taxon, a variation on orbital configuration is visible (Figures 3 and 4). ANOVA results on both convergence and verticality showed that orbit orientation differs significantly between taxa (Table 3). For these analyses, we excluded 2 species from the dataset, *Lestodelphys* and *Glironia*, due to their small sample sizes. Variance on their means was high, which could have led to errors on analysis due to great overlap with other species. *Caluromysiops* had small sample size as well. Nevertheless, this genus was retained on ANOVAs because the means of orbit orientation were so higher that even with great variance they did not overlap with other species.

Table 2. Correlations between orbital convergence and verticality. *Glironia venusta* was only included in the “all taxa” analysis due to low number of specimens

	<i>r</i>	<i>P</i>
<i>Caluromys lanatus</i>	-0.91	0.00
<i>Caluromysiops irrupta</i>	-0.95	0.00
<i>Chironectes minimus</i>	-0.88	0.00
<i>Didelphis albiventris</i>	-0.90	0.00
<i>Glironia venusta</i>	-	-
<i>Gracilinanus agilis</i>	-0.96	0.00
<i>Lestodelphys halli</i>	-0.96	0.00
<i>Lutreolina crassicaudata</i>	-0.88	0.00
<i>Marmosa (Micoureus) demerarae</i>	-0.90	0.00
<i>Marmosa (Marmosa) murina</i>	-0.93	0.00
<i>Marmosops incanus</i>	-0.95	0.00
<i>Metachirus nudicaudatus</i>	-0.96	0.00
<i>Monodelphis brevicaudata</i>	-0.94	0.00
<i>Philander opossum</i>	-0.95	0.00
<i>Thylamys elegans</i>	-0.93	0.00
<i>Tlacuatzin canescens</i>	-0.91	0.00
All taxa	-0.83	0.00

Table 3. ANOVA of orbital orientation parameters between species, performed with all taxa and excluding *Glironia* and *Lestodelphys*

	Convergence		Verticality	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
All taxa	26.4	0.00	26.0	0.00
Without <i>Glironia</i> and <i>Lestodelphys</i>	30.5	0.00	25.5	0.00

Tukey tests for convergence showed that *Lutreolina* and *Chironectes* are significantly different from 12 of 15 other genera (Figure 5). *Caluromysiops* and *Gracilinanus* distinguished from 9 of 15 genera. Results for verticality showed that *Caluromysiops* could be distinguished from all other genera; *Caluromys* and *Lutreolina* showed significant differences from 13 of 15 other genera, whereas *Marmosops* and *Tlacuatzin* are different from 9 and 8 genera, respectively.

Snout morphology

The rostrum parameters (RL and RPW) allow us to delineate snout form. For didelphid marsupials, there are visible differences in the morphologies described for snout shape in this study (Figure 6), and the snout shape of the studied species is not limited to “wide-short” or “long-narrow” mammal patterns.

Caluromysiops, *Caluromys*, and *Lutreolina* have short and wide snouts (low RL and high RPW), whereas *Philander* and *Marmosops* have long and narrow snouts (high RL and low RPW). *Glironia* differs from both pattern, has moderately long but one of the widest snouts (high RL and RPW). *Metachirus* and *Monodelphis* have longer but neither wide nor narrow snouts, with intermediate values to RPW. *Lestodelphys*, *Chironectes*, *Tlacuatzin*, *Marmosa (Micoureus)*, *Didelphis*, *Gracilinanus*, and *Marmosa* show intermediate values for both the rostrum parameters.

Matrix correlation tests

Results of the Mantel tests are presented in Table 4. Correlations between orbital parameters matrices and genetic distances matrices showed that there is no significant phylogenetic effect on orbit orientation.

Within ecological factors, locomotion was not significant correlated with both orbital parameters. In contrast, a significant effect of diet was found to orbit verticality ($r = 0.39$, $P < 0.001$), although not in convergence.

Within morphological factors, either cranium size (body size) or encephalization showed no significant correlations with any orbital parameter. The rostrum parameters are the only variables that shown a good, significant correlation with orbit orientation. The RL ($r = 0.53$, $P < 0.001$ with orbital convergence and $r = 0.67$, $P < 0.001$ with verticality) is most likely to influence orbits morphology, and RPW has significant correlation only with verticality ($r = 0.46$, $P = 0.01$).

In sum, convergence showed a significant relationship only with length of rostrum within all the variables tested here. Otherwise, verticality showed to be positively correlated with diet, relative length of rostrum and proportional width of rostrum.

Discussion

As a group, didelphid opossums have orbits with low verticality and high convergences, although orientation parameters vary across taxa, mostly related to rostrum aspects. Size, encephalization quotients, phylogeny, or locomotor habits are uncorrelated to orbit orientation, contrarily to diet, suggesting that orbit orientation in opossums is more likely related to feeding habits or behavior.

The negative correlation between convergence and verticality we found agrees with the horizontal/convergent condition previously described for marsupials (Heesy 2008) and, specifically, didelphids (Cartmill 1972) using smaller samples. This pattern is visible in species with highly convergent and horizontal orbits, such as *Glironia*,

