

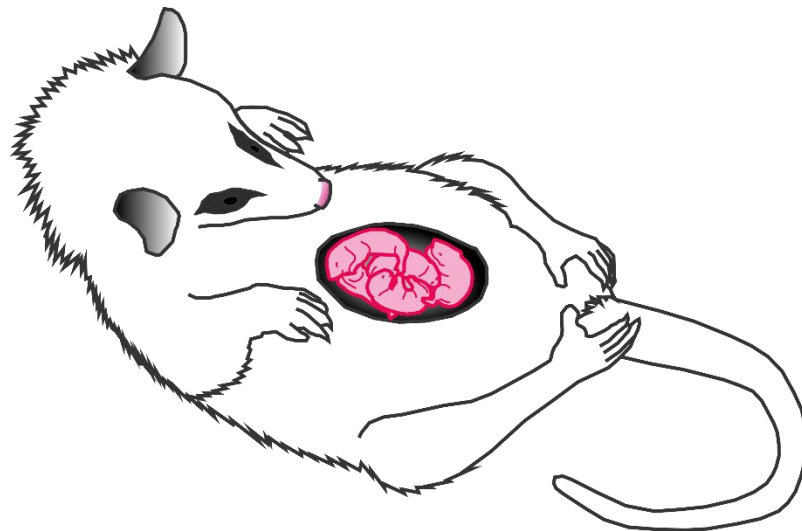
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UNIVERSIDADE FEDERAL DE MINAS GERAIS
Instituto de Ciências Biológicas
Programa de Pós-graduação em Zoologia

Mariah Milly Yoshikawa

BODY SIZE AND POUCH ASSOCIATION IN MARSUPIALS



Belo Horizonte

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Mariah Milly Yoshikawa

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Dissertação apresentada à Pós-graduação em Zoologia da Universidade Federal de Minas Gerais como parte dos requisitos necessários para obtenção do título de Mestre em Zoologia.

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ATA DE DEFESA DE DISSERTAÇÃO

MARIAH MILLY YOSHIKAWA

Ao quinto dia do mês de dezembro do ano de dois mil e vinte e dois, às nove horas, ocorreu a defesa de Mestrado da Pós-Graduação em Zoologia, de autoria da Mestranda Mariah Milly Yoshikawa intitulada: **“Body size and pouch association in marsupials”**. Abrindo a sessão, o Presidente da Comissão, Prof. Dr. Fernando Araújo Perini, após dar a conhecer aos presentes o teor das Normas Regulamentares do Trabalho Final, passou a palavra para a candidata para apresentação de seu trabalho.

Esteve presente a Banca Examinadora composta pelos membros: Diego Astúa, Jamille de Moura Bubadué, e demais convidados. Seguiu-se a arguição pelos examinadores, com a respectiva defesa da candidata.

Após a arguição, apenas a Comissão Examinadora permaneceu na sala para avaliação e deliberação acerca do resultado final, a saber: o trabalho foi APROVADO SEM ALTERAÇÕES.

Nada mais havendo a tratar, o Presidente da Comissão encerrou a reunião e lavrou a presente ata, que será assinada por todos os membros participantes da Comissão Examinadora.

Belo Horizonte, 05 de dezembro de 2022

Assinatura dos membros da banca examinadora:



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É 13!

RESUMO

A bolsa é uma característica variável dos marsupiais, mas poucos estudos abordaram sua evolução. A hipótese de que o tamanho do corpo prediz a presença de bolsa e, portanto, que todas as espécies maiores de marsupiais possuem bolsa é a mais amplamente aceita. Essa hipótese baseia-se na crença de que seria necessária uma estrutura física para proteger e auxiliar os filhotes na fixação das tetas, uma vez que espécies maiores passam mais tempo se desenvolvendo e atingem tamanhos maiores. No entanto, outras variáveis também podem prever a presença de bolsas, como o tamanho da ninhada. A suposição é que espécies com ninhadas pequenas investiriam mais na proteção de filhotes através das bolsas do que espécies com ninhadas maiores, assim, uma bolsa como estrutura de proteção e suporte beneficiaria as espécies. Neste trabalho, testamos a associação entre o tamanho do corpo e o tamanho da ninhada com a presença da bolsa em um contexto filogenético, estimamos o sinal filogenético da bolsa e reconstruímos o estado ancestral da bolsa, utilizando dados coletados da literatura. Para avaliar a associação de caracteres, realizamos uma análise de modelo linear generalizado misto filogenético (PGLMM) usando o tamanho do corpo e o tamanho da ninhada como preditores da presença de bolsa. Comparamos os escores de AIC de cinco modelos, e o modelo 'tamanho do corpo + tamanho da ninhada' foi selecionado, sugerindo que ambos os preditores influenciam a presença de bolsa de forma aditiva. O sinal filogenético foi estimado pela estatística D, e os resultados indicam que o estado da bolsa está altamente associado à filogenia em marsupiais. Para a reconstrução do estado ancestral, comparamos os desempenhos de seis modelos (três '*equal-rates*' e três '*all-rates-different*'). O modelo que melhor se ajustou aos nossos dados foi o '*equal-rates*' com um regime de taxa única entre ramos, sugerindo que o marsupial ancestral era mais provavelmente sem bolsa ou tinha uma bolsa intermediária, e que a bolsa surgiu independentemente muitas vezes durante a evolução dos marsupiais. A partir de nossas análises, propomos que o aumento do tamanho do corpo, o tamanho da ninhada pequena e a filogenia estão associados à presença de bolsas em marsupiais, mas cada fator tem efeitos diferentes ou adicionais nos clados internos.

Palavras-chave: bolsa marsupial; marsupiais; marsúpio; modelo linear generalizado misto filogenético; reconstrução de estado ancestral; sinal filogenético; tamanho corporal; tamanho de ninhada.

ABSTRACT

The pouch is a variable feature of marsupials, but few studies tackled its evolution. It is broadly hypothesized that body size predicts pouch presence, and therefore, that larger species of marsupial are all pouched. This hypothesis is based on the belief that a physical structure would be required to protect and to assist the young in teat attachment, since larger species have longer pouch lives and reach larger sizes. However, other variables could also predict pouch presence, such as litter size. The assumption is that species with small litter size would invest more in pouch young protection than species with larger litter sizes, thus, a pouch as a protective and supportive structure would benefit the species. Here, with data gathered from the literature, we test the association between body size and litter size with pouch presence in a phylogenetic context, estimate pouch's phylogenetic signal, and reconstruct ancestral pouch state. To assess character association, we conducted a phylogenetic generalized linear mixed model analysis (PGLMM) using body size and litter size as predictors of pouch presence. We compared the AIC scores of five models, and the 'body size + litter size' model was selected, suggesting that both predictors influence pouch presence additively. Phylogenetic signal was estimated by D statistic, and the result indicates that pouch state is highly associated with phylogeny in marsupials. For the ancestral state reconstruction, we compared the performances of six models (three 'equal-rates' and three 'all-rates-different'). The model that best fitted our data was the 'equal-rates' with a single rate regime across branches, suggesting that the marsupial ancestor was more likely pouchless or had an intermediate pouch, and that the pouch emerged independently many times during marsupial evolution. From our analyses, we propose that body size increase, small litter size, and phylogeny are all associated with pouch presence in marsupials, but each factor has different or additional effects on the inner clades.

Keywords: ancestral state reconstruction; body size; litter size; marsupial pouch; marsupials; marsupium; phylogenetic generalized linear mixed model; phylogenetic signal.

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1 INTRODUCTION

Currently, seven orders compose Marsupialia, the metatherian crown clade (Tyndale-Biscoe 2005; Wilson and Mittermeier 2015). Three orders occur in the New World, predominantly in Central and South Americas: Didelphimorphia (opossums), with 129 species; Paucituberculata (shrew-opossums), with seven species (Mammal Diversity Database 2022) and Microbiotheria, with a single species, *Dromiciops gliroides* (the monito del monte) (Palma and Valladares-Gómez 2015). In Australasia, four orders of marsupials are recognized: Notoryctemorphia (marsupial moles), with two species; Dasyuromorphia (carnivorous marsupials and numbats), with 77 species; Peramelemorphia (bilbies and bandicoots), with 22 species, and Diprotodontia (koalas, wombats, kangaroos, possums, and relatives), the largest marsupial order, with 150 species (Mammal Diversity Database 2022). Phylogenetically, the earliest lineages to branch in the clade were Didelphimorphia and Paucituberculata (Meredith et al. 2009b; Nilsson et al. 2010; Mitchell et al. 2014; Duchêne et al. 2018; Upham et al. 2019). Some authors gather these two orders in a paraphyletic group denominated “Ameridelphia” (Beck 2012). In 1982, Szalay proposed that Microbiotheria and the Australasian orders form a monophyletic clade, Australidelphia, based on the morphology of ankle bones. Later, several morphological and molecular studies corroborated this hypothesis (e.g., Amrine-Madsen et al. 2003; Horovitz and Sánchez-Villagra 2003; Nilsson et al. 2010).

Morphologically, many characteristics distinguish Marsupialia from other mammals (placentals and monotremes). Among them are reproductive traits such as the position of the ureters (Renfree 1993) and the presence of two lateral vaginae and two uteri in females (Tyndale-Biscoe and Renfree 1987a; Tyndale-Biscoe 2005). Although typically associated with female marsupials, the pouch is a reproductive feature that is not exclusive to them. It emerges independently in some male marsupials, as a protective scrotal pouch (e.g., water opossums (Enders 1935), marsupial moles (Sweet 1907), and in the recently extinct thylacines (Pocock 1926)) and in other vertebrate lineages (e.g., *Gastrotechia* marsupial frogs (del Pino 1980) and in mammals, such as echidnas (Augee et al. 2006)). Regardless, the female marsupial pouch could be an advantageous characteristic for their young. It provides them physical protection and support (expressly important during mother

movements), covert from predators, chemical defense, humidity that favors skin gas exchange, and warmth for the ectothermic newborn (Edwards et al. 2012; Edwards and Deakin 2013). It serves as a shelter where the neonates develop until weaning when they become precocious young: furred, endothermic, with the eyes open and high mobility (Russell 1982; Sobral and Guilhon 2016; Smith and Keyte 2020). However, the female pouch is absent in several marsupial genera, since most didelphids (e.g., *Marmosa*), all living caenolestids and some dasyurids (e.g., *Antechinus*) are pouchless or have intermediate pouches (Woolley 1974; Russell 1982; Tyndale-Biscoe and Renfree 1987a).

One of the main difficulties in unraveling the evolution of soft tissue structures is that fossils rarely preserve them (Purnell et al. 2018). Consequently, phylogenetic and evolutionary studies of marsupials seldom include pouch presence as a character or discuss its evolution. A few phylogenetic relationship analyses have included pouch presence as a character (Horovitz and Sánchez-Villagra 2003; Voss and Jansa 2009; Schneider and Gurovich 2017), but most of these studies refrain from making deeper evolutionary discussions, with only Schneider and Gurovich (2017) discussing pouch evolution at length. A way to overcome this limitation in marsupial evolution knowledge is by employing phylogenetic comparative methods to test old and new hypotheses about its evolution. Epipubic bones have already been proposed – and discarded – as a predictor of pouch presence in marsupials, since the pouch seems to be a relatively modern adaptation in contrast to these primitive bones (Szalay 1982; Tyndale-Biscoe and Renfree 1987b; White 1989). Another hypothesis is relative to body size. In large species females are pouched, while in small ones, pouch can frequently be absent (Tyndale-Biscoe 2005). In this way, many authors proposed that the increase in body size drove pouch emergence in marsupials in general (Tyndale-Biscoe and Renfree 1987b; Tyndale-Biscoe 2005), or at least in Didelphimorphia (Harder 1992; Astúa 2015; Voss and Jansa 2021). Body size is a selective aspect of all organisms, since as it increases, it may encourage structural innovations; at the same time, evolutionary novelties may also allow body size increase, so it is both a promoter and a consequence of evolution (Bonner 2011). Considering that young of larger species will achieve greater body size throughout a longer period of pouch life than smaller species, and that teat attachment is pivotal for young survival (Russell 1982), a physical structure that helps

in the protection of the young and promotes such attachment would be welcome, if not, necessary.

Here, we aim to test an extension of that hypothesis. Since we know that some small marsupials are also pouched, and because in a macroevolutionary timescale structural novelties hardly occur due to a single factor, we introduce another predictor variable that could help explain pouch presence in Marsupialia other than body size and phylogenetic relationships, litter size. This hypothesis assumes that in species with small litter size, the pouch would also improve young's survival rate, since it is considered a protective structure. Given that, the present work tests if body and litter size predict pouch presence in marsupials, considering a phylogenetic background, infers phylogenetic signal of the pouch, and reconstructs likely ancestral pouch states, through phylogenetic comparative methods.

2 MATERIAL AND METHODS

2.1 Data collection

The phylogenetic tree of Marsupialia used in this study was pruned from the node-dated major clade credibility tree of the DNA-only dataset of Upham *et al.* (2019), with 279 terminals. We gathered data from the literature on pouch presence, body mass, and litter size for 195 taxa, sampling all 19 extant families of marsupials (Table 1). We followed the taxonomy of the Mammal Diversity Database (2022), except for *Dromiciops gliroides*, which we consider as a single species following Palma and Valladares-Gómez (2015).

Table 1. Table with summary data of the 195 taxa used in this study (only species for which we found data on all three variables – pouch presence, body mass, and litter size – were included). Columns show sum of sampled species/clade, sum of pouched and pouchless taxa/clade, body mass (in grams) and litter size mean values/clade. Didelphimorphia, Paucituberculata, Microbiotheria and Notoryctemorphia have a single family so their data values are the same as their respective families.

Clade	Sum			Mean	
	Species	Pouched	Pouchless	Mass (g)	Litter size
Didelphimorphia	43	14	29	322.52	6.47

Didelphidae	43	14	29	322.52	6.47
Caluromyinae	4	3	1	326.25	3.26
Didelphinae	37	11	26	335.63	6.97
Didelphini	11	11	0	975.5	5.59
Marmosini	19	0	19	64.92	7.41
Metachirini	1	0	1	300	3.87
Thylamyini	6	0	6	25.71	8.64
Glironiinae	1	0	1	130	3
Hyladelphinae	1	0	1	15	4
Paucituberculata	2	0	2	36.5	2.81
Caenolestidae	2	0	2	36.5	2.81
Microbiotheria	1	0	1	24	3
Microbiotheriidae	1	0	1	24	3
Notoryctemorphia	1	1	0	55	1
Notoryctidae	1	1	0	55	1
Dasyuromorphia	40	13	27	379.29	6.08
Myrmecobiidae	1	0	1	459	3.68
Dasyuridae	39	13	26	377.25	6.14
Dasyurinae	14	3	11	991.89	5.51
Phascogalinae	14	0	14	46.62	6.54
Sminthopsinae	11	10	1	15.77	6.44
Planigalini	4	4	0	6.63	6.56
Sminthopsini	7	6	1	21	6.36
Peramelemorphia	14	14	0	906.11	1.99
Peramelidae	13	13	0	891.19	2.02
Echymiperinae	5	5	0	749.1	1.7
Peramelinae	6	6	0	686.7	2.47
Peroryctinae	2	2	0	1860	1.52
Thylacomyidae	1	1	0	1100	1.5
Diprotodontia	94	94	0	4922.97	1.29
Hypsiprymnodontidae	1	1	0	520	2.04
Macropodidae	44	44	0	7253.5	1
Lagostrophinae	1	1	0	1800	0.9
Macropodinae	43	43	0	7380.33	1
Potoroidae	8	8	0	1478.75	1
Bettongini	5	5	0	1592	1
Potoroini	3	3	0	1290	1
Acrobatidae	2	2	0	31	2.06
Petauridae	6	6	0	267.17	1.55
Dactylopsilinae	3	3	0	292	1.37
Petaurinae	3	3	0	242.33	1.74
Pseudocheiridae	12	12	0	1038.38	1.33
Hemibelideinae	2	2	0	1312.5	1
Pseudocheirinae	5	5	0	613.9	1.77
Pseudochiropsinae	5	5	0	1353.2	1.02
Tarsipedidae	1	1	0	9	2.93

Burramyidae	5	5	0	23.2	3.57
Phalangeridae	11	11	0	2545.91	1.17
Phalangerinae	8	8	0	2641,25	1.22
Trichosurinae	3	3	0	2291.67	1.02
Phascolarctidae	1	1	0	5100	1.01
Vombatidae	3	3	0	27966.67	1

2.1.1 Pouch presence

We used pouch presence for ancestral state reconstruction and character association analysis, in the latter as a response variable. Data was surveyed from the literature and through personal communications when information about a taxon was dubious or sparse (Table 1; detailed information for each species in Appendix 1). We based the presence or absence of pouch on Woolley's (1974) and Russell's (1982) descriptions (Figure 1). For analytic purposes, we considered type 1 pouch as "absent". This pouch type is also called "intermediate pouch", since it only develops in the breeding season, forming ridges of skin that often do not fully cover the young (Woolley 1974; Tyndale-Biscoe and Renfree 1987a). Types 2–6 were considered as "present".

We should clarify some important remarks about pouch state assessment. As it is a feature that suffers morphological and physiological changes during lifetime, the pouch may have its first appearance only when the female reaches sexual maturity and enters its first breeding season, so pouchless descriptions of some taxa may be inaccurate (Woolley 1974). Another consideration is that in cases of females with a type 1 pouch, if sample size is small or if it was only seen in the wild (with no laboratory husbandry), the accuracy of determination of pouch presence is also hampered (Woolley and Elliott 2014). In some cases, pouch presence or absence is based on phylogenetic relationships (i.e., species with many congeners with a well-known pouch state such as *Monodelphis iheringi*) (Astúa 2015), but since there is no direct evidence, we excluded those from analyses.

