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A phylogeny and timescale for the living genera of kangaroos and kin (Macropodiformes : Marsupialia) based on nuclear DNA sequences

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Abstract. Kangaroos and kin (Macropodiformes) are the most conspicuous elements of the Australasian marsupial fauna. The approximately 70 living species can be divided into three families: (1) Hypsiprymnodontidae (the musky rat kangaroo); (2) Potoroidae (potoroos and bettongs); and (3) Macropodidae (larger kangaroos, wallabies, banded hare wallaby and pademelons). Here we examine macropodiform relationships using protein-coding portions of the *ApoB*, *BRCA1*, *IRBP*, *Rag1* and *vWF* genes via maximum parsimony, maximum likelihood and Bayesian methods. We estimate times of divergence using two different relaxed molecular clock methods to present a timescale for macropodiform evolution and reconstruct ancestral states for grades of dental organisation. We find robust support for a basal split between Hypsiprymnodontidae and the other macropodiforms, potoroid monophyly and macropodid monophyly, with *Lagostrophus* as the sister-taxon to all other macropodids. Our divergence estimates suggest that kangaroos diverged from Phalangeroidea in the early Eocene, that crown-group Macropodiformes originated in the late Eocene or early Oligocene and that the potoroid–macropodid split occurred in the late Oligocene or early Miocene followed by rapid cladogenesis within these families 5 to 15 million years ago. These divergence estimates coincide with major geological and ecological changes in Australia. Ancestral state reconstructions for grades of dental organisation suggest that the grazer grade evolved independently on two different occasions within Macropodidae.

Introduction

Of the four Australasian marsupial orders, Diprotodontia is both the most taxonomically and ecologically diverse. Within Diprotodontia, the suborder Macropodiformes (kangaroos and kin) contains the largest number of species. Owing to their unique mode of locomotion and ability to live in almost any Australasian environment, kangaroos are one of the most conspicuous and diverse faunal elements of Australasia. Most kangaroos are terrestrial, herbivorous, foregut fermenters that range in size from 0.5 grams to 90 kilograms. However, there are arboreal forms (*Dendrolagus*) and carnivorous forms may have even once existed (Flannery 1989; Wroe *et al.* 1998). The breadth of the macropodiform radiation is striking (Eisenberg 1981; Flannery 1984, 1989; Springer *et al.* 1997). Among kangaroos there are ecological vicars of primates, cervids and lagomorphs (Flannery 1989). Despite being one of the most intensively studied groups of marsupials with one of the best-known fossil records of any marsupial group, their intergeneric relationships are still poorly understood. These interrelationships have previously been investigated using morphology, chromosomes, nuclear DNA, mitochondrial DNA, microcomplement fixation (MC'F) with albumin and single-copy DNA–DNA hybridisation. However, the nodes are often only poorly supported and different studies have yielded results that are sometimes contradictory. Furthermore, there has only been one molecular study to simultaneously address the interrelationships of all living kangaroo genera (Westerman *et al.* 2002; see below).

Wilson and Reeder (2005) recognise three extant families of kangaroos in the suborder Macropodiformes: Hypsiprymnodontidae, Potoroidae and Macropodidae. The musky rat-kangaroo (*Hypsiprymnodon moschatus*) is the only living representative of Hypsiprymnodontidae. Potoroidae includes four genera of bettongs, potoroos and small rat kangaroos (*Aepyprymnus*, *Bettongia*, *Caloprymnus*, *Potorous*). Macropodidae includes the larger kangaroos, wallabies and pademelons. These three major lineages have consistently been recognised in kangaroo systematics, although often at the subfamily or even tribal level.

Numerous authors have investigated relationships among the major kangaroo lineages, whether classified as families, subfamilies or even tribes. Bensley (1903) recognised a single family (Macropodidae) for all kangaroos. Raven and Gregory's (1946) discussion of kangaroo relationships was framed in the context of adaptive branching in relation to habitat. The ancestral habitat is "doubtless the rain forest" (p. 2) and of "this ancestral stage only the tiny musk kangaroo of north Queensland remains as a living witness" (p. 2). From the rain forest, Raven and Gregory (1946) speculated that (1) rat kangaroos evolved where "the forest