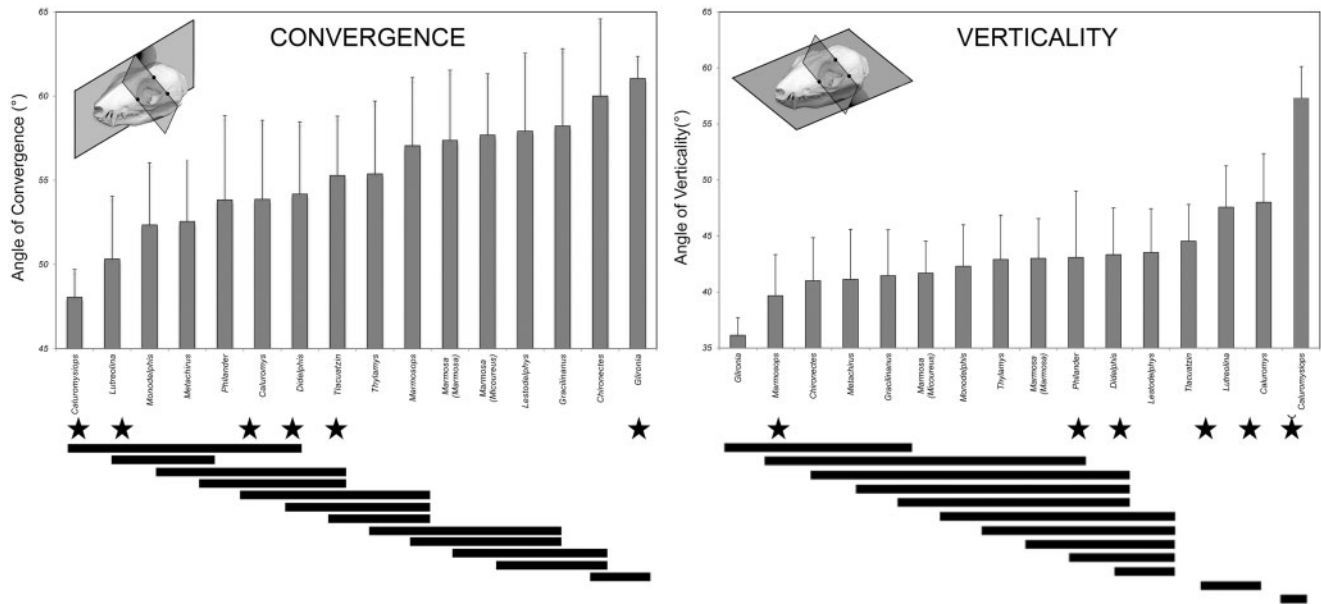


Figure 5. Means of orbit orientation parameters, in degrees, with standard deviations (SD) represented by error bars. Black bars below graph show results of Tukey test (taxa joined by a black bar were not found to be significantly different in Tukey tests). Stars indicate taxa with pouches. See text for details.

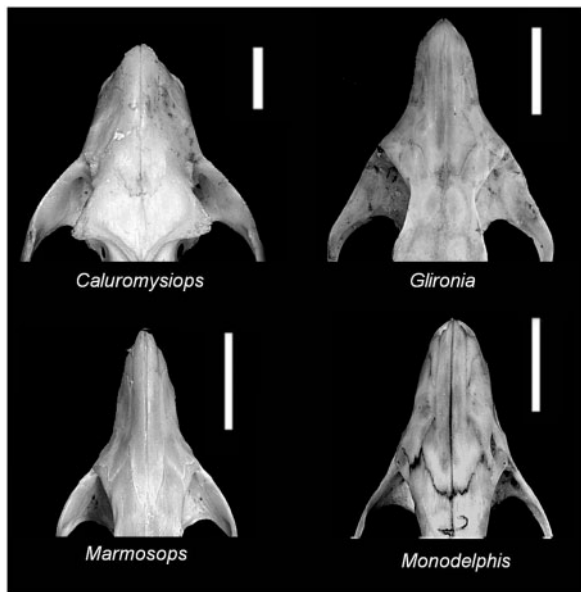


Figure 6. Examples of differences on rostrum shape between some of the didelphid species.

Chironectes, *Gracilimanus*, *Marmosa (Micoureus)*, and *Marmosops* (Figure 3).

However, this relation varies within the Didelphidae family, and even with a general group pattern, we detected considerable intergeneric variation within our more diverse Didelphidae sample. Some taxa have less convergent but more vertical orbits, such as *Caluromysiops* and *Lutreolina* (Figure 4), whereas others do not fit in the antagonistic pattern, such as *Metachirus* and *Monodelphis*, which have low vertical and less convergent orbits, *Caluromys* and *Tlacuatzin*, with highly vertical and moderately convergent orbits, or *Marmosa*, with highly convergent but moderately vertical orbits.

Taxa with different body sizes have similar orbits, as *Chironectes* (big size, 510–790 body weight, with 60° on

Table 4. Matrix correlations and respective results from Mantel tests between the factors of influence and orbit orientation

	Convergence		Verticality	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Phylogeny	0.118	0.11	0.139	0.05
Cranium size (=Body size)	0.128	0.09	0.015	0.36
Encephalization	0.184	0.14	0.198	0.12
RL	0.526	0.00	0.674	0.00
RPW	0.192	0.101	0.464	0.011
Locomotion	0.033	0.38	0.095	0.24
Diet	0.202	0.06	0.387	0.00

Significant results are highlighted in bold

convergence and 41° on verticality) and *Gracilimanus* (small size, 15–40 g, with 58° on convergence and 41° on verticality as well). The contrary is equally true: *Lutreolina* and *Chironectes* have a similar sizes, but they have distinct orbit orientations (50° on convergence and 47° on verticality; *Chironectes* mentioned above), whereas *Tlacuatzin* is small as *Gracilimanus* but also with distinct orbit orientation (55° convergence and 44° on verticality; *Gracilimanus* mentioned above). Thus, orbit orientation is seemingly not a product of allometric effects and cannot be explained as a simple consequence of the great variation on body size present in this group, unlike other features in Didelphidae, such as bite force (Bezerra 2009) and scapula morphology (Astúa 2009), nor as the result of the ontogenetic trajectory, as seen in cranial shape in large didelphids (Sebastião and Marroig 2013).