Two species we included in the dataset are noteworthy: *Caluromysiops irrupta* (Didelphidae: Caluromyinae), which has no formal pouch description traceable in literature, but has been reported as fully pouched (Astúa 2015; Voss and Jansa

2021), and *Planigale ingrami* (Dasyuridae: Sminthopsinae: Planigalini), which has more than one pouch morphology depending on population origin (Baker 2015). For the latter, we used the pouch state of *Planigale ingrami* (form *subtilissima*, from Kimberley in Western Australia's north) from Woolley (1974), that has a type 4 pouch.

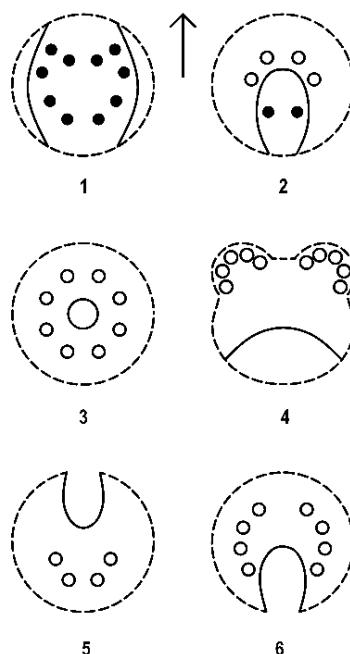


Figure 1. Pouch types: 1 – Intermediate pouch that develops during breeding season. 2 – The mammary area is partially covered by a crescentic antero-lateral fold of skin. 3 – The mammary area is covered by a circular fold of skin. 4 – The mammary area is covered by a crescentic antero-lateral fold of skin and the teats are inside two pockets. 5 – The mammary area is fully covered by a fold of skin that opens forward (“forward-facing pouch”). 6 – The mammary area is fully covered by a fold of skin that opens backward (“backward-facing pouch”). Arrow points cranially. Broken lines represent pouch area limits and black lines represent pouch openings. Small black circles represent exposed teats and small open circles represent enclosed teats. After Tyndale-Biscoe and Renfree (1987), in Inkscape version 1.2.

2.1.2 Body mass

We compiled data for the predictor variable body mass (in grams), a proxy for body size, mainly from the Weisbecker *et al.* (2013) dataset and from Wilson and Mittermeier (2015) (Table 1; detailed information in Appendix 2). When available, we used only female body mass ($n = 154$), to avoid size-related dimorphism. When body mass was sex-unspecified, we used the general taxon body mass ($n = 54$). Before

analysis, we log transformed the mean body mass, since these values comprise different orders of magnitude in marsupials. All values were also standardized as z-scores (i.e., with mean = 0 and standard deviation = 1) (Symonds and Blomberg 2014).

2.1.3 Litter size

Some data on litter size, the second predictor variable, were collected from papers and book chapters (Table 1; detailed information in Appendix 3), but mostly from the PanTheria database (Jones et al. 2009). To perform the character association analysis, we used litter size mean values. All values were also standardized as z-scores (i.e., with mean = 0 and standard deviation = 1) (Symonds and Blomberg 2014).

2.2 Phylogenetic comparative methods

Taxa that share a common ancestor are not independent, therefore, ignoring their phylogenetic relationships is a problematic approach in a comparative study (Felsenstein 1985). To better understand pouch evolution in marsupials, we conducted three analyses: (1) character association with body size and litter size as predictors of pouch presence, (2) phylogenetic signal estimation for the pouch, and (3) ancestral state reconstruction of the pouch.

Before performing the phylogenetic generalized linear mixed model analysis or PGLMM (Ives and Helmus 2011), we verified if body mass and litter size have individual effects (i.e., are independent) on the response variable estimating the Pearson's moment-correlation coefficient (r_{pearson}) between log transformed body mass and litter size, disregarding phylogenetic relationships, with 'cor.test' function. The Pearson's correlation is used to establish the relationship between two continuous variables, and its coefficient ranges from -1 to +1; when $r_{\text{pearson}} = 0$, we fail to reject the null hypothesis and there is no correlation; when $r_{\text{pearson}} \neq 0$, the alternative

hypothesis is accepted, and the correlation is true (Puth et al. 2014). If $r_{\text{pearson}} = -1$ or $+1$, then the variables would be perfectly correlated, imposing the same effect on the response variable (i.e., redundant); if it is a positive number $\neq +1$, then the variables are positively correlated, if it is a negative number $\neq -1$, the variables are negatively correlated (Puth et al. 2014).

To assess character association in a phylogenetic context, we performed the PGLMM. This method allows considering more than one predictor variable for a binary dependent variable, while including the phylogenetic information in the analysis as a random variable (Ives and Garland 2014). We tested five models against each other with 'pglm_compare' function in the 'phyr' package. (1) The 'null' model sets the standards for the comparisons. (2) The 'body mass' model gives the association between body mass only and pouch presence, and (3) the 'litter size' model gives the association between litter size only and pouch presence. (4) The 'body mass + litter size' model tests if both body mass and litter size are associated with pouch presence additively and (5) the 'body mass * litter size' model tests if both predictors are associated with pouch presence interactively.

The pouch's phylogenetic signal was estimated through D statistic, a measure proposed by Fritz and Purvis (2010) for binary traits, in our case, pouch presence or absence, using 'phylo.d' function in the 'caper' package. Revell et al. (2008) define phylogenetic signal as "the statistical nonindependence among species trait values due to their phylogenetic relatedness". Thus, we use it to evaluate if more closely related taxa are more likely to present a same pouch state. In D statistic, if $D = 0$, the trait is phylogenetically conserved as expected under a Brownian threshold model, if $D = 1$, it is random relative to phylogeny. If $D < 0$, the trait is highly conserved throughout phylogeny; if $D > 1$, it is overdispersed (Fritz and Purvis 2010).

For the ancestral state reconstruction, we fitted six models using 'corHMM' function in the 'corHMM' package and compared their performances. Models were 'equal-rates' and 'all-rates-different', with a single, two and three rate regimes across branches each. In 'equal-rates' models the rates of change in state transitions are symmetric (i.e., rates of change between two states are the same forwards and backwards), and in 'all-rates-different' models the rates of change in state transitions are unequal (i.e., rates of change between two states can be different in all transitions) (Harmon 2018).

To choose the best-fitting models for the PGLMM and ancestral state reconstruction, we compared their Akaike Information Criterion (AIC). The best-fitting model is the one with the lowest AIC score with a difference of 4 units to the second lowest score. If there is no 4 units' difference, the simplest model is the best choice (Harmon 2018). The AICc is the small sample size correction of AIC, but it can also be employed in large sample size analyses with little effect (Harmon 2018). The AICw is the AIC relative weight, which is used as model averaging when there is uncertainty of the best model choice in a set of models that share parameters of interest, in this case, the best-fitting model is the one with AICw closest to 1 (Harmon 2018).

All analysis were performed in R programming environment (R Core Team 2021, version 4.1.0), using the 'phytools' (Revell 2012, version 0.7-80), 'geiger' (Pennell et al. 2014, version 2.0.7), 'phyr' (Li et al. 2020, version 1.1.0), 'rr2' (Ives and Li 2018, version 1.0.2), 'DHARMA' (Hartig 2016, version 0.4.5), 'corHMM' (Beaulieu et al. 2013, version 2.7), and 'caper' (Orme et al. 2018, version 1.0.1) packages.

3 RESULTS

In our dataset, pouched species ($n = 136$) have mean body mass (mean_w) of 3665.85 g (4.3 g to 31900 g), and mean litter size (mean_{ls}) of 2.18 young/litter (0.9 to 8.62 young/litter). Pouchless species ($n = 59$) have mean weight of 103.07 g (8 g to 880 g), and mean litter size of 6.51 young/litter (2 to 12 young/litter). All species larger than 880 g are pouched. The largest pouchless species is *Dasyurus viverrinus*, and the smallest pouched species are *Planigale* dasyurids. Small, pouched taxa (< 880 g, $n = 48$) are found in Didelphimorphia, Notoryctemorphia, Peramelemorphia, Dasyuromorphia, but mostly in Diprotodontia ($n = 19$).

Pearson's correlation coefficient for the predictor variables (body mass and litter size) is significant (P-value $< 2.2e-16$, $\alpha = 0.05$, $r_{\text{pearson}} = - 0.672$). This result is evidence that the variables are not redundant, and the negative coefficient indicates the negative relationship between them. Thus, if log body mass is high, mean litter size is low and vice versa (Figure 2).

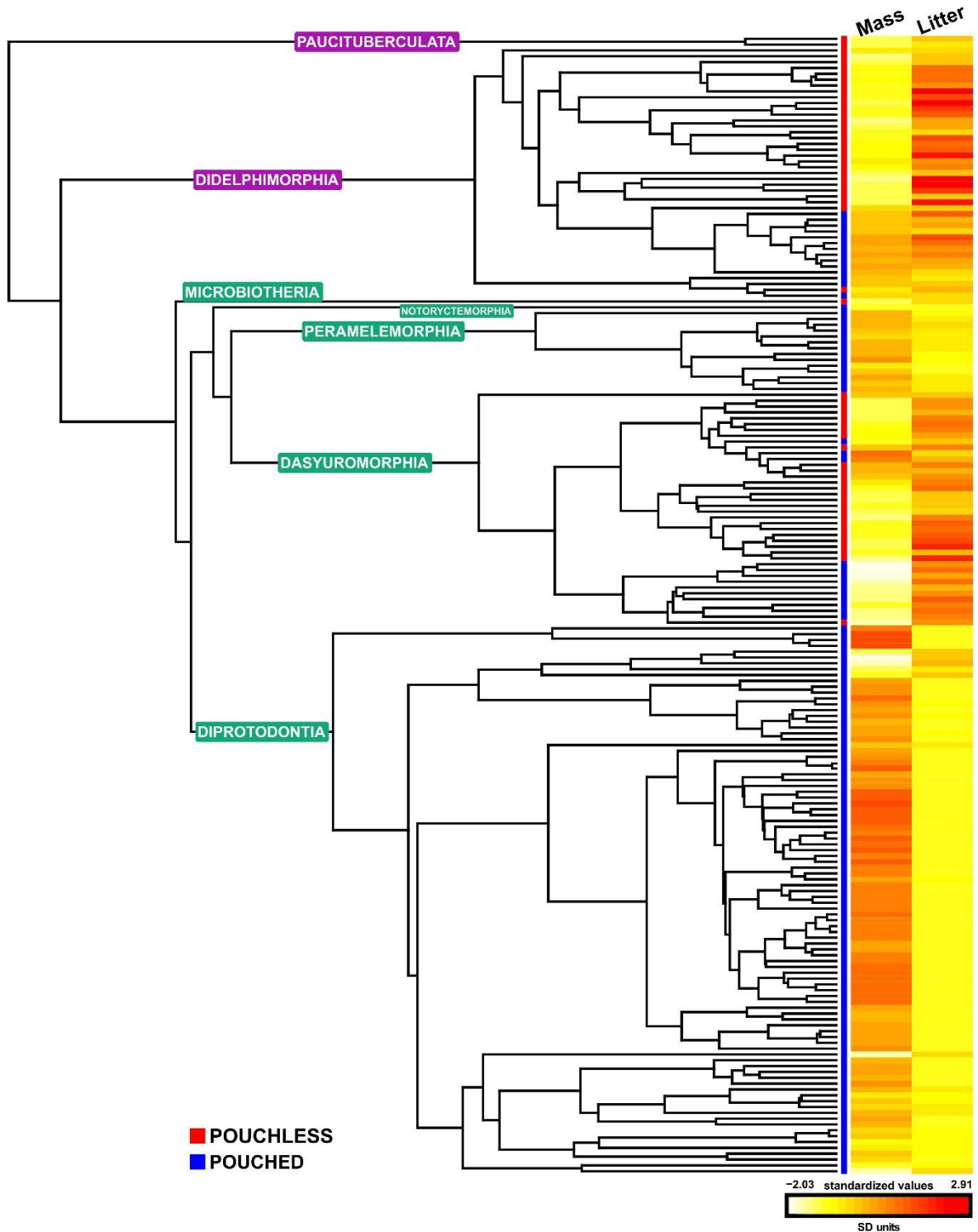


Figure 2. Phylogenetic heatmap created with 'phylo.heatmap' function in the 'phytools' package. It shows the marsupial subtree (after 'drop.tip' function for taxa with missing data; $n = 195$) with pouch presence (blue squares; $n = 136$) or absence (red squares; $n = 59$) at the tips. Wide columns show log-transformed mean body masses ('Mass', on the left) and mean litter sizes ('Litter', on the right) standardized values (z-scores). The lowest values are whitish/yellowish, and the highest values are

orange/reddish. Plotted side-by-side, the negative Pearson's correlation between the predictor variables is noticeable. Image editing made in Inkscape version 1.2.

For the PGLMM analysis, the best fitting model for our dataset was the 'body mass + litter size' (AIC = 109.97, AICw = 0.67; Table 2). This means that both predictor variables influence pouch emergence additively. Our results suggest that species with large body size and small litter size tend to be pouched, and species with small body size and large litter size tend to be pouchless, even though large body size does not imply in small litter size and small body size does not imply in large litter size. As suggested earlier, body size would affect pouch presence because larger species' young tend to have longer development and may achieve larger sizes, so they would benefit from a support structure during the period of teat attachment as they increase in size. Regarding litter size, we suggest that species with smaller litter sizes would invest more in fewer young, so a pouch would be beneficial for species continuity.

We found a strong phylogenetic signal for pouch presence in marsupials ($D = -0.48$). This means that the pouch state distribution is highly related to phylogeny, so that closely related taxa tend to share the same pouch state.

Table 2. Phylogenetic generalized linear mixed model scores. 'Body size + litter size' model has the best (lowest) AIC score.

Model	AIC	R ²	AICw
(1) 'Null'	226.81	0.8	0
(2) 'Body mass'	124.67	0.91	0
(3) 'Litter size'	146.54	0.8	0
(4) 'Body mass + litter size'	109.97	0.91	0.67
(5) 'Body mass * litter size'	111.37	0.91	0.33

For ancestral state reconstruction (Figure 3), the model that best fitted our data was the 'all-rates-different' with a single rate regime across branches (AICc = 83.44, AICw = 0.34; Table 3). Nevertheless, since this model showed a very similar fit to that of 'equal-rates' model with a single rate across branches (AICc = 83.96, AICw = 0.26), within a 4 AICc units, the 'equal-rates' model with a single rate regime across branches was selected. Models with two or three rate categories consistently

had worse fit than single rate models. According to our results, the lack of pouch or an intermediate pouch is plesiomorphic for Marsupialia (Table 4). The pouch emerged independently at least three times: in the ancestor of Australidelphia, in the ancestor of Caluromyinae, and in the ancestor of Didelphini. Reversals are seen in didelphids, microbiotheriids and dasyurids.

Table 3. Ancestral state reconstruction model scores. Model (4) fits best but lacks a 4 units' difference from the second-best model (1), so the simplest model (1) is the best choice for our dataset.

Model	AICc	AICw
(1) 'Equal-rates' with one rate regime across branches	83.96	0.26
(2) 'Equal-rates' with two rate regimes across branches	84.19	0.24
(3) 'Equal-rates' with three rate regimes across branches	93.93	0
(4) 'All-rates-different' with one rate regime across branches	83.44	0.34
(5) 'All-rates-different' with two rate regimes across branches	85.03	0.15
(6) 'All-rates-different' with three rate regimes across branches	97.29	0

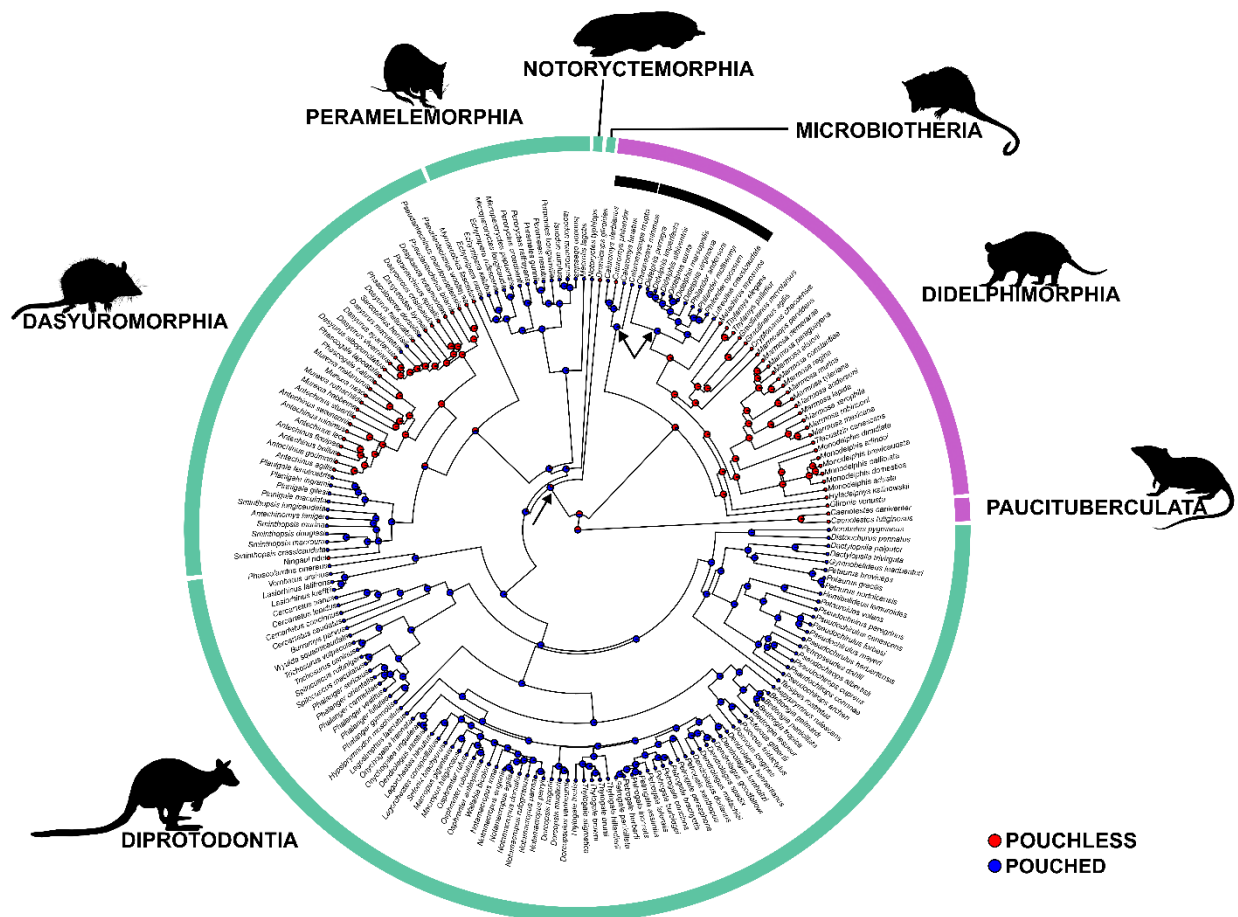


Figure 3. Ancestral state reconstruction of pouch presence across Marsupialia under ‘equal-rates’ with a single rate regime across branches model. Green arcs indicate australidelphian orders and pink arcs indicate “ameridelphian” orders. The marsupial ancestral is more likely pouchless (at the center). Pouch emergence indicated by arrows in the ancestor of Australidelphia (close to the center of the tree), in the ancestor of Caluromyinae (highlighted by left inner black arc at the top right of the tree), and in the ancestor Didelphini (highlighted by right inner black arc at the top right of the tree). Red circles mean pouchless, and blue circles mean pouched. Animal silhouettes were downloaded from PhyloPic.org with no modifications. *Caenolestes fuliginosus* (Paucituberculata), *Dromiciops gliroides* (Microbiotheria), and *Notoryctes typhlops* (Notoryctemorphia) by Sarah Werning are licensed under [CC BY 3.0](https://creativecommons.org/licenses/by/3.0/). *Antechinus flavipes* (Dasyuromorphia) by Robbi Bishop-Taylor, *Didelphis virginiana* (Didelphimorphia) by Margot Michaud, *Perameles nasuta* (Peramelemorphia) by Steven Traver, and *Wallabia bicolor* (Diprotodontia) by Michael Scroggie are all in public domain ([CC0 1.0](https://creativecommons.org/licenses/by/3.0/)). Tree annotation made in Inkscape version 1.2.