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leads out into the gullies and thickets" and that (2) "in other directions the rain forest leads into the open forest" and eventually into the "open plains" and that macropodines evolved and diversified along this ecological gradient. Raven and Gregory's (1946) fig. 1 depicts a sister-group relationship between potoroines and macropodines to the exclusion of *Hypsiprymnodon*. In contrast, Pearson (1946, 1950*a*, 1950*b*) and Tate (1948) concluded that hypsiprymnodontines are more closely related to potoroine than to macropodine kangaroos. Numerous authors have followed Pearson (1946, 1950*a*, 1950*b*) and Tate (1948) in grouping potoroines and *Hypsiprymnodon moschatus* together, either in the family Potoroidae with two subfamilies (Potoroinae, Hypsiprymnodinae) or in the subfamily Potoroinae with two tribes (Potoroini, Hypsiprymnodontini) (Ride 1964; Kirsch 1977; Archer 1984; Case 1984; Flannery 1984; Woodburne 1984; Flannery and Rich 1986; Aplin and Archer 1987; Flannery 1989; Baverstock *et al*. 1990; Marshall *et al.* 1990). In contrast to this arrangement, and more in keeping with Raven and Gregory's (1946) earlier work, Ride (1993) and Wroe and Archer (1995) grouped extinct propleopines and *H. moschatus* as sister-taxa in the family Hypsiprymnodontidae and further considered Hypsiprymnodontidae a sister-lineage to all other kangaroos. Szalay's (1994) detailed analysis of tarsal morphology also supports a basal split between *Hypsiprymnodon* and other living kangaroos based on the retention of primitive tarsal structures in *H. moschatus*, which are more similar to those of arboreal possums than to other macropodiforms. Among the living macropodiform genera, *Hypsiprymnodon* is unique in that its fast gait is the quadrepedal bound instead of the bipedal hop, the digital pads are striated, the first toe is retained, the second and third toes are robust and the hind and front limbs are about equivalent in length (Flannery 1989; Szalay 1994). Furthermore, it has a litter size of two instead of one (Johnson and Strahan 1982), possesses a simple stomach (non-sacculated) similar to that of the phalangerids (Langer 1979; 1980) and P_2 persists even after the eruption of P_3 (Flannery 1989; Ride 1993). DNA studies (Burk *et al.* 1998; Burk and Springer 2000; Osborne *et al.* 2002; Westerman *et al*. 2002; Baker *et al.* 2004; Kavanagh *et al.* 2004; Meredith *et al.* in press) provide robust support for a basal split between Hypsiprymnodontidae and other living macropodiform genera.

Determining the phylogenetic placement of *Lagostrophus* (banded hare wallaby) is often considered the most vexing problem in kangaroo systematics. Most authors have suggested that *Lagostrophus* is nested within Macropodidae (Gould 1842; Bensley 1903; Raven and Gregory 1946; Kirsch 1977; Archer 1984). However, Thomas (1888) suggested that it was distinct from all other macropodids based on craniodental and pelage differences. Anatomical features of the female reproductive system suggest that *Lagostrophus* retains primitive characters that are more similar to the potoroids and Australasian possums than to other macropodids (Tyndale-Biscoe 1964, 2005; Pearson 1950*b*). Baverstock *et al.*'s (1989) microcomplement fixation study of Macropodidae shows that *Lagostrophus* is "distinct from all other macropodines" and "perhaps closer to the potoroines than to macropodines" (p. 47).

The sthenurines were a successful group of kangaroos that originated in the late Miocene (Murray 1991; Prideaux 2004) and were thought to have gone extinct in the late Pleistocene (Flannery 1989; Prideaux 2004). In contrast, Flannery (1983, 1989) suggested that *Lagostrophus* is sister to all other macropodids and could be the last surviving member of Sthenurinae based on five dental traits. Murray (1991, 1995) and Prideaux (2004) suggested that these shared features are plesiomorphic or the result of adaptive convergence. Molecular studies (Westerman *et al*. 2002; Nilsson 2006) suggest that *Lagostrophus* is a distinct kangaroo lineage that has been separate from other living kangaroos from 16.1–23.4 million years (Westerman *et al.* 2002; Nilsson 2006).

The monophyly of all macropodids (*sensu* Wilson and Reeder 2005) except *Lagostrophus* is secure based on molecular studies (Burk and Springer 2000; Westerman *et al.* 2002). However, relationships among the remaining genera within this group are poorly known and studies based on different lines of evidence suggest contradictory sets of relationships. This lack of resolution has been attributed to a rapid and extensive radiation with little extinction of genera in the last 5–10 million years, coinciding with the drying out of Australia and concurrent with the spread of grasslands (Raven and Gregory 1946; Flannery 1989; Kirsch *et al.* 1995).