The lack of correlation of orbital parameters with phylogenetic data indicates that orbit orientation diversification in Didelphidae genera is not suffering strong influence from the phylogenetic inertia, unlike other cranial features (Astúa 2004). Although using a single measurement (orbit length), Flores et al. (2015) also found no phylogenetic signal in the allometric trend of the orbit on both marsupial phylogenies they tested. Thus, as significant correlations with other cranial morphological features were detected, orbit orientation

variation seems to be responding to functional factors rather than solely to shared variation between taxa due to common ancestry.

The verticality levels found here are highly variable across taxa, ranging from moderate to highly vertical orbits (28.7° to 65.0°), and the absence of correlation between encephalization quotient and orbital parameters means that the low encephalization seen in didelphids cannot be used as an explanation for its horizontal position (“low frontation”), as we hypothesized based on Primates. In primate cranial embryonic development, the neurocranium ossifies before the face, due to early differentiation of the neural system and a longer intrauterine time. In marsupials, however, it is the development of facial elements that is instead accelerated, with early ossification of the secondary palate, crucial in newborn marsupials for the oral apparatus to be precociously functional to enable suckling at a very early developmental stage relative to eutherian fetal stages (Smith 1997; 2001).

The facial and palate ossification results in early separation of oral and nasal cavities, enabling olfaction, breathing while suckling, and providing muscles attachments, thus allowing the newborn to attach to the mother’s nipple, whereas the neurocranium bones develop more lately (Smith 1997; 2001). These differences in timing and function priorities may explain why didelphids and primates show different patterns of relationships between orbit orientation and encephalization.

The marsupial cranium is also highly integrated (e.g., Porto et al. 2009), meaning that the correlation between cranial elements is so high that factors influencing one module will influence others as well. In Didelphidae, integration is even greater in the face region, mainly in the oral subregion, whereas in eutherians the facial integration decrease through ontogeny (Porto et al. 2009; Goswami et al. 2012). As we found a high association between rostrum shape and diet, and a weak but significant association between diet and verticality, we may presume an indirect effect of feeding ecology on orbit orientation. Thus, if the forces of masticatory muscles affect snout morphology, is likely that these forces also affect orbit orientation, as most bones forming the orbits are from the facial region. In addition, the neurocranium of didelphids forms a distinct module, uncorrelated with the face, as they respond to different growth factors (Porto et al. 2009; Shirai and Marroig 2010). This could explain why orbits are allowed to change independently of a change in encephalization, unlike in Primates. However, a proper evaluation of these putative interactions on orbits construction of didelphids is needed, to understand in how extent orbital morphology is a consequence of forces acting in early ontogeny or if habits of the adults override these interactions to some extent, or both.

When compared to other mammals, the limited verticality of didelphid orbits is evident, mainly at the frontal cranium, due their flattened shapes. Dabelow (1929) suggested that in marsupials, the chondrocranium suffers a flattening effect during early development in the pouch, and this would be the reason for the smaller size of marsupial crania. Subsequently, Cartmill (1972) proposed that the low frontation/verticality he found in *Didelphis* (and confirmed here for all Didelphidae) was due to spatial constrictions imposed to the cranium during the pouch phase. However, unlike *Didelphis*, not all didelphid species have pouches (Voss and Jansa 2009), and some of them were included in our dataset (*Glironia venusta*, *Gracilinanus agilis*, *Lestodelphys balli*, *Marmosa murina*, *Marmosops incanus*, *Metachirus nudicaudatus*, *Marmosa (Micoureus) demerarae*, *Monodelphis brevicaudata*, *Thylamys elegans*, and *Tlacuatzin canescens*; Carmignotto and Monfort 2006; Voss and Jansa 2009). Additional, those species with a marsupium vary in pouch configuration (Palma 1997; Voss and

Jansa 2009). Therefore, this hypothesis, based on a large-bodied taxon with a pouch, would not apply to all didelphids. In fact, our results showed that species with higher verticality degrees tend to be those with a marsupium (see Figure 5). The delay on marsupial neurocranium development mentioned before is thus more likely to be the cause of low encephalization quotient found by us than simply spatial constraint inside the pouch (if present).

As the didelphid braincase is markedly small when compared to most mammals (Hiemae and Jenkins 1969), the surface available to muscle attachments in its neurocranium is reduced in most species (although additional surface is available on the sagittal and lambdoid crest on the larger species as they age). Nevertheless, an increase in other cranial parts, such as the zygomatic arch or the maxilla, may compensate for such reduction. Indeed, in the young didelphids (as in all mammals) the major part of the cranium is formed by the braincase, whereas in the adult cranium, the zygomatic arch and the facial regions increase in proportion and relative importance of the cranial length and width (Sebastião and Marroig 2013). Although this has been quantified mostly for larger species, even in smaller didelphid species (particularly those with more globose braincases), adults have relatively smaller braincases than young. A more robust zygomatic arch breadth, mainly at its anterior (facial or jugal) portion, results in more surface for muscle origins or insertions, and could compensate for the lack of available surface on neurocranium to masticatory muscles (Hiemae and Jenkins 1969). The jugal portion of the zygomatic arches also forms the inferior margins of orbits. Thus, it seems reasonable to consider that modifications due to muscles acting on that structure would lead to modifications on orbit orientation, as increasing the constitution of zygomatic arches and consecutive restriction on verticality angle would culminate on high convergence angle, rather than modifications on neurocranium.

