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Mariana do Nascimento Brum

TAXAS EVOLUTIVAS, DISPARIDADE E ECOMORFOLOGIA DA MANDÍBULA DE MARSUPIAIS AMERICANOS

Santa Maria, RS
2022

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Dissertação apresentada ao Curso de Pós-Graduação em Biodiversidade Animal, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do título de **Mestre em Ciências Biológicas – Área Biodiversidade Animal**.

Orientador: Profº. Drº. Nilton Carlos Cáceres
Coorientadora: Drª. Jamile de Moura Bubadué

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2021

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RESUMO

TAXAS EVOLUTIVAS, DISPARIDADE E ECOMORFOLOGIA DA MANDÍBULA DE MARSUPIAIS AMERICANOS

AUTORA: Mariana do Nascimento Brum

ORIENTADOR: Nilton Carlos Cáceres

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Os marsupiais americanos constituem um grupo diverso de mamíferos que estão distribuídos ao longo de todo o continente. Atualmente as suas mais de ~120 espécies são classificadas em 3 ordens principais (Paucituberculata, Microbiotheria e Didelphimorphia). Devido à sua grande diversidade, o grupo é foco de numerosos estudos evolutivos, dentre os quais pode-se constatar que essas espécies possuem um alto grau de integração morfológica em suas estruturas cranianas. Todavia, os os padrões adaptativos do grupo ainda estão pouco claros. Como a grande maioria dos marsupiais americanos possuem hábitos noturnos e solitários, o estudo dos seus comportamentos de forrageio em ambiente natural é desafiador. Pesquisadores tem buscado descrever hipóteses de dieta com base em diferentes metodologias. Contudo, a relação morfofuncional da mandíbula dos marsupiais americanos com as principais hipóteses de dieta, sobretudo as mais recentes, ainda não foi amplamente estudada. Neste estudo, alto efeito do hábito alimentar no tamanho da mandíbula dos marsupiais americanos foi observado, porém não na sua forma. Estudos anteriores constaram que a variabilidade do crânio dos marsupiais é fortemente associada com a variação do tamanho (= alometria) tanto intra- quanto interespecificamente. Essa variabilidade morfológica alométrica pode auxiliar nas explicações morfofuncionais do crânio neste grupo. Constatamos que a alometria é um fator importante na diversidade morfológica do grupo, afetando as taxas evolutivas e de disparidade morfológica em alguns clados, possivelmente influenciando na heterogenia morfológica encontrada entre os clados. Além disso, a história biogeográfica dos diferentes clados de marsupiais americanos é um outro fator importante aqui discutido, que potencialmente explicam a heterogenia dos valores de taxas evolutivas e disparidade encontrados na forma e tamanho da mandíbula destes animais. Salientamos a importância da de melhorar a qualidade das hipóteses funcionais em estudos evolutivos no grupo, levando em consideração as propriedades dos itens alimentares (dureza e tamanho) consumidos por estes animais.

Palavras-chave: Caenolestidae, Microbiotheria, Didelphimorphia, alometria, dieta, morfologia

ABSTRACT

EVOLUTIONARY RATES, DISPARITY, AND ECOMORPHOLOGY OF THE MANDIBLE OF AMERICAN MARSUPIALS

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The American marsupials chosen a diverse group of mammals that are distributed throughout the entire continent. Currently its more than ~120 species are classified into 3 main orders (Paucituberculata, Microbiotheria and Didelphimorphia). Due to its great diversity, the group is the focus of numerous evolutionary studies, among which it can be seen that these species have a high degree of morphological integration in their cranial structures. However, the adaptive patterns of the group are still unclear. As the vast majority of marsupials have nocturnal and solitary habits, the study of their foraging behavior in a natural environment is challenging. Researchers have searched for hypothetical diet descriptors based on different methodologies. However, a morphofunctional relationship of the American marsupial mandible with the main diet hypotheses, especially the more recent ones, has not yet been widely studied. In this study, the high effect of eating habits on the mandible size of American marsupials was observed, but not on their shape. Previous studies have found that variability in marsupial variety is associated with variation in size (= allometry) both intra and interspecifically. This allometric morphological variability can help in morphofunctional explanations of blood in this group. We found that allometry is an important factor in the morphological diversity of the group, affecting the rates of evolution and morphological disparity in some clades, possibly influencing the morphological heterogeneity found among the clades. Furthermore, the biogeographic history of the different clades of American marsupials is another important factor discussed here, which potentially explains the heterogeneity of the values of evolutionary rates and disparities found in the shape and size of the mandible of these animals. We emphasize the importance of improving the quality of the pending hypotheses in evolutionary studies in the group, taking into account the properties of the food items (hardness and size) consumed by these animals.

Keywords: Caenolestidae, Microbiotheria, Didelphimorphia, allometry, diet, morphology

SUMÁRIO

INTRODUÇÃO

Os marsupiais se diversificaram no início do Cretáceo, estando hoje geograficamente restritos às Américas e Oceania (GOIN et al., 2016). Na América, os marsupiais estão distribuídos ao longo de todo o continente, principalmente na América do Sul e Central (ELDRIDGE et al., 2019). Atualmente, existem cerca de ~120 espécies de marsupiais americanos (BURGIN et al., 2018), classificados em três ordens: Paucituberculata, representada pelos pequenos marsupiais insetívoros endêmicos da região dos Andes (MARTIN e GONZÁLES-CHÁVEZ, 2016); Microbiotheria, representada apenas por uma espécie vivente, encontrada apenas nas florestas temperadas chilenas e argentinas (VALLADÁRES-GOMES et al, 2017); e Didelphimorphia, sendo a mais diversa, com espécies distribuídas ao longo de toda América (VOSS e JANSA, 2009). Desta forma, a diversidade de marsupiais americanos pode ser estudada a nível morfológico em relação ao seu contexto evolutivo e biogeográfico.

A disparidade morfológica é uma forma de avaliar a diversidade entre clados, uma vez que essa métrica quantifica a variedade morfológica entre grupos (GOSWAMI e POLLY, 2010). Em geral, os marsupiais são conhecidos por terem menor disparidade e menores taxas evolutivas que os demais mamíferos (FABRE et al., 2021). Além disso, em comparação com os australianos, as espécies americanas também apresentam menor variedade morfológica e menores taxas evolutivas (BUBADUÉ et al., 2022). Contudo, há uma lacuna sobre a variabilidade entre os clados das espécies americanas.

Os marsupiais possuem uma morfologia altamente integrada (SHIRAI e MARROIG, 2010; GOSWAMI et al., 2016). Isso significa que os componentes morfogenéticos do crânio se desenvolvem de forma coordenada, possuindo alta correlação entre si (MONTEIRO, 2005). Essas restrições podem afetar a diversificação morfológica dos clados, gerando maiores ou menores níveis de disparidade entre as espécies, e ao longo do tempo evolutivo (GOSWANI et al., 2016). Além da integração morfológica, a associação entre o tamanho e forma (= alometria) das estruturas ósseas pode estar intimamente relacionada com os padrões de disparidade e taxas evolutivas entre os clados (SANSALONE, et al., 2018). Essa associação em marsupiais é evidente, sendo uma importante questão a ser analisada quando se trata da evolução do grupo (CHEMISQUY et al., 2014; MAGNUS e CÁCERES, 2016; BUBADUÉ et al., 2019; CHEMISQUY et al., 2020).