Sanson (1989) recognised four grades of dental organisation within kangaroos: (1) potoroid and basal macropodoid grade; (2) browser grade; (3) intermediate browser/grazer grade; and (4) grazer grade. These grades are based on the ability to process grass, P_3 size and retention, molar link and loph height and arching of the molar row. The potoroid and basal macropodoid grade is considered ancestral in that the premolar is large, the molars are bunodont (i.e. no lophs or links) and the molar tooth row is twisted. The large premolar is used predominantly for seed crushing whereas the molars are used to process invertebrates. The browser grade is for low fibre browse and is characterised by a moderately large premolar for cutting/crushing stems and molars that are aligned in a row to process leaves. However, the molar lophs are small in size. The intermediate browser/grazer grade is for a mixed browse and grass diet and is characterised by a reduced premolar and a slightly curved molar tooth row that reduces occlusion. Molar teeth are lophodont with strong links and the premolar is occasionally lost allowing for the forward movement of molars as they become worn down. As a result, shearing occurs predominantly on the back molars and grass is processed by the premolar. The grazer grade is specialised for grass and is characterised by a very reduced P_3 and is often shed before all molars have erupted. The tooth rows are strongly curved to increase shearing of grass. Lophs and links are very well developed. The upper and lower molar rows curve in different directions, which allows only two molars to occlude at any time. The molars in grazers erupt sequentially, with worn molars being shed from the front.

Sanson (1989) suggested that the grazer grade, which occurs in kangaroos with a high fibre grass diet, represents the culmination of the evolution of the macropodoid dentition through earlier successive stages that were adapted to a mixed invertebrate and very low-fibre plant diet and then a low-fibre leaf diet. It is straightforward to hypothesise that these different grades are somehow tied to the spread of grasslands resulting from the progressive "drying" of Australia from the late Eocene onwards. If these grades are directional, then they should appear sequentially starting with the potoroid and basal macropodoid

grade in the ancestral forms and eventually ending with the grazer grade in the more recently diverged forms. However, no study to date has looked at the evolution of these grades via ancestral state reconstructions.

Molecular estimates of divergence times have usually accompanied the most recent molecular phylogenetic analyses. Burk *et al.* (1998) and Burk and Springer (2000) estimated divergence times of several macropodiform clades based on mitochondrial transversions. Kirsch *et al.* (1997) estimated macropodiform divergence dates based on single-copy DNA–DNA hybridisation data. Westerman *et al.* (2002) estimated dates of divergence for macropodiform genera based on three mitochondrial genes and one nuclear gene using the parametric programs of Thorne *et al.* (1998) and Kishino *et al.* (2001) with eight hard constraints. Meredith *et al.* (2008) used the Bayesian parametric approaches of Thorne and Kishino (2002) and Drummond *et al.* (2006) for a five gene marsupial nuclear concatenation. Results of previous molecular dating studies on macropodiform divergences are summarised in Table S1 in an Accessory Publication on the *Australian Journal of Zoology* website.

In this paper, we address the intergeneric relationships within the living Macropodiformes using a concatenation consisting of protein-coding portions of five nuclear genes (*ApoB*, *BRCA1*, *IRBP*, *Rag1* and *vWF*) and estimate their times of divergence using the relaxed Bayesian molecular clock methods of Thorne and Kishino (2002) and Drummond *et al.* (2006). This phylogeny and timescale of macropodiform evolution is then used to examine the evolution of grades of dental organisation as put forth by Sanson (1989) and determine environmental correlates of the macropodiform radiation.