Table 4. Ancestral pouch state of some clades and some taxa whose pouch state deviate from the rest of their clade.

Clade	Pouch state
Marsupialia	Pouchless
Didelphimorphia	Pouchless
Caluromyinae	Pouched
<i>Caluromys philander</i>	Pouchless
Didelphinae	Pouchless
(Metachirini + Didelphini)	Pouchless
Didelphini	Pouched
Paucituberculata	Pouchless
Australidelphia	Pouched
<i>Dromiciops gliroides</i>	Pouchless
((Notoryctemorphia + (Peramelemorphia + Dasyuromorphia)) + Diprotodontia)	Pouched
(Notoryctemorphia + (Peramelemorphia + Dasyuromorphia))	Pouched
Peramelemorphia	Pouched
Dasyuromorphia	Pouchless
((Dasyurinae + Phascogalinae) + Sminthopsinae)	Pouchless
(Dasyurinae + Phascogalinae)	Pouchless
Dasyurinae	Pouchless
<i>Phascosorex dorsalis</i> , <i>Sarcophilus harrisii</i> and <i>Dasyurus maculatus</i>	Pouched
Phascogalinae	Pouchless
Sminthopsinae	Pouched
<i>Ningauai ridei</i>	Pouchless
Diprotodontia	Pouched

4 DISCUSSION

Given our results, it seems that phylogeny, body size, and litter size are all associated with pouch state distribution in marsupials. We propose a general pattern in which body size increase in a lineage requires pouch presence, but smaller body size does not, necessarily, imply a pouchless state. We suggest that species with small litter size tend to be pouched, although this association is harder to see and demands further investigation. In a simplistic way, these hypotheses are related to increase in pouch young survival rate that leads to species stability. Generally, large species have longer periods of development and are heavier at weaning (Russell 1982). Since teat attachment is crucial for the young development, the pouch offers protection (among other benefits) and physical support for them to keep attached while suckling. In species with small litter size, the pouch would increase the odds for survival of the young, as a protective and supportive structure. Furthermore, in some clades, phylogeny seems to influence pouch presence distribution despite great variation in body and/or litter size, suggesting that, once acquired, phylogenetic inertia may have a strong role in preserving the pouch. As it is beyond the scope of this research to discuss phylogenetic concepts, we use the term “phylogenetic inertia” as an explanation for phenotypic stasis, regardless of the process (e.g., selection resistance, canalization) that lead to it (see Blomberg and Garland (2002) for a review on the subject).

In 1977, Kirsch already proposed that pouch emerged independently in different marsupial lineages. Here, we suggest that it emerged at least three times in the ancestor of higher clades. According to our study, pouchlessness or an intermediate pouch was likely the plesiomorphic state in the common marsupial ancestor, corroborating Schneider and Gurovich (2017). Our pouch emergence estimates in Didelphini and Caluromyinae agree with Voss and Jansa (2009), and again with Schneider and Gurovich (2017). However, in our reconstruction, the Australidelphia ancestor was likely pouched, contradicting Schneider and Gurovich (2017), as in their work, it was likely pouchless or had an intermediate pouch. This conflict may be due to differences in taxa sampling and phylogenetic hypothesis used in each study. Beyond that, with the independent emergence of such a complex trait in divergent lineages, it would be plausible to assume that the genetic and developmental apparatus necessary to develop a pouch in female marsupials are inherited from the marsupial common ancestor.

When the pouch is present, it comes in distinctive morphologies, but generally, closely related taxa share the same pouch type. Among the 136 pouched species of our dataset, the majority has a type 5 pouch (eleven didelphids and 88 diprotodonts). The second most common morphology is type 6, and it is present in *Notoryctes typhlops*, in two didelphids and three diprotodonts, and in all fourteen peramelemorphs. Pouch types 2, 3 and 4 are exclusive to dasyurids. Curiously, it seems that, in Didelphimorphia and Diprotodontia, type 6 pouch belong to taxa or lineages that diverged first within the clade. In Didelphini, *Lutreolina* and *Chironectes* diverged earlier than other taxa with type 5 pouch (Voss and Jansa 2009; Upham *et al.* 2019), and in Diprotodontia, Vombatiformes (koalas and wombats) diverged at the base of the order (Meredith *et al.* 2009; Upham *et al.* 2019), and all other diprotodonts have a type 5 pouch. In most of these cases, we highlight that a backward-facing pouch seems to be related to habitat specialization. *Chironectes minimus* is the only semi-aquatic marsupial (Astúa 2015), wombats are burrowing animals (Wells 2015), and koalas are large but lethargic arboreal creatures (Jackson 2015). Horovitz and Sánchez-Villagra (2003) included pouch type in their morphological higher-level phylogenetic analysis. Their results show that peramelemorphs evolved type 6 from an intermediate pouch, the majority of diprotodonts evolved a type 5 from an intermediate pouch, and Vombatiformes evolved type 6 from type 5. Our results disagree with the two first statements, since in our reconstruction both the ancestors of peramelemorphs and diprotodonts were fully pouched, so they could not have evolved their pouches from an intermediate type. It is important to say that their topology differs from ours, notably among Australidelphia's inner relationships. Still, it would be interesting to further investigate the reconstruction of ancestral pouch morphology states using a more refined analysis.

In Didelphimorphia, the largest American order of mammals, pouch can be present or absent (Astúa 2015), and the clade's ancestor was more likely pouchless. In this clade, body size increase and pouch presence association are strongly correlated, as previously proposed (Harder 1992; Astúa 2015; Voss and Jansa 2021). Body size range is large, from 14 g to 1800 g (128.6-fold difference). Metachirini + Didelphini and Caluromyinae show the highest increase in body mass during didelphid evolution (Amador and Giannini 2016). Indeed, species of Didelphini (*Chironectes*, *Lutreolina*, *Didelphis*, and *Philander*) are the largest in the order, with

mean body size of 1031.8 g, and they are all pouched (type 5 in *Didelphis* and *Philander* and type 6 in *Lutreolina* and *Chironectes*). Marmosini (*Marmosa*, *Tlacuatzin* and *Monodelphis*), Thylamyini (*Chacodelphys*, *Cryptonanus*, *Gracilinanus*, *Lestodelphys*, *Thylamys*, and *Marmosops*), Glironiinae (*Glironia venusta*), and Hyladelphinae (*Hyladelphys kalinowskii*) are the lightest didelphids, with mean body size of 56.8 g, and they are all truly pouchless. Metachirini, the sister group of Didelphini are the largest pouchless didelphids (mean_w = 300 g), and its relatively large size may have been preserved from the ancestor of Metachirini + Didelphini (Amador and Giannini 2016). We speculate that Metachirini also retained the ancestral pouchless condition by phylogenetic inertia, while the Didelphini lineage kept increasing in size, so the pouch emerged in its ancestor. Body size and pouch presence association are also evident in Caluromyinae (*Caluromys* and *Caluromyslops*), whose ancestor was pouched. The three larger species (mean_w = 301.7 g) possess a type 5 pouch, while the smallest species, *Caluromys philander* (mean_w = 187 g), lacks a true pouch (type 1). Because of this reversion to a plesiomorphic state in *C. philander*, we hypothesize that even at an intermediate state the pouch has a role in neonates' survival rate, as it would cover the newborn, at least right after their birth. Conversely, litter size does not seem to associate with pouch presence in Didelphidae, as it varies greatly within pouchless (3.0 to 12.0 young/litter) and pouched taxa (2.0 to 8.6 young/litter). In *C. philander*, litter size is higher (mean_{ls} = 4.2) than in the other three pouched Caluromyinae (mean_{ls} = 3.0), but the difference seems to be small to assume that this clade fits any general pattern regarding litter size. Nevertheless, association between pouch presence and litter size in didelphids has been already tested by Battistella *et al.* (2019), with litter size as a response variable. They did not find an association between the variables after phylogenetic correction, but they estimated a high phylogenetic signal for pouch presence in the clade ($\lambda = 1.023$; $P < 0.001$).

In the other "Ameridelphian" order, Paucituberculata, all species are truly pouchless (Patterson 2015) as was their ancestor. Due to a lack of litter size data for the clade, we collected data on the three variables for only two of the seven current species, *Caenolestes caniventer* and *C. fuliginosus* (mean_w = 36.5 g; mean_{ls} = 2.8), which may hamper our assumptions on pouch state association for the group. Notwithstanding, extant caenolestids retained a small body size from the paucituberculatan ancestor (Abello *et al.* 2018), and it is reasonable to infer that they

also kept pouchlessness due to a lack of body size increase during their evolution or due to phylogenetic inertia.

In the australidelphian ancestor, pouch is reconstructed as present, but it is difficult to hypothesize which factors may be responsible for this outcome. In the clade, body size range is extreme, from 4.3 g to 31900 g (a dramatic > 7400-fold difference), and mean litter sizes range from 0.9 to 10.8 (12-fold difference). It is feasible that the ancestor of Australidelphia could have been a relatively large pouched species, and pouch was retained in the remaining of the clade by phylogenetic inertia. However, independently of which factors encouraged pouch occurrence in this clade, many taxa reversed pouch state later in evolution, albeit not completely. All pouchless species in the group have an intermediate pouch (type 1) and belong exclusively to the Microbiotheria and Dasyuromorphia orders, except for *Myrmecobius fasciatus* (Dasyurimorphia: Myrmecobiidae), which seems to be truly pouchless.

The sole extant representative of Microbiotheria, the monito del monte, has an intermediate pouch (type 1) (Schneider and Gurovich 2017), weighs 24 g on average, and has a small litter size of three young per litter. Considering that the marsupial ancestor was pouchless and the australidelphian ancestor was pouched, the reversal to a plesiomorphic state in *Dromiciops* is comparable to the case of *Caluromys philander*, in which after the pouch emerged in a higher clade, it returned to an intermediate state as the taxon decreased in body size.

Notoryctemorphia has two species, *Notoryctes caurinus* and *N. typhlops*. These are rare small fossorial animals with a backward-facing pouch (Aplin 2015). We only found litter size data for *N. typhlops*, which has a single young per litter and weighs 55 g on average. However, since marsupial mole females have only two teats (Aplin 2015b), two young are the most they could raise at once. Since they live underground in sand (Aplin 2015b), a rear-opening pouch enables the young to keep suckling in a safer and cleaner space. Pouch presence was possibly retained from the ancestor they share with Peramelemorphia + Dasyuromorphia clade, but its morphology is unknown. Therefore, it is uncertain if the backward-facing pouch in marsupial moles is inherited from the ancestor, or if evolved from a different pouch type. Either way, considering that these species are fossorial, it is reasonable to infer that pouch young would die if only attached to the teat, without a physical protection.

All peramelemorphs have a backward-facing pouch, and despite the low disparity of its body plan, they display a considerably large body size range (Dickman 2015), from 164.5 g to 2920 g ($\text{mean}_w = 906.1$ g; 17.8-fold difference). The mean litter size is small, ranging from one to three but averaging two young per litter, which could be a factor for pouch presence in this group. Horovitz and Sánchez-Villagra (2003) suggested that the group evolved a type 6 pouch from an intermediate pouch, hence, a full pouch emerged in their ancestor. Our reconstruction suggests that the pouch is more likely kept from an already pouched ancestor that they share with Dasyuromorphia, possibly due to phylogenetic inertia.

In Dasyuromorphia, pouch evolution examination becomes more complicated. In this order, body size range is from 4.3 g to 7000 g ($\text{mean}_w = 379.3$ g; 1628-fold difference), and litter sizes range from 2.9 to 10.1 young/litter ($\text{mean}_{ls} = 6.1$). The pouch can be present (types 2, 3, and 4) or absent (truly pouchless or type 1). According to our reconstruction, the ancestor of Dasyuromorphia reverted to the plesiomorphic pouchless condition of Marsupialia, and most descendants retain this condition. The numbat (*Myrmecobius fasciatus*) is unique in many ways. It is the only marsupial specialized in termite-eating, the only truly pouchless australidelphian, and the only species of the family Myrmecobiidae, the sister lineage of Dasyuridae (Cooper 2011; Friend 2015). It weighs 489 g on average and has a mean litter size of 3.7 young/litter. If the dasyuromorphian ancestor was truly pouchless, the numbat possibly retained this character state due to phylogenetic inertia. If it had a type 1 pouch, the numbat pouchless condition is a novelty. Conversely, Dasyuridae is a speciose family, divided into three subfamilies. In Dasyurinae (*Dasycercus*, *Dasyuroides*, *Dasykaluta*, *Parantechinus*, *Myoictis*, *Pseudantechinus*, *Neophascogale*, *Phascolosorex*, *Dasyurus*, and *Sarcophilus*), body size range is from 25 g to 7000 g ($\text{mean}_w = 991.1$ g; 280-fold difference), and litter sizes range from 2.9 to 7.5 young/litter ($\text{mean}_{ls} = 5.5$). The ancestor of Dasyurinae preserved the pouchless dasyuromorphian state, and most taxa exhibit an intermediate pouch. Apparently, there is an association between body size and pouch presence in this subfamily. In general, pouchless taxa are smaller than pouched ones, with a mean weight of 258.6 g. The pouch reemerges with a type 2 in the large spotted-tailed quoll (*Dasyurus maculatus*), with 4000 g, and in the even larger Tasmanian devil (*Sarcophilus harrisi*), with 7000 g. However, the pouch also reappears with a type 3 in the small *Phascolosorex dorsalis*, that weighs 42 g. A possible explanation for this

oddity could be a small litter size of 3.4 young/litter, the second smaller of the subfamily, just behind the Tasmanian devil ($\text{mean}_{\text{ls}} = 2.9$). In addition, it seems that body size increase overcomes a phylogenetic constraint in *Dasyurus*, as only one of the six recognized species is pouched. In Phascogalinae (*Antechinus*, *Murexia*, and *Phascogale*), all species have an intermediate pouch (type 1), as their ancestor also possibly did. They are generally smaller than dasyurines, with a mean weight of 46.6 g and a mean litter size of 6.5 young/litter. The lack of variation in pouch presence and morphology in Phascogalinae is possibly due to phylogenetic inertia. Sminthopsinae is divided into Sminthopsini (*Ningau*, *Antechinomys*, and *Sminthopsis*) and Planigalini (*Planigale*). They have a pouched ancestor, which is a possible reversal to the australidelphian plesiomorphic state. In Sminthopsini, body size ranges from 8 g to 50 g, so we consider them small. Still, almost all species are pouched. *Antechinomys* has a type 2 pouch and *Sminthopsis* species, a type 3. The smallest species, *Ningau ridei*, with 8 g, has an intermediate pouch. We hypothesize that a size increase in the ancestor of Sminthopsini influenced pouch reemergence. The descendants may have decreased in size but kept the pouched condition by phylogenetic inertia. As for the loss of a full pouch in *Ningau*, body size decrease may have influenced it, but it requires more investigation. Finally, in Planigalini, we could infer that all planigales remained pouched as the ancestor of Sminthopsinae by phylogenetic inertia. But it is important to acknowledge that pouch state assessments of *P. tenuirostris* and *P. gilesi* may be inaccurate (Woolley 1974; Woolley and Elliott 2014). Besides, systematics of Dasyuridae changed since Woolley's 1974 work, in which she compiles a dataset of pouch types of many dasyurids. The case of *P. subtilissima* is a great example of how modern systematics affects the interpretation of phenotypic evolution in a clade. In 1974, when Woolley described the unusual type 4 pouch of *Planigale subtilissima*, it was regarded as a species and now it is considered a form of *P. ingrami* (Baker 2015). Later, Woolley and Elliott (2014) corrected pouch morphology assessment of *P. ingrami* form *brunneus* as a type 1 pouch. So, planigales may be more diverse in pouch morphology than we usually assume, and further investigation may change our view of pouch evolution in this taxon. The work of Woolley (1974) was seminal for pouch morphology evolution in dasyurids, but it seems that pouch evolution in this group may probably be the hardest to unravel.