Estudos de ecomorfologia em marsupiais americanos ainda possuem uma grande lacuna. Um dos principais temas que dificultam esse tipo de estudo é em relação aos hábitos alimentares do grupo. Por possuírem, em sua grande maioria, hábitos noturnos e solitários, as espécies são difíceis de serem visualizadas em campo, dificultando a descrição de seu comportamento alimentar (CASTRO, DAHUR e FERREIRA, 2021). Nos últimos anos, vários trabalhos tem buscado descrever e melhorar as classificações de dieta para o grupo (VIEIRA e ASTÚA, 2003; BUBADUÉ et al., 2022; AMADOR e GIANNINI, 2021). Além da dieta, o hábito locomotor pode ser utilizado como uma alternativa para estudos de morfologia funcional, visto que pode ser associado à forma com que as espécies forrageiam (DUMONT et al., 2016), embora na prática hipóteses de locomoção não foram bem sucedidas em explicar a morfologia do crânio e mandíbula destes animais (CHEMISQUY et al., 2021).

Considerando isso, o objetivo deste trabalho foi compreender os padrões de diversificação morfológica entre os clados de mamíferos americanos, avaliando aspectos morfofuncionais da mandíbula destes animais. Tivemos como objetivos específicos: 1) comparar e descrever o tamanho e a forma da mandíbula de marsupiais americanos de uma perspectiva evolutiva; 2) analisar o padrão alométrico da mandíbula do grupo e como isso afetou a sua diversificação; 3) verificar as diferenças na disparidade morfológica entre os clados; 4) identificar as diferenças de taxas evolutivas entre os clados de marsupiais americanos; 5) testar o efeito de diferentes hipóteses de dieta, categorias locomotoras e sua interação como alternativa para explicar a ecomorfologia do grupo.

Estrutura da Dissertação

Esta dissertação está estruturada em formato *Research Article*, conforme as normas da revista *Journal of Mammalian Evolution.*

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Evolutionary rates, disparity, and ecomorphology of the mandible of American marsupials

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Abstract

The American marsupials have been consistently reported to present a highly integrated and conserved morphology. Previous research have attempted to understand their evolution in relation their feeding and foraging ecology, and most recently, new dietary hypotheses have emerged. We found that the mandibular size variation varies in accordance to their feeding ecology. Smaller marsupials are mostly insectivores, while large marsupials have a more diversified diet, probably related to the increased nutritional value other food contents can add to support the largest body sizes. Keeping with previous research, we found that mandible shape variation is intrinsically related to the speciation processes of marsupials, and that allometry is a common path to increase morphological diversity in this group. Morphological variation of American marsupials is not homogeneously distributed, nor it is accumulated in the same tempo of evolution across clades. The lack of mandibular shape ecomorphs in American marsupials could be related to their overall more generalised morphology. However, we argue that this could also be because the dietary hypothesis at hand do not specify the properties of food items each species is capable of processing, which, from a biomechanical perspective, is more important in order to fully understand mandibular functional morphology.

Key-words: adaptation, Caenolestidae, Didelphidae, macroevolution, morphometrics

Introduction

The American marsupials morphology is highly integrated (Porto et al. 2008; Shirai and Marroig 2010; Goswami et al. 2012; Goswami et al. 2016). Integration patterns in morphology may affect clade phenotypic variability (Klingenberg 2010), but it can also promote coordinate morphological changes of traits, not necessarily affecting evolutionary rates (Goswami et al. 2014). The observed constraints in marsupial morphology are not completely detrimental to evolution because it can also facilitate variation trough paths of least resistance (Shirai and Marroig 2010). In American marsupials allometry constitutes one of these paths, leading to the variation of specific shape traits in relation to size, being important for morphological discrimination of clades (Chemisquy et al. 2014; Magnus and Cáceres 2017; Chemisquy et al. 2021). Still, marsupials allometric and evolutionary constraints tends to difficult the detection of ecomorphological patterns linked to their evolution (Goswami et al. 2016).

Many studies had attempted to understand how diet and morphology correlates in American marsupials (Prevosti et al. 2011; Chemisquy et al. 2021), but failed to report a clear relationship between the type of food item and the morphological evolution within the group. One of the main difficulties for studying American-marsupial functional morphological diversity is the lack of detailed dietary information for several species within the group (Chemisquy et al. 2021). This is due to the lack of behavioural studies, since most marsupial species have nocturnal and solitary habits, making it difficult to visualize them under field conditions (Castro et al. 2021). Hence, understanding and successfully classifying the food habits of American marsupials can be challenging (Lessa and Geise 2010).

Vieira and Astúa (2003) attempted to classify marsupials diets at the genus level according to the degree of herbivory (consuming mainly vegetation, with more carbohydrates) in relation to insectivory/carnivory (consuming mainly insects or small vertebrates, with more proteins). This attempt has been previously used in studies that investigated functional morphology in the skull of the American marsupials, not achieving statistical support to explain cranial shape differences among species (Chemisquy et al. 2021). More recently, other dietary hypothesis has been presented, like the three (animalivore, omnivore and herbivore) and five levels categorizations (carnivore, frugivore, insectivore, omnivore and carnivore-insectivore) of Amador and Giannini (2021) and the three levels approach (carnivore, insectivore, omnivore) of (Bubadué et al. 2022). As an alternative to diet, locomotor categories can be an important factor to consider (Dumont et al. 2016; Zelditch et al. 2017) because it gives us a better estimation on how these marsupials move and capture their food while foraging. In this regards, however, Chemisquy et al. (2021) found no significant cranial shape differences with different use of the vertical strata.

Marsupials in America represent nearly 1/3 of the number of species in the world and are organized into three main orders: Didelphimorphia, Paucituberculata, and Microbiotheria (Amador and Giannini 2016). Didelphimorphia has the largest number of extant species in America (N = ~120, (Burgin et al. 2018)), along with the greatest variability in size, varying from the smallest, weighting around 10g (genus *Gracilinanus*), to the largest species weighting ~1.5kg (genus *Didelphis*) (Amador and Giannini 2016; Burgin et al. 2018). The group is classified into 4 subfamilies (Glironiinae, Caluromyinae, Hyladelphinae, and Didelphinae) and 4 tribes (Marmosini, Metachirini, Didelphini, and Thylamini) (Voss and Jansa 2009). Meanwhile, Paucituberculata is represented by 6 species of small insectivorous marsupials (~100g) restricted to the Andes (Martin 2011; Abello 2013) and Microbiotheria which is represented by only one extant species, the "monito del monte" *Dromiciops gliroides*, distributed in Subantarctic forests of southern Chile and Argentina (Fontúrbel et al. 2014). The marsupials found in America present a high variability of morphological and ecological traits, generally related to the evolutionary histories of clades (Amador and Giannini 2016).

Evolutionary rates and morphological disparity patterns are not necessarily uniform through major clades evolutionary history (Sherratt et al. 2017; Siqueira et al. 2020). It has been reported that American marsupials tend to have lower evolutionary rates than the Australian clade, along with overall lower morphological disparity (Bubadué et al. 2022). It is still not completely clear, however, if the low disparity and evolutionary rates within specific American clades are uniform to the point where it could explain the lack of adherence of clear ecomorphotypes among them. Alternatively, the lack of high-quality dietary information on these species (Lessa and Geise 2010) could also be detrimental to test ecomorphological hypotheses in the whole group. Therefore, a more detailed comparison of morphological disparity and evolutionary rates among the clades of American marsupials along with a broader comparison of all existing dietary classifications for these animals (Vieira and Astúa 2003; Amador and Giannini 2021; Bubadué et al. 2022) is fundamental to understand the lack of adherence of ecomorphological patterns in these animals.