Materials and methods

Taxon sampling/gene sequences

This study includes representatives from all australidelphian orders, all kangaroo genera and all subgenera of *Macropus* (in Table S2 in an Accessory Publication on the *Australian Journal of Zoology* website). DNA was extracted following Kirsch *et al.* (1990) or Meredith *et al.* (2008). PCR was used to amplify segments of five nuclear genes with *Taq* DNA polymerase (Invitrogen) and the following temperature regime: initial denaturation at 94°C for 2 min; 35 cycles of 1 min at 94°C (denaturation), 1 min at 50°C (annealing), and 2 min at 72°C (extension); and a final extension for 10 min at 72°C. Primers used in this study are given in in aligned fasta files in an Accessory Publication on the *Australian Journal of Zoology* website. Gene segments that were amplified were from protein coding portions of *ApoB* (exon 26; Apolipoprotein B), *BRCA1* (exon 11; breast and ovarian cancer susceptibility gene), *IRBP* (exon 1; interphotoreceptor retinoid binding protein gene), *Rag1* (intronless; recombination activating gene-1) and *vWF* (exon 28; vonWillebrand factor gene). These genes were chosen because they have consistently shown their utility in resolving marsupial intergeneric relationships (e.g. Amrine-Madsen *et al.* 2003; Meredith *et al.* 2008, in press). PCR products were cleaned using the QIAquick Gel Extraction Kit (QIAGEN) or AccuPrep™ Gel Purification Kit (Bioneer Corporation). PCR products were sequenced in both directions at the UCR Core Instrumentation Facility with an automated DNA sequencer (ABI 3730x1). Westerman *et al.* (2002) used mitochondrial DNA and protamine P_1 sequences to evaluate phylogenetic relationships within Macropodiformes. These genes were excluded from this study because we aimed for an independent nuclear DNA data set with no missing data. Further, protamine P_1 sequences contain complex indels that are difficult to align (Burk and Springer 2000). Chromosome locations of the genes used in this study are listed in an Accessory Publication on the *Australian Journal of Zoology* website.

DNA alignments

DNA sequences were first translated into amino acids and then the program SOAP ver. 1.2a4 (Löytynoja and Milinkovitch 2001) was used to align the amino acid sequences and identify alignment-ambiguous regions. Gap opening (11–19) and gap extension (3–11) penalties in steps of two were employed. This resulted in 25 different alignments for each gene segment. The DNA sequences were then manually aligned to the amino acid alignment using the program Se-AL (Rambaut 1996). We retained 6065 bp for all subsequent analyses (*ApoB* = 766 sites; *BRCA1* = 2504 sites; *IRBP* = 1280 sites; *Rag1* = 544 sites; *vWF* = 971 sites). The aligned nexus file is given in an Accessory Publication on the *Australian Journal of Zoology* website.

Data compatibility

We performed a partition homogeneity test (Farris *et al.* 1994; Swofford 2002) using PAUP 4.0b10 (Swofford 2002) with 1000 replications and 10 taxon input orders per replicate. This test indicated that it was appropriate to combine the genes into one dataset ($P < 0.001$). In addition, we employed the bootstrap compatibility method (De Queiroz 1993; Teeling *et al.* 2002) using RAxML-VI-HPC (Stamatakis 2006). Five hundred bootstrap replicates were performed. All analyses were started from randomised maximum parsimony trees using the fast hill-climbing algorithm, the general time reversible (GTR) + Γ model of sequence evolution, with all free parameters being estimated. We found no conflicting nodes above 90% bootstrap support. Given these results, we elected to combine the individual genes into one data set.

Phylogenetic analyses

Maximum parsimony (MP) analyses were performed using PAUP 4.0b10 (Swofford 2002). Maximum likelihood (ML) analyses were performed using PhyML (Guindon and Gascuel 2003). The Akaike Information Criterion as implemented in Modeltest 3.06 (Posada and Crandall 1998) was used to determine the best-fit models of molecular evolution for the PhyML, Bayesian and molecular dating analyses (see below). The models that were selected were as follows: GTR + Γ + I (*ApoB*, *BRCA1* and concatenation); K81uf + Γ + I (*IRBP*); TIM + Γ + I (*Rag1*); and TVMef + Γ + I (*vWF*). For the MP analysis we implemented heuristic searches with 1000 randomised addition orders and tree-bisection-reconnectoin (TBR) branch swapping. The PhyML analysis was started from a neighbour joining tree. In all analyses, gaps were treated as missing data. Bootstrap analyses were performed with either 1000 (MP) or 500 (ML) replicates.

Bayesian analyses were carried out using MrBayes ver. 3.1.1 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). This program uses Metropolis-coupled Markov chain Monte Carlo sampling to calculate Bayesian posterior probabilities. The nuclear concatenation was analysed first as if it was a single gene under one model of molecular evolution (model as above) and in the second analysis, each gene was allowed its own model of molecular evolution (models as above). If Modeltest 3.06 suggested a model not implemented in MrBayes, we used the next most complex model. We used default priors, random starting trees and eight Markov chains (one cold and seven hot), with chain sampling every 1000 generations. Analyses were terminated when the average standard deviation of frequencies for the simultaneous analyses fell below 0.01 (~10 million generations). Bayesian posterior probabilities were calculated after discarding the first 25% of the sampled trees as burn-in.