In contrast to dasyurids, pouch variation is minimal in Diprotodontia. All species present a type 6 pouch, except for Vombatiformes, that display a type 5 pouch, as we discussed above. In our dataset, diprotodonts range from 7 g to 31900 g, more than a 4500-fold difference. Thus, the general pattern of large body sizes and pouch presence association is less clear. Burramyidae, Hypsiprymnodontidae, Macropodidae, Phalangeridae, Potoroidae, Pseudocheiridae, and Tarsipedidae all have a forward-facing pouch. Nonetheless, some taxa do not fit any previously established pouch type. In Acrobatidae, the pouch is a deep and weakly bilobate pocket (Aplin 2015a). In Petauridae, the pouch of *Dactylopsila palpator* is divided by a septum, with a nipple in each subunit (Jackson 2015a), but the remaining petaurids seem to have a usual type 5 pouch. Koalas and wombats have a backward-facing pouch (Jackson 2015b; Wells 2015). In wombats, the pouch morphology is probably related to their burrowing activity, as seen in marsupial moles. In koalas, it may be related to the change of food intake that the young experience at around six months old, when they start to feed on “pap”, special jelly-like feces of the mother (Jackson 2015b). Curiously, koalas and wombats belong to a lineage that diverged at the base of the order (Meredith *et al.* 2009a; Upham *et al.* 2019). According to our ancestral state reconstruction, the ancestral diprotodont was fully pouched, and all descendants retained the pouched state. Horovitz and Sánchez-Villagra (2003) suggest that diprotodonts evolved a type 5 pouch from an intermediate pouch, but in their tree koalas and wombats occupy a more derived position, changing character polarization. Pouch ubiquity in Diprotodontia, despite body size variation, raises two hypotheses, not necessarily mutually exclusive. One is that extant taxa preserved the pouch from their ancestor by phylogenetic inertia, while some lineages went through miniaturization in size, such as burramyids and acrobatids. The other hypothesis is that the small litter size may also influence pouch condition, since these animals usually have a single or two young per litter. Therefore, small litter sizes in the group, that are associated with increase in herbivory (Fisher *et al.* 2001), may also influence pouch presence in the group.

5 CONCLUSION

This research is the first to test an association between pouch presence and predictor variables across Marsupialia. In general, phylogeny seems to be the greatest influence on pouch presence in marsupials. Apparently, pouch absence in caenolestids and pouch presence in notoryctids, peramelemorphs, phascogalines, and diprotodonts, are due to phylogenetic inertia. Moreover, body size increase association fits well in didelphids and dasyurines. The reemergence of the pouch in Sminthopsinae is still difficult to understand given our results. The litter size association is probably of less influence on pouch presence in marsupials, requiring further investigation. A variation partitioning would help us to understand how much each variable affects each clade in the phylogeny. Furthermore, it would be interesting to reconstruct ancestral pouch morphology, so we could make a more thorough examination of pouch macroevolutionary patterns.

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SUPPLEMENTARY MATERIAL

APPENDIX 1 – POUCH DATA TABLE

Appendix 1. Pouch data for the 195 species used in this study (only species for which we found data on pouch presence, body mass, and litter size are included in the table) and their source material. ‘Pouch’ column shows pouch presence or absence. ‘Type’ column shows pouch type after Woolley’s (1974) and Russell’s (1982) descriptions.

Species	Pouch	Type	References
<i>Caluromys derbianus</i>	present	5	Astúa 2015
<i>Caluromys lanatus</i>	present	5	Astúa 2015; Voss and Jansa 2021
<i>Caluromys philander</i>	absent	1	Astúa 2015; Voss and Jansa 2021
<i>Caluromysiops irrupta</i>	present	?	Astúa 2015; Voss and Jansa 2021
<i>Chironectes minimus</i>	present	6	Astúa 2015
<i>Didelphis albiventris</i>	present	5	Tyndale-Biscoe and Renfree 1987; Astúa 2015
<i>Didelphis aurita</i>	present	5	Astúa 2015; Voss and Jansa 2021
<i>Didelphis imperfecta</i>	present	5	Astúa 2015; Voss and Jansa 2021
<i>Didelphis marsupialis</i>	present	5	Tyndale-Biscoe and Renfree 1987; Astúa 2015
<i>Didelphis pernigra</i>	present	5	Astúa 2015; Voss and Jansa 2021
<i>Didelphis virginiana</i>	present	5	Tyndale-Biscoe and Renfree 1987; Astúa 2015
<i>Lutreolina crassicaudata</i>	present	6	Astúa 2015
<i>Philander andersoni</i>	present	5	Astúa 2015
<i>Philander mcilhennyi</i>	present	5	Astúa 2015; Voss and Jansa 2021
<i>Philander opossum</i>	present	5	Astúa 2015
<i>Marmosa alstoni</i>	absent	0	Astúa 2015
<i>Marmosa andersoni</i>	absent	0	Astúa 2015
<i>Marmosa constantiae</i>	absent	0	Astúa 2015
<i>Marmosa demerarae</i>	absent	0	Astúa 2015
<i>Marmosa lepida</i>	absent	0	Astúa 2015
<i>Marmosa mexicana</i>	absent	0	Astúa 2015
<i>Marmosa murina</i>	absent	0	Astúa 2015
<i>Marmosa paraguayana</i>	absent	0	Astúa 2015
<i>Marmosa regina</i>	absent	0	Astúa 2015
<i>Marmosa robinsoni</i>	absent	0	Astúa 2015
<i>Marmosa tyleriana</i>	absent	0	Astúa 2015
<i>Marmosa xerophila</i>	absent	0	Astúa 2015
<i>Monodelphis adusta</i>	absent	0	Astúa 2015
<i>Monodelphis arlindoi</i>	absent	0	Astúa 2015
<i>Monodelphis breviceaudata</i>	absent	0	Astúa 2015
<i>Monodelphis dimidiata</i>	absent	0	Astúa 2015
<i>Monodelphis domestica</i>	absent	0	Astúa 2015
<i>Monodelphis palliolata</i>	absent	0	Astúa 2015
<i>Tlacuatzin canescens</i>	absent	0	Astúa 2015
<i>Metachirus myosuros</i>	absent	0	Astúa 2015
<i>Cryptonanus chacoensis</i>	absent	0	Astúa 2015
<i>Gracilinanus agilis</i>	absent	0	Astúa 2015
<i>Gracilinanus microtarsus</i>	absent	0	Astúa 2015
<i>Marmosops parvidens</i>	absent	0	Astúa 2015

<i>Thylamys elegans</i>	absent	0	Astúa 2015
<i>Thylamys pallidior</i>	absent	0	Astúa 2015
<i>Glironia venusta</i>	absent	0	Astúa 2015
<i>Hyladelphys kalinowskii</i>	absent	0	Astúa 2015
<i>Caenolestes caniventer</i>	absent	0	Patterson 2015
<i>Caenolestes fuliginosus</i>	absent	0	Patterson 2015
<i>Dromiciops gliroides</i>	absent	1	Palma and Valladares-Gómez 2015; Schneider and Gurovich 2017
<i>Notoryctes typhlops</i>	present	6	Tyndale-Biscoe and Renfree 1987; Aplin 2015c
<i>Myrmecobius fasciatus</i>	absent	0	Cooper 2011; Friend 2015
<i>Dasyercus cristicauda</i>	absent	1	Woolley 1974; Nowak 2018
<i>Dasykaluta rosamondae</i>	absent	1	Woolley 1974 as <i>Antechinus rosamondae</i> , 1991
<i>Dasyuroides byrnei</i>	absent	1	Woolley 1971a, 1974; Aslin 1974
<i>Dasyurus albopunctatus</i>	absent	1	Woolley 2001
<i>Dasyurus hallucatus</i>	absent	1	Oakwood 2000
<i>Dasyurus maculatus</i>	present	2	Woolley 1974; Baker 2015
<i>Dasyurus spartacus</i>	absent	1	Woolley 2001
<i>Dasyurus viverrinus</i>	absent	1	Fleay 1935; Woolley 1974
<i>Parantechinus apicalis</i>	absent	1	Woolley 1971b, 1974 as <i>Antechinus apicalis</i> , 1991b
<i>Phascosorex dorsalis</i>	present	3	Woolley 2003
<i>Pseudantechinus bilarni</i>	absent	1	Calaby and Taylor 1981 as <i>Antechinus bilarni</i> ; Tyndale-Biscoe and Renfree 1987 as <i>Parantechinus bilarni</i>
<i>Pseudantechinus macdonnellensis</i>	absent	1	Woolley 1974 as <i>Antechinus macdonnellensis</i> , 1991b
<i>Pseudantechinus woolleyae</i>	absent	1	Woolley 2018
<i>Sarcophilus harrisii</i>	present	2	Guiler 1970; Woolley 1974
<i>Antechinus agilis</i>	absent	1	Mason <i>et al.</i> 2017
<i>Antechinus bellus</i>	absent	1	Calaby and Taylor 1981; Mason <i>et al.</i> 2017
<i>Antechinus flavipes</i>	absent	1	Woolley 1974; Mason <i>et al.</i> 2017
<i>Antechinus godmani</i>	absent	1	Woolley 1974; Mason <i>et al.</i> 2017
<i>Antechinus leo</i>	absent	1	Mason <i>et al.</i> 2017
<i>Antechinus minimus</i>	absent	1	Woolley 1974; Wilson 1986 as <i>Antechinus minimus maritimus</i> ; Mason <i>et al.</i> 2017
<i>Antechinus stuartii</i>	absent	1	Woolley 1974; Mason <i>et al.</i> 2017
<i>Antechinus swainsonii</i>	absent	1	Woolley 1974; Mason <i>et al.</i> 2017
<i>Murexia habbema</i>	absent	1	Woolley 2003 as <i>Antechinus habbema</i> ; Baker 2015
<i>Murexia melanurus</i>	absent	1	Woolley 2003 as <i>Antechinus melanurus</i>
<i>Murexia naso</i>	absent	1	Woolley 2003 as <i>Antechinus naso</i>
<i>Murexia rothschildi</i>	absent	1	Woolley 2003
<i>Phascogale calura</i>	absent	1	Nowak 2018
<i>Phascogale tapoatafa</i>	absent	1	Woolley 1974; Baker 2015; Nowak 2018
<i>Planigale gilesi</i>	present	2	Woolley 1974; Baker 2015
<i>Planigale ingrami</i> (subtilissima form)	present	4	Woolley 1974 as <i>Planigale subtilissima</i> ; Baker 2015 as <i>Planigale ingrami subtilissima</i> form
<i>Planigale maculata</i>	present	2	Woolley 1974
<i>Planigale tenuirostris</i>	present	2	Woolley 1974
<i>Antechinomys laniger</i>	present	2	Woolley 1974; Baker 2015
<i>Ningau ridei</i>	absent	1	Tyndale-Biscoe and Renfree 1987
<i>Sminthopsis crassicaudata</i>	present	3	Woolley 1974
<i>Sminthopsis douglasi</i>	present	3	Archer 1979
<i>Sminthopsis longicaudata</i>	present	3	Baker 2015

<i>Sminthopsis macroura</i>	present	3	Tyndale-Biscoe and Renfree 1987; Baker 2015
<i>Sminthopsis murina</i>	present	3	Tyndale-Biscoe and Renfree 1987
<i>Echymipera clara</i>	present	6	Nowak 2018; pers. comm. Kenny
<i>Echymipera kalubu</i>	present	6	Travouillon nov. 2021
<i>Echymipera rufescens</i>	present	6	Dickman 2015; Nowak 2018; pers. comm. Kenny Travouillon nov. 2021
<i>Microperoryctes longicauda</i>	present	6	Nowak 2018; pers. comm. Kenny
<i>Microperoryctes papuensis</i>	present	6	Travouillon nov. 2021
<i>Isoodon auratus</i>	present	6	Nowak 2018; pers. comm. Kenny
<i>Isoodon macrourus</i>	present	6	Travouillon nov. 2021
<i>Isoodon obesulus</i>	present	6	Nowak 2018; pers. comm. Kenny
<i>Perameles bougainville</i>	present	6	Travouillon nov. 2021
<i>Perameles gunnii</i>	present	6	Dickman 2015; Nowak 2018; pers. comm. Kenny Travouillon nov. 2021
<i>Perameles nasuta</i>	present	6	Tyndale-Biscoe and Renfree 1987; Dickman 2015; Nowak 2018; pers. comm. Kenny Travouillon nov. 2021
<i>Peroryctes broadbenti</i>	present	6	Tyndale-Biscoe and Renfree 1987; Dickman 2015; Nowak 2018; pers. comm. Kenny Travouillon nov. 2021
<i>Peroryctes raffrayana</i>	present	6	Nowak 2018; pers. comm. Kenny
<i>Macrotis lagotis</i>	present	6	Travouillon nov. 2021
<i>Hypsiprymnodon moschatus</i>	present	5	Tyndale-Biscoe and Renfree 1987; Nowak 2018; pers. comm. Kenny Travouillon nov. 2021
<i>Lagostrophus fasciatus</i>	present	5	Lyne 1964; Tyndale-Biscoe and Renfree 1987; pers. comm. Kenny Travouillon nov. 2021
<i>Dendrolagus bennettianus</i>	present	5	Aplin <i>et al.</i> 2010; Dickman 2015; pers. comm. Kenny Travouillon nov. 2021
<i>Dendrolagus dorianus</i>	present	5	Dickman 2015; pers. comm. Kenny Travouillon nov. 2021
<i>Dendrolagus goodfellowi</i>	present	5	Southgate 2015; pers. comm. Kenny Travouillon nov. 2021
<i>Dendrolagus inustus</i>	present	5	Johnson and Strahan 1982; Nowak 2018
<i>Dendrolagus lumholtzi</i>	present	5	Eldridge and Coulson 2015
<i>Dendrolagus matschiei</i>	present	5	Eldridge and Coulson 2015
<i>Dendrolagus spadix</i>	present	5	Eldridge and Coulson 2015
<i>Dorcopsis hageni</i>	present	5	Eldridge and Coulson 2015
<i>Dorcopsis muelleri</i>	present	5	Eldridge and Coulson 2015
<i>Dorcopsulus vanheurni</i>	present	5	Eldridge and Coulson 2015
<i>Lagorchestes conspicillatus</i>	present	5	Eldridge and Coulson 2015
<i>Lagorchestes hirsutus</i>	present	5	Eldridge and Coulson 2015
<i>Macropus fuliginosus</i>	present	5	Eldridge and Coulson 2015
<i>Macropus giganteus</i>	present	5	Eldridge and Coulson 2015
<i>Notamacropus agilis</i>	present	5	Eldridge and Coulson 2015
<i>Notamacropus dorsalis</i>	present	5	Eldridge and Coulson 2015
<i>Notamacropus eugenii</i>	present	5	Eldridge and Coulson 2015
<i>Notamacropus irma</i>	present	5	Eldridge and Coulson 2015
<i>Notamacropus parma</i>	present	5	Eldridge and Coulson 2015
<i>Notamacropus parryi</i>	present	5	Eldridge and Coulson 2015
<i>Notamacropus rufogriseus</i>	present	5	Eldridge and Coulson 2015
<i>Onychogalea fraenata</i>	present	5	Eldridge and Coulson 2015
<i>Onychogalea unguifera</i>	present	5	Eldridge and Coulson 2015
<i>Osphranter antilopinus</i>	present	5	Eldridge and Coulson 2015
<i>Osphranter robustus</i>	present	5	Eldridge and Coulson 2015
<i>Osphranter rufus</i>	present	5	Eldridge and Coulson 2015

<i>Petrogale assimilis</i>	present	5	Eldridge and Coulson 2015
<i>Petrogale brachyotis</i>	present	5	Eldridge and Coulson 2015
<i>Petrogale burbidgei</i>	present	5	Eldridge and Coulson 2015
<i>Petrogale concinna</i>	present	5	Eldridge and Coulson 2015
<i>Petrogale herberti</i>	present	5	Eldridge and Coulson 2015
<i>Petrogale inornata</i>	present	5	Eldridge and Coulson 2015
<i>Petrogale lateralis</i>	present	5	Eldridge and Coulson 2015
<i>Petrogale penicillata</i>	present	5	Eldridge and Coulson 2015
<i>Petrogale persephone</i>	present	5	Eldridge and Coulson 2015
<i>Petrogale xanthopus</i>	present	5	Eldridge and Coulson 2015
<i>Setonix brachyurus</i>	present	5	Eldridge and Coulson 2015
<i>Thylogale billardieri</i>	present	5	Eldridge and Coulson 2015
<i>Thylogale browni</i>	present	5	Eldridge and Coulson 2015
<i>Thylogale brunii</i>	present	5	Eldridge and Coulson 2015
<i>Thylogale stigmatica</i>	present	5	Eldridge and Coulson 2015
<i>Thylogale thetis</i>	present	5	Eldridge and Coulson 2015
<i>Wallabia bicolor</i>	present	5	Eldridge and Coulson 2015
<i>Aepyprymnus rufescens</i>	present	5	Eldridge and Coulson 2015
<i>Bettongia gaimardi</i>	present	5	Eldridge and Coulson 2015
<i>Bettongia lesueur</i>	present	5	Eldridge and Coulson 2015
<i>Bettongia penicillata</i>	present	5	Eldridge and Coulson 2015
<i>Bettongia tropica</i>	present	5	Eldridge and Coulson 2015
<i>Potorous gilbertii</i>	present	5	Tyndale-Biscoe and Renfree 1987; Eldridge and Frankham 2015
<i>Potorous longipes</i>	present	5	Tyndale-Biscoe and Renfree 1987; Eldridge and Frankham 2015
<i>Potorous tridactylus</i>	present	5	Tyndale-Biscoe and Renfree 1987; Eldridge and Frankham 2015
<i>Acrobates pygmaeus</i>	present	other	Aplin 2015a
<i>Distoechurus pennatus</i>	present	other	Aplin 2015a
<i>Dactylopsila palpator</i>	present	other	Jackson 2015b
<i>Dactylopsila trivirgata</i>	present	5	McKENNA 2005; Nowak 2018
<i>Gymnobelideus leadbeateri</i>	present	5	Tyndale-Biscoe and Renfree 1987; Nowak 2018
<i>Petaurus breviceps</i>	present	5	Tyndale-Biscoe and Renfree 1987; Lindenmayer 2002
<i>Petaurus gracilis</i>	present	5	Lindenmayer 2002
<i>Petaurus norfolcensis</i>	present	5	Lindenmayer 2002
<i>Hemibelideus lemuroides</i>	present	5	Jackson 2015c
<i>Petauroides volans</i>	present	5	Jackson 2015c
<i>Pseudocheirus peregrinus</i>	present	5	Tyndale-Biscoe and Renfree 1987; Jackson 2015c
<i>Pseudochirulus canescens</i>	present	5	Jackson 2015c
<i>Pseudochirulus forbesi</i>	present	5	Jackson 2015c
<i>Pseudochirulus herbertensis</i>	present	5	Jackson 2015c
<i>Pseudochirulus mayeri</i>	present	5	Jackson 2015c
<i>Petropseudes dahlii</i>	present	5	Jackson 2015c
<i>Pseudochirops albertisii</i>	present	5	Jackson 2015c
<i>Pseudochirops archeri</i>	present	5	Jackson 2015c
<i>Pseudochirops corinnae</i>	present	5	Jackson 2015c
<i>Pseudochirops cupreus</i>	present	5	Jackson 2015c
<i>Tarsipes rostratus</i>	present	5	Tyndale-Biscoe and Renfree 1987; Wooller 2015
<i>Burramys parvus</i>	present	5	Tyndale-Biscoe and Renfree 1987
<i>Cercartetus caudatus</i>	present	5	Jackson 2015a; Nowak 2018
<i>Cercartetus concinnus</i>	present	5	Jackson 2015a; Nowak 2018