This study aims to test these emerging dietary hypotheses and locomotor categories in the American-marsupial mandible size and shape, along with the main patterns of diversification within and between taxonomic clades. Our specifics objectives are: 1) to compare and describe the morphological size and shape of the mandible of American marsupials from an evolutionary perspective; 2) to analyse the allometric pattern of the mandible of American marsupials and its role in morphological diversification; 3) to analyse the differences in morphological disparity among American marsupials clades; 4) to identify the evolutionary rate differences among clades; 5) to test for the effect of different dietary hypotheses, locomotor categories, and their interaction as an alternative to explain functional morphology in American marsupials.

Material and Methods

We collected 222 photos of the mandible in lateral view of 42 species of American marsupials, representing 38.2% of the existing species diversity and 77.3% of genera diversity (Table 1). The material studied belongs to the mammal collections of the Museu Argentino de Ciência Naturais (MACN), Museu de Ciências Naturais da Fundação Zoobotânica (MCNFZB), Museu de História Natural Capão da Imbuia (MHNCI), Museo de La Plata (MLP), Museu Nacional do Rio de Janeiro (MN), Museu Paraense Emílio Goeldi (MPEG), Museu de Zoologia da Universidade de São Paulo (MZUSP), Natural History Museum of London (NHN), Universidade Federal de Santa Catarina (UFSC), Universidade do Estado de Mato Grosso (UNEMAT), Universidade Federal de Santa Maria (UFSM), and World Museum Liverpool (WML).

Table 1. Number of extant species from 17 genera used in the study, as well as the number of collected specimens at scientific museums.

	Extant species	Collected species	%	Specimens
Caenolestidae	6	4	66.7	19
Caenolestes	4	2	50	11
Lestoros	1	1	100	6
Rhyncholestes	1	1	100	2
Didelphidae	120	37	37.8	188
Caluromys	3	3	100	19
Chironectes	1	1	100	4
Cryptonanus	5	2	40	16
Didelphis	6	4	66.7	31
Gracilinanus	G	3	50	15
Lestodelphys	1	1	100	7
Lutreolina	1	1	100	12

Marmosa	19	4	21.1	13
Marmosops	17	1	5.88	2
Metachirus	1	1	100	4
Monodelphis	21	5	23.8	23
Philander	7	2	28.6	6
Thylamys	10	8	80	36
Microbiotheriidae	1	1	100	5
Dromiciops	1	1	100	5
Total	112	47	42	212

We used tpsDig2 (Rohlf 2015) to position 8 anatomical landmarks and 3 sections of curvature semi-landmarks to capture the marsupial mandibular size and shape variations (Fig. 1). We then slide and aligned the raw coordinates with Generalized Procrustes Analysis (GPA) in the package 'geomorph' (Adams et al. 2021). With this process, size (log centroid size) and shape variables (Procrustes coordinates) were extracted from these raw coordinates. The centroid size of the structures was calculated from the square root of the sum of squared distances of each landmark from the centre of each configuration (Zelditch et al. 2012).

Figure 1. Position of landmarks and semi landmarks in the mandible of *Didelphis marsupialis* (MPEG 12810). 1 = base of first lower incisor; 2 = posterior base of the fourth lower incisor; 3 = posterior base of lower canine; 4 = midpoint between third premolar and first molar; 5 = posterior base of the fourth lower molar; 6 = junction between mandibular corpus and ramus; 7 = posterior end of the coronoid process; 8 = lateral tip of the condylar process; 9 = tip of the angular process; 10 = anteromedial end of the masseteric fossa; Semilandmarks: 1st curve: 14 points sliding between landmarks 1 and 9; 2nd curve: 10 points sliding between landmarks 6 and 7; 3rd curve: 10 landmarks sliding between landmarks 7 and 8.

We built a majority-rule consensus tree from 10k node-dated trees from Upham et al. (2019) in Mesquite 3.51 (Maddison and Maddison 2018) to perform all comparative analyses.

We used four different dietary hypotheses to describe morphological variation of American marsupials (Table 2). Vieira and Astúa (2003) that describes five levels (I to V) of diet, according to food specialization. Bubadué et al. (2022) that categorized species in 3 levels based on literature (I = insectivore, C = carnivore, O = omnivore). Amador and Giannini (2021) that used a compilation from different literature sources and divide in two schemes, a simpler one (A = animalivore, O = omnivore, H = herbivore) and a complete one (I = insectivory, O = omnivore, F = frugivore, C = carnivore, IC = insectivore-carnivore). We also used the four locomotor categories based in Bubadué et al. (2022) (T = terrestrial, A = arboreal, SA = semiaquatic and S = scansorial) as an alternative and interaction factor to dietary regimes.

Table 2. Diet hypotheses and locomotor category classifications to the species considered in this study.

Species	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**LC**
Caenolestidae					
Caenolestes caniventer (Anthony, 1921)	IV	I	I	A	T
Caenolestes fuliginosus (Tomes, 1863)	IV	O	I	A	T
Lestoros inca (Thomas, 1917)	IV	I	I	A	T
Rhyncholestes raphanurus (Osgood, 1924)	IV	I	I	A	T
Didelphidae					
Caluromys derbianus (Waterhouse, 1841)	IV	O	F	H	A
Caluromys lanatus (Olfers, 1818)	I	O	F	H	A
Caluromys philander (Linnaeus, 1758)	I	O	F	H	A
Chironectes minimus (Zimmermann, 1780)	V	C	C	A	SA
Cryptonanus agricolai (Moojen, 1943)	IV	O	I	A	A
Cryptonanus chacoensis (Tate, 1931)	IV	O	I	A	A
Cryptonanus guahybae (Tate, 1931)	IV	O	I	A	A
Didelphis albiventris (Lund, 1840)	III	C	O	O	S
Didelphis aurita (Wied-Neuwied, 1826)	III	O	O	O	S
Didelphis marsupialis (Linnaeus, 1758)	III	O	O	O	S
Didelphis virginiana (Kerr, 1792)	III	O	O	O	S
Gracilinanus agilis (Burmeister, 1854)	II	I	I	A	A
Gracilinanus marica (Thomas, 1898)	II	I	I	A	A
Gracilinanus microtarsus (Wagner, 1842)	II	I	I	A	A
Lestodelphys halli (Thomas, 1921)	V	C	C	A	T
Lutreolina crassicaudata (Desmarest, 1804)	V	O	C	A	T
Marmosa constantiae (O. Thomas, 1904)	II	I	C	A	A
Marmosa demerarae (O. Thomas, 1905)	II	I	I	A	A
Marmosa murina (Linnaeus, 1758)	II	I	I	A	A
Marmosa paraguayana (Tate, 1931)	II	I	I	A	A
Marmosops incanus (Lund, 1840)	II	I	I	A	S

Metachirus myosuros (Temminck, 1824)	IV	O	I	A	T
Monodelphis americana (Müller, 1776)	IV	O	I	A	T
Monodelphis dimidiata (Wagner, 1847)	IV	O	I	A	T
Monodelphis domestica (Wagner, 1842)	IV	O	I	A	T
Monodelphis kunsi (Pine, 1975)	IV	O	I	A	T
Monodelphis *scalops* (Thomas, 1888)	IV	O	I	A	T
Philander quica (Temminck, 1824)	IV	O	IC	A	S
Philander opossum (Linnaeus, 1758)	IV	O	O	O	S
Thylamys cinderella (Thomas, 1902)	IV	I	I	A	T
Thylamys citellus (Thomas, 1912)	IV	I	I	A	T
Thylamys elegans (Waterhouse, 1839)	IV	I	I	A	S
Thylamys macrurus (Olfers, 1818)	IV	I	I	A	T
Thylamys pallidior (O. Thomas, 1902)	IV	I	I	A	T
Thylamys pulchellus (Cabrera, 1934)	IV	I	I	A	T
Thylamys pusillus (Desmarest, 1804)	IV	I	I	A	T
Thylamys venustus (Thomas, 1902)	IV	I	I	A	T
Microbiotheriidae					
Dromiciops gliroides (Thomas, 1894)	IV	I	I	A	A

In 'phytools' (Revell 2011), we used the function 'phenogram' to project our phylogenetic tree in a space defined by log centroid size in order to fully describe its variation across clades and dietary categories. We used a phylogenetic linear model with randomized 9,999 residual permutation implemented with function procD.pgls in "geomorph" (Adams et al. 2021) to test for the different dietary hypotheses and locomotor categories in size variation.

