Body size and litter size as predictors of pouch presence in marsupials

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Data availability

The R script, data, and tree files necessary to reproduce the analyses and plots are available at https://github.com/danielmcasali/Pouch_evolution

Author contributions

All authors designed the research, D.M.C. and M.M.Y. compiled the data., D.M.C and R.V.M. performed the analyses; all authors interpreted the results, wrote the manuscript, and critically reviewed its content.

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Conflict of interest

The authors declare no conflict of interest.

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ABSTRACT

The marsupial pouch is a key adaptation for offspring protection and development, yet its evolutionary drivers remain unclear. While body size matters, the role of litter size is less understood. Using phylogenetic comparative methods, we investigated the evolutionary relationship between pouch presence, body mass, and litter size across 195 marsupial species. Our results reveal that pouch presence is strongly phylogenetically conserved and positively correlated with larger body size, with all large-bodied species possessing a pouch. By contrast, pouch presence is negatively associated with litter size, with species with larger litters typically lacking a pouch, while those with smaller litters retain one. We found that body mass evolves faster in pouched lineages. Ancestral state reconstructions suggest multiple independent origins of the pouch, although the ancestral marsupial condition remains uncertain, but most likely corresponding to pouch absence. These findings support the hypothesis that the pouch evolves in response to trade-offs between offspring quantity and maternal investment, aligning with broader patterns of parental care strategies. Our work provides a new vision for the evolutionary trajectory of one of the most conspicuous marsupial features.

KEYWORDS: Mammals, evolution, marsupium, offspring

INTRODUCTION

Protective pouches for offspring have evolved across multiple vertebrate lineages, showcasing their broad evolutionary significance as a successful reproductive strategy for securing the development of young (Tyndale-Biscoe & Renfree, 1987a; 1987b). Protective pouches are seen in disparate groups such as marsupial frogs (Hemiphractidae) (del Pino 1980; 2018), but also in fishes, as seahorses and pipefish (Syngnathidae)

(Dudley et al., 2022; Harada et al., 2022), where they serve as critical adaptations that increase offspring survival. These structures provide various benefits, including physical protection, stability during parental movement, concealment from predators, and environmental regulation for the developing young. In mammals, it is commonly thought that protective pouches have evolved independently in monotremes, like echidnas (Tachyglossidae) (Augee et al., 2006), and many times in marsupials (Kirsch, 1977; Tyndale-Biscoe, 2005; Voss and Jansa, 2009).

In marsupial mammals, pouches (or marsupium) are folds of skin in the abdomen with varying degrees of enclosure across species, working as a cutaneous pocket enveloping the mammary teats and newborns. Marsupial pouches show a large diversity of shapes and forms, but overall, they can be classified as open folds, temporary pouches (breeding-season only), and permanent pouches, having functional differences in protection and lactation access (Russell, 1982; Tyndale-Biscoe, 2005). For these reasons, pouches are typically associated with females, where they provide protection and support to the altricial neonates; however, they are also present in males of some species as scrotal pouches, such as in water opossums (*Chironectes minimus*), marsupial moles (Notoryctidae), and the recently extinct thylacines (*Thylacinus cynocephalus*) (Enders, 1935; Pocock, 1926; Sweet, 1907). In marsupials, the pouch serves multiple reproductive functions, with its importance varying across different species, as reflected by the diversity of pouch structures. One primary role is direct physical protection from the external environment (Russell, 1982). Additionally, the pouch ensures warmth and stable humidity, which facilitates effective integumental gas exchange (Kubota et al., 1989) and supports the survival of ectothermic newborns (Edwards et al., 2012; Edwards & Deakin, 2013). The pouch is also involved in chemical defense, as pouch fluids contain antimicrobial proteins that inhibit bacterial growth (Ambatipudi et al., 2008; Bobek &

Deane, 2001; Edwards et al., 2012). By maintaining a constant microenvironment, the pouch enables neonates to develop safely until weaning, at which point they gain fur, endothermy, and mobility (Russell, 1982; Smith & Keyte, 2020; Sobral & Guilhon, 2016). Because the pouch is directly associated with the mother, it allows for stable maternal contact, ensuring that developing young remain at a temperature comparable to that of the adult, thus addressing the challenges of ectothermy in early development (Hulbert, 1988). However, not all marsupials possess pouches. Many didelphids, all living caenolestids, and some dasyurids are pouchless (Russell, 1982; Tyndale-Biscoe & Renfree, 1987a; Woolley, 1974).

Evolutionary hypotheses regarding pouch presence in marsupials have varied over time. For a long time, pouch presence was linked to epipubic bones, suggesting that these bones would serve as a functional pouch support (Coues, 1872; Elftman, 1929; Tyson, 1698). However, this hypothesis has fallen out of favor, as the pouch appears to be a relatively recent adaptation compared to the epipubic bones, which are thought to be a plesiomorphic characteristic of mammals, besides the pouch also lacking direct muscular attachment with the epipubic bones (Guilhon et al., 2021; Szalay, 1982; Tyndale-Biscoe & Renfree, 1987b; White, 1989). Another hypothesis posits that body size plays a key role, with larger marsupials generally having pouches, while smaller species often do not (Tyndale-Biscoe, 2005). This suggests that body size may drive the evolution of the pouch in marsupials, or at least in some clades like Didelphimorphia (Astúa, 2015; Harder, 1992; Voss & Jansa, 2021). Body size is frequently considered a crucial factor influencing the evolutionary history of animals, as increases in size often demand structural innovations while simultaneously allowing further growth, being both a driver and outcome of the evolutionary process (Bonner, 2011). For larger marsupial species, whose young require extended periods of pouch life, attaining larger sizes and needing to maintain teat

attachment for longer periods (Russell, 1982), a pouch is thought to provide essential protection and stability.

However, pouch presence in marsupials cannot be solely explained by body size. Some smaller marsupials also possess pouches, suggesting that other factors may play a role in their presence. Given that evolutionary novelties rarely arise due to a single selective pressure, it seems likely that other factors, such as litter size, could also influence pouch evolution in marsupials. In species with smaller litters, the presence of a pouch may increase the survival rate of the young by providing additional protection. This aligns with different patterns of parental care associated with pouch types, as described by Russell (1982). Species with large litters and small pouches (Pattern A) leave their underdeveloped young in nests early, whereas those with fewer young and well-developed pouches (Pattern B) retain them in the pouch for longer before transferring them to a nest. In species with large pouches and typically a single offspring (Pattern C), the young remain in the pouch until they are mobile enough to follow the mother. These patterns suggest that both litter size and developmental strategy contribute to pouch evolution. Moreover, a strong phylogenetic signal was recovered for life history traits such as pouch presence, body size, and litter size in Didelphidae (Battistella et al., 2019), suggesting that evolutionary constraints may also shape the evolution of these traits in marsupials. Thus, this study aims to determine whether body size and litter size predict pouch presence in marsupials, while accounting for phylogenetic relationships.

MATERIALS AND METHODS

Data

We gathered data on pouch presence, body mass, and litter size for 195 taxa, sampling all 19 extant families of marsupials (Supplementary Material Tables S1-S3). We followed the taxonomy of the Mammal Diversity Database (2022), except for *Dromiciops gliroides*, which we consider as the single species of the genus following Palma and Valladares-Gómez (2015).