Statistical tests

We used CONSEL (Shimodaira 2002) to implement Kishino–Hasegawa (KH), Shimodaira–Hasegawa (SH) and approximately unbiased (AU) statistical tests (Kishino and Hasegawa 1989; Shimodaira and Hasegawa 1999; Shimodaira 2002) to compare alternative phylogenetic hypotheses. The KH and SH tests are biased and can place overconfidence in the incorrect tree (KH) or yield results that are too conservative (SH). The AU test is meant to overcome the deficiencies of the aforementioned tests but is only approximately unbiased (Shimodaira 2002).

Molecular dating analyses

We employed the likelihood ratio statistic to test the molecular clock hypothesis. The molecular clock was rejected ($P < 0.001$) for each gene and for the concatenation. Therefore, we employed two different methods, BEAST ver. 1.4.6 and *Multidivtime*, that allow for a relaxed molecular clock (Thorne *et al.* 1998; Kishino *et al.* 2001; Thorne and Kishino 2002; Drummond *et al.* 2006; Drummond and Rambaut 2007). These methods also allow for the incorporation of multiple fossil constraints. By contrast with *Multidivtime*, BEAST ver. 1.4.6 (Drummond *et al.* 2006; Drummond and Rambaut 2007) simultaneously estimates both the phylogeny and divergence time estimates, allows for more complex models of molecular evolution (e.g. GTR + Γ + I), does not assume autocorrelation of rates among lineages and permits both "soft" and "hard" node constraints (Hedges and Kumar 2004; Yang and Rannala 2006).

Multidivtime

Multidivtime (version 9-25-03) (Thorne *et al.* 1998; Kishino *et al.* 2001; Thorne and Kishino 2002) requires a rooted tree topology with a specified outgroup that is subsequently removed from the dating analysis. The most complex model of sequence evolution allowable is F84 + Γ, lineage rates are assumed to be autocorrelated and only "hard" bounds are allowable. We used the Bayesian phylogeny obtained in the five gene partitioned analysis (Fig. 1). *Estbranches* was used to estimate branch lengths, which were then used by *Multidivtime* to estimate times of divergence (Thorne *et al.* 1998; Kishino *et al.* 2001; Thorne and Kishino 2002). The five-gene dataset was treated both as a single gene (each gene changed rate by a common factor) and as a concatenation of individual genes, which allows gene-specific rate trajectories (Thorne and Kishino 2002). In the *Multidivtime* analyses, we implemented the F84 model of sequence evolution with a Γ distribution modelled by four discrete categories given that the models suggested by Modeltest were at least as complicated as the F84 + Γ model. The tree shown in Fig. 1 was used in PAUP 4.0b10 (Swofford 2002) to estimate the transition/transversion parameters and the rate category estimates of the Γ distribution. We set the mean of the prior distribution for the root of the Australasian taxa at 70 million years ago, which is approximately 15 million years older than the oldest known Australian australidelphian fossils from Murgon (Godthelp *et al.* 1999; Beck *et al.* 2008). We chose to add approximately 15 million years to the upper bound because the fossil record will usually underestimate the actual time of divergence. The median amount of evolution from the ingroup root node to the ingroup tips was divided by the mean of the prior for the root of Australidelphia. This value was used as the mean of the prior distribution for the rate of molecular evolution at the ingroup root node. All analyses were run for one million generations with burn-in set to 10%; chains were sampled every 100 generations. All analyses were performed at least twice to check for convergence among runs.

BEAST

We implemented the uncorrelated lognormal distribution (UCLN) model. In this model the rate of each lineage is independently drawn from a lognormal distribution. Like the *Multidivtime* analyses, we treated the dataset both as a partitioned dataset and as a single gene (non-partitioned). The mean substitution rate for the concatenated BEAST analyses was calculated as in the non-partitioned *Multidivtime* analyses. However, the model implemented to estimate the rate was the one suggested by Modeltest 3.06 (Posada and Crandall 1998; see above). This same methodology was applied to each gene to estimate rates of molecular evolution for the partitioned BEAST analyses. We used the model of sequence evolution suggested by Modeltest 3.06 (Posada and Crandall 1998) as a guide for choosing the model implemented in BEAST. If more than two substitution rates were suggested, we used the GTR model. Otherwise, the HKY model was implemented.

