Chapter 11

Evolution and Diversification of Faunivorous Marsupials Skull

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Abstract

Marsupials have a long evolutionary history of diversification in the southern hemisphere, where they expanded geographic distribution from America through Antarctica, reaching Australasia. American and Australasian marsupials have mostly evolved and diversified indepently, albeit sharing some evolutionary patterns of morphological variation. Based on morphological traits of the cranium and the mandible, we found that marsupial phenotypic variation significantly correlates with dietary adaptions along a size gradient from small insectivorous taxa, towards intermediate omnivores, then larger carnivores. This variation is phylogenetically structured in both biogeographical groups. Evolutionary rates in cranial

morphology do not differ between American and Australasian marsupials, however differences occur in the mandible with Australasian species evolving at faster rates than American ones. This is probably the result of larger size variation associated to functional demands in producing stronger bite force by the largest carnivorous taxa within this clade.

Key-Words: Adaptation. Comparative methods. Ecomorphology. Faunivory. Feeding specialization. Morphometry.

Introduction

Marsupials emerged in the late Cretaceous (Goin et al. 2016) and today comprise ~379 extant species (Burgin et al. 2018), which are confined and isolated geographically to the Australasian region and the Americas (Sánchez-Villagra 2013Burgin et al. 2018). Compared to the more diverse Placentalia (~6111 extant spp., Upham et al. 2019), marsupial species are born at a much earlier developmental stage, and for that reason they present different ontogenetic growth patterns. Examples include the precocial development of the forelimb bones in order to climb and reach their mothers nipples and the early ossification of the palatal region to allow suckling while growing in the mother's pouch (Shirai and Marroig 2010; Sánchez-Villagra 2013).

Such a difference in the ontogenetic patterns has been regarded as a key to explain morphological diversity especially in the skull of marsupials compared to placental mammals. Marsupial morphology is highly integrated, with size playing an important role in morphological divergence among species (Chemisquy et al. 2020; Shirai and Marroig 2010). Size variation provides the basis for a higher phenotypic plasticity in marsupials, facilitating the appearance of novel adaptive morphologies (Goswami 2006; Shirai and Marroig 2010). This may eventually explains why marsupials evolved a great number of feeding and locomotory adaptations across the Australasian region than in the Americas (Mitchell et al. 2014).

Most of the American marsupial diversity is concentrated in South America, where three orders are found: Didelphimorphia (opossum and mouse opossums), Paucituberculata

(shrew opossums) and Microbiotheria, represented by the 'monito del monte' *Dromiciops gliroides* (Sánchez-Villagra 2013). Nowadays, most of North America has only one extant marsupial genus (*Didelphis*), which dispersed north from the southern clade after the Great American Biotic Interchange (GABI) (Palma 2003).

The oldest known metatherians are from the Lower Cretaceous, in North America, while the origin of Marsupialia appears to be a more recent event, during Late Cretaceous or early Paleocene, that most likely happened in the Gondwana (Eldridge et al. 2019). Dispersal of metatherians from South America to Australia through Antarctica has probably happened prior to the Eocene (Goin et al. 2016). Supporting this hypothesis, the earliest methaterians in Australasia, which had plesiomorphic features, originated in the Oligocene (Palma 2003). Extant Australidelphia, which are today Australasian residents, are within the orders Diprotodontia (the kangaroos, possums, wombats, and koala), Peramelemorphia (bandicoots and bilbies), Dasyuromorphia (mostly small faunivorous animals, but including the thylacine and the Tasmanian devil), and Notoryctemorphia (marsupial moles). Interestingly, the South American Microbiotheria order is also within Australidelphia clade, with supported monophyly (Mitchell et al. 2014).

Among extant marsupials, Diprotodontia is the most recent clade, having evolved highly specialised herbivorous species, with distinguishable cranial adaptations, including the loss of canines (Crompton 2011). In comparison, all other marsupial clades show more similarities in morphology. Most of the comparative work on marsupials often assessed morphological convergence between them and placentals. Previous focus on carnivores showed similarities in the skull (= cranium + mandible) adaptations to catch prey and to cut and slice meat, traits that might be quite stable among mammalian species (Wroe and Milne 2007; Goswami et al. 2010; Prevosti et al. 2012). The work of Prevosti et al. (2012) explored the mandible shape variation and demonstrated that it is possible to discriminate carnivorous marsupials from placentals, although some convergence occurs among specialised predators and scavengers. Wroe and Milne (2007) equally found convergent cranial morphologies between metatherian and eutherian carnivores while Goswami et al. (2010) identified an even greater disparity in skull variation of living and fossil metatherian

carnivores when compared to eutherian species. This would challenge the hypothesis that marsupials should exhibit higher levels of morphological constraints in the skull, which can be evaluated by assessing their evolutionary rates (i.e. the accumulation rate of phenotypic variation over time; Adams 2014) and morphological disparity (i.e. the total amount of morphological variation). Still, the degree of morphological overlap between Ameridelphia and Australidelphia species is unclear since studies have focused more on the placental vs. marsupial morphological comparison, rather than the biogeographical differences within marsupials.

Chemisquy et al. (2020) recently demonstrated that allometry is the leading pathway of cranial shapediversification in didelphid marsupials. However, some specific traits of the marsupial skull show different levels of adaptive responses. Marsupial molars, for instance, are highly integrated, and therefore, morphological shifts between species occur in all teeth together as a unity (Werdelin 1987). In comparison, other functional features of the mandible of marsupials are potentially more adaptable, such as the angular process whose shape variation is functionally related to different dietary adaptations (Sánchez-Villagra and Smith 1997). Sánchez-Villagra and Smith (1997) reported that marsupial herbivores (Diprotodontia) tend to have a shelf-like angular process, while faunivorous ones (peramelids, dasyurids, *Dromiciops*) have a rod-like or intermediate angular process (didelphids, *Caenolestes*). This pattern apparently evolved multiple times in marsupial evolution, supporting an adaptive role for such a trait. Additionally, marsupial herbivores present greater size variation than faunivorous forms, showing more specific and adaptive traits in the skull to exploit multiple phytophagous behaviours (Goswami 2006). To this date, no comparative study has been conducted to compare the skull morphological variation between American and Australasian faunivorous lineages while using a comprehensive analytical framework.

The aim of this study is to identify similarities and differences in the skull morphology of the American and Australasian lineages of marsupials in relation to their ecological adaptations (i.e., diet and locomotion). Phylogenetic comparative methods were

employed to quantify phylogenetic signal, morphological disparity, and evolutionary rates in functional skull metrics exhibited by these two groups.