Data for the presence of a pouch was obtained from the literature, complemented by personal communications when information about a taxon was dubious or sparse (Supplementary Material Table S1). We based the presence or absence of the pouch on Woolley's (1974) and Russell's (1982) descriptions. 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 (Tyndale-Biscoe & Renfree, 1987a; Woolley, 1974). Types 2–6 were considered "present". We compiled body mass data (in grams), a proxy for body size, from Wilson and Mittermeier (2015) and Weisbecker et al. (2013) (Supplementary Material Table S2). When available, we considered the entire range of body mass variation per species, and considered the mid-point of the range as the mean body mass value. For four of the 195 species (~2%), only the mean body mass value was available. Litter size data (mean and range) were obtained from the literature (Supplementary Material Table S3) and the PanTheria database (Jones et al., 2009). For 91 of 195 species (~47%), only the mean litter size was available, either because the litter size is invariably

one for the species (58 species, ~30%), or because ranges were not available (33 species, ~17%).

In order to account for trait uncertainty in analyses, we randomly sampled body mass and litter size values from uniform distributions of the range of variation of each species, using the mean trait value when the range was not available. In addition to these 100 simulated data sets, which were applied to calculate phylogenetic signal and perform phylogenetic regressions, we also gathered a dataset of mean trait values, which was used in model fitting analyses. Body mass data was converted to a $\log_{10}$ scale, to account for the fact that it spans five orders of magnitude. For the regressions, $\log_{10}$ body mass and litter size values were standardized as Z-scores (i.e., mean = 0 and standard deviation = 1) (Symonds & Blomberg, 2014).

Trees

We obtained 100 time-calibrated trees from the tip-dated, DNA-only, Bayesian posterior sample from Upham et al. (2019, https://vertlife.org/data/mammals) and its respective major clade credibility tree (MCC). The trees were pruned to the taxonomic sample for which data were available.

Phylogenetic signal

We inspected the phylogenetic signal for individual traits ¬¬– pouch, body mass, and litter size – and for all their combinations, with the M statistic, a metric that provides a unified framework to assess phylogenetic signal of discrete, continuous, and combined sets of traits, based on distance matrices obtained with Gower's distance (Yao & Yuan, 2025a). M statistic ranges from 0 to 1, representing the lowest and highest degree of phylogenetic signal, respectively. These tests were conducted in the R programming environment (R Core Team, 2025), with the function *phylosignal_M* of the package

180 *phylosignalDB* (Yao & Yuan, 2025b), utilizing 999 random permutations to assess
181 significance ($\alpha = 0.05$). The M statistic was calculated over 100 paired combinations of
182 simulated datasets and posterior sample trees, and the results were summarized by the
183 mean and the 95% confidence interval (CI).

184

185 ***Phylogenetic regressions***

186 To assess the degree of multicollinearity between body mass and litter size data, we
187 calculated the variance inflation factor (VIF) after conducting preliminary logistic
188 regressions. For these procedures, we used the functions *glm* and *vif* from the packages
189 *stats* and *car* (Fox & Weisberg, 2019), respectively. The VIF values for these variables
190 and their interaction were only moderate (< 3), allowing them to be included together in
191 subsequent regressions. We then carried out logistic regressions using phylogenetic
192 generalized linear mixed models (PGLMM; Ives & Helmus, 2011), treating pouch
193 presence as the response variable, body mass and litter size as fixed effects, and
194 phylogeny as a random effect. For the regressions, we utilized the function
195 *pglmm_compare* from the R package *phyr* (Ives et al., 2020). We explored five alternative
196 models: i) a null model, regressing the pouch presence over its intercept, to represent the
197 absence of influence of the continuous variables, ii) adding body mass as an independent
198 variable, iii) adding litter size as an independent variable, iv) adding both body mass and
199 litter size as independent variables, but not allowing for an interaction between them, and
200 finally, v) adding both variables and allowing for their interaction. Model fitting was
201 assessed with the Bayesian information criterion (BIC) (Schwarz, 1978), comparing the
202 relative weights of models (BICw) and ΔBIC (≤ 2). As for the analysis of phylogenetic
203 signal, all regressions were conducted across 100 paired combinations of simulated
204 datasets and posterior sample trees, with model parameters, BIC, and BICw summarized

by their means and 95% CIs. In addition to that, for the regression coefficients, we also calculated a second confidence interval based on Rubin's rules (Rubin, 1987). This confidence interval, originally developed in the context of multiple imputation, accounts for both within- and among-dataset estimation uncertainty.

Correlated evolution of the pouch with body mass

Recently, Boyko et al. (2023) proposed a novel method for jointly modelling the evolution of discrete and continuous traits. Different from previous approaches in which the discrete trait is painted to the tree and the parameters of the continuous trait are estimated assuming fixed regimes, this new approach considers that both traits evolve synergistically, affecting one another. For that, the discrete trait is evolved into the tree using stochastic character maps, and discrete and continuous trait parameters are jointly inferred. When both traits evolve in a correlated fashion, fixing any of the traits *a priori* could potentially mislead the inferences (Boyko et al., 2023). This new implementation can be used with alternative continuous and discrete models of trait evolution, including discrete hidden state models, which allows for a more rigorous assessment of alternative hypotheses based on both character-dependent and character-independent models (Boyko et al., 2023). Currently, it is not possible to include both continuous traits as correlated variables in a single analysis, so we focused on the correlation of pouch presence with body mass, which received stronger support in regression analyses. Initial attempts to correlate pouch presence and litter size led to unstable results and indicated parameter unidentifiability, possibly related to the complexity of the models associated with the reduced variability of litter size data, if compared to that of body mass. Moreover, these are very time-consuming analyses. Therefore, they were conducted solely considering the dataset with trait means and the MCC tree. We used 100 stochastic character maps to account for mapping uncertainty, and conducted the analyses with 10 random starts to try

to avoid local optima. For character-independent models, we sampled nodes and used adaptive sampling, as recommended in the function documentation.

We considered 28 models in total, which can be separated into four sets. The first set comprises character-dependent models (CD), in which the discrete trait evolves either according to the equal rates (ER), or to the all rates different (ARD) model (Revell et al., 2025), whereas the continuous trait evolves according to one of the following models: multi-rate Brownian motion model (BMV), multi-rate Ornstein-Uhlenbeck (OUV), multi-optima OU (OUM), or an OU model that allows both rates and optima values to vary (OUMV) (Beaulieu et al., 2012; Felsenstein, 1985; Hansen, 1997). For CD models, different parameters of the continuous models are associated with the alternative discrete trait states, and represent the case of correlation between the pouch presence/absence with the properties (rate of evolution or optimum value) of the continuous trait. Considering all possible combinations of discrete and continuous models, this first set included eight models in total (Supplementary Material Table S4).

A second set of models considered character-independent null hypotheses (CID), in which the continuous trait evolves according to either a uniform BM or a uniform OU model, with changes in their parameter values being independently estimated from the observed states of the discrete character, which evolve uniformly as well. These models represent the total absence of correlation between the evolution of the pouch and that of the continuous variable. A total of four CID models were evaluated (Supplementary Material Table S4).