For shape, the Procrustes coordinates were used in a Principal Component Analysis (PCA), mapping the phylogeny, to view and describe the overall variation in marsupial mandibular shape. To assess the effects of size-related shape variation (allometry) and its interaction with clades as a factor we first performed a Procrustes linear model with randomized 9,999 residual permutation ((Monteiro 1999); implemented with function procD.lm in "geomorph" (Adams et al. 2021). We then performed the phylogenetic model, also randomizing residuals with 9,999 permutations procedure, to estimate the strength of allometry in the mandible when phylogenetic relationships are accounted for. Finally, we tested for the different dietary hypotheses and locomotor categories in shape variation (Adams et al. 2021).

To compare the morphological variation between clades, we calculated Procrustes disparity, based in the sum of the diagonal elements of the group covariance matrix (Zelditch et al. 2012), using the function "morphol.disparity" of 'geomorph' (Adams et al. 2021). We also computed the size and shape net rates of morphological change over time in the phylogeny (= evolutionary rate, (Adams 2014)), represented by the σ^2, of all American marsupials and between clades. These rates were estimated using the "compare.evol.rates' function, also in geomorph (Adams et al. 2021).

Results

We found a large effect of all dietary hypotheses, but not of locomotor categories, in mandibular size, where diet hypotheses 1 ($R^2 = 0.342$) and 3 ($R^2 = 0.325$) presented the largest effects (Table 3). We highlighted the 3rd hypothesis, which is the 5 level hypothesis of Amador and Giannini (2021) in our phenogram (Fig. 2) to describe differences in size in relation to dietary categories. The largest mandibles are from the omnivore, carnivore, insectivores/carnivores Didelphini and the omnivores/frugivores Caluromyinae, while the smallest mandibles were from insectivores from various clades (Fig. 2).

The first principal component (PC1) summarised 30.99% of mandible variation and separates the frugivorous/omnivorous Caluromyinae from other clades (Fig. 3, positive scores), given their shorter and wider dentary, with an advantageous and posteriorly oriented coronoid process and smaller condylar process, if compared to other clades. PC2 (23.73% of variation) separates the Caenolestinae from the other clades (Fig. 3, negative scores), due to their shorter coronoid process and more pronounced condylar process. PC3 (15.95% of variation) separates Thylamyini, Microbiotheriidae and Caenolestinae, all small insectivores, due to their overall thinner and longer mandible (Fig. 4 positive scores) from Didelphini, with opposite deformations. PC4 also represents high values of variation (9.48%), but clades largely overlap in this dimension (Fig. 4). The main variation along the PC4 is in the size of the coronoid process and condylar process set. In the lowest values of PC4 are the species with thinner coronoid process and largest condylar process, being the highest values of the axis presenting opposite deformations.

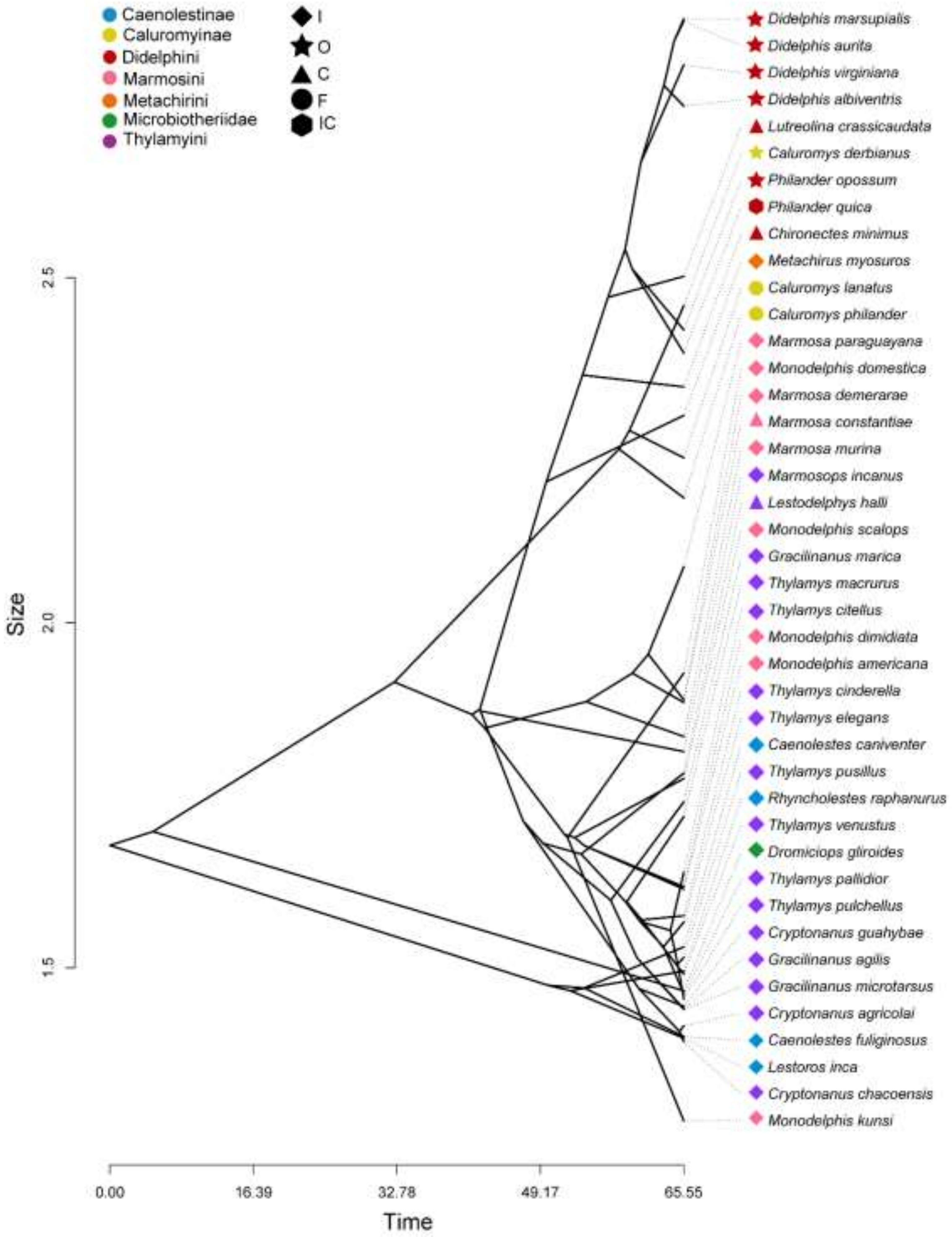

Figure 2. Phenogram of size variation using Upham et al. (2019) American marsupial majority-rule consensus tree with clades and classification of diet 3 hypothesis, by Amador and Giannini (2021).