Abbreviations

A = Arboreal

BAM = Breadth between molars

C = Carnivore

FI = Family inference

GI = Genus inference

I = Insectivore

JL = Jaw length

LC = Locomotion categories

MACN = *Museo Argentino de Ciencias Naturales "Bernardino Rivadavia"*

MAM = Moment arm of masseter

MAT = Moment arm of temporalis

MCNFZB = *Museu de Ciências Naturais da Fundação Zoobotânica do Rio Grande do Sul*

MD = Morphological Disparity

MF = Inference based on morphological features

MFL = Masseteric fossa length

MHNCI = *Museu de História Natural Capão do Imbuia*

MLP = *Museo de La Plata*

MN = Museu Nacional

MPEG = *Museu Paraense Emílio Goeldi*

MS = Length of upper molar series

MZUSP = *Museu de Zoologia da Universidade de São Paulo*

NHM = Natural History Museum

O = Omnivore

PC = Principal component

PCA = Principal components analysis

PL = Palatal length

PMS = Length of upper pre-molar series

SAq = Semiaquatic

SC = Total number of specimens with cranium

Sc =Scansorial

SI = Subfamily inference

SL = Skull length

SM = Total number of specimens with mandible

T = Terrestrial

UFSC = *Universidade Federal de Santa Catarina*

UFSM = *Universidade Federal de Santa Maria*

UNEMAT = *Universidade do Estado de Mato Grosso*

WML = World Museum of Liverpool

ZAW = Zygomatic arm width

Materials and Methods

Faunivorous marsupials' orders that possess canine teeth were selected as representative of the Ameridelphia (Paucituberculata and Didelphimorphia) and Australidelphia groups (Dasyuromorphia, Microbiotheria, Notoryctemorphia, and Peramelemorphia). Specimens were collected from the scientific collections of the *Museo Argentino de Ciencias Naturales "Bernardino Rivadavia"* (MACN, Buenos Aires, Argentina), *Museo de La Plata* (MLP, La Plata, Argentina), *Museu de Ciências Naturais da Fundação Zoobotânica do Rio Grande do Sul* (MCNFZB, Porto Alegre, Brazil), *Museu de História Natural Capão do Imbuia* (MHNCI, Curitiba, Brazil), *Museu Nacional* (MN, Rio de Janeiro, Brazil), *Museu Paraense Emílio Goeldi* (MPEG, Belém, Brazil), *Museu de Zoologia da Universidade de São Paulo* (MZUSP, São Paulo, Brazil), *Universidade Federal de Santa Catarina* (UFSC, Florianópolis, Brazil), *Universidade Federal de Santa Maria* (UFSM, Santa Maria, Brazil), *Universidade do Estado de Mato Grosso* (UNEMAT, Nova Xavantina, Brazil), Natural History Museum (NHM, London, UK), World Museum

of Liverpool (WML, Liverpool, UK). Our data comprise cranial and mandible photographs of 40 Ameridelphian (~ 33% of living species) and 47 Australidelphian species (~ 50% of the living faunivorous species) (see Table 1 for number of species and specimens collected for each group). The cranium was photographed in ventral view, with the palate positioned parallel to the camera. The mandible was primarily photographed on the left side, positioned in lateral view. In case the left side of the mandible was damaged, we photographed the right side instead (~20% of the specimens). Both structures were photographed at the fixed distance of1 metre with a scale bar positioned parallel to the tooth row. This procedure allowed the conversion of pixels to millimetres. Linear measurements were taken using tpsDig2 ver. 2.26 (Rohlf 2015). The cranium and mandible measurements were selected using Radinsky (1982) as a primary reference, as they have been shown to be good descriptors of functional morphology in faunivorous mammals. These include: zygomatic arm width (ZAW), skull length (SL), moment arm of temporalis (MAT), moment arm of masseter (MAM), masseteric fossa length (MFL), and jaw length (JL). Additionally, we followed Cerqueira (1980) to include cranial measurements that were specifically informative for the marsupials: breadth between molars (BAM, measured by the distance between the first upper molars), palatal length (PL), length of upper molar series (MS), length of upper pre-molar series (PMS). MS and PMS was the calculated average between the measurements of both sides of the cranium (Fig. 1). These measurements were all log transformed for further analyses.

Upham et al. (2019) tree (Fig. 2) was used for all comparative analyzes. The consensus tree included the marsupials within this study and a separate one for each group (Ameridelphia and Australidelphia). Each species was classified according to three dietary (insectivorous, carnivorous, and omnivorous) and four locomotory categories (arboreal, scansorial, semiaquatic, and terrestrial) (Tables 2 and 3). Because marsupials in general tend to have a more opportunistic diet and are difficult to classify, a simplified three-level categorization of their diet was used. Species that consistently rely on vertebrate or invertebrate food items at any time of the year in greater proportion (> 50%) were considered within the carnivorous or insectivores categories, while the remaining species

were considered omnivorous when presenting a a more balanced diet, often varing between animal and vegetal items frequency in accordance to seasonal variation and/or resource availability (Santori et al. 2012). Dietary classification found in original studies were prioritized, whilst using the general classification of other studies that did not focus primarily on dietary behaviour of species to fill in gaps *a posteriori*.

Principal Component Analysis (PCA) was first performed in order to explore morphological variation between species without any a priori hypothesis in the data structure. Although different corrections can be applied to linear measurements before PCA, we opted to employ a simple log transformation that allowed less spread in data variance. Phylogenetic relationships were mapped in the PC morphospaces using phylomorphospace that provided a graphical depiction of species departure from their hypothetical ancestors estimated via squared changed parsimony algorithm (Revell 2012). Traditionally, the first principal component can be interpreted as size component when all measurements show similar correlation to it, while the other PCs can be interpreted as shape (Adams et al. 2009). Therefore, we did all our subsequent analyses using these separate components.

Using the *K*-statistics available in the R package 'geomorph' (Adams and Otárola-Castillo 2013), the phylogenetic signal for the first two principal components, which explained together above 95% of morphological variation, were quantified. We did this on both PCs separately, and for all marsupials and for each group, using 9,999 permutations. The *K* value measures the degree of phylogenetic signal in a dataset in relation to what is expected by Brownian motion (Adams 2014). The procedure also gives a comparative value, the effect size (Z), which allows the interpretation on which dataset possess the strongest signal.

To test if skull morphology (PC1=size, PC2 = shape) differs between dietary and locomotory categories, a randomised linear model with 9,999 permutations was performed with the function 'lm.rrpp' of the R package 'RRPP' (Collyer and Adams 2018). The procedure was than repeated by adding a phylogenetic covariance matrix as a covariate into the model in order to account for phylogenetic dependency. The morphological disparity of

size and shape was measured for Ameridelphia and Australidelphia in order to quantify and compare the amount of morphological variation between these groups and between diets for all Marsupialia. Evolutionary rates in skull morphology for Ameridelphia and Australidelphia marsupials were described by the σ^2 static, which quantifies the rate of accumulation of variance in a single or in multivariate traits over time while accounting for phylogenetic relationships (Adams 2014). A comparison between the evolutionary rates between carnivorous, omnivores and insectivorous marsupials was additionally performed. This was assessed using the function 'compare.evol.rates' in the package 'geomorph' (Adams and Otárola-Castillo 2013) that test statistically if evolutionary rates between groups differ from random expectation. All statistical procedures were performed on the cranium and the mandible measurements separately.

Results

Morphological Variation Description

Morphological variation of the cranium functional measurements was summarised by the combination of the first two principal components, which cumulatively explained 98.58% of total variation. PC1 (93.67%), interpreted as a "size" vector, was positively loaded on all the cranium measurements (coefficients: BAM = 0.378, MS = 0.399, PMS = 0.448, PL = 0.434, SL = 0.408, ZAW = 0.378), separating small (negative scores, mostly insectivores) from large species (positive scores, carnivores and omnivores). PC2 explained 4.91% of morphological variation and it was positively loaded on ZAW (0.441), BAM (coef = 0.308) and MS (coef = 0.296) and negatively on PMS (coef = -0.778), separating larger carnivorous dasyurids (positive scores) from more specialised insectivores with longer rostrum and PMS series, such as caenolestids and peramelids. Small sized didelphids and dasyurids overlap in the centre of both PCs, while the thylacine occupies an extreme position in the first PC (size) but not in the second (shape) (Fig. 3).