A third set comprises the CID+ models, in which the properties of the continuous trait vary according to changes between observable and hidden pairs of states of the discrete character. These models allow for variation in rates or optimum values in the continuous trait that are unrelated to the presence or absence of the pouch. It is noteworthy that, for

CID+ (as well as for HYB, see below) models, ER and ARD definitions apply only for back-and-forth transitions between observed states or between hidden states, but these two pairs of rates are assumed to be different, as much as the back-and-forth transitions between observed and hidden pairs of states. These total 8 models (Supplementary Material Table S4).

Lastly, the fourth set comprises hybrid models (HYB), in which both observed and hidden states are associated with variable rates or optimum values estimated for the continuous trait, corresponding to the scenario that additional variation, beyond the simple presence or absence of the pouch, is also correlated with the evolutionary patterns of the continuous variable. A total of eight HYB models were considered (Supplementary Material Table S4).

Model fitting was carried out with the function hOUwie of the package OUwie (Beaulieu & O'Meara, 2025), and model support was assessed with BICw, with all models with BICw $\geq$ 0.1 regarded as best-fitting models. The function *dent_walk* of the package *dentist* (Boyko & O'Meara, 2024) was used to calculate the 95% CIs for the parameters of the best-fitting models, using 2,000 steps, and monitoring the stabilization of the estimated intervals. After that, we estimated the ancestral states for pouch presence under the best-fitting models. To make plots, we relied on functions of the packages phytools (Revell, 2024) and ggplot2 (Wickham, 2016).

RESULTS

Phylogenetic signal

Pouch presence, body mass, litter size, and their combinations all exhibited significant phylogenetic signals across the 100 combinations of datasets and trees (Table 1). Among

individual traits, body mass showed the strongest phylogenetic signal, closely followed by pouch presence, while litter size displayed a slightly weaker signal. The combination of the two continuous predictors, body mass and litter size, yielded the highest overall phylogenetic signal. Combinations of pouch presence with either or both predictors also showed strong signals, comparable to those of the individual traits (Table 1).

Association of the pouch with body size and litter size

The results of the phylogenetic logistic regressions indicated that the two models that include both body mass and litter size as predictors provided a better explanation of the data, with the sum of the mean BICw values for the other three models being 0.01.(Table 2). The model lacking an interaction term was best supported relative to that including this parameter, with mean BICw values of 70 and 29, respectively, but being equivalent per the ΔBIC threshold. Since the model without an interaction explains the data slightly better and is less parametrized, it was favored here. The slope was positive for body mass, indicating that large-sized marsupials are more frequently associated with the presence of the pouch (Figure 1A, B), even after accounting for variation across dataset–tree combinations and estimation uncertainties. (Table 2). Although the pouch is present in species across the entire size range sampled here, its absence is observed only in relatively smaller species, while all relatively larger species possess a pouch (Figure 1A). In contrast, pouch presence is, on average, negatively correlated with litter size (Figure 1A, C), with species at the higher end of the range of litter sizes never presenting a pouch, whereas those with very small litters always have it (Figure 1A). The uncertainty estimated for regression coefficients across the 100 combinations of datasets and trees is small, reinforcing the pattern of a negative correlation. However, when estimation uncertainty is also accounted for, the CI of the slope values for litter also includes small positive values (Table 2).

The correlated evolution of the pouch presence and body mass is further supported by the hOUwie analyses. Two best-fitting models were nearly tied in terms of BICw support– CD_ER_OUMV (BICw = 0.47; Table 3) and CD_ER_BMV (BICw = 0.46; Table 3). All other models were associated with BICw values < 0.1, and were not further considered. Parameter values for the best-fitting models and their uncertainty are summarized in Table 4. The OUMV model led to a very low attraction (α) estimate, implying an average half-live of more than 600 million years, many times longer than the tree height. In terms of parameter interpretation, this makes this model collapse to a BMV model. Moreover, the OUMV model recovered unreasonably high values of θ associated with the presence of a pouch (Table 4). For those reasons, we focus on the parameter estimates of BMV here. This model indicates that, although gains and losses of the pouch occur at equal rates (0.004), rates of body mass evolution are dependent on the presence or absence of the pouch. When the pouch was present, body mass evolved, on average, about three times faster (0.017) than when the pouch was absent (0.005).

Ancestral state estimations

The ancestral pouch condition and subsequent evolution slightly varied depending on which of the two best-fitting models are considered. However, as stated above, there are clear indications that the OUVM model collapses to a BMV in our analyses; therefore, we focus our results on the patterns obtained by the latter model (Figure 2A). The ancestral states obtained under the OUMV model are depicted for completeness (Figure 2B).

The ancestral marsupial condition and the condition in the node uniting Didelphimorphia and Australidelphia are nearly undefined, with pouch absence being slightly more likely (Figure 2A). Pouch absence is maintained in Paucituberculata and Didelphimorphia, with

328 independent acquisitions of the pouch in Didelphini, Caluromyinae (lost again in
329 *Caluromys philander*), and Australidelphia.

330 Among australidelphians, the pouch was lost in Microbiotheria (i.e., *Dromiciops*
331 *gliroides*), but retained as the most likely ancestral condition of Eomarsupialia,
332 Diprotodontia, and Agreodontia. Within the latter clade, the ancestral condition is also
333 retained in Notoryctemorphia (i.e., *Notoryctes typhlops*),
334 Peramelemorphia+Dasyuromorphia, Peramelemorphia, and Dasyuromorpha (Figure
335 2A).

336 The ancestral condition in Dasyuridae and Sminthopsinae (lost in *Ningaui ridei*) is also
337 the retention of the pouch, which would have been lost independently in Myrmecobiidae
338 and in Dasyurinae, to be regained in the clade uniting the genera *Phascolosorex*, *Dasyurus*
339 and *Sarcophilus*, and lost again two times among some of its species (Figure 2A).

340

341 **DISCUSSION**

342 Our findings provide new insights into the evolutionary dynamics of the marsupial pouch,
343 highlighting its complex relationship with life-history traits. Larger marsupials are more
344 likely to have a pouch, while smaller species may lack one. Conversely, pouch presence
345 is negatively correlated with litter size, as species with larger litters usually do not have a
346 pouch, whereas those with smaller litters do. Pouch presence is associated with both body
347 mass and litter size, and body mass evolves more rapidly in species with a pouch. The
348 ancestral marsupial condition is still uncertain, but it was most likely pouchless.

349 Kirsch (1977) already proposed that pouches emerged independently in different
350 marsupial lineages. Our findings support this idea, suggesting that the pouch emerged at
351 least three times in the ancestors of major marsupial clades. Despite the uncertainty

related to the basal-most nodes, our results indicate that the pouch was clearly absent in the ancestor of Didelphimorphia, but was gained independently in Didelphini and Caluromyinae, in addition to the acquisition in the ancestor of Australidelphia, being subsequently retained in most taxa of this clade. Among Dasyuromorphia, notably, Myrmecobiidae and most Dasyurinae lacked the pouch, despite a few secondary acquisitions (e.g., *Dasyurus maculatus* and *Sarcophilus harrisii*), whereas Sminthopsinae have a pouch, with only one exception (*Ningaui ridei*).