For each dataset we performed three independent runs of ten million generations with a burn-in of 10% and sampling every 1000 generations. These were then combined using Log-Combiner (Rambaut and Drummond 2007) and Tracer 1.2 (Rambaut and Drummond 2003) was used to check for both stationarity/mixing and estimated sample sizes greater than 200. This was repeated for each analysis. We employed both hard-bounded-only and soft-bounded-only node constraints. For the soft-bounded-only analyses, the node constraints followed a normal distribution, with 95% of the normal distribution between the specified minima and maxima and 2.5% of the distribution in each tail.

Fossil constraints

Forty-six hard bounded constraints were chosen based on the fossil record (in an Accessory Publication on the *Australian Journal*

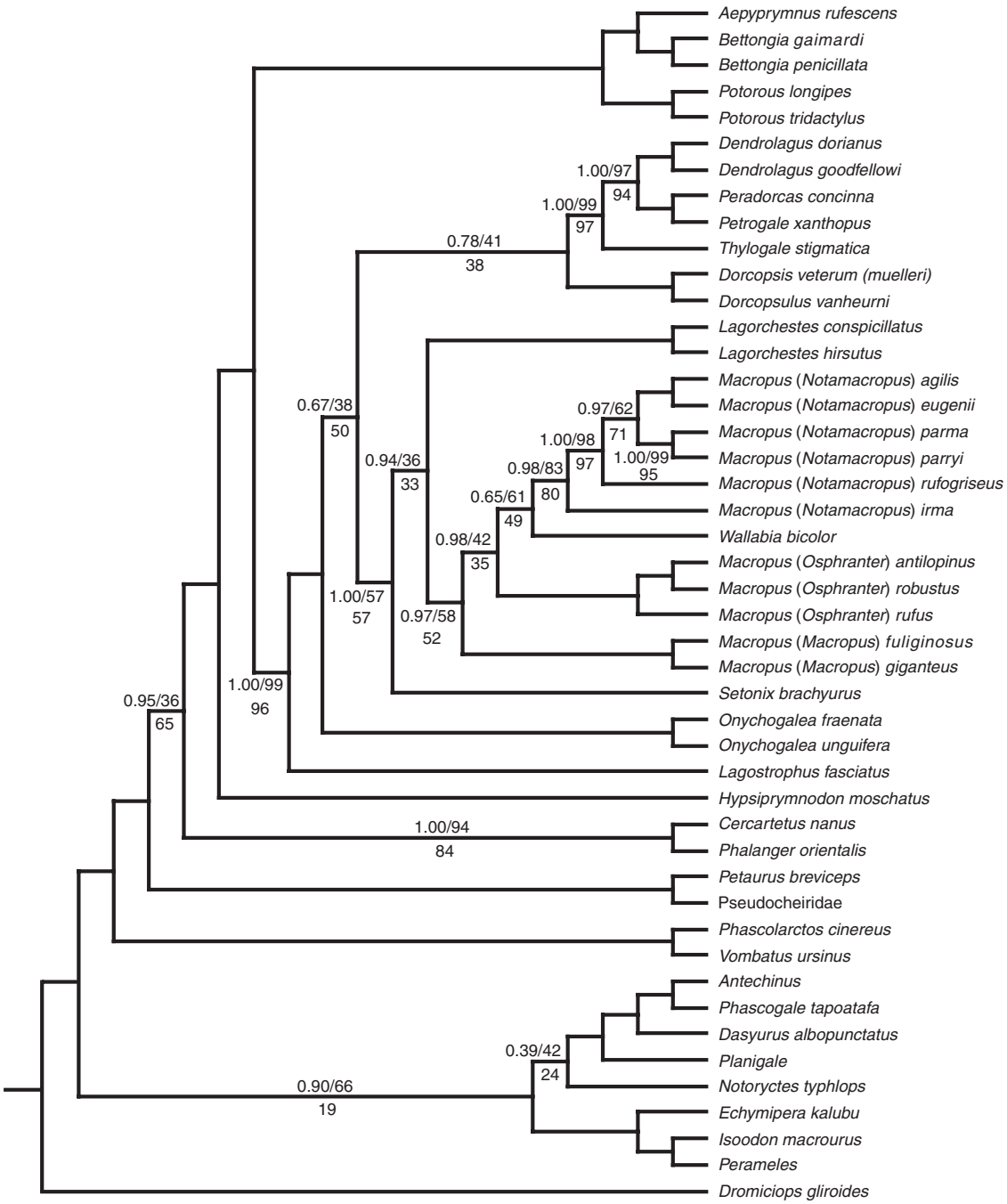

Fig. 1. Bayesian tree obtained from the partitioned Bayesian analysis. Values above branches and before slashes are mean Bayesian posterior probabilities based on two simultaneous runs. Values above branches and after slashes are maximum likelihood bootstrap support percentages. Values below branches are maximum parsimony bootstrap support percentages. Branches without support values were supported in all analyses with 100% bootstrap support and 1.00 Bayesian posterior probabilities.

of Zoology website). We utilised both stratigraphic bounding (Benton and Donoghue 2007) and phylogenetic bracketing (Reisz and Müller 2004; Müller and Reisz 2005) to determine minimum and maximum constraints following the reasoning set forth by Benton and Donoghue (2007). Stratigraphic bounding is the "consideration of the absence of fossils from underlying deposits" (Benton and Donoghue 2007: p. 28) and phylogenetic bracketing is "bracketing the next node below and above" a divergence event (Benton and Donoghue 2007: p. 28) to estimate a minimum and/or maximum. Given the patchy Australasian fossil record, stratigraphic bounding was not restricted to the immediate next-oldest-fossil-bearing deposit not containing any fossils from the lineage of interest. Instead we used the fossil-bearing deposit, which was one fossil-bearing unit older than the immediate next-oldest-

fossil-bearing layer not containing the lineage of interest. For example, the majority of fossil sites in Riversleigh can be placed into one of three systems A (24.7–25.0 Mya), B (15.97–23.03 Mya) or C (11.61–15.57 Mya). Suppose that the oldest known fossil belonging to a given clade is from a System C deposit. We would assign a minimum age of 11.61 million years and a maximum age of 25.0 million years (base of Zone A) using stratigraphic bounding.