<i>Cercartetus lepidus</i>	present	5	Nowak 2018
<i>Cercartetus nanus</i>	present	5	Tyndale-Biscoe and Renfree 1987; Nowak 2018
<i>Phalanger carmelitae</i>	present	5	Helgen and Jackson 2015
<i>Phalanger gymnotis</i>	present	5	Shoemaker and Croxton 1982; Helgen and Jackson 2015
<i>Phalanger lullulae</i>	present	5	Helgen and Jackson 2015
<i>Phalanger orientalis</i>	present	5	Helgen and Jackson 2015
<i>Phalanger sericeus</i>	present	5	Helgen and Jackson 2015
<i>Phalanger vestitus</i>	present	5	Helgen and Jackson 2015
<i>Spilocuscus maculatus</i>	present	5	Shoemaker and Croxton 1982; Helgen and Jackson 2015
<i>Spilocuscus rufoniger</i>	present	5	Helgen and Jackson 2015
<i>Trichosurus caninus</i>	present	5	Tyndale-Biscoe and Renfree 1987; Helgen and Jackson 2015
<i>Trichosurus vulpecula</i>	present	5	Tyndale-Biscoe and Renfree 1987; Helgen and Jackson 2015
<i>Wyulda squamicaudata</i>	present	5	Helgen and Jackson 2015
<i>Phascolarctos cinereus</i>	present	6	Tyndale-Biscoe and Renfree 1987
<i>Lasiorhinus krefftii</i>	present	6	Nowak 2018
<i>Lasiorhinus latifrons</i>	present	6	Tyndale-Biscoe and Renfree 1987
<i>Vombatus ursinus</i>	present	6	Tyndale-Biscoe and Renfree 1987

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APPENDIX 2 – BODY MASS DATA TABLE

Appendix 2. Body mass data for the 195 species used in this study (only species for which we found data on pouch presence, body mass, and litter size are included in the table) and their source material. 'MIN', 'MAX' and 'Mean' columns show minimum, maximum, and mean weights of each species, respectively. 'Sex' specifies if body mass data is female-only or sex-unspecified (general).

Species	MIN	MAX	Mean	Sex	References
<i>Caluromys derbianus</i>	–	–	308	female	Weisbecker <i>et al.</i> 2013
<i>Caluromys lanatus</i>	300	520	410	general	Astúa 2015
<i>Caluromys philander</i>	–	–	187	female	Weisbecker <i>et al.</i> 2013
<i>Caluromysiops irrupta</i>	300	500	400	general	Astúa 2015
<i>Chironectes minimus</i>	510	790	650	general	Astúa 2015
<i>Didelphis albiventris</i>	500	2500	1500	general	Astúa 2015
<i>Didelphis aurita</i>	700	1500	1100	general	Astúa 2015
<i>Didelphis imperfecta</i>	600	1200	900	general	Astúa 2015
<i>Didelphis marsupialis</i>	570	2400	1485	general	Astúa 2015
<i>Didelphis pernigra</i>	720	2000	1360	general	Astúa 2015
<i>Didelphis virginiana</i>	–	–	1800	female	Weisbecker <i>et al.</i> 2013
<i>Lutreolina crassicaudata</i>	200	910	555	general	Astúa 2015
<i>Philander andersoni</i>	225	600	412.5	general	Astúa 2015
<i>Philander mcilhennyi</i>	396	640	518	general	Astúa 2015
<i>Philander opossum</i>	–	–	450	female	Weisbecker <i>et al.</i> 2013
<i>Marmosa alstoni</i>	60	150	105	general	Astúa 2015
<i>Marmosa andersoni</i>	28	38	33	general	Astúa 2015
<i>Marmosa constantiae</i>	68	90	79	general	Astúa 2015
<i>Marmosa demerarae</i>	62	130	96	general	Astúa 2015
<i>Marmosa lepida</i>	10	18	14	general	Astúa 2015
<i>Marmosa mexicana</i>	–	–	35	female	Weisbecker <i>et al.</i> 2013
<i>Marmosa murina</i>	19	100	59.5	general	Astúa 2015
<i>Marmosa paraguayana</i>	56	230	143	general	Astúa 2015
<i>Marmosa regina</i>	76	164	120	general	Astúa 2015
<i>Marmosa robinsoni</i>	–	–	60	female	Weisbecker <i>et al.</i> 2013
<i>Marmosa tyleriana</i>	30	56	43	general	Astúa 2015
<i>Marmosa xerophila</i>	29	57	43	female	Astúa 2015
<i>Monodelphis adusta</i>	15	35	25	general	Astúa 2015
<i>Monodelphis arlindoi</i>	33	96	64.5	female	Astúa 2015
<i>Monodelphis brevicaudata</i>	–	–	70	female	Weisbecker <i>et al.</i> 2013
<i>Monodelphis dimidiata</i>	40	84	62	general	Astúa 2015
<i>Monodelphis domestica</i>	58	110	84	general	Astúa 2015
<i>Monodelphis palliolata</i>	32	63	47.5	female	Astúa 2015
<i>Tlacuatzin canescens</i>	30	70	50	general	Astúa 2015
<i>Metachirus myosuroides</i>	–	–	300	female	Weisbecker <i>et al.</i> 2013
<i>Cryptonanus chacoensis</i>	10	19	14.5	general	Astúa 2015
<i>Gracilinanus agilis</i>	15	40	27.5	general	Astúa 2015
<i>Gracilinanus microtarsus</i>	12	58	35	general	Astúa 2015
<i>Marmosops parvidens</i>	21	32	26.5	general	Astúa 2015
<i>Thylamys elegans</i>	–	–	25	female	Weisbecker <i>et al.</i> 2013
<i>Thylamys pallidior</i>	13	38.5	25.75	general	Astúa 2015

<i>Glironia venusta</i>	–	–	130	general	Astúa 2015
<i>Hyladelphys kalinowskii</i>	10	20	15	general	Astúa 2015
<i>Caenolestes caniventer</i>	29	47	38	general	Patterson 2015
<i>Caenolestes fuliginosus</i>	–	–	35	female	Weisbecker <i>et al.</i> 2013
<i>Dromiciops gliroides</i>	–	–	24	female	Weisbecker <i>et al.</i> 2013
<i>Notoryctes typhlops</i>	50	60	55	general	Aplin 2015c
<i>Myrmecobius fasciatus</i>	–	–	459	female	Weisbecker <i>et al.</i> 2013
<i>Dasyercus cristicauda</i>	–	–	80	female	Weisbecker <i>et al.</i> 2013
<i>Dasykaluta rosamondae</i>	–	–	30	female	Weisbecker <i>et al.</i> 2013
<i>Dasyuroides byrnei</i>	–	–	100	female	Weisbecker <i>et al.</i> 2013
<i>Dasyurus albopunctatus</i>	–	–	520	female	Baker 2015
<i>Dasyurus hallucatus</i>	–	–	400	female	Weisbecker <i>et al.</i> 2013
<i>Dasyurus maculatus</i>	–	–	4000	female	Weisbecker <i>et al.</i> 2013
<i>Dasyurus spartacus</i>	–	–	684	female	Baker 2015
<i>Dasyurus viverrinus</i>	–	–	880	female	Weisbecker <i>et al.</i> 2013
<i>Parantechinus apicalis</i>	–	–	58	female	Weisbecker <i>et al.</i> 2013
<i>Phascosorex dorsalis</i>	–	–	42	female	Weisbecker <i>et al.</i> 2013
<i>Pseudantechinus bilarni</i>	15	35	25	female	Baker 2015
<i>Pseudantechinus macdonnellensis</i>	–	–	30	female	Weisbecker <i>et al.</i> 2013
<i>Pseudantechinus woolleyae</i>	30	45	37.5	female	Baker 2015
<i>Sarcophilus harrisii</i>	–	–	7000	female	Weisbecker <i>et al.</i> 2013
<i>Antechinus agilis</i>	–	–	18	female	Weisbecker <i>et al.</i> 2013
<i>Antechinus bellus</i>	–	–	34	female	Weisbecker <i>et al.</i> 2013
<i>Antechinus flavipes</i>	–	–	34	female	Weisbecker <i>et al.</i> 2013
<i>Antechinus godmani</i>	42	67	54.5	female	Baker 2015
<i>Antechinus leo</i>	–	–	54	female	Weisbecker <i>et al.</i> 2013
<i>Antechinus minimus</i>	–	–	42	female	Weisbecker <i>et al.</i> 2013
<i>Antechinus stuartii</i>	–	–	20	female	Weisbecker <i>et al.</i> 2013
<i>Antechinus swainsonii</i>	–	–	41	female	Weisbecker <i>et al.</i> 2013
<i>Murexia habbema</i>	22.7	31.2	26.95	female	Baker 2015
<i>Murexia melanurus</i>	17	44	30.5	female	Baker 2015
<i>Murexia naso</i>	29	48.5	38.75	female	Baker 2015
<i>Murexia rothschildi</i>	–	–	60	female	Weisbecker <i>et al.</i> 2013
<i>Phascogale calura</i>	–	–	43	female	Weisbecker <i>et al.</i> 2013
<i>Phascogale tapoatafa</i>	–	–	156	female	Weisbecker <i>et al.</i> 2013
<i>Planigale gilesi</i>	–	–	6.9	female	Weisbecker <i>et al.</i> 2013
<i>Planigale ingrami</i>	–	–	4.3	female	Weisbecker <i>et al.</i> 2013
<i>Planigale maculata</i>	–	–	10	female	Weisbecker <i>et al.</i> 2013
<i>Planigale tenuirostris</i>	–	–	5.3	female	Weisbecker <i>et al.</i> 2013
<i>Antechinomys laniger</i>	–	–	20	female	Weisbecker <i>et al.</i> 2013
<i>Ningau ridei</i>	–	–	8	female	Weisbecker <i>et al.</i> 2013
<i>Sminthopsis crassicaudata</i>	–	–	15	female	Weisbecker <i>et al.</i> 2013
<i>Sminthopsis douglasi</i>	–	–	50	female	Weisbecker <i>et al.</i> 2013
<i>Sminthopsis longicaudata</i>	15	25	20	female	Baker 2015
<i>Sminthopsis macroura</i>	–	–	20	female	Weisbecker <i>et al.</i> 2013
<i>Sminthopsis murina</i>	–	–	14	female	Weisbecker <i>et al.</i> 2013
<i>Echymipera clara</i>	–	–	983	female	Weisbecker <i>et al.</i> 2013
<i>Echymipera kalubu</i>	–	–	600	female	Weisbecker <i>et al.</i> 2013
<i>Echymipera rufescens</i>	–	–	1400	female	Weisbecker <i>et al.</i> 2013
<i>Microperoryctes longicauda</i>	–	–	598	female	Weisbecker <i>et al.</i> 2013
<i>Microperoryctes papuensis</i>	145	184	164.5	general	Dickman 2015
<i>Isoodon auratus</i>	300	670	485	general	Dickman 2015
<i>Isoodon macrourus</i>	–	–	1100	female	Weisbecker <i>et al.</i> 2013
<i>Isoodon obesulus</i>	–	–	700	female	Weisbecker <i>et al.</i> 2013

<i>Perameles bougainville</i>	–	–	226	female	Weisbecker <i>et al.</i> 2013
<i>Perameles gunnii</i>	–	–	750	female	Weisbecker <i>et al.</i> 2013
<i>Perameles nasuta</i>	–	–	859	female	Weisbecker <i>et al.</i> 2013
<i>Peroryctes broadbenti</i>	940	4900	2920	general	Dickman 2015
<i>Peroryctes raffrayana</i>	–	–	800	female	Weisbecker <i>et al.</i> 2013
<i>Macrotis lagotis</i>	–	–	1100	female	Weisbecker <i>et al.</i> 2013
<i>Hypsiprymnodon moschatus</i>	360	680	520	general	Dennis 2015
<i>Lagostrophus fasciatus</i>	–	–	1800	female	Weisbecker <i>et al.</i> 2013
<i>Dendrolagus bennettianus</i>	–	–	9300	female	Weisbecker <i>et al.</i> 2013
<i>Dendrolagus dorianus</i>	–	–	8800	female	Weisbecker <i>et al.</i> 2013
<i>Dendrolagus goodfellowi</i>	–	–	7400	female	Weisbecker <i>et al.</i> 2013
<i>Dendrolagus inustus</i>	–	–	11400	female	Weisbecker <i>et al.</i> 2013
<i>Dendrolagus lumholtzi</i>	–	–	6475	female	Weisbecker <i>et al.</i> 2013
<i>Dendrolagus matschiei</i>	–	–	9200	female	Weisbecker <i>et al.</i> 2013
<i>Dendrolagus spadix</i>	7000	9100	8050	general	Eldridge and Coulson 2015
<i>Dorcopsis hageni</i>	–	–	5500	female	Weisbecker <i>et al.</i> 2013
<i>Dorcopsis muelleri</i>	–	–	5000	female	Weisbecker <i>et al.</i> 2013
<i>Dorcopsulus vanheurni</i>	–	–	1893	female	Weisbecker <i>et al.</i> 2013
<i>Lagorchestes conspicillatus</i>	–	–	3000	female	Weisbecker <i>et al.</i> 2013
<i>Lagorchestes hirsutus</i>	–	–	1310	female	Weisbecker <i>et al.</i> 2013
<i>Macropus fuliginosus</i>	–	–	16000	female	Weisbecker <i>et al.</i> 2013
<i>Macropus giganteus</i>	–	–	17800	female	Weisbecker <i>et al.</i> 2013
<i>Notamacropus agilis</i>	–	–	11000	female	Weisbecker <i>et al.</i> 2013
<i>Notamacropus dorsalis</i>	–	–	6500	female	Weisbecker <i>et al.</i> 2013
<i>Notamacropus eugenii</i>	–	–	5500	female	Weisbecker <i>et al.</i> 2013
<i>Notamacropus irma</i>	–	–	8000	female	Weisbecker <i>et al.</i> 2013
<i>Notamacropus parma</i>	–	–	3550	female	Weisbecker <i>et al.</i> 2013
<i>Notamacropus parryi</i>	–	–	11000	female	Weisbecker <i>et al.</i> 2013
<i>Notamacropus rufogriseus</i>	–	–	13800	female	Weisbecker <i>et al.</i> 2013
<i>Onychogalea fraenata</i>	–	–	3400	female	Weisbecker <i>et al.</i> 2013
<i>Onychogalea unguifera</i>	–	–	5800	female	Weisbecker <i>et al.</i> 2013
<i>Osphranter antilopinus</i>	–	–	17500	female	Weisbecker <i>et al.</i> 2013
<i>Osphranter robustus</i>	–	–	15600	female	Weisbecker <i>et al.</i> 2013
<i>Osphranter rufus</i>	–	–	26500	female	Weisbecker <i>et al.</i> 2013
<i>Petrogale assimilis</i>	–	–	4300	female	Weisbecker <i>et al.</i> 2013
<i>Petrogale brachyotis</i>	–	–	3700	female	Weisbecker <i>et al.</i> 2013
<i>Petrogale burbidgei</i>	900	1400	1150	general	Eldridge and Coulson 2015
<i>Petrogale concinna</i>	–	–	1350	female	Weisbecker <i>et al.</i> 2013
<i>Petrogale herberti</i>	–	–	4300	female	Weisbecker <i>et al.</i> 2013
<i>Petrogale inornata</i>	–	–	4200	female	Weisbecker <i>et al.</i> 2013
<i>Petrogale lateralis</i>	–	–	4200	female	Weisbecker <i>et al.</i> 2013
<i>Petrogale penicillata</i>	–	–	6300	female	Weisbecker <i>et al.</i> 2013
<i>Petrogale persephone</i>	–	–	5200	female	Weisbecker <i>et al.</i> 2013
<i>Petrogale xanthopus</i>	–	–	7000	female	Weisbecker <i>et al.</i> 2013
<i>Setonix brachyurus</i>	–	–	2900	female	Weisbecker <i>et al.</i> 2013
<i>Thylogale billardierii</i>	–	–	3900	female	Weisbecker <i>et al.</i> 2013
<i>Thylogale browni</i>	–	–	4338	female	Weisbecker <i>et al.</i> 2013
<i>Thylogale brunii</i>	–	–	4338	female	Weisbecker <i>et al.</i> 2013
<i>Thylogale stigmatica</i>	–	–	4100	female	Weisbecker <i>et al.</i> 2013
<i>Thylogale thetis</i>	–	–	3800	female	Weisbecker <i>et al.</i> 2013
<i>Wallabia bicolor</i>	–	–	13000	female	Weisbecker <i>et al.</i> 2013
<i>Aepyprymnus rufescens</i>	–	–	2500	female	Weisbecker <i>et al.</i> 2013
<i>Bettongia gaimardi</i>	–	–	1660	female	Weisbecker <i>et al.</i> 2013
<i>Bettongia lesueur</i>	–	–	1300	female	Weisbecker <i>et al.</i> 2013