The mandible PCA summarised 99.24% of total variation in the first two components. All measurements increased at the positive scores of PC1 (97.85%), therefore larger species have larger PC1 scores (JL coef. = 0.504; MAM coef. = 0.465; MAT coef. =

0.478; MFL = 0.547). Along PC2 (1.39%), which was positively correlated with MAT (coef. = 0.805) and negatively correlated with JL (coef. = -0.408) and MFL (coef. = -0.418), carnivores and insectivores showed larger MAT (top of the plot) and smaller JL and MFL than omnivores (bottom of the plot) (Fig. 4). In the mandible morphospace, the Ameridelphian group completely overlapped with the Australidelphian clade.

Size component

The size component (PC1 in figures 3 and 4) of both cranium (K = 0.721, Z = 8.327, P < 0.001) and mandible (K = 0.752, Z = 8.327, P < 0.001) showed similar and significant phylogenetic signals. This signal in the Ameridelphia group was stronger (cranium: K = 1.307, Z = 7.598, P < 0.001; mandible: K = 1.488, Z = 8.090, P < 0.001) than in the Australidelphia (cranium: K = 0.763, Z = 5.758, P < 0.001; mandible: K = 0.748, Z = 5.879, P < 0.001).

Diet was a statistically significant factor in Marsupialia size (cranium: $R^2 = 0.391$, $F_{2, 84} = 27.005$, P < 0.001; mandible: $R^2 = 0.377$, $F_{2, 84} = 25.396$, P < 0.001), Ameridelphia (cranium: $R^2 = 0.642$, $F_{2, 37} = 33.212$, P < 0.001; mandible: $R^2 = 0.632$, $F_{2, 37} = 31.786$, P < 0.001), and Australidelphia (cranium: $R^2 = 0.249$, $F_{2, 45} = 7.295$, P < 0.001; mandible: $R^2 = 0.207$, $F_{2, 45} = 5.751$, P = 0.006). It remains significant when phylogeny was accounted for all marsupials (cranium: $R^2 = 0.079$, $F_{2, 84} = 3.624$, P = 0.035; mandible: $R^2 = 0.095$, $F_{2, 84} = 4.394$, P = 0.020), but not when separating the biogeographical groups (P > 0.05, Table 4). Locomotor categories were not significant in the Marsupialia and Australidelphia cranium and mandible sizes (P > 0.05), but they were in the Ameridelphia sample (cranium: $R^2 = 0.416$, $F_{3, 36} = 8.532$, P < 0.001; mandible: $R^2 = 0.399$, $F_{3, 36} = 7.959$, P < 0.001). Locomotor categories did not have a significant effect after phylogeny was accounted for (P > 0.05, Table 4).

Ameridelphia (cranium MD = 0.185, mandible MD = 0.126) and Australidelphia (cranium MD = 0.306, mandible MD = 0.191) were not statistically different in regards to size morphological disparity (Pairwise absolute differences between variances: cranium = 0.120, P = 0.104; mandible = 0.065, P = 0.223) but they differed in evolutionary rates

(cranium P = 0.012; mandible P = 0.014). Australidelphia rates were higher (cranium rate = 0.010, mandible rate = 0.007) than in Ameridelphia (cranium rate = 0.003, mandible rate = 0.002).

Carnivores (cranium MD = 0.348; mandible MD = 0.232) showed higher and significantly different morphological disparity than both insectivores (cranium MD = 0.064; mandible MD = 0.039) and omnivores (cranium MD = 0.071; mandible MD = 0.050), but the last two were not different between each other (see pairwise comparisons at Table 5). Evolutionary rates were also statistically different between diets for the cranium and the mandible size components (cranium P value = 0.044; mandible P = 0.038). Carnivorous marsupials had the highest rates (cranium rate = 0.012, mandible rate = 0.008), followed by omnivores (cranium rate = 0.006, mandible rate = 0.003) and insectivores with the lowest rates (cranium rate = 0.003, mandible rate = 0.002).

Shape component

The shape component (PC2 at figures 3 and 4) of both cranium (K = 0.749, Z = 7.371, P < 0.001) and mandible (K = 0.515, Z = 5.572, P < 0.001) showed significant phylogenetic signal, although the cranium had a higher effect. The discrepancy of the signal between cranium and mandible was larger in the Ameridelphia group (cranium: K = 0.925, Z = 4.432, P < 0.001; mandible: K = 0.414, Z = 3.289, P < 0.001) than in the Australidelphia (cranium: K = 0.910, Z = 6.429, P < 0.001; mandible: K = 0.843, Z = 5.879, P < 0.001).

Diet was significantly correlated with skull shape in Marsupialia (cranium: R^2 = 0.261, $F_{2, 84}$ = 14.827, P < 0.001; mandible: R^2 = 0.298, $F_{2, 84}$ = 17.816, P < 0.001) and Australidelphia (cranium: R^2 = 0.509, $F_{2, 45}$ = 22.837, P < 0.001; mandible: R^2 = 0.207, $F_{2, 45}$ = 5.750, P < 0.001), but not in Ameridelphia (cranium: R^2 = 0.047, $F_{2, 37}$ = 0.911, P = 0.356; mandible: R^2 = 0.013, $F_{2, 37}$ = 0.246, P = 0.737). The diet effect in cranium and mandible shapes was not significant when phylogeny was accounted for (P > 0.05, Table 6).

Locomotor categories differed significantly in the cranium shape of Marsupialia (R^2 = 0.145, $F_{3, 83}$ = 4.673, P = 0.015), but not in the mandible (R^2 = 0.085, $F_{3, 83}$ = 2.559, P =

0.079). In Ameridelphia, locomotory categories differed significantly for the mandible sample (cranium: $R^2 = 0.182$, $F_{3, 36} = 2.680$, $P = 0.061$; mandible: $R^2 = 0.268$, $F_{3, 36} = 4.394$, $P = 0.011$), while in Australidelphia it was the opposite (cranium: $R^2 = 0.221$, $F_{2, 45} = 6.231$, $P = 0.004$; mandible: $R^2 = 0.113$, $F_{2, 45} = 2.805$, $P = 0.073$). Locomotor categories did not have a significant effect after phylogeny was accounted for ($P > 0.05$, Table 6).

Ameridelphia (cranium MD = 0.007, mandible MD = 0.001) and Australidelphia skull morphology (cranium MD = 0.019, mandible MD = 0.004) were statistically different in regards to morphological disparity (Pairwise absolute differences between variances: cranium = 0.012, $P < 0.001$; mandible = 0.003, $P = 0.002$) while evolutionary rates did not differ between these groups for both the cranium and the mandible shape components (cranium P value = 0.201; mandible P = 0.204). Australidelphia rates were slightly higher (cranium rate = 0.0005, mandible rate = 0.00010) than those in Ameridelphia (cranium rate = 0.0002, mandible rate = 0.00005).