Along with zygomatic arch development, the early ossification of the palate is crucial to newborn marsupials, as mentioned above, and the use of oral apparatus at very early stages of marsupial development would constitute an important shape-molding agent for the cranial bones included the orbits. The effect of increasing palate length on decreasing of verticality is expected (Noble et al. 2000). As palate length increases, orbital margins are forced into a dorsal orientation, and then orbits become less vertical, and vice versa. Similarly, influences of rostrum shape on orbit orientation have been reported in Eutheria (Cox 2008). Short and wide snouts imply in high convergent orbits, whereas long and narrow snouts restrict orbit convergence to lower degrees (Cox 2008).

Our data suggest within the Didelphidae family, longer snouts correspond to more convergent and less vertical orbits (Figure 7A and 7B), and wider snouts are related to more vertical orbit but show little effect on orbital convergence (Figure 7C and 7D). As a result, the stretching of the rostrum drives orbit position to a more dorsal alignment and a more convergent orientation in the cranium, whereas the widening implies in a more vertical (towards the rostrum) and, by extension, to a little divergent orbit orientation, as the interorbital distance also increases and shifts the orbit to a more lateral position. It is important to notice that there is considerable intergeneric variation in this pattern, just as for the convergence/verticality correlation and length/width rostrum patterns. The main pattern of relationship between orbits and rostrum seems to be particularly driven by those taxa that are morphologically more distinctive, such as *Lutreolina*, *Caluromys*, *Caluromysiops*, and *Glironia* (Figure 7; Table 1).

The work of the masticatory muscles is one of the main molding agents of the maxilla. In Eutheria, shorter rostra reflects a

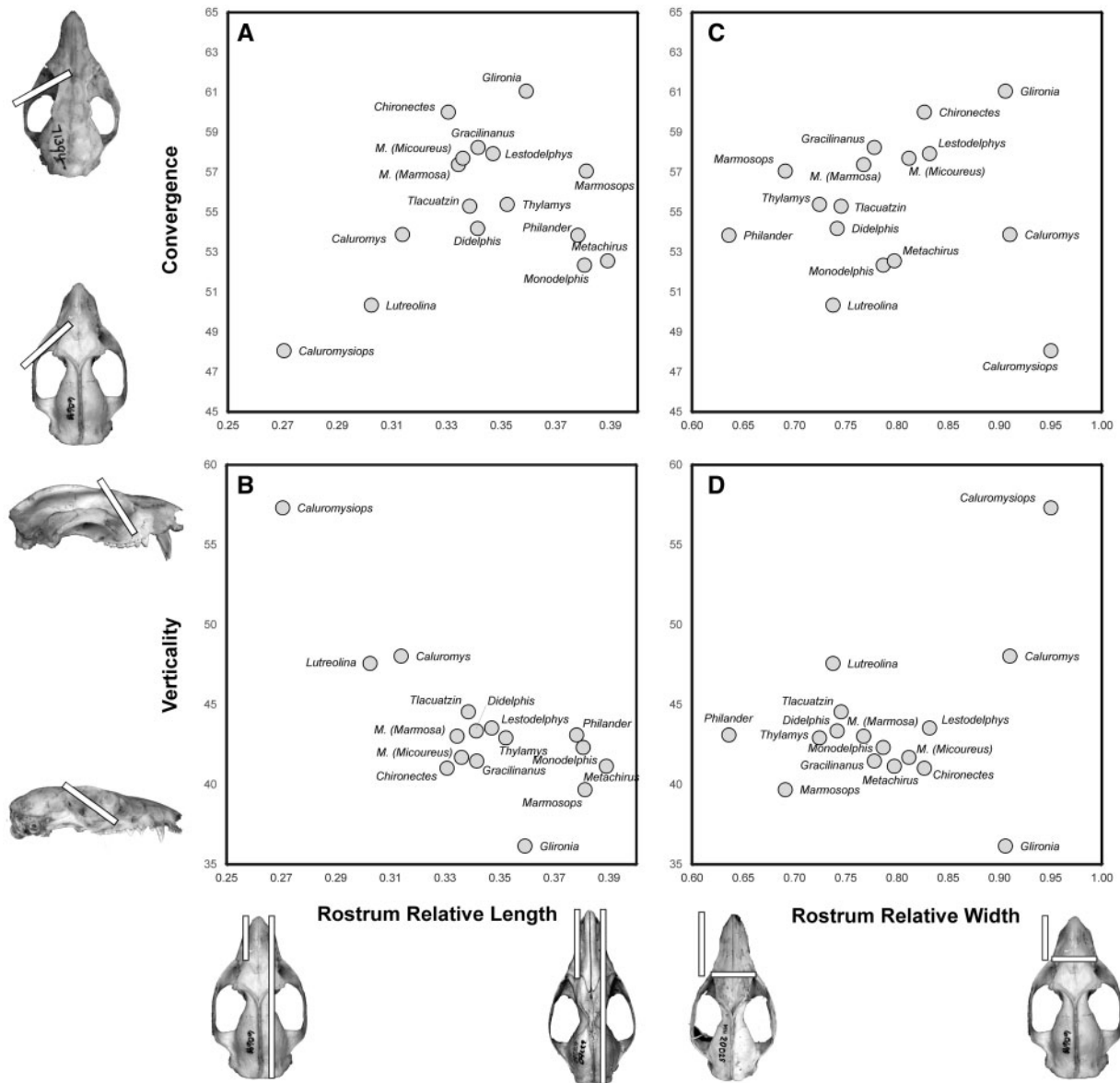


Figure 7. Scatterplots of RL and width versus convergence and verticality. Crania depicted on the x-axes refer to genera with the highest and lowest values for each axis, with the measurements used for calculations of RL and width indicated, whereas crania along the y-axes represent genera with the highest and lowest verticality and convergence, with bars as presented in Figures 3 and 4.