<i>Bettongia penicillata</i>	–	–	1300	female	Weisbecker <i>et al.</i> 2013
<i>Bettongia tropica</i>	–	–	1200	female	Weisbecker <i>et al.</i> 2013
<i>Potorous gilbertii</i>	700	1200	950	general	Eldridge and Frankham 2015
<i>Potorous longipes</i>	1600	2200	1900	general	Eldridge and Frankham 2015
<i>Potorous tridactylus</i>	–	–	1020	female	Weisbecker <i>et al.</i> 2013
<i>Acrobates pygmaeus</i>	–	–	12	female	Weisbecker <i>et al.</i> 2013
<i>Distoechurus pennatus</i>	–	–	50	female	Weisbecker <i>et al.</i> 2013
<i>Dactylopsila palpator</i>	–	–	320	female	Weisbecker <i>et al.</i> 2013
<i>Dactylopsila trivirgata</i>	–	–	423	female	Weisbecker <i>et al.</i> 2013
<i>Gymnobelideus leadbeateri</i>	–	–	133	female	Weisbecker <i>et al.</i> 2013
<i>Petaurus breviceps</i>	–	–	115	female	Weisbecker <i>et al.</i> 2013
<i>Petaurus gracilis</i>	310	454	382	female	Jackson 2015b
<i>Petaurus norfolcensis</i>	–	–	230	female	Weisbecker <i>et al.</i> 2013
<i>Hemibelideus lemuroides</i>	–	–	925	female	Weisbecker <i>et al.</i> 2013
<i>Petauroides volans</i>	–	–	1700	female	Weisbecker <i>et al.</i> 2013
<i>Pseudocheirus peregrinus</i>	–	–	900	female	Weisbecker <i>et al.</i> 2013
<i>Pseudochirulus canescens</i>	–	–	365	female	Weisbecker <i>et al.</i> 2013
<i>Pseudochirulus forbesi</i>	–	–	600	female	Weisbecker <i>et al.</i> 2013
<i>Pseudochirulus herbertensis</i>	–	–	1050	female	Weisbecker <i>et al.</i> 2013
<i>Pseudochirulus mayeri</i>	–	–	154.5	female	Weisbecker <i>et al.</i> 2013
<i>Petropseudes dahlia</i>	–	–	2000	female	Weisbecker <i>et al.</i> 2013
<i>Pseudochirops albertisii</i>	–	–	756	female	Weisbecker <i>et al.</i> 2013
<i>Pseudochirops archeri</i>	–	–	1120	female	Weisbecker <i>et al.</i> 2013
<i>Pseudochirops corinnae</i>	–	–	1190	female	Weisbecker <i>et al.</i> 2013
<i>Pseudochirops cupreus</i>	–	–	1700	female	Weisbecker <i>et al.</i> 2013
<i>Tarsipes rostratus</i>	–	–	9	female	Weisbecker <i>et al.</i> 2013
<i>Burramys parvus</i>	–	–	42	female	Weisbecker <i>et al.</i> 2013
<i>Cercartetus caudatus</i>	–	–	30	female	Weisbecker <i>et al.</i> 2013
<i>Cercartetus concinnus</i>	–	–	13	female	Weisbecker <i>et al.</i> 2013
<i>Cercartetus lepidus</i>	–	–	7	female	Weisbecker <i>et al.</i> 2013
<i>Cercartetus nanus</i>	–	–	24	female	Weisbecker <i>et al.</i> 2013
<i>Phalanger carmelitae</i>	–	–	1000	female	Weisbecker <i>et al.</i> 2013
<i>Phalanger gymnotis</i>	–	–	2400	female	Weisbecker <i>et al.</i> 2013
<i>Phalanger lullulae</i>	–	–	1770	female	Weisbecker <i>et al.</i> 2013
<i>Phalanger orientalis</i>	–	–	2500	female	Weisbecker <i>et al.</i> 2013
<i>Phalanger sericeus</i>	–	–	1967	female	Weisbecker <i>et al.</i> 2013
<i>Phalanger vestitus</i>	–	–	1933	female	Weisbecker <i>et al.</i> 2013
<i>Spilocuscus maculatus</i>	–	–	3060	female	Weisbecker <i>et al.</i> 2013
<i>Spilocuscus rufoniger</i>	–	–	6500	female	Weisbecker <i>et al.</i> 2013
<i>Trichosurus caninus</i>	–	–	2900	female	Weisbecker <i>et al.</i> 2013
<i>Trichosurus vulpecula</i>	–	–	2300	female	Weisbecker <i>et al.</i> 2013
<i>Wyulda squamicaudata</i>	–	–	1675	female	Weisbecker <i>et al.</i> 2013
<i>Phascolarctos cinereus</i>	–	–	5100	female	Weisbecker <i>et al.</i> 2013
<i>Lasiorhinus krefftii</i>	–	–	31900	female	Wells 2015
<i>Lasiorhinus latifrons</i>	–	–	26000	female	Weisbecker <i>et al.</i> 2013
<i>Vombatus ursinus</i>	–	–	26000	female	Weisbecker <i>et al.</i> 2013

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APPENDIX 3 – LITTER SIZE DATA TABLE

Appendix 3. Litter size data for the 195 species used in this study (only species for which we found data on pouch presence, body mass, and litter size are included in the table) and their source material. 'MIN', 'MAX', and 'Mean' columns show minimum, maximum, and mean litter size per species, respectively. Some data are based on punctual observations described in the 'Observation' column.

Species	MIN	MAX	Mean	Reference	Observation
<i>Caluromys derbianus</i>	–	–	3.14	Jones <i>et al.</i> 2009	–
<i>Caluromys lanatus</i>	–	–	3.7	Battistella <i>et al.</i> 2019	–
<i>Caluromys philander</i>	–	–	4.18	Jones <i>et al.</i> 2009	–
<i>Caluromysiops irrupta</i>	–	–	2	Jones <i>et al.</i> 2009	–
<i>Chironectes minimus</i>	–	–	3.24	Jones <i>et al.</i> 2009	–
<i>Didelphis albiventris</i>	–	–	6.9	Jones <i>et al.</i> 2009	–
<i>Didelphis aurita</i>	–	–	6.11	Jones <i>et al.</i> 2009	–
<i>Didelphis imperfecta</i>	–	–	4.9	Battistella <i>et al.</i> 2019	–
<i>Didelphis marsupialis</i>	–	–	7.09	Jones <i>et al.</i> 2009	–
<i>Didelphis pernigra</i>	–	–	4.2	Battistella <i>et al.</i> 2019	–
<i>Didelphis virginiana</i>	–	–	8.62	Jones <i>et al.</i> 2009	–
<i>Lutreolina crassicaudata</i>	–	–	7.71	Jones <i>et al.</i> 2009	–
<i>Philander andersoni</i>	–	–	3	Battistella <i>et al.</i> 2019	–
<i>Philander mcilhennyi</i>	–	–	5	Battistella <i>et al.</i> 2019	–
<i>Philander opossum</i>	–	–	4.75	Jones <i>et al.</i> 2009	–
<i>Marmosa alstoni</i>	–	–	11	Astúa 2015	"A litter of eleven young has been recorded." (Astúa 2015 p.153)
<i>Marmosa andersoni</i>	–	–	5	Astúa 2015	"One of the seven known specimens of Anderson's Mouse Opossum was a female carrying five attached young" (Astúa 2015 p.141)
<i>Marmosa constantiae</i>	–	–	8	Jones <i>et al.</i> 2009	–
<i>Marmosa demerarae</i>	–	–	6.29	Jones <i>et al.</i> 2009	–
<i>Marmosa lepida</i>	–	–	4.99	Jones <i>et al.</i> 2009	–
<i>Marmosa mexicana</i>	–	–	11.6	Jones <i>et al.</i> 2009	–
<i>Marmosa murina</i>	–	–	8.4	Jones <i>et al.</i> 2009	–
<i>Marmosa paraguayana</i>	–	–	5.56	Jones <i>et al.</i> 2009	–
<i>Marmosa regina</i>	6	8	7	Astúa 2015	–
<i>Marmosa robinsoni</i>	–	–	9.5	Jones <i>et al.</i> 2009	–
<i>Marmosa tyleriana</i>	–	–	3	Jones <i>et al.</i> 2009	–
<i>Marmosa xerophila</i>	–	–	7.9	Battistella <i>et al.</i> 2019	–
<i>Monodelphis adusta</i>	–	–	4	Astúa 2015	"A female trapped in

					August had four suckling young" (Astúa 2015 p.147)
<i>Monodelphis arlindoi</i>	–	–	6	Catzefflis <i>et al.</i> 2018	–
<i>Monodelphis brevicaudata</i>	–	–	7.24	Jones <i>et al.</i> 2009	–
<i>Monodelphis dimidiata</i>	–	–	12	Jones <i>et al.</i> 2009	–
<i>Monodelphis domestica</i>	–	–	7.29	Jones <i>et al.</i> 2009	–
<i>Monodelphis palliolata</i>	7	8	7.5	Astúa 2015	"In northern Venezuela, 7–8 young/litter were reported (mean litter size 7.5 young), with 1.5 litters/year on average." (Astúa 2015 p.150)
<i>Tlacuatzin canescens</i>	–	–	8.49	Jones <i>et al.</i> 2009	–
<i>Metachirus myosuros</i>	–	–	3.87	Jones <i>et al.</i> 2009	–
<i>Cryptonanus chacoensis</i>	–	–	12	Astúa 2015	"They have been reported to have litters of up to twelve young, although they have only nine mammae." (Astúa 2015 p.170)
<i>Gracilinanus agilis</i>	–	–	12	Jones <i>et al.</i> 2009	–
<i>Gracilinanus microtarsus</i>	–	–	9.7	Battistella <i>et al.</i> 2019	–
<i>Marmosops parvidens</i>	–	–	4.05	Jones <i>et al.</i> 2009	–
<i>Thylamys elegans</i>	–	–	10.6	Jones <i>et al.</i> 2009	–
<i>Thylamys pallidior</i>	3	4	3.5	Astúa 2015	–
<i>Glironia venusta</i>	–	–	3	Astúa 2015	"A female with three fully furred young was captured in December in south-western Pará, Brazil." (Astúa 2015 p.131)
<i>Hyladelphys kalinowskii</i>	–	–	4	Battistella <i>et al.</i> 2019	–
<i>Caenolestes caniventer</i>	–	–	2	Jones <i>et al.</i> 2009	–
<i>Caenolestes fuliginosus</i>	–	–	3.62	Jones <i>et al.</i> 2009	–
<i>Dromiciops gliroides</i>	–	–	3	Jones <i>et al.</i> 2009	–
<i>Notoryctes typhlops</i>	–	–	1	Jones <i>et al.</i> 2009	–
<i>Myrmecobius fasciatus</i>	–	–	3.68	Jones <i>et al.</i> 2009	–
<i>Dasyercus cristicauda</i>	–	–	6.5	Jones <i>et al.</i> 2009	–
<i>Dasykaluta rosamondae</i>	–	–	6.64	Jones <i>et al.</i> 2009	–
<i>Dasyuroides byrnei</i>	–	–	5.14	Jones <i>et al.</i> 2009	–
<i>Dasyurus albopunctatus</i>	–	–	6.19	Jones <i>et al.</i> 2009	–
<i>Dasyurus hallucatus</i>	–	–	6.39	Jones <i>et al.</i> 2009	–
<i>Dasyurus maculatus</i>	–	–	4.47	Jones <i>et al.</i> 2009	–
<i>Dasyurus spartacus</i>	–	–	6.78	Jones <i>et al.</i> 2009	–
<i>Dasyurus viverrinus</i>	–	–	4.7	Jones <i>et al.</i> 2009	–
<i>Parantechinus apicalis</i>	–	–	7.5	Jones <i>et al.</i> 2009	–
<i>Phascolosorex dorsalis</i>	–	–	3.39	Jones <i>et al.</i> 2009	–
<i>Pseudantechinus bilarni</i>	–	–	4.75	Jones <i>et al.</i> 2009	–

<i>Pseudantechinus macdonnellensis</i>	–	–	5.88	Jones <i>et al.</i> 2009	–
<i>Pseudantechinus woolleyae</i>	–	–	5.99	Jones <i>et al.</i> 2009	–
<i>Sarcophilus harrisii</i>	–	–	2.88	Jones <i>et al.</i> 2009	–
<i>Antechinus agilis</i>	–	–	10	Jones <i>et al.</i> 2009	–
<i>Antechinus bellus</i>	–	–	10.08	Jones <i>et al.</i> 2009	–
<i>Antechinus flavipes</i>	–	–	8.54	Jones <i>et al.</i> 2009	–
<i>Antechinus godmani</i>	–	–	4.58	Jones <i>et al.</i> 2009	–
<i>Antechinus leo</i>	–	–	8.19	Jones <i>et al.</i> 2009	–
<i>Antechinus minimus</i>	–	–	7.19	Jones <i>et al.</i> 2009	–
<i>Antechinus stuartii</i>	–	–	6.89	Jones <i>et al.</i> 2009	–
<i>Antechinus swainsonii</i>	–	–	8.24	Jones <i>et al.</i> 2009	–
<i>Murexia habbema</i>	2	4	3	Baker 2015	–
<i>Murexia melanurus</i>	–	–	3.49	Jones <i>et al.</i> 2009	–
<i>Murexia naso</i>	–	–	3.68	Jones <i>et al.</i> 2009	–
<i>Murexia rothschildi</i>	–	–	3.39	Jones <i>et al.</i> 2009	–
<i>Phascogale calura</i>	–	–	7.5	Jones <i>et al.</i> 2009	–
<i>Phascogale tapoatafa</i>	–	–	6.79	Jones <i>et al.</i> 2009	–
<i>Planigale gilesi</i>	–	–	5.23	Jones <i>et al.</i> 2009	–
<i>Planigale ingrami</i>	–	–	7.48	Jones <i>et al.</i> 2009	–
<i>Planigale maculata</i>	–	–	7.64	Jones <i>et al.</i> 2009	–
<i>Planigale tenuirostris</i>	–	–	5.99	Jones <i>et al.</i> 2009	–
<i>Antechinomys laniger</i>	–	–	5.99	Jones <i>et al.</i> 2009	–
<i>Ningau ridei</i>	–	–	5.99	Jones <i>et al.</i> 2009	–
<i>Sminthopsis crassicaudata</i>	–	–	6.52	Jones <i>et al.</i> 2009	–
<i>Sminthopsis douglasi</i>	–	–	6.78	Jones <i>et al.</i> 2009	–
<i>Sminthopsis longicaudata</i>	–	–	4.24	Jones <i>et al.</i> 2009	–
<i>Sminthopsis macroura</i>	–	–	7	Jones <i>et al.</i> 2009	–
<i>Sminthopsis murina</i>	–	–	8	Jones <i>et al.</i> 2009	–
<i>Echymipera clara</i>	–	–	2.2	Jones <i>et al.</i> 2009	–
<i>Echymipera kalubu</i>	–	–	2.07	Jones <i>et al.</i> 2009	–
<i>Echymipera rufescens</i>	–	–	2.18	Jones <i>et al.</i> 2009	–
<i>Microperoryctes longicauda</i>	–	–	1.03	Jones <i>et al.</i> 2009	–
<i>Microperoryctes papuensis</i>	–	–	1.01	Jones <i>et al.</i> 2009	–
<i>Isoodon auratus</i>	–	–	2.6	Jones <i>et al.</i> 2009	–
<i>Isoodon macrourus</i>	–	–	3.13	Jones <i>et al.</i> 2009	–
<i>Isoodon obesulus</i>	–	–	2.33	Jones <i>et al.</i> 2009	–
<i>Perameles bougainville</i>	–	–	2	Jones <i>et al.</i> 2009	–
<i>Perameles gunnii</i>	–	–	2.31	Jones <i>et al.</i> 2009	–
<i>Perameles nasuta</i>	–	–	2.43	Jones <i>et al.</i> 2009	–
<i>Peroryctes broadbenti</i>	–	–	1.76	Jones <i>et al.</i> 2009	–
<i>Peroryctes raffrayana</i>	–	–	1.27	Jones <i>et al.</i> 2009	–
<i>Macrotis lagotis</i>	–	–	1.5	Jones <i>et al.</i> 2009	–
<i>Hypsiprymnodon</i>	–	–	2.04	Jones <i>et al.</i> 2009	–