In the cranium shape, carnivores (MD = 0.010) and omnivores (MD = 0.011) showed similar morphological shape disparities, while insectivores had the lowest (MD = 0.009), these comparisons were not significantly different (see pairwise comparisons at Table 5). For mandible shape, carnivores presented the highest disparity (MD = 0.013), followed by insectivores (MD = 0.009) and then omnivores (MD = 0.003). In this case, only omnivores MD was significantly different from the other categories (Table 6). No difference in evolutionary rates occurred for skull shape between dietary groups (cranium P value = 0.705; mandible P = 0.253). Cranium (carnivorous rate = 0.0006, omnivorous rate = 0.0003, insectivorous rate = 0.0003) and mandible rates were of comparable sizes between categories (carnivorous rate = 0.00010, omnivorous rate = 0.00010, insectivorous rate = 0.00004), but carnivores presented the highest rate in the cranium and insectivores the lowest in the mandible.

Discussion

The overall skull morphological variation of faunivorous marsupials is phylogenetically structured. Previous studies on the cranium, the mandible, and teeth

morphology of these clades consistently described marsupials as evolutionarily constrained in regards to these morphological structures (Goswami 2006; Chemisquy et al. 2020). This study showed that the Ameridelphia group shares a moderate (cranium measurements, Fig. 3) to complete overlap area within the morphospace of Australidelphia (mandible, Fig. 4). During most of the Cenozoic period, these groups evolved and diversified in isolation from each other (Goin et al. 2016), so it is conceivable to hypothesize that they may share convergent morphologies.

The morphological skull variation of marsupials reflects adaptations to distinct dietary niches along a size gradient, from small insectivorous taxa towards intermediate size omnivores than larger carnivores (Figs. 3 and 4). This variation is phylogenetically structured so that diet differences occur only if Ameridelphia and Australidelphia are analysed together. This means that diet categories are constrained within families for both American and Australasian marsupials. Therefore, the association between skull morphology and diet becomes weaker within the two groups because the size variation that is associated with diet is smaller within rather than between them. This intrinsic relationship between skull size and shape throughout marsupials evolutionary history has been discussed before (Flores et al. 2018; Chemisquy et al. 2020). Marsupials have an inherent and strong size vs. shape association, which is favoured by the ontogenetic contingency (see Shirai and Marroig 2010; Flores et al. 2018). This phenomenon is important for their early development outside the placenta and is key to understand their evolutionary histories (Sánchez-Villagra 2013). Thus, it is through the association between size and shape that most of their morphological variation accumulates through evolutionary time (Shirai and Marroig 2010; Chemisquy et al. 2020).

Australidelphia generally presents higher values of morphological disparity than Ameridelphia, especially in the shape component, where the morphological distances between the groups are significant (Fig. 3 and 4). The evolutionary rates of the cranium and the mandible size components are also higher in the Australidelphia. This latter clade, in comparison to Ameridelphia, has accumulated more variance in skull morphology over time (Adams 2014), suggesting that faunivorous marsupial evolution was held at different

strengths between the biogeographical groups. That is: Australidelphia became larger at a faster rate than Ameridelphia, explaining the higher disparity in this group. Regarding shape, evolutionary rates are similar and therefore we can argue there is a higher contingency in overall marsupial skull shape rather than size. In this sense, the higher morphological disparity (and faster rate of size evolution) in the Australasian region could be interpreted as a result of its longer isolation time, where marsupials evolved without the coexistence (and potential competition) of other mammalian groups, like the eutherians and especially other metatherians (Mitchell et al 2014; Eldridge et al. 2019).

The Ameridelphian cranium morphospace does not reach the specialised carnivorous morphospace, as presented in Australidelphia clade (increase of zygomatic arch width, breadth between molars and molar series, and decrease of pre-molar series; see PC2 of Fig. 3), and neither their larger sizes (PC1 of Fig. 3 and 4). Furthermore, the Australasian clade includes larger predatory forms, like the Tasmanian devil and the recently extinct thylacine, which are significantly larger than any living opossum (Amador and Giannini 2016; Rose et al. 2017; this chapter). Meanwhile, insectivorous adaptations seem to be consistent in both groups that evolved a large proportion of small sized taxa (Figs 3 and 4; Fisher and Dickman 1993; Prevosti et al. 2012).

The mandible shows similar patterns to the cranium in relation to overall function and size variation. Australasian marsupials present large variation in jaw length relative to the moment arm of the temporalis muscle. Species with shorter mandible exhibit larger moment arm of temporalis that increase bite force, a trait allometrically dependent (Goswami et al. 2010. The Australidelphia clade evolved extreme adaptive morphologies that range from the small insectivores such as *Planigale ingrami* and insectivorous morphotypes (e.g., *Antechinomys laniger* and *Antechinus flavipes),* somewhat morphologically similar to some American insectivorous species (e.g., *Monodelphis kunsi*), to the mid-sized insectivorous (#71, Fig. 4) and omnivorous species (#84, Fig. 4), which are comparable to *Rhyncholestes raphanurus* in shape (#4, Fig. 4), and finally, the large carnivorous morphotypes of *Sarcophilus harrisii* (#64, Fig. 4) and *Thylacinus*

cynocephalus (#85, Fig. 4) that do not overlap with any of the American marsupials morphospace (Fig. 4).

Carnivores and/or omnivores showed higher size and/or shape disparities than insectivores. These results could be related to the higher size range within the first two dietary groups. Because the allometric component is a consistently strong factor associated with shape variation (Chemisquy et al. 2020), largest size variation can also correlate to the highest shape variation. The evolutionary rates of carnivores are consistently higher than omnivores (especially size related) which are higher than the rates of insectivores. Carnivores potentially have a higher disparity than the other dietary groups because this phenotype evolved at faster rates than the other two. It is important to highlight that the carnivores group presents a higher range of size variation. For instance, the smallest carnivore we recorded was *Planigale ingrami*, with a mean skull length of 1.5 cm, while the largest was the thylacine *T. cynocephalus*, mean skull length of 19.2 cm (total variance of SL = 17.7 cm). This supports the interpretation that dietary partitioning within marsupials is primarily associated with size variation (Goswami 2006; Shirai and Marroig 2010).

This study is the first to encompass a large morphological diversity of Ameridelphian and Australidelphian marsupials using a comparative approach. More importantly, we bring novel results regarding the association between diet and skull morphology in these animals. We note that the full evolutionary history of faunivorous marsupials in regards to feeding adaptations can only be fully understood when looking at both biogeographical groups simultaneously, because of their shared ancestry and common morphology, that, when analysed together, can reveal clearer evolutionary adaptive trends.

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Tables

Table 1 Number of genera, species and specimens analyzed for each group, with information also divided by orders and families. #SC = total number of cranial specimens; #SM = total number of mandibular specimens

	# Genera	# Species	# SC	#SM
Marsupialia	41	87	437	480
Ameridelphia	16	40	202	207
Order Paucituberculata	3	4	19	19
Caenolestidae	3	4	19	19
Order Didelphimorphia	13	36	183	188
Didelphidae	13	36	183	188
Australidelphia	25	47	235	273
Order Dasyuromorphia	16	31	138	165
Dasyuridae	14	29	120	143
Myrmecobiidae	1	1	6	6
Thylacinidae	1	1	12	16
Order Microbiotheria	1	1	6	6
Microbiotheriidae	1	1	6	6
Order Peramelemorphia	7	14	88	100
Peramelidae	6	12	80	92
Thylacomyidae	1	2	8	8
Order Notoryctemorphia	1	1	3	2
Notoryctidae	1	1	3	2

Table 2 Classifications of diet and locomotor categories (LC) for American species. In diet: I = insectivore, C = carnivore, O = omnivore. In LC: T = terrestrial, SAq = Semiaquatic, Sc = scansorial, A = arboreal. In References (= Ref): GI = genus inference.