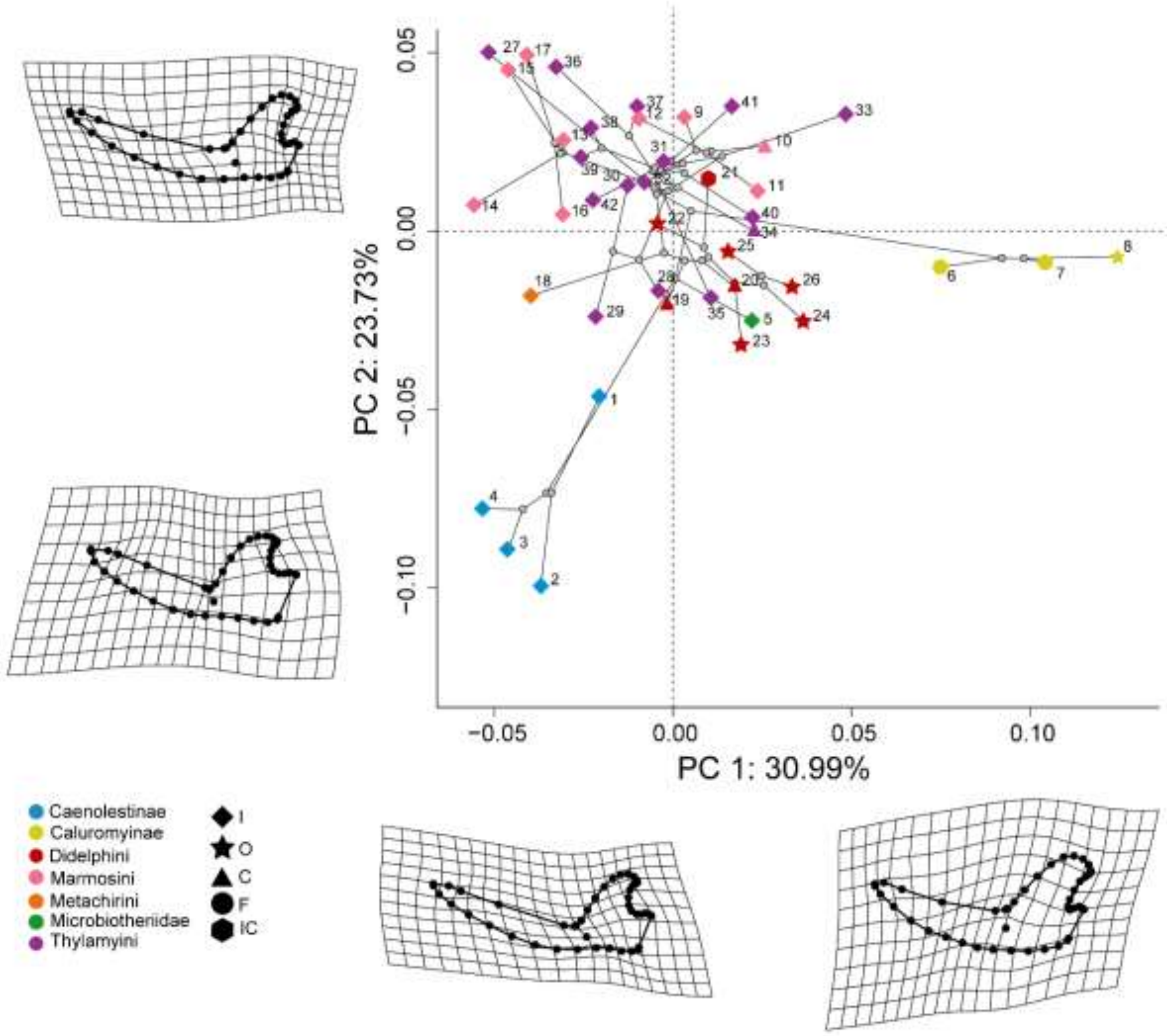

Figure 3. Phylomorphospace of first and second axis of mandible shape variation of American marsupials, with deformations according to each principal component. The colors represent the clades and the symbols the different dietary categories, according to diet 3 hypothesis. The species are numbered as: (1) *Rhyncholestes raphanurus*; (2) *Lestoros inca*; (3) *Caenolestes fuliginosus*; (4) *Caenolestes caniventer*; (5) *Dromiciops gliroides*; (6) *Caluromys philander*; (7) *Caluromys lanatus*; (8) *Caluromys derbianus*; (9) *Marmosa murina*; (10) *Marmosa constantiae*; (11) *Marmosa paraguayana*; (12) *Marmosa demerarae*; (13) *Monodelphis domestica*; (14) *Monodelphis kunsi*; (15) *Monodelphis scallops*; (16) *Monodelphis dimidiata*; (17) *Monodelphis americana*; (18) *Metachirus myosuros*; (19) *Chironectes minimus*; (20) *Lutreolina crassicaudata*; (21) *Philander quica*; (22) *Philander opossum*; (23) *Didelphis virginiana*; (24) *Didelphis albiventris*; (25) *Didelphis marsupialis*; (26) *Didelphis aurita*; (27) *Marmosops incanus*; (28) *Cryptonanus guahybae*; (29) *Cryptonanus agricolai*; (30) *Cryptonanus chacoensis*; (31) *Gracilinanus microtarsus*; (32) *Gracilinanus agilis*; (33) *Gracilinanus marica*; (34) *Lestodelphys halli*; (35) *Thylamys macrurus*; (36) *Thylamys venustus*; (37) *Thylamys cinderella*; (38) *Thylamys elegans*; (39) *Thylamys pallidior*; (40) *Thylamys citellus*; (41) *Thylamys pulchellus*; (42) *Thylamys pusillus*.