dominance of the *temporalis* muscles group over other masticatory muscles, whereas increased rostrum length is related to a dominance of the *masseter* group (Cox 2008). In “masseter-dominants” eutherians, a long snout restricts the convergence angle, with orbits confined to more lateral positions and increased interorbital distance. In “temporalis-dominants,” the great orbital convergence is associated to rostrum shortening (Cox 2008). Marsupials are a generalist group according to the musculature arrangement scheme used by Cox (2008), when there is no dominance of a muscle group over the others. Their arrangement is nonetheless comparable to the carnivorous pattern (“temporalis-dominant”) due to the predominance of *temporalis* muscles over masseter and pterygoid (Turnbull 1970), although lacking specialized dentition. In conclusion, rostral bones participating in the orbit margins (i.e., lacrimal, maxilla), and molding forces by muscles working on these bones might account for the differentiation on orbital orientation in Didelphidae and its

correlation with rostrum form. Additionally, the correlation between diet and verticality we found suggests multiple interactions between masticatory muscles use and rostrum shape, resulting in an indirect effect of didelphid feeding ecology on diversification of orbit orientation.

Cartmill (1972) hypothesized orbital morphology of arboreal didelphids would differ from that of terrestrial ones, just like arboreal squirrels with divergent orbits differ from their terrestrial counterparts with orbits relatively even more divergent. We rejected the hypothesis that arboreal didelphids have more convergent orbits than the non-arboreal species because we discard an effect of locomotion on orbit orientation in Didelphids, as there is no significant correlation between them. Our data show that orbital convergence in the terrestrial didelphid *Monodelphis*, for example, is not significantly lower than on the arboreal didelphid *Marmosa*, the scansorial *Didelphis*, or even the canopy specialist *Caluromys*.

More convergent orbits provide better visual acuity through the overlap of visual fields of each eye, resulting in better definition of contrasts and better depth perception, which are essential features for a mammal moving in an arboreal niche (Heesy 2004; Volchan et al. 2004). Several features of the didelphid postcranial skeleton are consistent with varying levels of adaptations for arboreal locomotion, particularly in larger species (Argot 2001, 2002, 2003; Astúa 2009, Flores and Díaz 2009). For smaller-sized opossums (and for all small-bodied mammals), however, locomotion on the ground for 10-g individual might be as challenging as the locomotion on the branches above the ground because they need to do some climbing on any fallen logs, rocks, or similar obstacles (Astúa 2009). Therefore, increased binocular vision due to high convergence levels could not be related to increased locomotor performance for small arboreal didelphids as compared to terrestrial ones. It is thus unclear if Arboreal Theory explains orbit orientation in Didelphimorphia: how species move seems to be more relevant than the stratum used.

If the Arboreal Theory were to be discarded for the group, the correlation between diet and verticality (along with the earlier discussion on rostrum shape) would then provide stronger support for the evolutionary history of orbit orientation in Didelphimorphia to be more reasonably tracked out to the NVPH instead. However, as assessed here, the significant correlation between diet and verticality does not allow to assess which feeding habits is related to which orbital orientation pattern, and how feeding behavior is impacted by the consequences of an improvement on visual perception of environment headed by orbital orientation. A deep evaluation of the diet preferences may help to explore the differences on orbit orientation between didelphid species, improving the resolution analysis to beyond the explanation to the whole family. If a better vision is useful to animalivorous species to pursue and catch moving prey, as much as to distinguish camouflaged preys, it could be just as important to frugivorous and folivorous species to distinguish green from ripe fruits, or to distinguish immature self-defended light-green leaves or deciduous indigestible brown leaves from good-tasting/non-toxic mature green leaves as well.

In conclusion, there is a general pattern of orbit orientation for all didelphids that agrees with previous hypotheses based on less diverse samples. Yet not all Didelphidae species fall in this pattern, showing that the commonly held idea of didelphids as a homogeneous groups is not so true. There are morphological differences between taxa beyond body size, and are associated with variance on function and behavioral aspects of each species. Recent detailed analyses are showing that some of the taxa used as models (usually the larger taxa such as *Didelphis*) may not always be the more adequate ones (Astúa 2009), as they have several derived character states in skeletal features (e.g., Flores 2009, Giannini et al. 2011). Even within a single genus, cranial shape has features that are strongly linked to size, whereas others seem to vary regardless of size (Astúa 2015b). Therefore, if didelphid taxa are to be used as “models” of primitive mammals, it is evident that several taxa should be included, as the existing morphological variation found within the group precludes the use of a single or a few taxa as representative morphologies for such primitive mammals. Additionally, it is important to mention that although our sample is the most diverse and representative of Didelphimorphia in orbit orientation studies, we have little resolution of the variation within each genus, and the pattern of variation on orbit orientation may differ between congeneric species. An adequate understanding of the existing morphological diversity in this purportedly morphologically conservative group

and its evolutionary causes and consequences still requires detailed and taxon-rich analyses.

Acknowledgments

We are grateful to all of the following institutions and professionals (curators and collection managers) for access to collections under their care and help during visits: R. Voss (AMNH); B. Patterson and M. Schulenberg (FMNH); R. Timm (KU); J.A. Oliveira, L.F. Oliveira, L. Salles and S. Franco (MN); J. Salazar-Bravo and W. Gannon (MSB); V. Pacheco and E. Vivar Pinares (MUSM); C. Conroy (MVZ); M. de Vivo and J. Barros (MZUSP); J. Braun and M. Revez (OMNH); L. Costa, Y. Leite and B. Andrade (UFMG); A. Gardner, L. Gordon and C. Ludwig (USNM) and J. Kirsch (in memoriam) and P. Holahan (UWZM). R. Voss also granted access to material he personally was studying, including rare specimens. For loans of several important specimens, we are also indebted to M. Hafner (LSUMZ) and P.C.A. Simões-Lopes and M. Graipel (UFSC). C. Heesy and the Centre de Coordination Ouest pour l'Etude et la Protection des Chauves-Souris provided us with important literature. We are grateful to Roberto Ferreira Artoni (UEPG), Juliana Quadros (UFPR) e Liliani Marília Tiepolo (UFPR) for suggestions on earlier versions of this article, as well as 2 anonymous reviewers whose suggestions helped improve the text.