<i>moschatus</i>					
<i>Lagostrophus fasciatus</i>	–	–	0.9	Jones <i>et al.</i> 2009	–
<i>Dendrolagus bennettianus</i>	–	–	1	Jones <i>et al.</i> 2009	–
<i>Dendrolagus dorianus</i>	–	–	1.01	Jones <i>et al.</i> 2009	–
<i>Dendrolagus goodfellowi</i>	–	–	1	Jones <i>et al.</i> 2009	–
<i>Dendrolagus inustus</i>	–	–	1.11	Jones <i>et al.</i> 2009	–
<i>Dendrolagus lumholtzi</i>	–	–	1	Jones <i>et al.</i> 2009	–
<i>Dendrolagus matschiei</i>	–	–	1.01	Jones <i>et al.</i> 2009	–
<i>Dendrolagus spadix</i>	–	–	1	Jones <i>et al.</i> 2009	–
<i>Dorcopsis hageni</i>	–	–	1.01	Jones <i>et al.</i> 2009	–
<i>Dorcopsis muelleri</i>	–	–	1	Jones <i>et al.</i> 2009	–
<i>Dorcopsulus vanheurni</i>	–	–	1.18	Jones <i>et al.</i> 2009	–
<i>Lagorchestes conspicillatus</i>	–	–	1	Jones <i>et al.</i> 2009	–
<i>Lagorchestes hirsutus</i>	–	–	1	Jones <i>et al.</i> 2009	–
<i>Macropus fuliginosus</i>	–	–	1	Jones <i>et al.</i> 2009	–
<i>Macropus giganteus</i>	–	–	1	Jones <i>et al.</i> 2009	–
<i>Notamacropus agilis</i>	–	–	1.01	Jones <i>et al.</i> 2009	–
<i>Notamacropus dorsalis</i>	–	–	1	Jones <i>et al.</i> 2009	–
<i>Notamacropus eugenii</i>	–	–	1.01	Jones <i>et al.</i> 2009	–
<i>Notamacropus irma</i>	–	–	1	Jones <i>et al.</i> 2009	–
<i>Notamacropus parma</i>	–	–	0.9	Jones <i>et al.</i> 2009	–
<i>Notamacropus parryi</i>	–	–	1	Jones <i>et al.</i> 2009	–
<i>Notamacropus rufogriseus</i>	–	–	1.01	Jones <i>et al.</i> 2009	–
<i>Onychogalea fraenata</i>	–	–	1	Jones <i>et al.</i> 2009	–
<i>Onychogalea unguifera</i>	–	–	1	Jones <i>et al.</i> 2009	–
<i>Osphranter antilopinus</i>	–	–	1	Jones <i>et al.</i> 2009	–
<i>Osphranter robustus</i>	–	–	1	Jones <i>et al.</i> 2009	–
<i>Osphranter rufus</i>	–	–	1	Jones <i>et al.</i> 2009	–
<i>Petrogale assimilis</i>	–	–	1	Jones <i>et al.</i> 2009	–
<i>Petrogale brachyotis</i>	–	–	1	Jones <i>et al.</i> 2009	–
<i>Petrogale burbidgei</i>	–	–	1	Jones <i>et al.</i> 2009	–
<i>Petrogale concinna</i>	–	–	1	Jones <i>et al.</i> 2009	–
<i>Petrogale herberti</i>	–	–	1	Eldridge and Coulson 2015	–
<i>Petrogale inornata</i>	–	–	1	Jones <i>et al.</i> 2009	–
<i>Petrogale lateralis</i>	–	–	1	Jones <i>et al.</i> 2009	–
<i>Petrogale penicillata</i>	–	–	1	Jones <i>et al.</i> 2009	–
<i>Petrogale persephone</i>	–	–	1	Jones <i>et al.</i> 2009	–
<i>Petrogale xanthopus</i>	–	–	1.02	Jones <i>et al.</i> 2009	–
<i>Setonix brachyurus</i>	–	–	1	Jones <i>et al.</i> 2009	–
<i>Thylogale billardierii</i>	–	–	1	Jones <i>et al.</i> 2009	–
<i>Thylogale browni</i>	–	–	1.02	Jones <i>et al.</i> 2009	–
<i>Thylogale brunii</i>	–	–	1	Jones <i>et al.</i> 2009	–
<i>Thylogale stigmatica</i>	–	–	1	Jones <i>et al.</i> 2009	–
<i>Thylogale thetis</i>	–	–	1	Jones <i>et al.</i> 2009	–

<i>Wallabia bicolor</i>	–	–	1.04	Jones <i>et al.</i> 2009	–
<i>Aepyprymnus rufescens</i>	–	–	1.03	Jones <i>et al.</i> 2009	–
<i>Bettongia gaimardi</i>	–	–	1	Jones <i>et al.</i> 2009	–
<i>Bettongia lesueur</i>	–	–	1	Jones <i>et al.</i> 2009	–
<i>Bettongia penicillata</i>	–	–	1	Jones <i>et al.</i> 2009	–
<i>Bettongia tropica</i>	–	–	1	Jones <i>et al.</i> 2009	–
<i>Potorous gilbertii</i>	–	–	1	Jones <i>et al.</i> 2009	–
<i>Potorous longipes</i>	–	–	1	Jones <i>et al.</i> 2009	–
<i>Potorous tridactylus</i>	–	–	1	Jones <i>et al.</i> 2009	–
<i>Acrobates pygmaeus</i>	–	–	2.8	Jones <i>et al.</i> 2009	–
<i>Distoechurus pennatus</i>	–	–	1.31	Jones <i>et al.</i> 2009	–
<i>Dactylopsila palpator</i>	–	–	1.02	Jones <i>et al.</i> 2009	–
<i>Dactylopsila trivirgata</i>	–	–	1.54	Jones <i>et al.</i> 2009	–
<i>Gymnobelideus leadbeateri</i>	–	–	1.54	Jones <i>et al.</i> 2009	–
<i>Petaurus breviceps</i>	–	–	1.82	Jones <i>et al.</i> 2009	–
<i>Petaurus gracilis</i>	–	–	1.75	Jones <i>et al.</i> 2009	–
<i>Petaurus norfolcensis</i>	–	–	1.64	Jones <i>et al.</i> 2009	–
<i>Hemibelideus lemuroides</i>	–	–	1	Jones <i>et al.</i> 2009	–
<i>Petauroides volans</i>	–	–	1	Jones <i>et al.</i> 2009	–
<i>Pseudocheirus peregrinus</i>	–	–	1.89	Jones <i>et al.</i> 2009	–
<i>Pseudochirulus canescens</i>	–	–	2.57	Jones <i>et al.</i> 2009	–
<i>Pseudochirulus forbesi</i>	–	–	1.26	Jones <i>et al.</i> 2009	–
<i>Pseudochirulus herbertensis</i>	–	–	1.89	Jones <i>et al.</i> 2009	–
<i>Pseudochirulus mayeri</i>	–	–	1.26	Jones <i>et al.</i> 2009	–
<i>Petropseudes dahlia</i>	–	–	1	Jones <i>et al.</i> 2009	–
<i>Pseudochirops albertisii</i>	–	–	1.02	Jones <i>et al.</i> 2009	–
<i>Pseudochirops archeri</i>	–	–	1.03	Jones <i>et al.</i> 2009	–
<i>Pseudochirops corinnae</i>	–	–	1.02	Jones <i>et al.</i> 2009	–
<i>Pseudochirops cupreus</i>	–	–	1.01	Jones <i>et al.</i> 2009	–
<i>Tarsipes rostratus</i>	–	–	2.93	Jones <i>et al.</i> 2009	–
<i>Burramys parvus</i>	–	–	3.6	Jones <i>et al.</i> 2009	–
<i>Cercartetus caudatus</i>	–	–	2.37	Jones <i>et al.</i> 2009	–
<i>Cercartetus concinnus</i>	–	–	4.6	Jones <i>et al.</i> 2009	–
<i>Cercartetus lepidus</i>	–	–	3.33	Jones <i>et al.</i> 2009	–
<i>Cercartetus nanus</i>	–	–	3.96	Jones <i>et al.</i> 2009	–
<i>Phalanger carmelitae</i>	–	–	1.01	Jones <i>et al.</i> 2009	–
<i>Phalanger gymnotis</i>	–	–	1.01	Jones <i>et al.</i> 2009	–
<i>Phalanger lullulae</i>	–	–	1.75	Jones <i>et al.</i> 2009	–
<i>Phalanger orientalis</i>	–	–	1.71	Jones <i>et al.</i> 2009	–
<i>Phalanger sericeus</i>	–	–	1.02	Jones <i>et al.</i> 2009	–
<i>Phalanger vestitus</i>	–	–	1.01	Jones <i>et al.</i> 2009	–
<i>Spilocuscus maculatus</i>	–	–	1.26	Jones <i>et al.</i> 2009	–
<i>Spilocuscus rufoniger</i>	–	–	1.02	Jones <i>et al.</i> 2009	–
<i>Trichosurus caninus</i>	–	–	1.02	Jones <i>et al.</i> 2009	–

<i>Trichosurus vulpecula</i>	–	–	1.02	Jones <i>et al.</i> 2009	–
<i>Wyulda squamicaudata</i>	–	–	1.01	Jones <i>et al.</i> 2009	–
<i>Phascolarctos cinereus</i>	–	–	1.01	Jones <i>et al.</i> 2009	–
<i>Lasiorhinus krefftii</i>	–	–	1	Jones <i>et al.</i> 2009	–
<i>Lasiorhinus latifrons</i>	–	–	1	Jones <i>et al.</i> 2009	–
<i>Vombatus ursinus</i>	–	–	1	Jones <i>et al.</i> 2009	–

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APPENDIX 4 – PHYLOGENETIC TREE

This is the subtree pruned from Upham *et al.* (2019), with 279 marsupial taxa and monotremes as outgroup. Some names of species used in this study were updated in the tree to match the Mammal Diversity Database taxonomy.

((Ornithorhynchus_anatinus:55.74627101,(Zaglossus_bruijnii:19.54880599,Tachyglossus_aculeatus:19.54880599):36.19747601):123.461509,(((Rhyncholestes_raphanurus:10.34387388,Lestoros_inca:10.34387388):3.542926268,(Caenolestes_convelatus:9.417250236,(Caenolestes_fuliginosus:8.726425432,(Caenolestes_sangay:3.021848845,Caenolestes_caniventer:3.021848845):5.704575903):0.6908268577):4.469549915):65.41086758,(((Glironia_venusta:31.94078391,(Hyladelphys_kalinowski:30.07328829,(((Monodelphis_emiliae:14.60331574,((Monodelphis_kunsi:11.12686797,(Monodelphis_reigi:5.734386521,((Monodelphis_osgoodi:2.425373004,Monodelphis_handleyi:2.425373004):0.7749717073,(Monodelphis_peruviana:2.532158799,Monodelphis_adusta:2.532158799):0.6681859122):2.534042836):5.392478029):1.886044789,(((Monodelphis_sanctaerosae:2.226665319,Monodelphis_glirina:2.226665319):1.025985187,Monodelphis_domestica:3.252650507):0.991179443,((Monodelphis_palliolata:1.957908157,Monodelphis_brevicaudata:1.957908157):0.449853246,Monodelphis_arlindoi:2.407761403):1.836068205):8.126664674,(((Monodelphis_theresa:0.2816248994,Monodelphis_scalops:0.2816248994):9.738018921,Monodelphis_dimidiata:10.01964577):1.477164376,(Monodelphis_gardneri:8.53969161,((Monodelphis_umbristriatus:2.810622206,Monodelphis_americana:2.810622206):4.976812895,Monodelphis_iheringi:7.787432022):0.7522585621):2.957116826):0.8736837933):0.6424181367):1.590403322):11.87997959,(Tlacuatzin_canescens:24.65417848,((Marmosa_rubra:13.24870168,((Marmosa_mexicana:3.862860036,Marmosa_robinsoni:3.862860036):2.329752846,Marmosa_xerophila:6.192611856):7.056089824):4.607766202,((Marmosa_lepida:9.844669213,Marmosa_andersoni:9.844669213):5.883771525,((Marmosa_tyleriana:4.415981821,Marmosa_murina:4.415981821):9.534049552,((Marmosa_regina:3.708752965,Marmosa_constantiae:3.708752965):2.896431071,(Marmosa_alstoni:4.990756682,(Marmosa_paraguayana:3.600443105,Marmosa_demerarae:3.600443105):1.390313577):1.614426327):7.34484768):1.778409022):2.128025775):6.797714024):1.829115474):2.020022166,(((Marmosops_parvidens:8.004677928,(Marmosops_pinheiroi:4.95806834,Marmosops_pakaraimae:4.95806834):3.046609588):9.460501638,((Marmosops_invictus:10.5881964,(Marmosops_paulensis:0.858251871,Marmosops_incanus:0.858251871):9.729943501):4.119548894,(Marmosops_noctivagus:4.32439424,Marmosops_impavidus:4.32439424):10.38335105):2.757436327):7.179311836,(((Cryptonanus_guahybae:4.462973238,(Cryptonanus_undaviensis:3.098088337,(Cryptonanus_chacoensis:0.5809453709,Cryptonanus_agricolai:0.5809453709):2.517142624):1.364883874):14.20089635,((Gracilinanus_aceramarcae:9.439443954,(Gracilinanus_peruanus:8.712262651,(Gracilinanus_agilis:6.983291281,Gracilinanus_microtarsus:6.983291281):1.728972054):0.7271813034):2.858731178,Gracilinanus_emiliae:12.29817684):6.365692743):1.620121209,(((Thylamys_tatei:5.371006309,(Thylamys_pallidior:2.816526159,Thylamys_elegans:2.816526159):2.554479808):2.57387416,((Thylamys_macrurus:6.700665269,(Thylamys_venustus:4.84317496,(Thylamys_sponsorius:0.4286323426,Thylamys_cinderella:0.4286323426):4.414541249):1.857493389):0.3848153191,((Thylamys_pusillus:0.9439334457,Thylamys_pulchellus:0.9439334457):0.7476052724,Thylamys_citellus:1.691538376):5.393942555):0.8593974857):2.790724508,(Thylamys_velutinus:7.359044679,Thylamys_karimii:7.359044679):3.376560983):3.707480059,Lestodelphys_halli:14.44308367):5.840906785):4.360500949):2.075859931,(Metachirus_myosuros:17.63988238,(((Lutreolina_massoia:1.17312721,Lutreolina_crassicaudata:1.17312721):7.330024494,((Philander_frenatus:5.615869467,((Philander_opossum:2.761192081,Philander_mcilhennyi:2.761192081):0.2137945197,Philander_andersoni:2.97498643):2.640881326):0.6823630647,(Didelphis_virginiana:4.338752063,((Didelphis_marsupialis:1.194923658,Didelphis_aurita:1.194923658):1.784561397,(Didelphis_albiventris:1.893617891,(Didelphis_imperfecta:1.440557403,Didelphis_ernesti:1.440557403):0.453060488):1.085867164):1.359266324):1.959479784):2.204921909):3.15896229,Chironectes_minimus:11.66211434):5.977768045):9.080467584):1.782964791):1.56997114):1.867494934):2.710771691,(Caluromysiops_irrupta:14.02248432,(Caluromysiops_lanatus:8.523561301,(Caluromysiops_philander:6.893541201,Caluromysiops_derbianus:6.893541201):1.630021127):5.498926436):20.62906752):39.66933722,(Dromiciops_gliroides:63.31118995,(((Notoryctes_typhlops:6.036533834,Notoryctes_caurinus:6.036533834):53.66581937,((Macrotis_lagotis:28.82844861,((Isodon_obesulus:2.747451891,(Isodon_macrourus:2.096708202,Isodon_auratus:2.096708202):0.6507436894):4.835277473,(Perameles_bougainville:7.170288581,(Perameles_nasuta:2.687648958,Perameles_gunnii:2.687648958):4.482641676):0.4124414677):7.276090269,((Peroryctes_raffrayana:5.

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APPENDIX 5 – DATA MATRIX

This is the initial matrix used for all analyses in the study (N = 240).

species,pouch,type,mass,litter
 Caluromys_derbianus,present,5,308,3.14
 Caluromys_lanatus,present,5,410,3.7
 Caluromys_philander,absent,1,187,4.18
 Caluromysiops_irrupta,present,?,400,2
 Chironectes_minimus,present,6,650,3.24
 Didelphis_albiventris,present,5,1500,6.9
 Didelphis_aurita,present,5,1100,6.11
 Didelphis_imperfecta,present,5,900,4.9
 Didelphis_marsupialis,present,5,1485,7.09
 Didelphis_pernigra,present,5,1360,4.2
 Didelphis_virginiana,present,5,1800,8.62
 Lutreolina_crassicaudata,present,6,555,7.71
 Lutreolina_massoia,present,6,284,NA
 Philander_andersoni,present,5,412.5,3
 Philander_mcilhennyi,present,5,518,5
 Philander_opossum,present,5,450,4.75
 Marmosa_alstoni,absent,0,105,11
 Marmosa_andersoni,absent,0,33,5
 Marmosa_constantiae,absent,0,79,8
 Marmosa_demerarae,absent,0,96,6.29
 Marmosa_lepida,absent,0,14,4.99
 Marmosa_mexicana,absent,0,35,11.6
 Marmosa_murina,absent,0,59.5,8.4
 Marmosa_paraguayana,absent,0,143,5.56
 Marmosa_regina,absent,0,120,7
 Marmosa_robinsoni,absent,0,60,9.5
 Marmosa_rubra,absent,0,61,NA
 Marmosa_tyleriana,absent,0,43,3
 Marmosa_xerophila,absent,0,43,7.9
 Monodelphis_adusta,absent,0,25,4
 Monodelphis_americana,absent,0,34.5,NA
 Monodelphis_arlindoi,absent,0,64.5,6
 Monodelphis_brevicaudata,absent,0,70,7.24
 Monodelphis_dimidiata,absent,0,62,12
 Monodelphis_domestica,absent,0,84,7.29
 Monodelphis_emiliae,absent,0,40,NA
 Monodelphis_gardneri,absent,0,10.3,NA
 Monodelphis_glirina,absent,0,62.5,NA
 Monodelphis_kunsi,absent,0,18.75,NA
 Monodelphis_palliolata,absent,0,47.5,7.5