Building on previous work, our findings emphasize the important association between pouch presence and parental care strategies, particularly in species with small litters. While Battistella et al. (2019) reported that litter size in didelphid marsupials is shaped by body size, climate, and phylogenetic proximity, with no significant role for pouch presence, we found a broader, contrasting pattern across marsupials. Specifically, our results suggest a negative correlation between litter size and pouch presence: large-litter species (Pattern A) often lack a pouch, while species with small litters (Patterns B and C) consistently possess one. This supports the framework proposed by Russell (1982), which links the presence of a well-developed pouch to species that invest more heavily in individual offspring (K-selection strategy; Pianka, 1970), maintaining them in the pouch through critical stages of development. In these cases, the pouch acts as a functional extension of maternal care, substituting for behaviors such as nest building and retrieving young, which are more prominent in species with reduced or absent pouches. Thus, the evolution of the pouch appears to be an adaptive response to the energetic and developmental demands associated with producing and rearing fewer altricial young. However, as size is also highly correlated with the presence of pouch in marsupials (Russell, 1982), and since both showed relatively stronger phylogenetic signals, size-mediated phylogenetic inertia possibly also shaped the overall pattern of the marsupium

evolution of major clades. Litter size, on the other hand, being more labile, may be a driver of particular life-history adaptations within these major clades. Moreover, an even stronger phylogenetic signal was recovered for the combination of body mass and litter size, and the combinations of these two predictors with pouch presence also yielded significant phylogenetic signal, suggesting a complex interplay of these variables shaping and being shaped by marsupial patterns of phylogenetic inertia.

The pattern observed among New World marsupials, is highly consistent with the relationship between body size and pouch presence: larger species invariably retain pouches, even with larger litters (e.g., species of *Didelphis,* with up to 21 young), while small species lack pouches altogether, even species with relatively small litters (e.g., *Marmosa tyleriana*, with an average of three, and *Monodelphis adusta*, with an average of four young) (Astúa, 2015; Jones et al., 2009). Most didelphids seem to follow r-selected strategies independently of pouch presence or body size (Fisher et al., 2001). This highlights the complexity of the relationships identified by our analyses and the importance of different evolutionary histories in shaping and directing evolutionary patterns of phenotypic characteristics such as the pouch.

Some noteworthy partial exceptions to the general rule can also be observed among australidelphians. In Diprotodontia, even smaller species (e.g., Burramyidae, Acrobatidae, Petauridae) retain well-developed pouches (Wilson & Mittermeier, 2015), a likely result of phylogenetic inertia. Despite their reduced body size, including some of the smallest species among marsupials, these species have proportionally few young compared to other tiny marsupials, typically producing 1-4 offspring per litter. This seems to be the maximum litter size in Diprotodontia, mirroring the "quality over quantity" strategy seen in other K-selected diprotodontian marsupials (e.g., koalas, *Phascolarctos cinereus*, 1-2 offspring). This suggests that phylogenetic constraints could maintain both

pouch retention and low fecundity in these lineages, despite miniaturization in some species, overriding typical r-selected expectations for small-bodied taxa.

The same cannot be said about other australidelphian marsupials, such as dasyurids. Our results indicate that the pouch appears to be an early acquisition in Australidelphia, lost in small-bodied dasyurids with large litters. This pattern is comparable to the one found in some small New World marsupials, sometimes associated with semelparous reproductive patterns (e.g., *Marmosops*) (Leiner et al., 2008). Among australidelphians (e.g., the genus *Antechinus*), the same pattern is observed (Braithwaite & Lee, 1979), in which adults live for very brief periods, sometimes less than a year, yet produce a large number of offspring. This suggests that, in Australidelphia, there is an inertial phylogenetic tendency to maintain the pouch, even in small species, as long as the number of young is relatively small. The evolution of highly committed r-strategists, with a high number of young, is likely a strong pressure to break this inertia and allow the loss of the pouch in these taxa. Nevertheless, we did find exceptions in dasyurids like *Planigale ingrami*, one of the smallest species of marsupials, with a well-developed pouch, but with up to 12 teats and 8 young per litter (Baker, 2015).

However, it should be noted that for every partial exception highlighted above, when the relationship with body mass does not strictly hold, the one related to litter size does. This indicates that although both variables more often than not acted synergistically to predict pouch presence, in some instances a single variable, associated with phylogenetic inertia, is already a good predictor of the observed pouch pattern. Moreover, these exceptions do not overrule the general pattern observed for most species, which was supported by the results of the phylogenetic comparative analyses presented here.

One of the main challenges in studying the evolution of soft tissue structures, such as the marsupial pouch, is their poor preservation in the fossil record (Purnell et al., 2018). As

a result, most phylogenetic and evolutionary studies of marsupials do not include pouch presence as a character or discuss the evolutionary history of this structure in depth. Although a few phylogenetic analyses have incorporated pouch presence (Horovitz & Sánchez-Villagra, 2003; Schneider & Gurovich, 2017; Voss & Jansa, 2009), only Schneider and Gurovich (2017) provide a detailed discussion of pouch evolution. Another caveat of our study is the simplified categorization of pouch presence and absence, which does not fully capture the extensive variation in pouch morphology among marsupials. Pouches vary in depth, teat arrangement and quantity, and orientation (Tyndale-Biscoe, 2005), and Russell (1982) classified them into six distinct types. These range from species with no permanent pouch but seasonal skin folds (Type 1) to those with fully enclosed pouches opening either anteriorly or posteriorly (Types 5 and 6). Also, the pouch is a feature that undergoes morphological and physiological changes during the lifetime; its first appearance may only happen when the female reaches sexual maturity and enters her first breeding season, so the classification of some taxa as pouchless may be inaccurate (Woolley, 1974). By focusing only on pouch presence or absence, our study does not account for this morphological complexity, which may influence both the function and evolutionary dynamics of the pouch and associated traits. Despite that, and at least for body mass, correlation analyses indicated that models accounting for hidden states, which could capture the effects of further detailing pouch variation, are not preferred over models using the simpler binary classification adopted here. This suggests that considering only the presence or absence of the marsupium may be sufficient to describe how these structures evolved in concert with body size.

The survival of marsupial neonates is remarkable, considering their altricial condition at birth. These young are born with only a few functional features, such as the forelimbs and mouthparts, while important systems like lung function, thermoregulation, and immunity

are still underdeveloped (Tyndale-Biscoe & Renfree, 1987a). The presence of the pouch plays a crucial role by providing a safe environment that supports continued development after birth. Our results offer new insights into the evolution of this important trait. We found that the presence of a pouch is closely related to life-history traits, especially body mass and litter size. Larger species are more likely to have a pouch, while smaller species with larger litters often lack one. Also, body mass evolves at different rates depending on whether the pouch is present or not. The pouch has been gained and lost multiple times during marsupial evolution, showing that it is a flexible adaptation that changes in different lineages. These findings highlight the complex role of the pouch in marsupial biology and its importance for their evolutionary history.

REFERENCES

Ambatipudi, K., Joss, J., Raftery, M., & Deane, E. (2008). A proteomic approach to analysis of antimicrobial activity in marsupial pouch secretions. *Developmental and Comparative Immunology*, 32(2), 108–120. https://doi.org/10.1016/j.dci.2007.04.009

Astúa, D. (2015). Family Didelphidae (opossums). In: D. E. Wilson & R. A. Mittermeier (Eds). *Handbook of the Mammals of the World - Vol. 5. Monotremes and Marsupials* (pp. 70–186). Lynx Edicions.