Caenolestidae	Diet	Ref	LC	Ref
1. *Caenolestes caniventer* (Anthony, 1921)	I	1	T	1
2. *Caenolestes fuliginosus* (Tomes, 1863)	O	1	T	1
3. *Lestoros inca* (Thomas, 1917)	I	1	T	1
4. *Rhyncholestes raphanurus* (Osgood, 1924)	I	1	T	1
Didelphidae				
5. *Caluromys derbianus* (Waterhouse, 1841)	O	1	A	1
6. *Caluromys lanatus* (Linnaeus, 1758)	O	2, 5	A	4
7. *Caluromys philander* (Linnaeus, 1758)	O	1	A	3
8. *Chironectes minimus* (Zimmermann, 1780)	C	2	SAq	3
9. *Cryptonanus chacoensis* (Tate, 1931)	I	6	A	4
10. *Cryptonanus guahybae* (Tate, 1931)	I	6	A	3
11. *Didelphis albiventris* (Lund, 1840)	O	2	Sc	4
12. *Didelphis aurita* (Wied-Neuwied, 1826)	O	1	Sc	4
13. *Didelphis marsupialis* (Linnaeus, 1758)	O	1	Sc	4
14. *Didelphis virginiana* (Kerr, 1792)	O	1	Sc	1
15. *Gracilinanus agilis* (Burmeister, 1854)	I	1	A	4
16. *Gracilinanus marica* (Thomas, 1898)	I	1	A	1
17. *Gracilinanus microtarsus* (Wagner, 1842)	I	1	A	4
18. *Lestodelphys halli* (Thomas, 1921)	I	6	T	1
19. *Lutreolina crassicaudata* (Desmarest, 1804)	O	7	T	4
20. *Marmosa constantiae* (O. Thomas, 1904)	I	GI (1)	A	4
21. *Marmosa demerarae* (O. Thomas, 1905)	I	GI (1)	A	4
22. *Marmosa murina* (Linnaeus, 1758)	I	GI (1)	A	3
23. *Marmosa paraguayana* (Tate, 1931)	I	GI (1)	A	3
24. *Marmosops incanus* (Lund, 1840)	I	1	Sc	4
25. *Metachirus myosuros* (Temminck, 1824)	I	2	T	3
26. *Monodelphis americana* (Müller, 1776)	I	6	T	4

27. *Monodelphis dimidiata* (Wagner, 1847)	I	2	T	4
28. *Monodelphis domestica* (Wagner, 1842)	I	6	T	4
29. *Monodelphis kunsi* (Pine, 1975)	I	6	T	4
30. *Monodelphis scalops* (Thomas, 1888)	I	6	T	4
31. *Philander quica* (Olfers, 1818)	O	1	Sc	4
32. *Philander opossum* (Linnaeus, 1758)	O	1	Sc	4
33. *Thylamys cinderella* (Thomas, 1902)	I	GI	T	8
34. *Thylamys citellus* (Thomas, 1912)	I	GI	T	8
35. *Thylamys elegans* (Waterhouse, 1839)	I	1	T	8
36. *Thylamys macrurus* (Olfers, 1818)	I	1	Sc	8
37. *Thylamys pallidior* (O. Thomas, 1902)	I	1	T	8
38. *Thylamys pulchellus* (Cabrera, 1934)	I	GI	T	8
39. *Thylamys pusillus* (Desmarest, 1804)	I	2	T	8
40. *Thylamys venustus* (Thomas, 1902)	I	1	T	8

References: 1 – Myers et al. 2020 (ADW); 2 – Prevosti et al. 2012; 3 – Bubadué et al. 2019; 4 – Paglia et al. 2012; 5 – Cáceres 2005; 6 – Vieira and Camargo 2012; 7 – Cáceres et al. 2002; 8 – Palma and Vieira 2012.

Table 3 Classifications of diet and locomotor categories (LC) for Australasian species. In diet: I = insectivore, C = carnivore, O = omnivore. In LC: T = terrestrial, Sc = scansorial, A = arboreal. In References (= Ref): GI = genus inference, FI = family inference, SI = subfamily inference, MF = inference based on morphological features.

Dasyuridae	Diet	Ref	LC	Ref
41. *Antechinomys laniger* (Gould, 1856)	I	2	T	8
42. *Antechinus flavipes* (Waterhouse, 1838)	I	2	Sc	9/12
43. *Antechinus godmani* (Thomas, 1923)	C	3	A	10
44. *Antechinus swainsonii* (Waterhouse, 1840)	C	3	A	11
45. *Dasycercus cristicauda* (Krefft, 1867)	C	3	T	1
46. *Dasyuroides byrnei* (Spencer, 1896)	C	2	T	1
47. *Dasyurus albopunctatus* (Schlegel, 1880)	C	6	Sc	5
48. *Dasyurus geoffroii* (Gould, 1841)	C	2	T	12
49. *Dasyurus hallucatus* (Gould, 1842)	C	2	T	12
50. *Dasyurus maculatus* (Kerr, 1792)	C	2	Sc	1
51. *Dasyurus viverrinus* (Shaw, 1800)	C	2	Sc	1
52. *Murexia habbema* (Tate & Archbold, 1941)	C	4	T	13
53. *Murexia longicaudata* (Schlegel, 1866)	C	4	T	13
54. *Murexia melanurus* (Thomas, 1899)	C	4	Sc	4
55. *Murexia naso* (Jentink, 1911)	C	4	T	13
56. *Murexia rothschildi* (Tate, 1938)	C	GI	T	13
57. *Myoictis melas* (Müller, 1840)	C	7	Sc	14
58. *Neophascogale lorentzii* (Jentink, 1911)	C	5	A	14
59. *Phascogale calura* (Gould, 1844)	C	3	A	3
60. *Phascolosorex doriae* (Thomas, 1886)	C	GI	A	12
61. *Phascolosorex dorsalis* (Peters & Doria, 1876)	C	4	A	15
62. *Planigale ingrami* (Thomas, 1906)	C	3	T	3
63. *Pseudantechinus macdonnellensis* (Spencer, 1895)	I	2	Sc	12
64. *Sarcophilus harrisii* (Boitard, 1841)	C	2	T	1
65. *Sminthopsis archeri* (Van Dyck, 1986)	C	GI	T	GI
66. *Sminthopsis crassicaudata* (Gould, 1844)	C	3	T	12