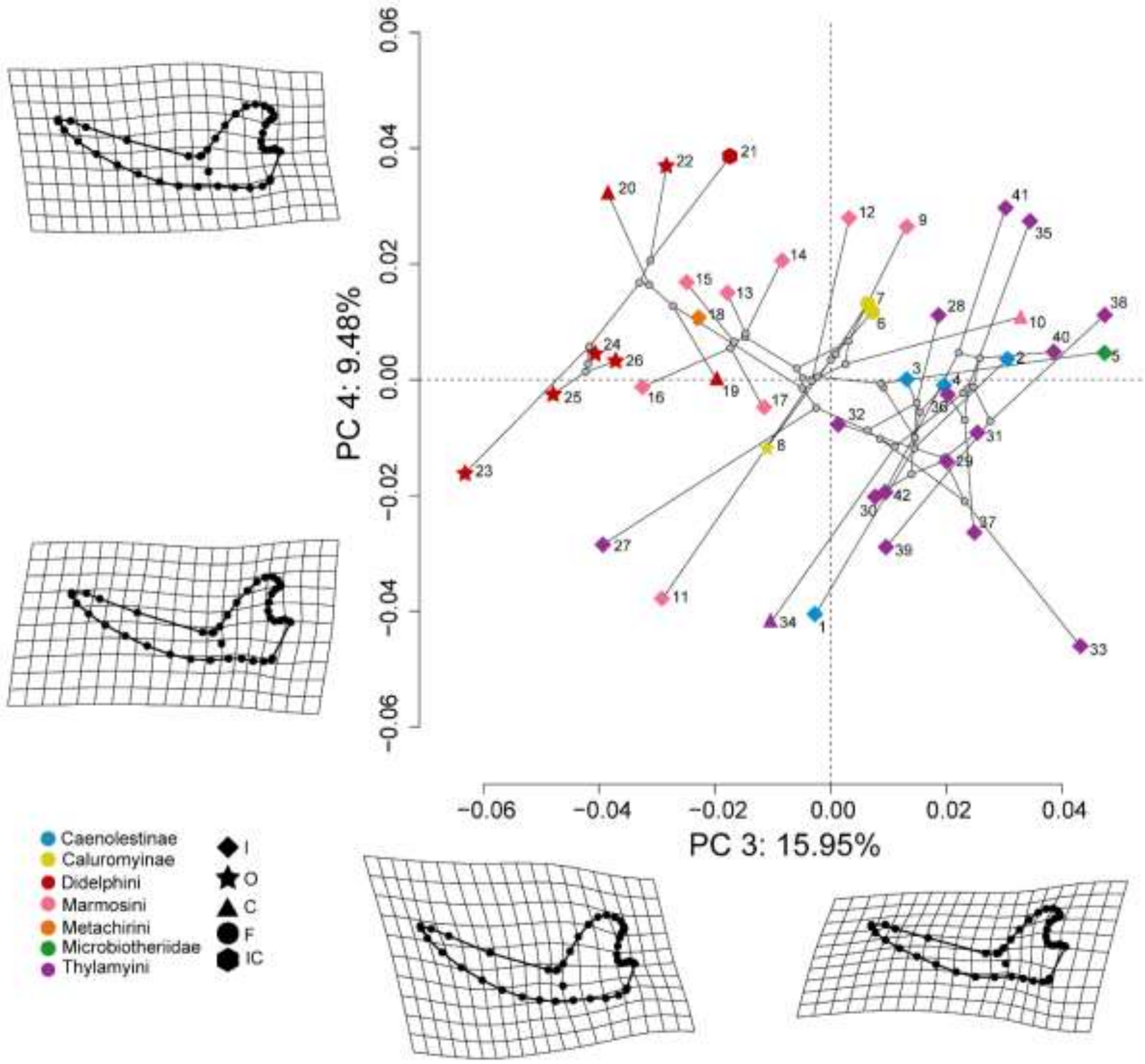

Figure 4. Phylomorphospace of third and fourth axis of mandible shape variation of American marsupials, with deformations according to each principal component. The colors represent the clades and the symbols the different diet categories, according diet 3. The species are numbered as: (1) *Rhyncholestes raphanurus*; (2) *Lestoros inca*; (3) *Caenolestes fuliginosus*; (4) *Caenolestes caniventer*; (5) *Dromiciops gliroides*; (6) *Caluromys philander*; (7) *Caluromys lanatus*; (8) *Caluromys derbianus*; (9) *Marmosa murina*; (10) *Marmosa constantiae*; (11) *Marmosa paraguayana*; (12) *Marmosa demerarae*; (13) *Monodelphis domestica*; (14) *Monodelphis kunsi*; (15) *Monodelphis scallops*; (16) *Monodelphis dimidiata*; (17) *Monodelphis americana*; (18) *Metachirus myosuros*; (19) *Chironectes minimus*; (20) *Lutreolina crassicaudata*; (21) *Philander quica*; (22) *Philander opossum*; (23) *Didelphis virginiana*; (24) *Didelphis albiventris*; (25) *Didelphis marsupialis*; (26) *Didelphis aurita*; (27) *Marmosops incanus*; (28) *Cryptonanus guahybae*; (29) *Cryptonanus agricolai*; (30) *Cryptonanus chacoensis*; (31) *Gracilinanus microtarsus*; (32) *Gracilinanus agilis*; (33) *Gracilinanus marica*; (34) *Lestodelphys halli*; (35) *Thylamys macrurus*; (36) *Thylamys venustus*; (37) *Thylamys cinderella*; (38) *Thylamys elegans*; (39) *Thylamys pallidior*; (40) *Thylamys citellus*; (41) *Thylamys pulchellus*; (42) *Thylamys pusillus*.

Clades explain 43% of mandibular shape ($R^2 = 0.432$, $F = 5.960$, $P < 0.001$) while size explains 17% ($R^2 = 0.169$, $F = 14.04$, $P < 0.001$). We found no interaction between allometry and clades ($R^2 = 0.037$, $F = 0.766$, $P = 0.755$), suggesting the pattern is overall the same across the American marsupials. The phylogenetic regression of shape and size of mandible was also significant, but presenting a weaker effect ($R^2 = 0.057$, $F = 2.423$, $P = 0.041$). The mandibular allometric pattern also favours the separation of clades, where largest marsupial clades, such as Didelphini and Caluromyinae tend to overlap due to their wider coronoid process and an overall more robust mandible, in contrast to all smaller clades, Marmosini, Thylamini, Caenolestidae and Microbiotheria, presenting a more gracile morphology (Fig. 5). We found no significant effect of dietary hypotheses or locomotor categories in mandibular shape (Table 3).