Augee, M., Gooden, B., & Musser, A. (2006). *Echidna: extraordinary egg-laying mammal*. CSIRO Publishing.

Baker, A. M. (2015). Family Dasyuridae (carnivorous marsupials). In: D. E. Wilson & R. A. Mittermeier (Eds). *Handbook of the Mammals of the World - Vol. 5. Monotremes and Marsupials* (pp. 232–348). Lynx Edicions.

Battistella, T., Cerezer, F., Bubadué, J., Melo, G., Graipel, M., & Cáceres, N. (2019). Litter size variation in didelphid marsupials: evidence of phylogenetic constraints and adaptation. *Biological Journal of the Linnean Society*, 126(1), 40–54. https://doi.org/10.1093/biolinnean/bly157

Beaulieu, J. M., Jhwueng, D. C., Boettiger, C., & O'Meara, B. C. (2012). Modeling stabilizing selection: expanding the Ornstein–Uhlenbeck model of adaptive evolution. *Evolution*, 66(8), 2369–2383. https://doi.org/10.1111/j.1558-5646.2012.01619.x

Beaulieu, J. M., & O'Meara, B. (2025). *OUwie: Analysis of evolutionary rates in an OU framework* (R package version 2.14). GitHub https://github.com/thej022214/OUwie

Bobek, G., & Deane, E. M. (2001). Possible antimicrobial compounds from the pouch of the koala, *Phascolarctos cinereus*. *Letters in Peptide Science*, 8, 133–137. https://doi.org/10.1023/A:1016257920016

Bonner, J. T. (2011). *Why Size Matters: From Bacteria to Blue Whales*. Princeton University Press.

Boyko, J. D., & O'Meara, B. C. (2024). dentist: Quantifying uncertainty by sampling points around maximum likelihood estimates. *Methods in Ecology and Evolution*, 15(4), 628-638. https://doi.org/10.1111/2041-210X.14297

Boyko, J. D., O'Meara, B. C., & Beaulieu, J. M. (2023). A novel method for jointly modeling the evolution of discrete and continuous traits. *Evolution*, 77(3), 836–851. https://doi.org/10.1093/evolut/qpad002

497 Braithwaite, R. W., & Lee, A. K. (1979). A mammalian example of semelparity. *The*
498 *American Naturalist*, 113(1), 151–155. https://doi.org/10.1086/283372

499 Coues, E. (1872). On the osteology and myology of *Didelphys virginiana. Memoirs of*
500 *the Boston Society of Natural History*, 2(1–III), 41–154.

501 del Pino, E. M. (1980). Morphology of the pouch and incubatory integument in marsupial
502 frogs (Hylidae). *Copeia*, 1, 10–17. https://doi.org/10.2307/1444129.

503 del Pino, E. M. (2018). The extraordinary biology and development of marsupial frogs
504 (Hemiphractidae) in comparison with fish, mammals, birds, amphibians and other
505 animals. *Mechanisms of development*, 154, 2–11.
506 https://doi.org/10.1016/j.mod.2017.12.002

507 Dudley, J. S., Paul, J. W, Teh, V., Mackenzie, T. E., Butler, T. A., Tolosa, J. M., Smith,
508 R., Foley, M., Dowland, S., Thompson, M.B., & Whittington, C. M. (2022).
509 Seahorse brood pouch morphology and control of male parturition in
510 *Hippocampus abdominalis*. *Placenta*, 127, 88–94.
511 https://doi.org/10.1016/j.placenta.2022.07.015

512 Edwards, M. J., & Deakin, J. E. (2013). The marsupial pouch: implications for
513 reproductive success and mammalian evolution. *Australian Journal of Zoology*,
514 61(1), 41–47. https://doi.org/10.1071/ZO12088

515 Edwards, M. J., Hinds, L. A., Deane, E. M., & Deakin, J. E. (2012). A review of
516 complementary mechanisms which protect the developing marsupial pouch
517 young. *Developmental & Comparative Immunology*, 37(2), 213-220.
518 https://doi.org/10.1016/j.dci.2012.03.013

519 Elftman, H.O. (1929) Functional adaptations of the pelvis in marsupials. *Bulletin of the*
520 *American Museum of Natural History*, 58(5), 189–232.

521 Enders, R. K. (1935). Mammalian life histories from Barro Colorado Island, Panama.
522 *Bulletin of the Museum of Comparative Zoology*, 78, 385–502.

523 Felsenstein, J. (1985). Phylogenies and the Comparative Method. *The American*
524 *Naturalist*, 125(1), 1–15. https://doi.org/10.1086/284325

525 Fisher, D. O., Owens, I. P. F., & Johnson, C. N. (2001). The ecological basis of life history
526 variation in marsupials. *Ecology,* 82(12), 3531–3540.
527 https://doi.org/10.1890/0012-9658(2001)082[3531:TEBOLH]2.0.CO;2

528 Fox, J., & Weisberg, S. (2019). *An R Companion to Applied Regression, Third edition*.
529 Sage.

530 Guilhon, G., Braga, C., & Cerqueira, R. (2021). Musculoskeletal anatomy and
531 nomenclature of the mammalian epipubic bones. *Journal of Anatomy*, 239(5),
532 1096–1103. https://doi.org/10.1111/joa.13489

533 Hansen, T. F. (1997). Stabilizing selection and the comparative analysis of adaptation.
534 *Evolution*, 51(5), 1341-1351. https://doi.org/10.1111/j.1558-
535 5646.1997.tb01457.x

536 Harada, A., Shiota, R., Okubo, R., Yorifuji, M., Sogabe, A., Motomura, H., Hiroi, J.,
537 Yasumasu, S., & Kawaguchi, M. (2022). Brood pouch evolution in pipefish and
538 seahorse based on histological observation. *Placenta*, 120, 88–96.
539 https://doi.org/10.1016/j.placenta.2022.02.014

Harder, J. D. (1992). Reproductive Biology of South American Marsupials. In: W. C. Hamlett (Ed). *Reproductive Biology of South American Vertebrates* (pp. 211–228). Springer New York.

Horovitz, I., & Sánchez-Villagra, M. R. (2003). A morphological analysis of marsupial mammal higher-level phylogenetic relationships. *Cladistics*, 19(3), 181–212. https://doi.org/10.1111/j.1096-0031.2003.tb00363.x

Hulbert, A. J. (1988). Metabolism and the development of endothermy. In: C. H. Tyndale-Biscoe & P. A. Janssens (Eds). *The developing marsupial: models for biomedical research* (pp. 148–161). Springer Berlin Heidelberg.