67. *Sminthopsis leucopus* (Gray, 1842)	C	3	T	12
68. *Sminthopsis murina* (Waterhouse, 1838)	C	3	T	12
69. *Sminthopsis virginiae* (Tarragon, 1847)	C	3	T	12
Microbiotheriidae				
70. *Dromiciops gliroides* (Thomas, 1894)	I	2	A	18
Myrmecobiidae				
71. *Myrmecobius fasciatus* (Waterhouse, 1836)	I	2	T	1
Notoryctidae				
72. *Notoryctes typhlops* (Stirling, 1889)	I	1/3	T	1
Peramelidae				
73. *Echymipera clara* (Stein, 1932)	O	GI	T	GI (16)
74. *Echymipera kalubu* (Fischer, 1829)	O	1	T	GI (16)
75. *Isoodon auratus* (Ramsay, 1887)	O	1/3	T	1
76. *Isoodon macrourus* (Gould, 1842)	O	1/3	T	GI
77. *Isoodon obesulus* (Shaw, 1797)	O	1/3	T	GI
78. *Microperoryctes longicauda* (Peters & Doria, 1876)	O	GI (7)	T	GI (7)
79. *Microperoryctes papuensis* (Laurie, 1952)	O	GI (7)	T	GI (7)
80. *Perameles bougainville* (Quoy & Gaimard, 1824)	O	1/3	T	1
81. *Perameles gunnii* (Gray, 1838)	O	3	T	12
82. *Perameles nasuta* (É. Geoffroy, 1804)	O	1/3	T	1
83. *Peroryctes raffrayana* (Milne-Edwards, 1878)	O	SI	T	MF
84. *Rhynchomeles prattorum* (Thomas, 1920)	O	FI	T	MF
Thylacinidae				
85. *Thylacinus cynocephalus* (Harris, 1808)	C	1/3	T	1
Thylacomyidae				
86. *Macrotis lagotis* (Reid, 1837)	O	1/3	T	1/3
87. *Macrotis leucura* (Thomas, 1887)	O	1	T	1

References: 1 – Myers et al. 2020 (ADW); 2 – Prevosti et al. 2012; 3 – ALA 2020; 4 – Grossek et al. 2010; 5 - Helgen and Opiang 2011; 6 – Flores et al. 2006; 7 – Wooley 2005; 8 - Stannard and Old 2010; 9 – Lada et al. 2007; 10 – Smith et al. 2017; 11 – Watchorn et al. 2020; 12– Jones et al. 2003; 13 – Cornelio 2011; 14 – Abdala et al. 2006; 15 – Kelly and Sears 2011; 16 – Shevill and Johnson 2008; 17 - Helgen and Flannery 2004; 18 - Amico et al. 2009.

Table 4 Linear models evaluating the size component association with diet and locomotory categories (LC) with and without phylogenetic control. Results are presented for cranium and mandible morphologies in Marsupialia, Ameridelphia and Australidelphia. Significance is highlighted in **bold**.

Marsupialia	Df	R^2	F	P
Cranium Size ~ diet	2, 84	0.391	27.005	**0.000**
+phylogeny	2, 84	0.079	3.624	**0.035**
Cranium Size ~ LC	3, 83	0.067	1.999	0.110
+phylogeny	3, 83	0.018	0.511	0.629
Mandible Size ~ diet	2, 84	0.377	25.396	**0.000**
+phylogeny	2, 84	0.095	4.394	**0.021**
Mandible Size ~ LC	3, 83	0.074	2.202	0.086
+phylogeny	3, 83	0.014	0.404	0.707
Ameridelphia				
Cranium Size ~ diet	2, 37	0.642	33.212	**0.000**
+phylogeny	2, 37	0.091	1.843	0.180
Cranium Size ~ LC	3, 36	0.416	8.532	**0.000**
+phylogeny	3, 36	0.091	1.843	0.180
Mandible Size ~ diet	3, 37	0.632	31.786	**0.000**
+phylogeny	3, 37	0.117	2.451	0.115
Mandible Size ~ LC	3, 36	0.399	7.959	**0.000**
+phylogeny	3, 36	0.036	0.445	0.704
Australidelphia				
Cranium Size ~ diet	2, 45	0.249	7.295	**0.002**
+phylogeny	2, 45	0.090	2.169	0.124
Cranium Size ~ LC	2, 45	0.046	1.056	0.356
+phylogeny	2, 45	0.034	0.771	0.469
Mandible Size ~ diet	2, 45	0.207	5.751	**0.006**
+phylogeny	2, 45	0.103	2.517	0.088
Mandible Size ~ LC	2, 45	0.033	0.761	0.473

+phylogeny	2, 45	0.022	0.486	0.620

Table 5 Pairwise comparison of morphological cranium and mandible size and shape disparities between carnivorous, insectivorous, and omnivorous marsupials. Above the diagonals are the pairwise absolute differences between variables; below are the P values which were computed under 9,999 permutations procedure. C = carnivores, I = insectivores, O = omnivores. Significance is highlighted in **bold**.

	Cranium size				**Cranium shape**		
	C	I	O		C	I	O
C		0.284	0.277	C		0.002	0.001
I	**0.000**		0.007	I	0.713		0.002
O	**0.001**	0.952		O	0.905	0.641	
	Mandible size				**Mandible shape**		
	C	I	O		C	I	O
C		0.193	0.182	C		0.000	0.002
I	**0.000**		0.011	I	0.545		0.002
O	**0.001**	0.888		O	**0.017**	**0.001**	

Table 6 Linear models evaluating shape component association with diet and locomotory categories (LC) with and without phylogenetic control. Results are presented for cranium and mandible morphologies in Marsupialia, Ameridelphia and Australidelphia. Significance is highlighted in **bold**.

Marsupialia	Df	R^2	F	P
Cranium Shape ~ diet	2, 84	0.261	14.828	**0.000**
+phylogeny	2, 84	0.005	0.222	0.790
Cranium Shape ~ LC	3, 83	0.144	4.673	**0.015**
+phylogeny	3, 83	0.030	0.853	0.426
Mandible Shape ~ diet	2, 84	0.298	17.816	**0.000**
+phylogeny	2, 84	0.002	0.079	0.919
Mandible Shape ~ LC	3, 83	0.085	2.559	0.079
+phylogeny	3, 83	0.025	0.698	0.520
Ameridelphia				
Cranium Shape ~ diet	2, 37	0.047	0.911	0.356
+phylogeny	2, 37	0.043	0.831	0.386
Cranium Shape ~ LC	3, 36	0.183	2.680	0.061
+phylogeny	3, 36	0.043	0.831	0.386
Mandible Shape ~ diet	3, 37	0.013	0.246	0.737
+phylogeny	3, 37	0.034	0.661	0.478
Mandible Shape ~ LC	3, 36	0.268	4.394	**0.011**
+phylogeny	3, 36	0.063	0.803	0.486
Australidelphia				
Cranium Shape ~ diet	2, 45	0.509	22.837	**0.000**
+phylogeny	2, 45	0.028	0.637	0.518
Cranium Shape ~ LC	2, 45	0.221	6.231	**0.004**
+phylogeny	2, 45	0.056	1.313	0.277
Mandible Shape ~ diet	2, 45	0.637	38.612	**0.000**
+phylogeny	2, 45	0.051	1.175	0.310
Mandible Shape ~ LC	2, 45	0.113	2.805	0.073