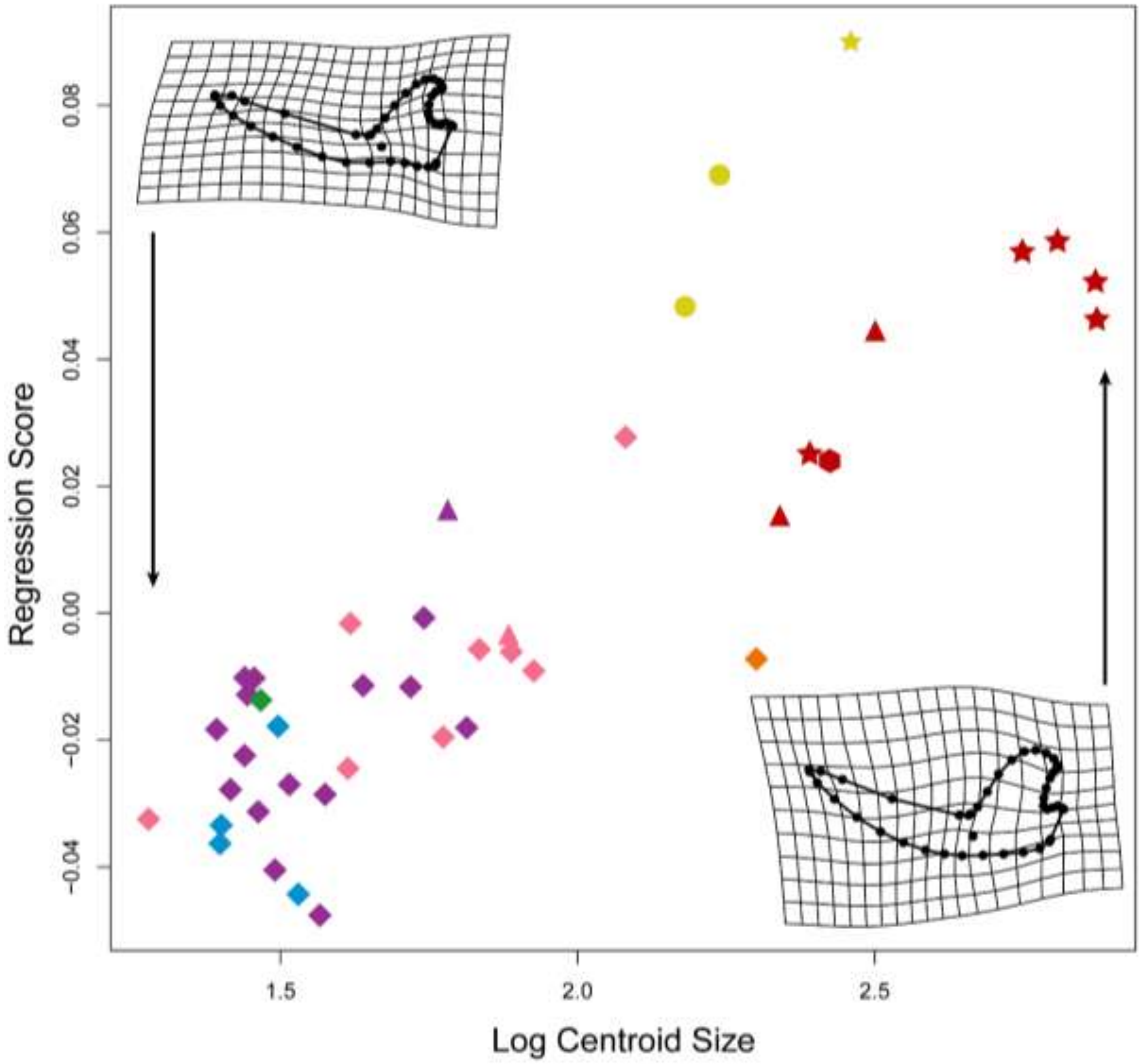

Figure 5. Plot of allometric effects in American marsupial mandible, with deformations of the smallest and largest species in the right and left corners of the graph, respectively. The colors represent the clades.

Table 3. Phylogenetic analysis of variance for size and Procrustes shape variables for influence of diet hypotheses and locomotor categories (LC) on size of marsupial mandible.

	Diet 1	Diet 2	Diet 3	Diet 4	LC
Size					
R^2	0.342	0.213	0.325	0.246	0.094
F	4.814	5.308	5.027	6.364	1.328
p value	**0.007**	**0.013**	**0.008**	**0.011**	0.266
Shape					
R^2	0.070	0.023	0.062	0.031	0.042
F	0.70	0.463	0.616	0.644	0.558
p value	0.758	0.882	0.765	0.671	0.812

We observed that Didelphini presented the highest values of size morphological disparity, while the other clades all presented low scores in comparison. Caluromyinae, followed by Caenolestidae and Microbiotheriidae were the clades that presented the highest values of shape disparity, while other clades presented consistently lower values (Fig. 6).

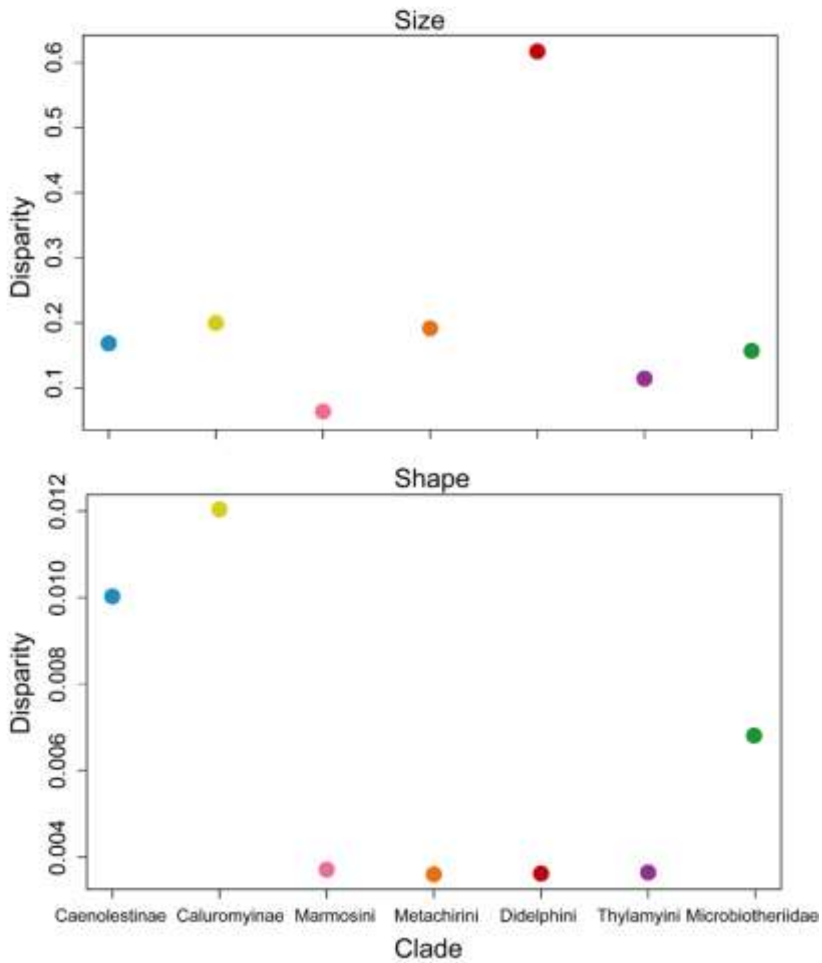

Figure 6. Morphological disparity of mandibular size and shape through the marsupial clades.

Didelphini have the highest size evolutionary rate, followed by Caluromyinae and Marmosini. For shape, Thylamyini presented the highst evolutionary rate, while all other clades fell below the marsupials overall mean (Fig. 7).

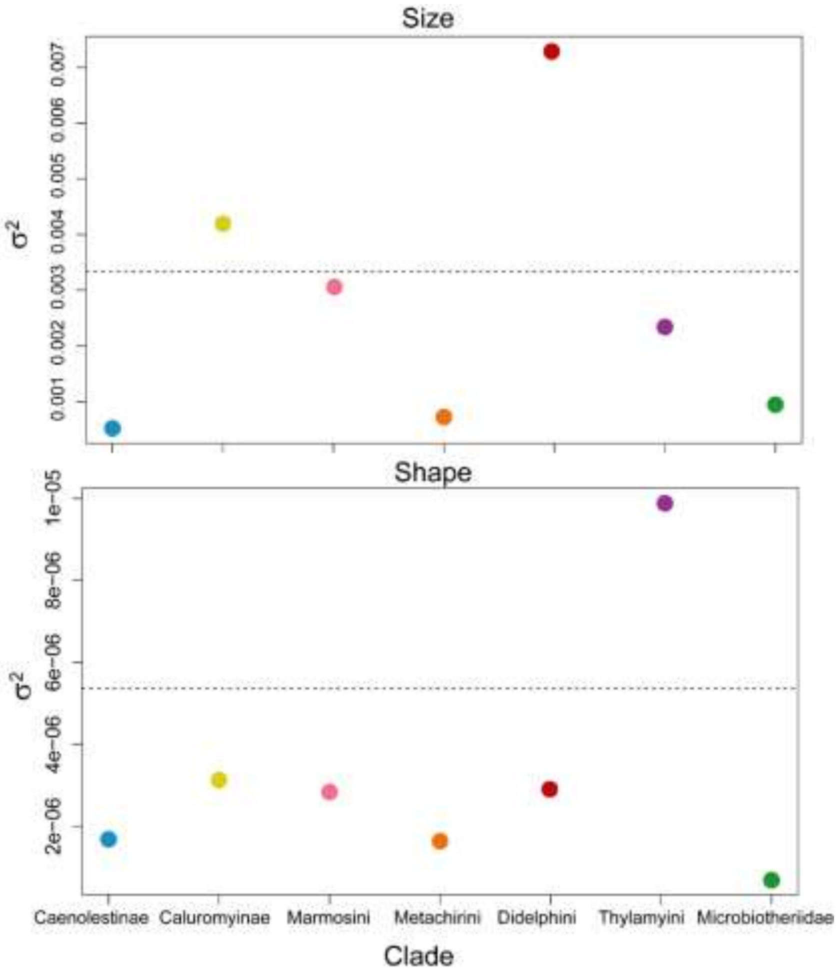

Figure 7. Evolutionary rates of marsupial mandibular size and shape through the marsupial clades. Trajected line represents the evolutionary rate computed for the full tree.