Ives, A. R., Dinnage, R., Helmus, M. R., & Li, D. (2020). phyr: Model based phylogenetic analysis (R package version 1.1.0). CRAN. https://CRAN.R-project.org/package=phyr

Ives, A. R., & Helmus, M. R. (2011). Generalized linear mixed models for phylogenetic analyses of community structure. *Ecological Monographs*, 81(3), 511–525. https://doi.org/10.1890/10-1264.1

Jones, K. E., Bielby, J., Cardillo, M., Fritz, S. A., O'Dell, J., Orme, C. D. L., Safi, K., Sechrest, W., Boakes, E. H., Carbone, C., Connolly, C., Cutts, M. J., Foster, J. K., Grenyer, R., Habib, M., Plaster, C. A., Price, S. A., Rigby, E. A., Rist, J., Teacher, A., Bininda-Emonds, O. R. P., Gittleman, J. L., Mace, G. M., & Purvis, A. (2009). PanTHERIA: A species-level database of life history, ecology, and geography of extant and recently extinct mammals. *Ecology*, 90(9), 2648. https://doi.org/10.1890/08-1494.1

562 Kirsch, J. A. W. (1977). The six-percent solution: second thoughts on the adaptedness of
563 the Marsupialia. *American Scientist*, 65(3), 276–288.
564 https://www.jstor.org/stable/27847837

565 Kubota, K., Shimizu, T., Shibanai, S., Nagae, K., & Nagata, S. (1989). Histological
566 properties and biological significance of pouch in red kangaroo (*Macropus rufus*).
567 *Anatomischer Anzeiger*, 168(2), 169–179.
568 https://europepmc.org/article/med/2523677

569 Leiner, N. O., Setz, E. Z. F., Silva, W. R. (2008). Semelparity and Factors Affecting the
570 Reproductive Activity of the Brazilian Slender Opossum (Marmosops paulensis)
571 in Southeastern Brazil. Journal of Mammalogy, 89(1), 153–158.
572 https://doi.org/10.1644/07-MAMM-A-083.1

573 Mammal Diversity Database. (2022). *Mammal Diversity Database (1.9)* [Dataset].
574 Zenodo. https://doi.org/10.5281/zenodo.6407053

575 Palma, R. E., & Valladares-Gómez, A. (2015). Family Microbiotheriidae (monito del
576 monte). In D. E. Wilson & R. A. Mittermeier (Eds). *Handbook of the Mammals*
577 *of the World - Vol. 5. Monotremes and Marsupials* (pp. 200–208). Lynx Edicions.

578 Pianka, E. R. (1970). On r-and K-selection. *The American Naturalist*, 104(940), 592–597.
579 https://doi.org/10.1086/282697

580 Pocock, R. I. (1926). The external characters of *Thylacinus*, *Sarcophilus* and some related
581 marsupials. *Proceedings of the Zoological Society of London*, 96(4), 1037–1084.
582 https://doi.org/10.1111/j.1096-3642.1926.tb02233.x

583 Purnell, M. A., Donoghue, P. J. C., Gabbott, S. E., McNamara, M. E., Murdock, D. J. E.,
584 & Sansom, R. S. 2018. Experimental analysis of soft-tissue fossilization: opening
585 the black box. *Palaeontology,* 61(3), 317–323. https://doi.org/10.1111/pala.12360

586 R Core Team. (2025). *R: A language and environment for statistical computing*
587 [Computer software]. R Foundation for Statistical Computing. https://www.R-
588 project.org/

589 Revell, L. J. (2024). phytools 2.0: an updated R ecosystem for phylogenetic comparative
590 methods (and other things). *PeerJ*, 12, e16505.
591 https://doi.org/10.7717/peerj.16505

592 Revell, L. J. (2025). Ancestral state reconstruction of phenotypic characters. *Evolutionary*
593 *Biology*, 52, 1–25. http://doi.org/10.1007/s11692-025-09645-y

594 Rubin, D. B. (1987). *Multiple imputation for nonresponse in surveys*. John Wiley & Sons.

595 Russell, E. M. (1982). Patterns of parental care and parental investment in marsupials.
596 *Biological Reviews*, 57(3), 423–486. https://doi.org/10.1111/j.1469-
597 185X.1982.tb00704.x

598 Schneider, N. Y., & Gurovich, Y. (2017). Morphology and evolution of the oral shield in
599 marsupial neonates including the newborn monito del monte (*Dromiciops*
600 *gliroides*, Marsupialia Microbiotheria) pouch young. *Journal of Anatomy*, 231(1),
601 59–83. https://doi.org/10.1111/joa.12621

602 Schwarz, G. (1978). Estimating the dimension of a model. *The Annals of Statistics*, 6(2),
603 461–464. https://www.jstor.org/stable/2958889

Smith, K. K., & Keyte, A. L. (2020). Adaptations of the marsupial newborn: birth as an extreme environment. *Anatomical Record*, 303(2), 235–249. https://doi.org/10.1002/ar.24049

Sobral, G., & Guilhon, G. (2016). Problemas no uso dos termos altricial e precoce na Mastozoologia. *Boletim da Sociedade Brasileira de Mastozoologia*, 76, 61–67.

Sweet, G. (1907). The Skin, Hair, and Reproductive Organs of *Notoryctes*: Contributions to our Knowledge of the Anatomy of *Notoryctes typhlops*, Stirling—Parts IV and V. *Journal of Cell Science*, 2(202), 325-344. https://doi.org/10.1242/jcs.s2-51.202.325

Symonds, M. R. E., & Blomberg, S. P. (2014). A Primer on Phylogenetic Generalised Least Squares. In: L. Z. Garamszegi (Ed). *Modern Phylogenetic Comparative Methods and Their Application in Evolutionary Biology* (pp. 105–130). Springer Berlin Heidelberg.

Szalay, F. S. (1982). A new appraisal of marsupial phylogeny and classification. In: M. Archer (Ed). *Carnivorous Marsupials* (pp. 621–640). Royal Zoological Society of New South Wales.

Tyndale-Biscoe, H. (2005). *Life of Marsupials*. CSIRO Publishing.

Tyndale-Biscoe, H., & Renfree, M. (1987a). Breeding biology of marsupials by family. In: *Reproductive physiology of marsupials* (pp. 14–94). Cambridge University Press.

Tyndale-Biscoe, H., & Renfree, M. (1987b). Marsupials and the evolution of mammalian reproduction. In: *Reproductive physiology of marsupials* (pp. 396–423). Cambridge University Press.

Tyson, E. (1698). Carigueya, seu Marsupiale Americanum. or, The anatomy of an opossum, dissected at Gresham-College by Edw. Tyson, M. D. Fellow of the College of Physicians, and of the Royal Society, and reader of anatomy at the Chyrurgeons-Hall, in London. *Philosophical Transactions of the Royal Society of London*, 20(239), 105–164. https://doi.org/10.1098/rstl.1698.0023

Upham, N. S., Esselstyn, J. A., & Jetz, W. (2019). Inferring the mammal tree: Species-level sets of phylogenies for questions in ecology, evolution, and conservation. *PLoS Biology*, 17(12), e2006903. https://doi.org/10.1371/journal.pbio.3000494

Voss, R. S., & Jansa, S. A. (2009). Phylogenetic relationships and classification of didelphid marsupials, an extant radiation of New World metatherian mammals. *Bulletin of the American Museum of Natural History*, 2009(322), 1–177. https://doi.org/10.1206/322.1

Voss, R. S., & Jansa, S. A. (2021). Morphology. In: *Opossums: An Adaptive Radiation of New World Marsupials* (pp. 80–116). Johns Hopkins University Press.