+phylogeny	2, 45	0.057	1.319	0.278

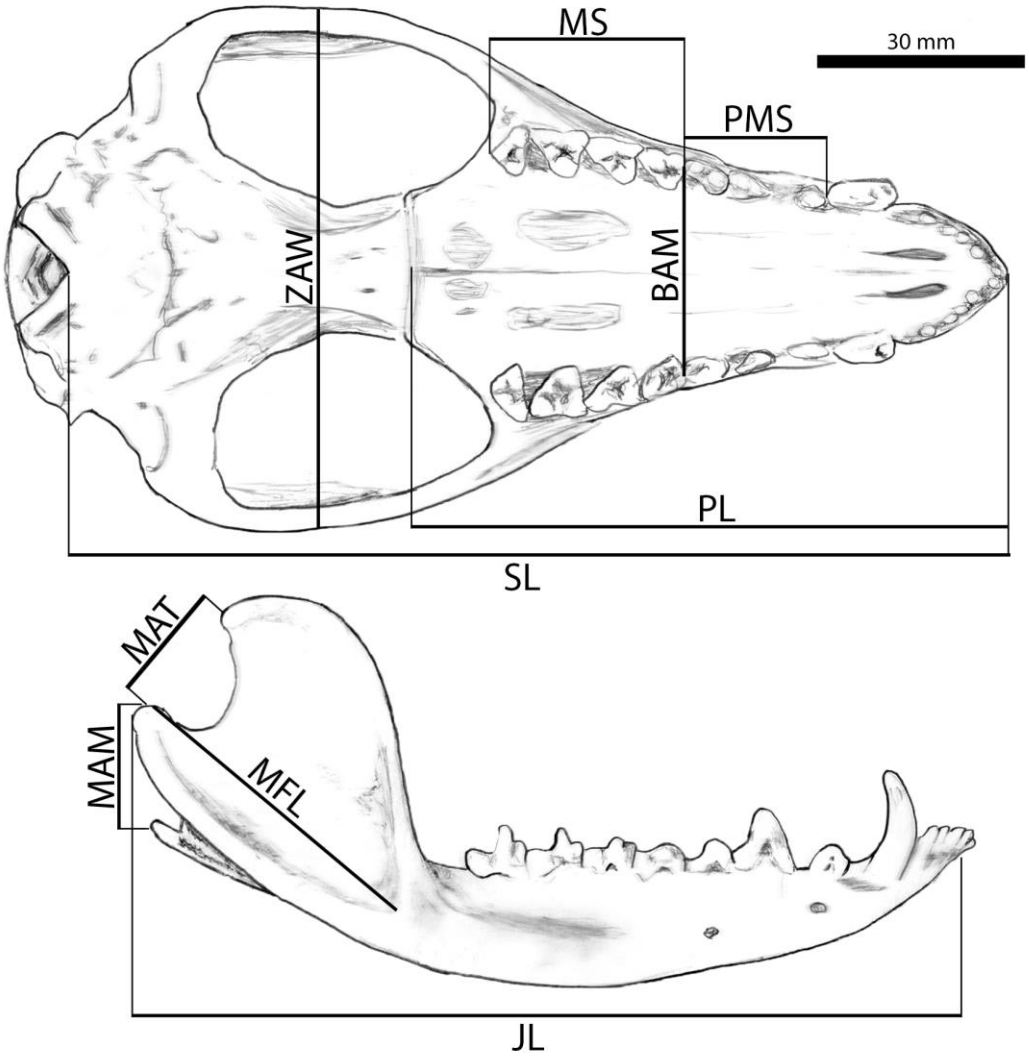

Fig. 1 Schematic drawing of *Didelphis marsupialis*, based on specimen MPEG 20747, with the measurements used in this study. Skull: zygomatic arm width (ZAW), palatal length (PL), skull length (SL), breadth between molars (BAM), length of upper molar series (MS), length of upper pre-molar series (PMS); Mandible: moment arm of temporalis (MAT), moment arm of masseter (MAM), masseteric fossa length (MFL), jaw length (JL).

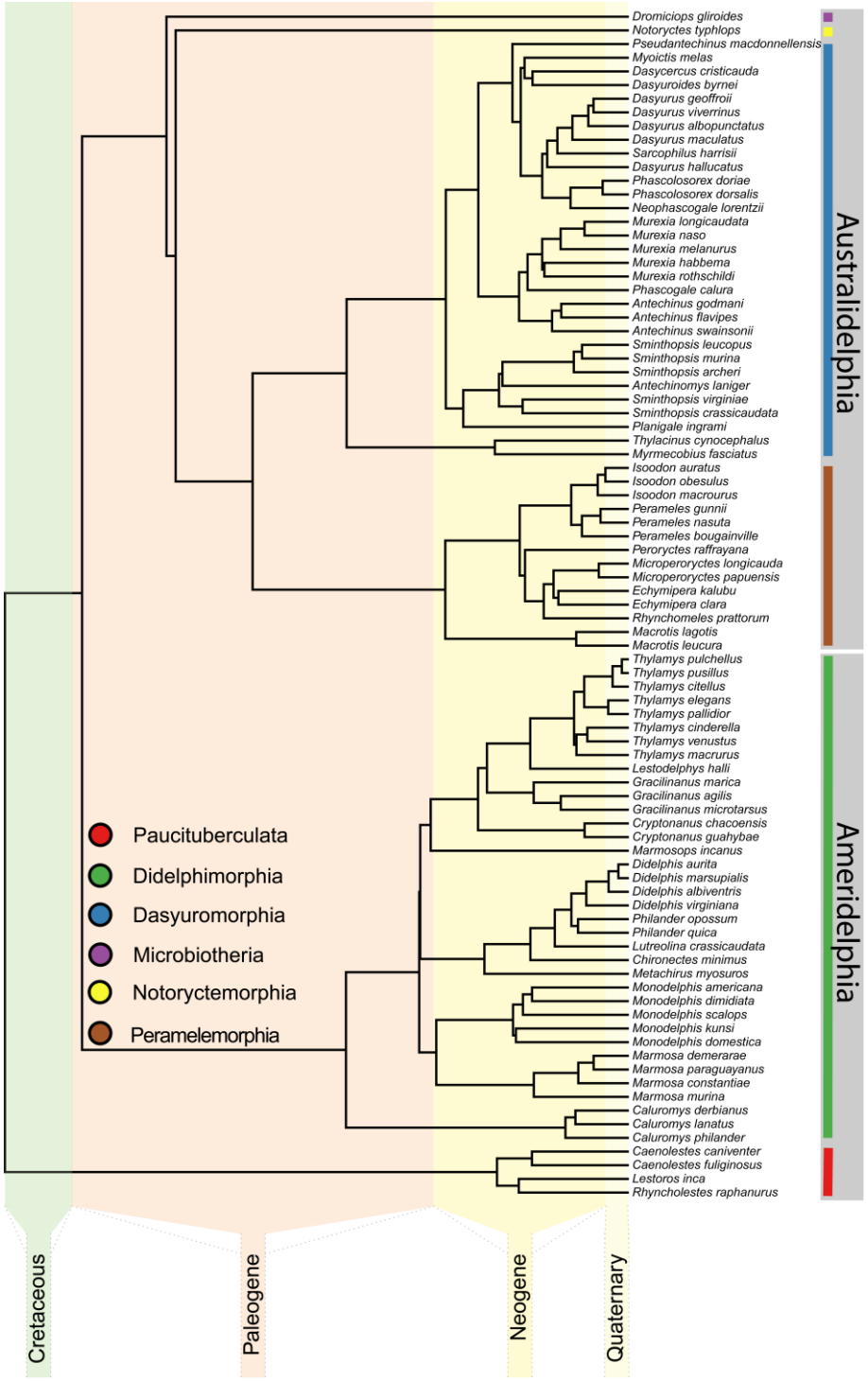

Fig. 2 Upham et al. (2019) majority consensus phylogenetic topology for Australidelphia + Ameridelphia.