Funding

Data collection was supported by a doctoral fellowship to D.A. from Fundação de Amparo à Pesquisa do Estado de São Paulo, (00/11444-7), by grants from Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (APQ-0351-2.04/06), and a Grant-in-Aid of Research from the American Society of Mammalogists, and is currently supported by a Research Fellowship from Conselho Nacional de Desenvolvimento Científico e Tecnológico (306647/2013-3).

Supplementary material

Supplementary material can be found at <http://www.cz.oxfordjournals.org/>.

References

- Amrine-Madsen H, Scally M, Westerman M, Stanhope MJ, Krajewski C et al., 2003. Nuclear gene sequences provide evidence for the monophyly of australidelphian marsupials. *Mol Phylogeny Evol* 28:186–196.
- Argot C, 2001. Functional-adaptive anatomy of the forelimb in the Didelphidae, and the paleobiology of the Paleocene marsupials *Mayulestes ferox* and *Pucadelphys andinus*. *J Morphol* 247:51–79.
- Argot C, 2002. Functional-adaptive analysis of the hindlimb anatomy of extant marsupials and the paleobiology of the paleocene marsupials *Mayulestes ferox* and *Pucadelphys andinus*. *J Morphol* 253:76–108.
- Argot C, 2003. Functional-adaptive anatomy of the axial skeleton of some extant marsupials and the paleobiology of the Paleocene marsupials *Mayulestes ferox* and *Pucadelphys andinus*. *J Morphol* 255:279–300.
- Asher RJ, Horowitz I, Sanchez-Villagra MR, 2004. First combined cladistic analysis of marsupial mammal interrelationships. *Mol Phylogeny Evol* 33:240–250.
- Astúa D, 2004. Evolução morfológica do crânio e elementos pós-cranianos dos marsupiais americanos (Didelphimorphia, Paucituberculata, Microbiotheria). PhD Dissertation. São Paulo, Brazil: Universidade de São Paulo, 462+iv.
- Astúa D, 2009. Evolution of scapula size and shape in didelphid marsupials (Didelphimorphia: Didelphidae). *Evolution* 63:2438–2456.
- 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 D, 2015a. Family Didelphidae (Opossums). In: Wilson DE, Mittermeyer RA, editors. *Handbook of the Mammals of the World*. Barcelona, Spain: Lynx Edicions, 70–186.