Weisbecker, V., Ashwell, K., & Fisher, D. (2013). An improved body mass dataset for the study of marsupial brain size evolution. *Brain Behavior and Evolution*, 82(2), 81-82. https://doi.org/10.1159/000348647

White, T. D. (1989). An analysis of epipubic bone function in mammals using scaling theory. *Journal of Theoretical Biology*, 139(3), 343-357. https://doi.org/10.1016/S0022-5193(89)80213-9

Wickham, H. (2016). *ggplot2: Elegant graphics for data analysis* (R package version 3.3.6). https://ggplot2.tidyverse.org

649 Wilson, D. E., & Mittermeier, R. A. (2015). *Handbook of Mammals of the World, Vol. 5:*
650 *Monotremes and Marsupials. 1st ed*. Lynx Edicions.

651 Woolley, P. A. (1974). The pouch of *Planigale subtilissima* and other dasyurid
652 marsupials. *Journal of the Royal Society of Western Australia*, 57(1), 11–15

653 Yao, L., & Yuan, Y. (2025a). Yao, L., & Yuan, Y. (2025). A Unified Method for
654 Detecting Phylogenetic Signals in Continuous, Discrete, and Multiple Trait
655 Combinations. *Ecology and Evolution*, 15(3), e71106.
656 https://doi.org/10.1002/ece3.71106

657 Yao, L., & Yuan, Y. (2025b). *phylosignalDB: Explore phylogenetic signals using*
658 *distance-based methods* (R package version 0.2.2). https://CRAN.R-
659 project.org/package=phylosignalDB

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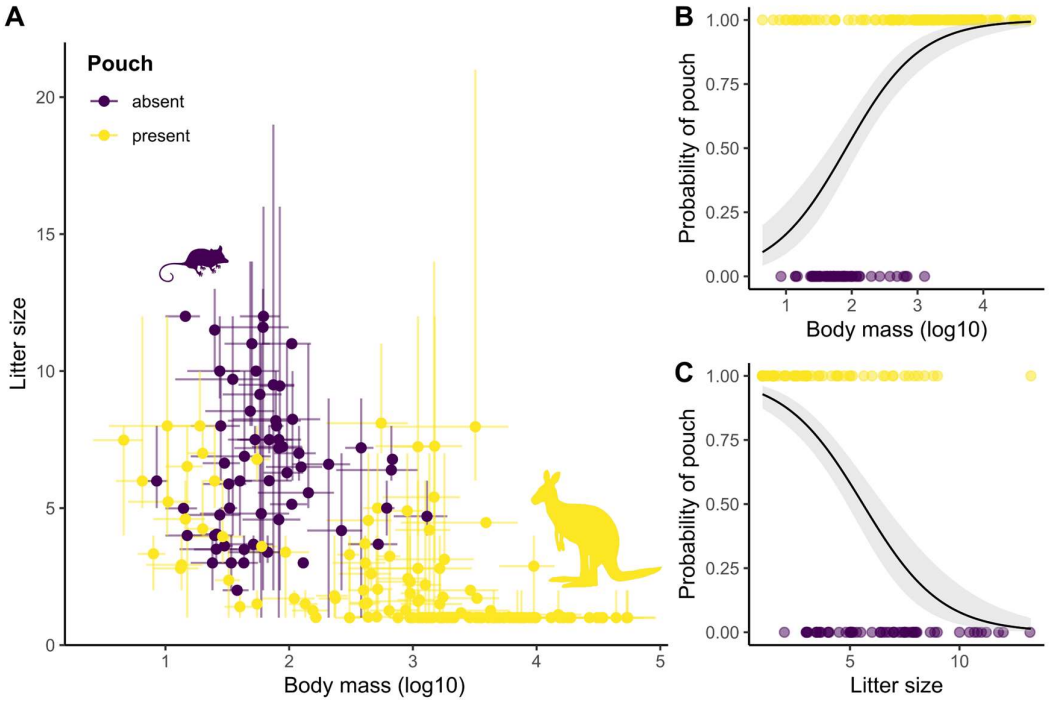

Fig 1 Relationship of $\log_{10}$ body mass, litter size, and pouch presence across the 195 sampled marsupial species. Biplot depicting the mean values (dots), and the range of variation (bars) across the 100 simulated datasets (A). Silhouettes illustrating extreme opposite combinations of traits: *Cryptonanus chacoensis* (purple) and *Osphranter rufus* (yellow) obtained in PhyloPic (www.phylopic.org). Logistic regression curve illustrating the probability of pouch presence as a function of $\log_{10}$ body mass (B). Logistic regression curve illustrating the probability of pouch presence as a function of litter size (C). Trait values and pouch probabilities were averaged across the 100 datasets for plotting B and C.

Alt text Fig 1 Plots showing the relationship among body mass, litter size, and pouch presence in 195 marsupial species. Silhouettes depict species with extreme trait combinations. Logistic curves illustrate how the probability of pouch presence increases with body mass and decreases with litter size.