Monodelphis_sanctaerosae,absent,0,23,NA
 Monodelphis_scalops,absent,0,56.5,NA
 Tlacuatzin_canescens,absent,0,50,8.49
 Metachirus_myosuros,absent,0,300,3.87
 Cryptonanus_agricolai,absent,0,18,NA
 Cryptonanus_chacoensis,absent,0,14.5,12
 Cryptonanus_guahybae,absent,0,19.5,NA
 Cryptonanus_unduaviensis,absent,0,27.5,NA
 Gracilinanus_aceramarcae,absent,0,20.75,NA
 Gracilinanus_agilis,absent,0,27.5,12
 Gracilinanus_emiliae,absent,0,12,NA
 Gracilinanus_microtarsus,absent,0,35,9.7
 Lestodelphys_halli,absent,0,80,NA
 Marmosops_impavidus,absent,0,38,NA
 Marmosops_incanus,absent,0,46,NA
 Marmosops_invictus,absent,0,29.2,NA
 Marmosops_noctivagus,absent,0,60,NA
 Marmosops_parvidens,absent,0,26.5,4.05
 Marmosops_paulensis,absent,0,31.5,NA
 Marmosops_pinheiroi,absent,0,26,NA
 Thylamys_citellus,absent,0,60.5,NA
 Thylamys_elegans,absent,0,25,10.6
 Thylamys_karimii,absent,0,29.5,NA
 Thylamys_macrurus,absent,0,43.5,NA
 Thylamys_pallidior,absent,0,25.75,3.5
 Thylamys_pulchellus,absent,0,17.9,NA
 Thylamys_pusillus,absent,0,21.5,NA
 Thylamys_sponsorius,absent,0,38.75,NA
 Thylamys_tatei,absent,0,35,NA
 Thylamys_velutinus,absent,0,24.45,NA
 Thylamys_venustus,absent,0,19.25,NA
 Glironia_venusta,absent,0,130,3
 Hyladelphys_kalinowskii,absent,0,15,4
 Caenolestes_caniventer,absent,0,38,2
 Caenolestes_convelatus,absent,0,42.5,NA
 Caenolestes_fuliginosus,absent,0,35,3.62
 Caenolestes_sangay,absent,0,41.5,NA
 Lestoros_inca,absent,0,27.5,NA
 Rhyncholestes_raphanurus,absent,0,21,NA
 Dromiciops_gliroides,absent,1?????,24,3
 Notoryctes_caurinus,present,6,55,NA
 Notoryctes_typhlops,present,6,55,1
 Myrmecobius_fasciatus,absent,0,459,3.68
 Dasycercus_cristicauda,absent,1,80,6.5
 Dasikaluta_rosamondae,absent,1,30,6.64
 Dasyuroides_byrnei,absent,1,100,5.14

Dasyurus_albopunctatus,absent,1,520,6.19
Dasyurus_hallucatus,absent,1,400,6.39
Dasyurus_maculatus,present,2,4000,4.47
Dasyurus_spartacus,absent,1,684,6.78
Dasyurus_viverrinus,absent,1,880,4.7
Myoictis_melas,absent,1,72,NA
Myoictis_wallacii,absent,1,192,NA
Neophascogale_lorentzii,absent,1,212,NA
Parantechinus_apicalis,absent,1,58,7.5
Phascosorex_dorsalis,present,3,42,3.39
Pseudantechinus_bilarni,absent,1,25,4.75
Pseudantechinus_macdonnellensis,absent,1,30,5.88
Pseudantechinus_woolleyae,absent,1,37.5,5.99
Sarcophilus_harrisii,present,2,7000,2.88
Antechinus_agilis,absent,1,18,10
Antechinus_bellus,absent,1,34,10.08
Antechinus_flavipes,absent,1,34,8.54
Antechinus_godmani,absent,1,54.5,4.58
Antechinus_leo,absent,1,54,8.19
Antechinus_minimus,absent,1,42,7.19
Antechinus_stuartii,absent,1,20,6.89
Antechinus_swainsonii,absent,1,41,8.24
Murexia_habbema,absent,1,26.95,3
Murexia_longicaudata,absent,1,54,NA
Murexia_melanurus,absent,1,30.5,3.49
Murexia_naso,absent,1,38.75,3.68
Murexia_rothschildi,absent,1,60,3.39
Phascogale_calura,absent,1,43,7.5
Phascogale_tapoatafa,absent,1,156,6.79
Planigale_gilesi,present,2,6.9,5.23
Planigale_ingrami,present,4,4.3,7.48
Planigale_maculata,present,2,10,7.64
Planigale_tenuirostris,present,2,5.3,5.99
Antechinomys_laniger,present,2,20,5.99
Ningau_ridei,absent,1,8,5.99
Sminthopsis_crassicaudata,present,3,15,6.52
Sminthopsis_douglasi,present,3,50,6.78
Sminthopsis_longicaudata,present,3,20,4.24
Sminthopsis_macroura,present,3,20,7
Sminthopsis_murina,present,3,14,8
Echymipera_clara,present,6,983,2.2
Echymipera_kalubu,present,6,600,2.07
Echymipera_rufescens,present,6,1400,2.18
Microperoryctes_longicauda,present,6,598,1.03
Microperoryctes_papuensis,present,6,164.5,1.01
Isoodon_auratus,present,6,485,2.6

Isoodon_macrourus,present,6,1100,3.13
 Isoodon_obesulus,present,6,700,2.33
 Perameles_bougainville,present,6,226,2
 Perameles_gunnii,present,6,750,2.31
 Perameles_nasuta,present,6,859,2.43
 Peroryctes_broadbenti,present,6,2920,1.76
 Peroryctes_raffrayana,present,6,800,1.27
 Macrotis_lagotis,present,6,1100,1.5
 Hypsiprymnodon_moschatus,present,5,520,2.04
 Lagostrophus_fasciatus,present,5,1800,1
 Dendrolagus_bennettianus,present,5,9300,1
 Dendrolagus_dorianus,present,5,8800,1.01
 Dendrolagus_goodfellowi,present,5,7400,1
 Dendrolagus_inustus,present,5,11400,1.11
 Dendrolagus_lumholtzi,present,5,6475,1
 Dendrolagus_matschiei,present,5,9200,1.01
 Dendrolagus_spadix,present,5,8050,1
 Dorcopsis_hageni,present,5,5500,1.01
 Dorcopsis_muelleri,present,5,5000,1
 Dorcopsulus_vanheurni,present,5,1893,1.18
 Lagorchestes_conspicillatus,present,5,3000,1
 Lagorchestes_hirsutus,present,5,1310,1
 Macropus_fuliginosus,present,5,16000,1
 Macropus_giganteus,present,5,17800,1
 Notamacropus_agilis,present,5,11000,1.01
 Notamacropus_dorsalis,present,5,6500,1
 Notamacropus_eugenii,present,5,5500,1.01
 Notamacropus_irma,present,5,8000,1
 Notamacropus_parma,present,5,3550,1
 Notamacropus_parryi,present,5,11000,1
 Notamacropus_rufogriseus,present,5,13800,1.01
 Onychogalea_fraenata,present,5,3400,1
 Onychogalea_unguifera,present,5,5800,1
 Osphranter_antilopinus,present,5,17500,1
 Osphranter_robustus,present,5,15600,1
 Osphranter_rufus,present,5,26500,1
 Petrogale_assimilis,present,5,4300,1
 Petrogale_brachyotis,present,5,3700,1
 Petrogale_burbidgei,present,5,1150,1
 Petrogale_concinna,present,5,1350,1
 Petrogale_herberti,present,5,4300,1
 Petrogale_inornata,present,5,4200,1
 Petrogale_lateralis,present,5,4200,1
 Petrogale_mareeba,present,5,3800,NA
 Petrogale_penicillata,present,5,6300,1
 Petrogale_persephone,present,5,5200,1

Petrogale_purpureicollis,present,5,4200,NA
 Petrogale_rothschildi,present,5,4350,NA
 Petrogale_xanthopus,present,5,7000,1.02
 Setonix_brachyurus,present,5,2900,1
 Thylogale_billardierii,present,5,3900,1
 Thylogale_browni,present,5,4338,1.02
 Thylogale_brunii,present,5,4338,1
 Thylogale_stigmatica,present,5,4100,1
 Thylogale_thetis,present,5,3800,1
 Wallabia_bicolor,present,5,13000,1.04
 Aepyprymnus_rufescens,present,5,2500,1.03
 Bettongia_gaimardi,present,5,1660,1
 Bettongia_lesueur,present,5,1300,1
 Bettongia_penicillata,present,5,1300,1
 Bettongia_tropica,present,5,1200,1
 Potorous_gilbertii,present,5,950,1
 Potorous_longipes,present,5,1900,1
 Potorous_tridactylus,present,5,1020,1
 Acrobates_pygmaeus,present,?,12,2.8
 Distoechurus_pennatus,present,?,50,1.31
 Dactylopsila_palpator,present,?,320,1.02
 Dactylopsila_trivirgata,present,5,423,1.54
 Gymnobelideus_leadbeateri,present,5,133,1.54
 Petaurus_breviceps,present,5,115,1.82
 Petaurus_gracilis,present,5,382,1.75
 Petaurus_norfolcensis,present,5,230,1.64
 Hemibelideus_lemuroides,present,5,925,1
 Petauroides_volans,present,5,1700,1
 Pseudocheirus_peregrinus,present,5,900,1.89
 Pseudochirulus_canescens,present,5,365,2.57
 Pseudochirulus_caroli,present,5,440,NA
 Pseudochirulus_forbesi,present,5,600,1.26
 Pseudochirulus_herbertensis,present,5,1050,1.89
 Pseudochirulus_mayeri,present,5,154.5,1.26
 Petropseudes_dahlii,present,5,2000,1
 Pseudochirops_albertisii,present,5,756,1.02
 Pseudochirops_archeri,present,5,1120,1.03
 Pseudochirops_corinnae,present,5,1190,1.02
 Pseudochirops_cupreus,present,5,1700,1.01
 Tarsipes_rostratus,present,5,9,2.93
 Burramys_parvus,present,5,42,3.6
 Cercartetus_caudatus,present,5,30,2.37
 Cercartetus_concinnus,present,5,13,4.6
 Cercartetus_lepidus,present,5,7,3.33
 Cercartetus_nanus,present,5,24,3.96
 Ailurops_ursinus,present,5,6500,NA

Strigocuscus_celebensis,present,5,750,NA
Phalanger_carmelitae,present,5,1000,1.01
Phalanger_gymnotis,present,5,2400,1.01
Phalanger_lullulae,present,5,1770,1.75
Phalanger_orientalis,present,5,2500,1.71
Phalanger_sericeus,present,5,1967,1.02
Phalanger_vestitus,present,5,1933,1.01
Spilocuscus_maculatus,present,5,3060,1.26
Spilocuscus_rufoniger,present,5,6500,1.02
Trichosurus_caninus,present,5,2900,1.02
Trichosurus_vulpecula,present,5,2300,1.02
Wyulda_squamicaudata,present,5,1675,1.01
Phascolarctos_cinereus,present,6,5100,1.01
Lasiorhinus_krefftii,present,6,31900,1
Lasiorhinus_latifrons,present,6,26000,1
Vombatus_ursinus,present,6,26000,1

APPENDIX 6 – SCRIPTS

Pearson's correlation script by Rafaela Missagia

```

data<-read.csv("marsupial.csv", header=TRUE, row.names = 1)
data

mass<-as.vector(data$mass)
litter<-as.vector(data$litter)
#log_mass
log_mass<-log(mass)

#pearson
cor.test(mass, litter, method=c("pearson"))

#spearman
cor.test(mass, litter, method=c("spearman"))

#pearson
cor.test(log_mass, litter, method=c("pearson"))

#spearman
cor.test(log_mass, litter, method=c("spearman"))

```

Ancestral state reconstruction and phylogenetic generalized linear mixed model script by Daniel Casali

```

#####OPEN LIBRARIES#####

library(phytools)
library(geiger)
library(corHMM)
library(phyr)
library(rr2)
library(DHARMA)

#####SET DATA AND TREE READY#####

#read the complete data
raw_data<-read.csv("marsupial_data.csv",row.names=1)

#filter to species with litter size information

```

```

data<-raw_data[is.na(raw_data$litter)==FALSE,]
data

#read the complete tree
tree<-read.tree("marsupial_tree.tre")
tree

#check mismatches
chk<-name.check(tree,data)
chk

#drop tips without data and ultrametricize the tranformed tree
tr<-drop.tip(tree,chk$tree_not_data)
tr<-force.ultrametric(tr)

#####ANCESTRAL STATES RECONSTRUCTIONS (ASR)#####

#prepare pouch data to corHMM format
pouch<-data.frame(row.names(data),data$pouch)
colnames(pouch)<-c("species","pouch")
pouch

#fit alternative models for state exchangeability and evolutionary rates across
branches, for the discrete trait (presence/absence of pouch)

##equal rate model (ER) and a single rate regime across branches
ER1<-corHMM(tr,pouch,model="ER",rate.cat=1)

##equal rate model (ER) and two rate regimes across branches
ER2<-corHMM(tr,pouch,model="ER",rate.cat=2)

##equal rate model (ER) and three rate regimes across branches
ER3<-corHMM(tr,pouch,model="ER",rate.cat=3)

##different rate model (ARD) and a single rate regime across branches
ARD1<-corHMM(tr,pouch,model="ARD",rate.cat=1)

##different rate model (ARD) and two rate regimes across branches
ARD2<-corHMM(tr,pouch,model="ARD",rate.cat=2)

##different rate model (ARD) and three rate regimes across branches
ARD3<-corHMM(tr,pouch,model="ARD",rate.cat=3)

ALL_POUCH<-list(ER1,ER2,ER3,ARD1,ARD2,ARD3)
saveRDS(ALL_POUCH,"pouch_ASR_results.rds")

###evaluate the best-fitting model with AICc
Model<-c("ER1","ER2","ER3","ARD1","ARD2","ARD3")
AICc<-
round(c(ER1$AICc,ER2$AICc,ER3$AICc,ARD1$AICc,ARD2$AICc,ARD3$AICc),2)

```

```

AICw<-round(as.numeric(aic.w(AICc)),2)
summary_pouch<-data.frame(Model,AICc,AICw)
summary_pouch

write.csv(summary_pouch,"Summary_Pouch_ASR.csv")

#plot ASR and heatmap

pdf("Plots_marsupials.pdf")

#discrete characater plot (pouch) - no model fitted better according to delta AICc and
AICc weights, so the simplest was applied (ER1)
  cols<-c("red","blue")
  tip_data<-as.factor(setNames(data$pouch,rownames(data)))
  plotTree(tr,type="fan",fsize=0.4,ftype="i",lwd=1)
  title(main="", adj=0.35, line = -1.0, cex.main=1.5)
  nodelabels(node=1:tr$Nnode+Ntip(tr), pie=ER1$states, piecol=cols, cex=0.2)
  tiplabels(pie=to.matrix(tip_data[tr$tip.label],levels(tip_data)),piecol=cols,cex=0.
1)
  legend(x="topleft",c("absent","present"),cex=1.0, pt.cex=1.5, pch=21, pt.bg =
cols, bty="n")

#continuous characters plot (body mass and litter size) - heatmaps with trait values
standardized in sd units
  cont<-data.frame(log(data[3]),data[4])
  rownames(cont)<-rownames(data)
  phylo.heatmap(tr,cont,standardize=TRUE,split=c(0.8,0.2),fsize=0.2)

dev.off()

#####LOGISTIC REGRESSIONS#####

#prepare data (mass was put in log scale and both independent variable were
rescaled as Z-scores - mean 0 and sd 1)

data_mod<-data
data_mod$pouch[data_mod$pouch=="absent"]<-0
data_mod$pouch[data_mod$pouch=="present"]<-1
data_mod$pouch<-as.numeric(data_mod$pouch)
data_mod$mass<-log(data_mod$mass)
data_mod$zmass<- (data_mod$mass -
mean(data_mod$mass))/(sd(data_mod$mass))
data_mod$zlitter<- (data_mod$litter - mean(data_mod$litter))/(sd(data_mod$litter))

##perform logistic regression analyses with mixed-models (body mass and litter size
are fixed effects for which we are interested to evaluate correlation (each separted,
both additively and both interactively)
##phylogeny as random effects

```

```

null<-pglmm_compare(pouch~1,data=data_mod,family="binomial",phy=tr)
mass<-pglmm_compare(pouch~zmass,data=data_mod,family="binomial",phy=tr)
litt<-pglmm_compare(pouch~zlitter,data=data_mod,family="binomial",phy=tr)
both<-
pglmm_compare(pouch~zmass+zlitter,data=data_mod,family="binomial",phy=tr)
inte<-
pglmm_compare(pouch~zmass*zlitter,data=data_mod,family="binomial",phy=tr)

```

```

#####save and summarize results
results<-list(null,mass,litt,both,inte)
saveRDS(results,"regression_results.rds")

```

```

summary<-as.data.frame(matrix(nrow=length(results),ncol=3))
Model<-c("Null","Body mass","Litter size","Body mass + Litter Size","Body mass *
Litter Size")
colnames(summary)<-c("Model","AIC","R2")
for (i in 1:length(Model))
{
  class(results[[i]])<-"communityPGLMM"
  summary[i,1]<-Model[i]
  summary[i,2]<-round(results[[i]]$AIC,2)
  summary[i,3]<-round(R2(results[[i]]),2)
}
summary$AICw<-round(as.numeric(aic.w(summary$AIC)),2)
summary_regression<-summary
summary_regression
write.csv(summary_regression,"Summary_Regressions.csv")

```

```

#####quality_check

```

```

pdf("Quality_check.pdf")
for (i in 1:length(results))
{
  plot(simulateResiduals(results[[i]]))
  title(paste(Model[i],line = 1))
}
dev.off()

```

Phylogenetic signal (D statistic) script by Daniel Casali

```

#Load packages
library(caper)
library(phytools)
library(corHMM)
def.par<-par()

```

```
##Load data  
data <-read.csv("marsupial_data.csv")
```

```
#Load trees  
tr <-read.tree("marsupial_tree.tre")  
tr<-drop.tip(tr,tr$tip.label[9])
```

```
#####D_statistic#####
```

```
##Set comparative data format  
comp_data<-comparative.data(tr, data, names.col = species, vcv = TRUE, na.omit =  
FALSE, warn.dropped = FALSE)
```

```
##Phylogenetic signal  
D_statistic<-phylo.d(data=comp_data, binvar = pouch, permut = 10000)  
D_statistic  
D_statistic$Parameters
```


APPENDIX 7 – RESIDUALS CHECKS OF PGLMM MODELS

Charts of simulated residuals extracted from five fitted models (PGLMM) using 'simulateResiduals' function in the 'DHARMA' package.