- Astúa D, 2015b. Morphometrics of the largest New World marsupials, opossums of the genus *Didelphis* (Didelphimorphia: Didelphidae). *Oecol Austr* 19:117–142.
- Astúa D, Leiner NO, 2008. Tooth eruption sequence and replacement pattern in woolly opossums, genus *Caluromys* (Didelphimorphia: Didelphidae). *J Mammal* 89:244–251.
- Astúa de Moraes D, Santori RT, Finotti R, Cerqueira R, 2003. *Nutritional and Fibre Contents of Laboratory-Established Diets of Neotropical Opossums (Didelphidae). Predators with Pouches: The Biology of Carnivorous Marsupials*. Collingwood: CSIRO, 229–237.
- Bezerra AC, 2009. Modelagem da força de mordida em marsupiais neotropicais (Didelphimorphia, Didelphidae). Undergraduate Thesis. Recife, Brazil: Universidade Federal de Pernambuco, 78.
- Cáceres NC, Ghizoni IR, Graipel ME, 2002. Diet of two marsupials, *Lutreolina crassicaudata* and *Micoureus demerarae*, in a coastal Atlantic Forest island of Brazil. *Mammalia* 66:331–339.
- Cardillo M, Bininda-Emonds ORP, Boakes E, Purvis A, 2004. A species-level phylogenetic supertree of marsupials. *J Zool* 264:11–31.
- Carmignotto AP, Monfort T, 2006. Taxonomy and distribution of the Brazilian species of *Thylamys* (Didelphimorphia: Didelphidae). *Mammalia* 70:126–144.
- Cartmill M, 1972. Arboreal adaptations and the origin of the order Primates. In: Tuttle R, editor. *The Functional and Evolutionary Biology of Primates*. Chicago: Aldine, 97–122.
- Cartmill M, 1997. Explaining primate origins. In: Ember CR, Ember M, editors. *Research Frontiers in Anthropology*. New Jersey: Prentice-Hall, Englewood Cliffs, 31–46.
- Cox PG, 2008. A quantitative analysis of the Eutherian orbit: correlations with masticatory apparatus. *Biol Rev* 83:35–69.
- Cunha AA, Vieira MV, 2002. Support diameter, incline, and vertical movements of four didelphid marsupials in the Atlantic forest of Brazil. *J Zool* 258:419–426.
- Dabelow A, 1929. Über korrelationen un der phylogenetischen Entwicklung der Schädelform I. *Morph. Jahrb* 67:84–133.
- Delciellos AC, Vieira MV, 2006. Arboreal walking performance in seven didelphid marsupials as an aspect of their fundamental niche. *Aust Ecol* 31:449–457.
- Eisenberg JF, Wilson DE, 1981. Relative brain size and demographic strategies in didelphid marsupials. *Amer Natur* 118:1–15.
- Emerson SB, Bramble DM, 1993. Scaling, allometry and skull design. In: Hanken J, Hall BK, editors. *The Skull, Volume 3*. Chicago: The University of Chicago Press, 384–416.
- Emmons LH, Feer F, 1997. *Neotropical Rainforest Mammals: A Field Guide*. Chicago and London: The University of Chicago Press.
- Finarelli JA, Goswami A, 2009. The evolution of orbit orientation and encephalization in the Carnivora (Mammalia). *J Anat* 214:671–678.
- Fish FE, 1993. Comparison of swimming kinematics between terrestrial and semiaquatic opossums. *J Mammal* 74:275–284.
- Flores DA, 2009. Phylogenetic analyses of postcranial skeletal morphology in didelphid marsupials. *Bull Amer Mus Nat Hist* 320:1–81.
- Flores DA, Abdala F, Martin GM, Giannini N, Martinez JM, 2015. Postweaning cranial growth in shrew opossums (Caenolestidae): a comparison with bandicoots (Peramelidae) and carnivorous marsupials. *J Mammal Evol* 22:285–303.
- Flores DA, Díaz MM, 2009. Postcranial skeleton of *Glironia venusta* (Didelphimorphia, Didelphidae, Caluromyinae): description and functional morphology. *Zoosyst Evol* 85:311–339.
- Freitas SR, Astúa de Moraes D, Santori RT, Cerqueira R, 1997. Habitat preference and food use by *Metachirus nudicaudatus* e *Didelphis aurita* (Didelphimorphia, didelphidae) in a restinga forest as Rio de Janeiro. *Revis Brasil De Biolo* 57:93–98.
- Gardner AL, editor, 2008. *Mammals of South America*. Chicago: The University of Chicago Press, 669.
- Giannini NP, Gaudioso P, Flores DA, Gaudin TJ, 2011. A possible function for an enigmatic synapomorphy of *Didelphis*. *Mammal Biol* 76: 512–514.
- Goswami A, Polly PD, Mock OB, Sánchez-Villagra MR, 2012. Shape, variance and integration during craniogenesis: contrasting marsupial and placental mammals. *J Evol Biol* 25:862–872.
- Grelle CEV, 2003. Forest structure and vertical stratification of small mammals in a secondary Atlantic forest, southeastern Brazil. *Stud Neotrop Fau Envir* 38:81–85.
- Heesy CP, 2004. On the relationship between orbit orientation and binocular visual field overlap in mammals. *Anat Rec A Discov Mol Cell Evol Biol* 281A:1104–1110.
- Heesy CP, 2005. Function of the mammalian postorbital bar. *J Morphol* 264:363–380.
- Heesy CP, 2008. Ecomorphology of orbit orientation and the adaptative significance of binocular vision in primates and other mammals. *Bra Behav Evol* 71:54–67.
- Hiiemae K, Jenkins FA, 1969. The anatomy and internal architecture of the muscles of mastication in *Didelphis marsupialis*. *Postilla* 140:1–49.
- Hokoç JN, Lima SMA, Moraes AMM, Ahnelt P, 2006. A Visão em Marsupiais: Características e Evolução. In: Cáceres NC, Monteiro-Filho ELA, editors. *Os Marsupiais do Brasil - Biologia, Ecologia e Evolução*. Campo Grande - MS: Editora UFMS, 364.
- Horovitz I, Sanchez-Villagra MR, 2003. A morphological analysis of marsupial mammal higher-level phylogenetic relationships. *Cladistics* 19:181–212.
- Iwaniuk AN, Heesy CP, Hall MI, Wylie DRW, 2008. Relative Wulst volume is correlated with orbit orientation and binocular visual field in birds. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* 194:267–282.
- Iwaniuk AN, Nelson JE, 2002. Can endocranial volume be used as an estimate of brain size in birds? *Canad J Zool* 80:16–23.
- Jones FW, 1916. *Arboreal Man*. London: Edward Arnold.
- Kumar S, Tamura K, Jakobsen IB, Nei M, 2001. MEGA2: Molecular Evolutionary Genetics Analysis software. *Bioinformatics* 17:1244–1245.
- Lemelin P, 1999. Morphological correlates of substrate use in didelphid marsupials: implications for primate origins. *J Zool* 247:165–175.
- Lemelin P, Schmitt D, Cartmill M, 2003. Footfall patterns and interlimb coordination in opossums (Family Didelphidae): evidence for the evolution of diagonal-sequence walking gaits in primates. *J Zool* 260:423–429.
- Marroig G, Cheverud JM, 2001. A comparison of phenotypic variation and covariation patterns and the role of phylogeny, ecology, and ontogeny during cranial evolution of new world monkeys. *Evolution* 55:2576–2600.
- Monteiro LR, Reis SF, 1999. *Princípios de Morfometria Geométrica*. Ribeirão Preto: Holos Editora.
- Noble VE, Kowalski EM, Ravosa MJ, 2000. Orbit orientation and the function of the mammalian postorbital bar. *J Zool* 250:405–418.
- Oliveira EV, Goin FJ, 2006. Marsupiais do início do terciário do Brasil: origem, irradiação e história biogeográfica. In: Cáceres NC, Monteiro-Filho ELA, editors. *Os Marsupiais do Brasil: Biologia, Ecologia e Evolução*. Campo Grande, MS: Editora UFMS, 299–320.
- Palma RE, 1997. *Thylamys elegans*. *Mammal Spec* 572:1–4.
- Passamani M, 1995. Vertical stratification of small mammals in Atlantic hill forest. *Mammalia* 59:276–279.
- Pettigrew JD, Dreher B, Hopkins CS, McCall MJ, Brown M, 1988. Peak density and distribution of ganglion cells in the retinae of Microchiropteran Bats: implications for visual acuity. *Bra Behav Evol* 32:39–56.
- Pires AD, Fernandez FAD, 1999. Use of space by the marsupial *Micoureus demerarae* in small Atlantic Forest fragments in south-eastern Brazil. *J Trop Ecol* 15:279–290.
- Pirlot P, 1981. A quantitative approach to the marsupial brain in an eothological perspective. *Revue Canadienne De Biologie* 40:229–250.
- Porto A, de Oliveira FB, Shirai LT, De Conto V, Marroig G, 2009. The evolution of modularity in the mammalian skull I: morphological integration patterns and magnitudes. *Evol Biol* 36:118–135.
- Rasmussen DT, 2005. Primate origins: lessons from a neotropical marsupial. *Amer J Primatol* 22:263–277.
- Ravosa MJ, Noble VE, Hylander WL, Johnson KR, Kowalski EM, 2000. Masticatory stress, orbital orientation and the evolution of the primate postorbital bar. *J Hum Evol* 38:667–693.