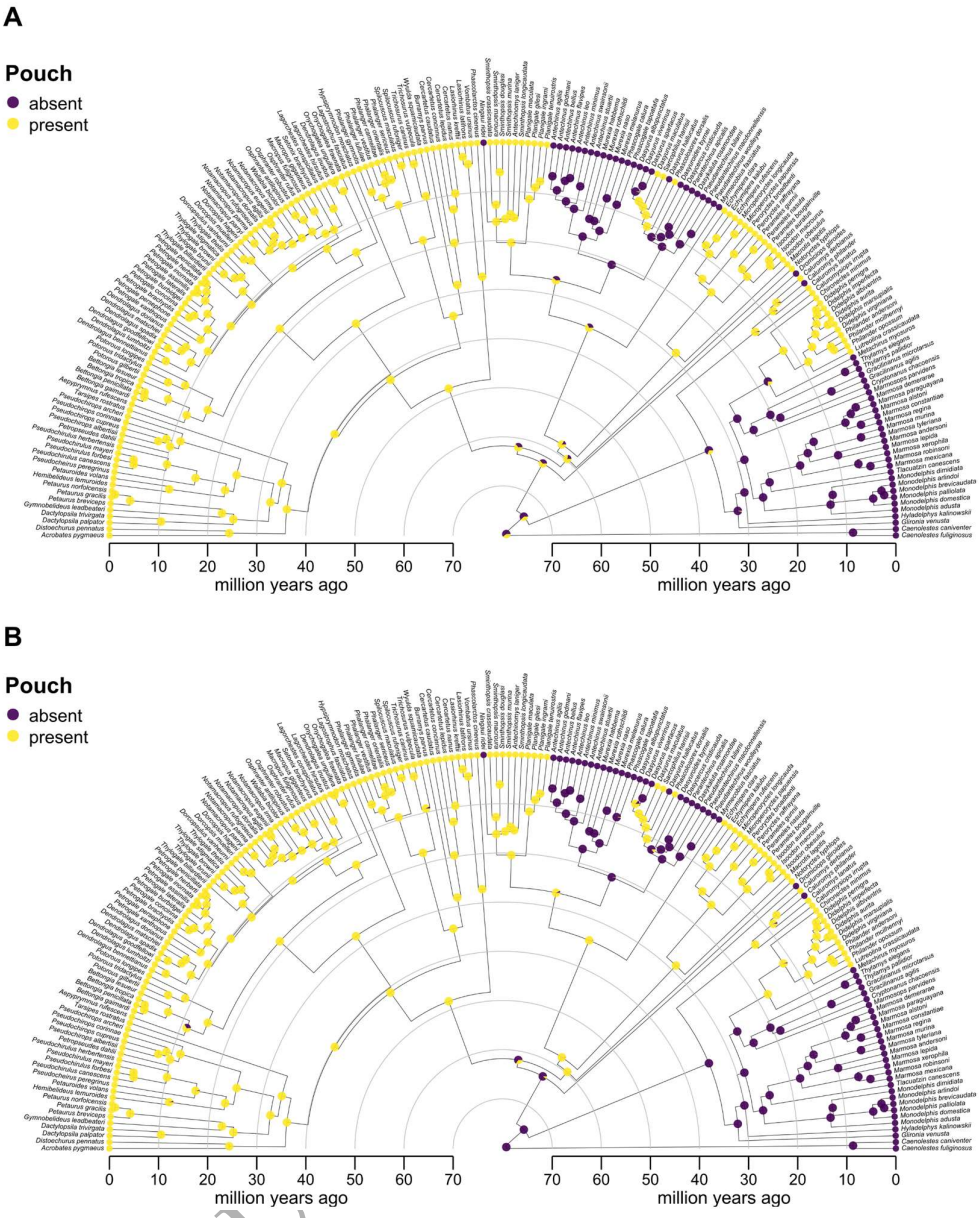

Fig 2 Ancestral state estimations for pouch presence evolving in correlation with body size. Ancestral states estimated under the CD_ER_BMV model (A). Ancestral states estimated under the CD_ER_OUMV model (B). Pie charts at the nodes represent the marginal probabilities of each ancestral state, and colors at the tips represent the observed state for extant species.

683 **Alt text Fig 2** Ancestral state reconstructions of pouch presence in marsupials modelled
684 in correlation with body size under alternative best-fitting models. Pie charts at nodes
685 indicate probabilities of each ancestral state, while tip colors denote observed states in
686 extant species.

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Table 1 Phylogenetic signal results for inidividual traits and their combinations. M statistic and associated p-values are summarized by the mean and confidence intervals (CIs) across the 100 combinations of tree and datasets. *Significant at α = 0.05.

Data set	M statistic	p-value
Pouch	0.72 (0.71 – 0.72)	0.001 (0.001 – 0.001)*
Body mass	0.74 (0.73 – 0.74)	0.001 (0.001 – 0.001)*
Litter size	0.69 (0.68 – 0.69)	0.001 (0.001 – 0.001)*
Body mass x litter size	0.95 (0.95 – 0.95)	0.001 (0.001 – 0.001)*
Pouch x body mass	0.70 (0.70 – 0.71)	0.001 (0.001 – 0.001)*
Pouch x litter size	0.73 (0.72 – 0.73)	0.001 (0.001 – 0.001)*
Pouch x body mass x litter size	0.70 (0.69 – 0.70)	0.001 (0.001 – 0.001)*

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Table 2 PGLMM parameters summarized by the mean and confidence intervals (CIs). CI for the mean across the 100 combinations of tree and datasets shown in parentheses, and Rubin's CI, which account for both within- and among-dataset uncertainty, shown in brackets. BIC = Bayesian information criterion, BICw = BIC weights.

Model	Intercept	Slope: body mass	Slope: litter size	Slope: interaction	BIC	BICw
Null	0.48 (0.46 – 0.49) [-2.33 – 3.28]	–	–	–	209.56 (208.97 – 210.15)	0.00 (0.00 – 0.00)
Body mass	1.37 (1.35 – 1.38) [-1.60 – 4.33]	1.48 (1.46 – 1.49) [0.27 – 2.68]	–	–	141.70 (141.21 – 142.18)	0.01 (0.01 – 0.02)
Litter size	0.50 (0.49 – 0.52) [-2.31 – 3.32]	–	-0.69 (-0.72 – -0.66) [-1.64 – 0.26]	–	170.05 (168.18 – 171.92)	0.00 (0.00 – 0.00)
Body mass + litter size	1.35 (1.33 – 1.37) [-1.65 – 4.36]	1.38 (1.37 – 1.40) [0.16 – 2.61]	-0.58 (-0.61 – -0.55) [-1.58 – 0.42]	–	129.84 (128.98 – 130.69)	0.70 (0.65 – 0.74)
Body mass * litter size	1.45 (1.43 – 1.47) [-1.63 – 4.53]	1.56 (1.53 – 1.59) [0.16 – 2.96]	-0.79 (-0.85 – -0.74) [-2.04 – 0.45]	-0.38 (-0.44 – -0.33) [-1.62 – 0.85]	131.85 (130.88 – 132.82)	0.29 (0.25 – 0.33)

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Table 3 Results of model fitting using hOUwie for the joint evolution of pouch and body mass. The best-fitting models (BICw ≥ 0.1) are indicted in bold. BIC - Bayesian information criterion, BICw - BIC weights

Model	BIC	BICw
CD_ER_BMV	**288.81**	**0.46**
CD_ER_OUV	294.24	0.03
CD_ER_OUM	302.98	< 0.01
CD_ER_OUMV	**288.74**	**0.47**
CD_ARD_BMV	295.05	0.02
CD_ARD_OUV	298.86	< 0.01
CD_ARD_OUM	304.28	< 0.01
CD_ARD_OUMV	298.20	< 0.01
CID_ER_BM1	296.67	0.01
CID_ER_OU1	301.30	< 0.01
CID_ARD_BM1	299.48	< 0.01
CID_ARD_OU1	304.73	< 0.01
CIDP_ER_BMV	308.14	< 0.01
CIDP_ER_OUV	315.61	< 0.01
CIDP_ER_OUM	321.79	< 0.01
CIDP_ER_OUMV	323.26	< 0.01
CIDP_ARD_BMV	317.63	< 0.01
CIDP_ARD_OUV	321.93	< 0.01
CIDP_ARD_OUM	314.17	< 0.01
CIDP_ARD_OUMV	313.36	< 0.01
HYB_ER_BMV	302.68	< 0.01
HYB_ER_OUV	306.97	< 0.01
HYB_ER_OUM	331.60	< 0.01
HYB_ER_OUMV	326.51	< 0.01
HYB_ARD_BMV	311.80	< 0.01
HYB_ARD_OUV	313.06	< 0.01
HYB_ARD_OUM	337.50	< 0.01
HYB_ARD_OUMV	324.04	< 0.01

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Table 4 Best estimate and 95% CI of the parameters of the best-fitting hOUwie models (BICw ≥ 0.1) for the joint evolution of pouch and body mass. 0 - pouch absent, 1 - pouch present.

Parameter/Model	CD_ER_OUMV	CD_ER_BMV
q0,1 = q1,0	0.004 (0.002 – 0.011)	0.004 (0.002 – 0.011)
α	0.001 (0.000 – 0.002)	-
σ^2_0	0.003 (0.002 – 0.009)	0.005 (0.003 – 0.010)
σ^2_1	0.019 (0.012 – 0.025)	0.017 (0.012 – 0.026)
θ	-	1.993 (0.702 – 3.087)
θ_0	1.423 (0.286 – 2.434)	-
θ_1	47.095 (12.829 – 122.238)	-

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