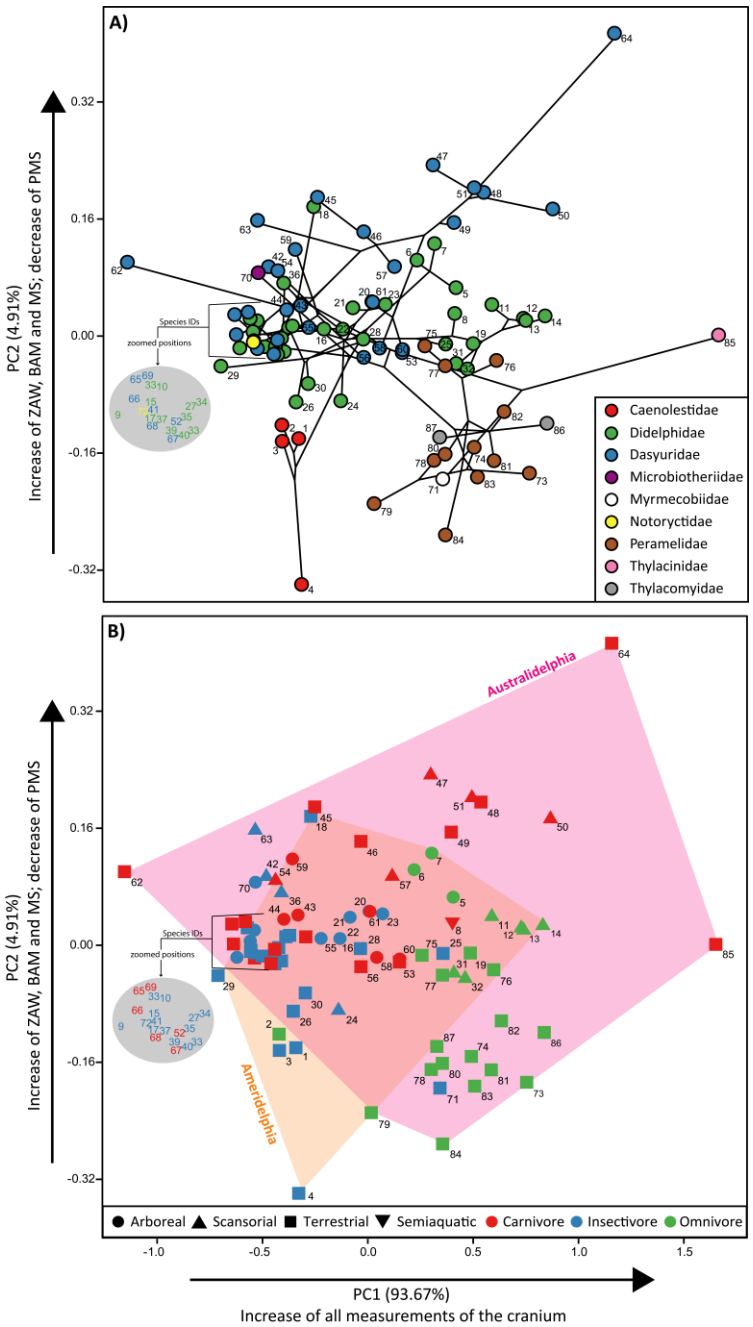

Fig. 3 Scatter plot of PC1 versus PC2 for the log transformed morphological measurements of the cranium of marsupials. A) Phylogeny mapped into morphospace with families labelled by different colours. B) Same but, separating Ameridelphia and Australidelphia with convex hulls, with species being coloured by different diet categories (C = carnivorous, O = omnivorous, I = insectivorous) and symbols representing different locomotion (T = terrestrial, Sc = scansorial, A = arboreal, SAq = semi-aquatic) categories. ZAW = zygomatic arm width, BAM = breadth between molars, MS = length of upper molar series, PMS = length of upper pre-molar series.

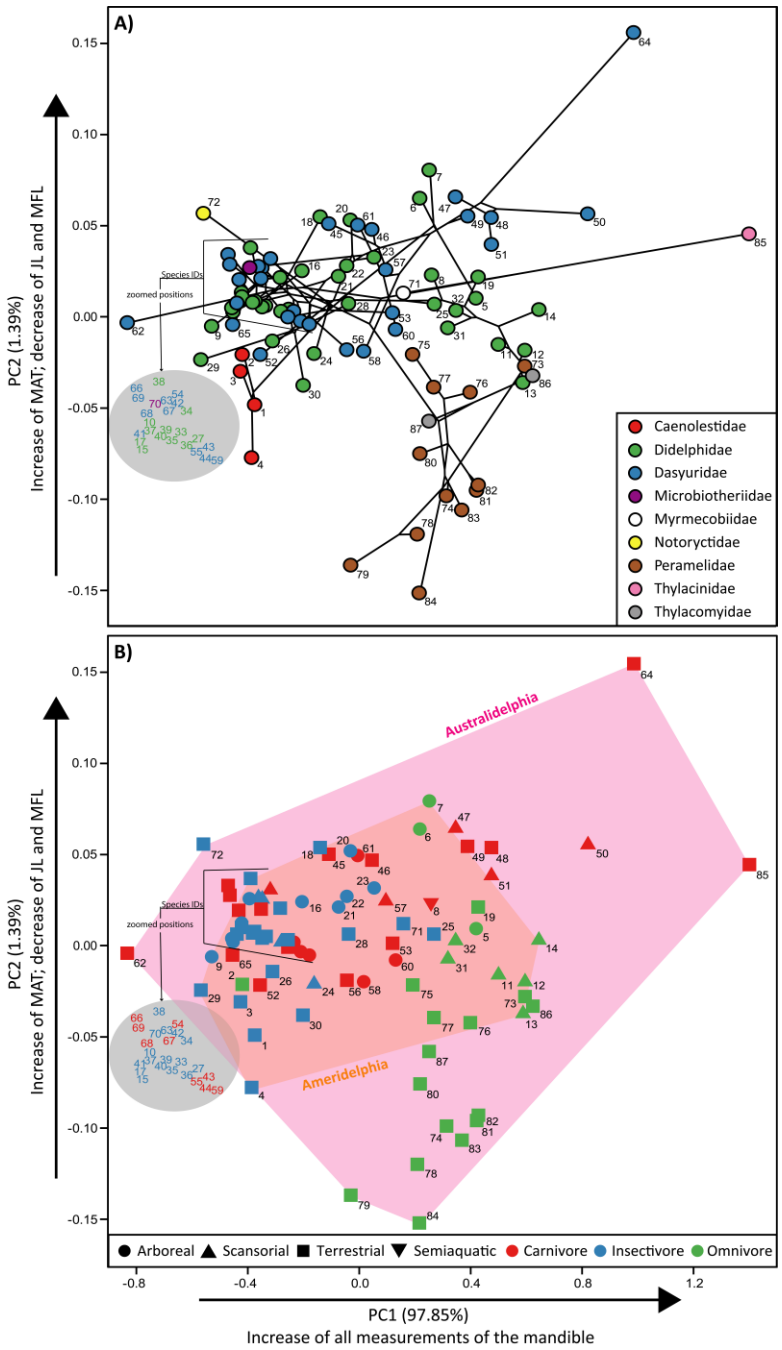

Figure 4 Scatter plot of PC1 versus PC2 for the log transformed morphological measurements of the mandible of marsupials. A) Phylogeny mapped into morphospace with families labelled by different colours. B) Same but, separating Ameridelphia and Australidelphia with convex hulls, with species being coloured by different diet categories (C = carnivorous, O = omnivorous, I = insectivorous) and symbols representing different locomotion (T = terrestrial, Sc = scansorial, A = arboreal, SAq = semi-aquatic) categories. JL = jaw Length, MAT = moment arm of temporalis, MFL = masseteric fossa length.