Discussion

We found that the American marsupial mandible has broadly diversified between subfamilies and tribes and at different rates of evolution. The path of least resistance for their morphological diversification is through allometry, which is a common pattern in Mammaliaformes (Krone et al. 2019) and a way to increase morphological disparity rapidly during evolution (Wilson and Sánchez-Villagra 2009). These results are consistent with previous studies on marsupial skull and molar evolution (Chemisquy et al. 2014; Magnus and Cáceres 2017; Chemisquy et al. 2021). Also congruent with these studies, we show that the available dietary and locomotor classifications of marsupials are not enough to separate them into distinct ecomorphs in respect to mandibular shape, which we will explain further. In contrast, we found a dietary effect for mandibular size variation. We also observed that some clades present higher morphological disparity and accumulate it more rapidly with evolutionary time than others, showing that American marsupials did not evolve uniformly.

The strength of the dietary effect in mandibular size varies depending of the category used. The hypotheses that had the strongest effect on size variation were the ranked genus level from Vieira and Astúa (2003) ($R^2 = 0.342$) and the five categories' species level classification of (Amador and Giannini 2021) ($R^2 = 0.325$). These categories are similar, but we chose here to describe size variation in accordance to the later because it is the most current and detailed one available in the literature. The ancestral marsupial was most likely an insectivore, a common state for several mammalian clades (Amador and Giannini 2021). From this point forward, American marsupials have diversified their food habits to incorporate more energetic food types (e.g. vertebrates, fruits), favouring the increase of their body size, while also keeping some degree of opportunistic behaviour (Bubadué et al. 2021a; Amador and Giannini 2021). Our results show this clearly, as insectivorous marsupials are all small-sized, followed by carnivores, frugivores, and omnivores (Fig. 2). From a functional perspective, larger marsupials are better capable of capturing and processing vertebrates and large fruits more easily than smaller ones, both being more energetic items than insects (Bubadué et al. 2021a; Santori et al. 2012). While vertebrates are an effective source of protein and lipids, fruits are also good sources of carbohydrates (Santori et al. 2012), both food categories supporting larger body sizes (Bubadué et al. 2021a). Omnivorous species are known to feed opportunistically on different items, being able to successfully incorporate the most available and energetic food items in the environment, regardless of seasonal effects (Santori et al. 2012).

The dietary effect in marsupials is congruent with what is expected by the Resource's Rule (McNab 2010), which states that both the availability and type of resource can have an important impact on body size and this can be viewed from both evolutionary and ecological perspectives. From an evolutionary perspective, there is greater overlap in size among marsupial clades than dietary categories (Fig. 2), suggesting that size determines the frequency of each type of food item is consumed. Furthermore, the largest sizes are restricted to specific clades, Didelphinae and Caluromyinae. These large clades also presented the highest morphological disparities in size, that is, large amount of morphological variation within them, along with high evolutionary rates. While high disparities indicate large size variation, high evolutionary rates mean that this morphological variation accumulates more rapidly in

Didelphinae and Caluromyinae in comparison to the others where rates are much smaller (Adams 2014).

The mandible shape morphospace separate clades in accordance to their taxonomy, showing that the jaw shape variation is following species group divergence patterns, that is, each shape component in the multidimensional mandible morphospace shows a specific clade divergence in regards to morphology, especially from more gracile to robust mandibles; but also varying in regards to specific regions of the mandible, like the condylar and coronoid processes (Figs 2 and 4); all features of taxonomic relevance (Voss and Jansa 2009). These findings are not specific to our study, but contribute to a body of evidence that have been consistently and extensively reported in the literature (Porto et al. 2008; Shirai and Marroig 2010; Chemisquy et al. 2014; Magnus and Cáceres 2017; Chemisquy et al. 2021). Functionally, allometry favours stronger bite forces, consistent with the patterns already observed in other mammalian clades. This allometric pattern is conservative across different comparative levels in marsupials, being previously documented both inter- (Chemisquy et al. 2021) and intraspecifically (Bubadué et al. 2021b; Magnus et al. 2017). At multiple shape dimensions, American marsupials are grouping, at some extent, in relation to their taxonomy, being that adaptation to specific feeding habits are all dependent of the speciation processes within the clade.

While mandible shape is dependent on the taxonomic diversity, that is, it is intimately related to the process of species diversification throughout American marsupial evolutionary history, the mandible shape evolutionary rates and disparities are variant between clades, especially in Thylamyini, which presented the highest values. Thylamyini is a noteworthy case of diversification within marsupials in respect to its ecogeographical history, being composed of several genus, including the open area specialists *Thylamys* and closed environment specialists *Marmosops* (Giarla and Jansa 2014; Díaz-Nieto et al. 2016). Thylamyini diversified and expanded their range into several extreme ecoregions, such as the Andes, Chaco, Cerrado woodlands and the Amazonian and Atlantic forests (Voss et al. 2013; Giarla and Jansa 2014; Díaz-Nieto et al. 2016; Fegies et al. 2021). The speciation events in this group have largely been influenced by lowland rivers and in situ diversification in the Andes, like in the case of *Thylamys* (Giarla and Jansa 2014), the recent open formations of the Pleistocene (*Cryptonanus*, (Fegies et al. 2021)), and by lowland to highland speciation processes (*Marmosops*, (Voss et al. 2013)), being both physical and ecological barriers important for their diversification (Voss et al. 2013; Giarla and Jansa 2014; Giarla et al. 2014; Fegies et al. 2021). This rapid diversification within Thylamyini supports our findings of fast morphological evolutionary rates.

The insectivorous ecomorph in marsupials is the ancestral state (Amador and Giannini 2021), being ultimately the most predominant form (48-69% of marsupials species included in this study are within this category). Our findings do not mean that the American-marsupial mandible morphology is not adaptive, it may just be that the way to study morphological changes in these animals must change. The mandible is a complex morphological structure that functions as a lever, being important for food processing (Zelditch et al. 2008; Monteiro and Nogueira 2010). From our point of view, American marsupials should be further studied through better identification of the food properties rather than food categorization, that is, food items hardness and size, since it is these features that will be important from a biomechanical perspective (Zelditch et al. 2008; Monteiro and Nogueira 2010; Santana et al. 2012; Marcé-Nogué et al. 2017). For instance, animals that often incorporate larger food items need a larger gap, while bite force is most likely related to the processing of harder food contents (Monteiro and Nogueira 2010; Santana et al. 2012), and this is regardless of being fruits or animal content. Studies on primates, bats and squirrels have clearly reported this biomechanical component of mandibular shape (Dumont and O'Neal 2004; Zelditch et al. 2008; Monteiro and Nogueira 2010; Santana et al. 2012; Marcé-Nogué et al. 2017), showing that, in order to further comprehend the adaptiveness of mandibular morphology in marsupials, researches need to go back to basic natural history in order to better incorporate food properties instead of type into macroevolutionary morphological studies. Furthermore, it is possible that the overall generalized morphology of marsupials (Chemisquy et al. 2021) is sufficient to process their food items, so that the size-oriented disparity would be enough for marsupials to incorporate harder and larger items. To this date, there are no extreme feeding behaviour specialisations reported to American marsupials, given their more opportunistic overall characteristic.

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