- Retief JD, Krajewski C, Westerman M, Winkfein RJ, Dixon GH, 1995. Molecular phylogeny and evolution of marsupial protamine P1 genes. *Proc Roy Soc B Biol Sci* 259:7–14.
- Rohlf FJ, 2016a. tpsDig [cited 2016 June 14]. Available from: <http://life.bio.sunysb.edu/morph/>.
- Rohlf FJ, 2016b. tpsRegr [cited 2016 June 14]. Available from: <http://life.bio.sunysb.edu/morph/>.
- Ross CF, Hall MI, Heesy CP, 2007. Were basal primates nocturnal? Evidence from eye and orbit shape. In: Ravosa MJ, Dagosto M, editors. *Primate Origins: Adaptation and Evolution*. New York: Springer.
- Ross CF, Kirk EC, 2007. Evolution of eye size and shape in primates. *J Hum Evol* 52:294–313.
- Santori RT, Astúa de Moraes D, Cerqueira R, 1995. Diet composition of *Metachirus nudicaudatus* and *Didelphis albiventris* (Marsupialia, Didelphoidea) in Southeastern Brazil. *Mammalia* 59:511–516.
- Santori RT, Lessa LG, Astúa D, 2012. Alimentação, nutrição e adaptações alimentares de marsupiais brasileiros. In: Cáceres NC, editor. *Os Marsupiais do Brasil: Biologia, Ecologia E Conservação*. Campo Grande: UFMS, 385–406.
- Santori RT, Rocha-Barbosa O, Vieira MV, Magnan-Neto JA, Loguercio MFC, 2005. Locomotion in aquatic, terrestrial, and arboreal habitat of thick-tailed opossum *Lutreolina crassicaudata* (Desmarest, 1804). *J Mammal* 86:902–908.
- Schmitt D, Lemelin P, 2002. Origins of primate locomotion: gait mechanics of the woolly opossum. *Amer J Phy Anthropol* 118:231–238.
- 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.
- Shirai LT, Marroig G, 2010. Skull modularity in neotropical marsupials and monkeys: size variation and evolutionary constraint and flexibility. *J Exp Zool* 314B:663–683.
- Smith GE, 1924. *The Evolution of Man*. London: Oxford University Press.
- Smith KK, 1997. Comparative patterns of craniofacial development in eutherian and metatherian mammals. *Evolution* 51:1663–1678.
- Smith KK, 2001. Early development of the neural plate, neural crest and facial region of marsupials. *J Anat* 199:121–131.
- Tribe CJ, 1990. Dental age classes in *Marmosa incana* and other didelphids. *J Mammal* 71:566–569.
- Turnbull WD, 1970. Mammalian masticatory apparatus. *Fieldiana Geol* 18:149–356.
- Tyndale-Biscoe CH, Mackenzie RB, 1976. Reproduction in *Didelphis marsupialis* and *Didelphis albiventris* in Colombia. *J Mammal* 57:249–265.
- Vieira EM, 2006a. Padrões de uso vertical do habitat por marsupiais brasileiros. *Os Marsupiais do Brasil: Biologia, Ecologia e Evolução*. Campo Grande, MS: Editora UFMS, 443–466.
- Vieira EM, Astúa D, 2003. Carnivory and insectivory in Neotropical marsupials. In: Jones M, Dickman C, Archer M, editors. *Predators with Pouches: The Biology of Carnivorous Marsupials*. Collingwood, Australia: CSIRO Publishing, 271–284.
- Vieira EM, Monteiro ELA, 2003. Vertical stratification of small mammals in the Atlantic rain forest of south-eastern Brazil. *J Trop Ecol* 19:501–507.
- Vieira MV, 2006b. Locomoção, morfologia e uso do habitat em Marsupiais Neotropicais: uma abordagem ecomorfológica. *Os Marsupiais do Brasil: Biologia, Ecologia e Evolução*. Campo Grande, MS: Editora UFMS, 299–321.
- Volchan E, Vargas CD, Da Franca JG, Pereira A, Da Rocha-Miranda CE, 2004. Tooled for the task: vision in the opossum. *Bioscience* 54:189–194.
- Voss RS, Gutiérrez EE, Solari S, Rossi RV, Jansa SA, 2014. Phylogenetic relationships of mouse opossums (Didelphidae, *Marmosa*) with a revised subgeneric classification and notes on sympatric diversity. *Amer Mus Novit* 3817:1–27.
- Voss RS, Jansa SA, 2003. Phylogenetic studies on didelphid marsupials II. Nonmolecular data and new IRBP sequences: separate and combined analyses of didelphine relationships with denser taxon sampling. *Bull Amer Mus Nat His* 276:1–82.
- Voss RS, Jansa SA, 2009. Phylogenetic relationships and classification of didelphid marsupials, an extant radiation of New World metatherian mammals. *Bull Amer Mus Nat His* 322:1–177.