Likewise, for phylogenetic bracketing, we did not bracket using the immediate sister-group, but instead used the next-most-distant sister-group. If we were interested in using phylogenetic bracketing to place a maximum on the (C,D) node for the following phylogenetic relationship (A,(B,(C,D))), we used the age of the oldest known fossil belonging to lineage A or B. If stratigraphic bounding and phylogenetic bracketing yielded different maxima, we used the older estimate. Fossils chosen were based on well documented and/or unequivocal specimens from directly dated and/or biocorrelated deposits. We followed Archer *et al.* (1999, 2006) and Travouillon *et al.* (2006) for biocorrelation and ages of Australasian deposits. The Riversleigh deposits were given ages in millions of years based on their presumed epoch following the timescale given by Gradstein *et al.* (2004). For example, System C deposits are thought to be middle Miocene (11.61–15.57), so if we used a fossil in a System C deposit as a maximum, an age of 15.57 million years was used, whereas if the fossil was used as a minimum, an age of 11.61 million years was assigned.

Ancestral state reconstructions

SIMMAP ver. 1.0 B2.3.2 (Bollback 2006) was used to estimate ancestral states for the four grades of dental organisation defined by Sanson (1989): (1) potoroid and basal macropodoid grade; (2) browser grade; (3) intermediate browser/grazer grade; and (4) grazer grade. Nielsen (2002) devised a simulation procedure to stochastically map discrete characters onto multiple trees, which is implemented in the program SIMMAP. The program explains the distribution of character states found in the terminal taxa by calculating the posterior probability distribution of the rate of change as well as the total number of character state changes. Samples from the posterior distribution are sampled by averaging over multiple trees and the number of changes is proportional to branch lengths while maintaining constant character states at the tips. Morphological priors must be specified. The rate prior has a gamma distribution and is described by the parameters α and β: α and β describe the mean (α/β) and variance (α/β^2). Branch length proportionality was maintained by rescaling branch lengths before applying the prior and 50 categories were used to make the gamma distribution discrete. Given the difficulty in determining appropriate morphological priors, multiple combinations of priors were investigated to determine the robustness of our ancestral state reconstructions. We chose the following three sets of priors: $\alpha = 1, \beta = 1$; $\alpha = 3, \beta = 2$; and $\alpha = 5, \beta = 5$. Analyses were run treating the grades of dental organisation as unordered and ordered character states. We used all post-burn-in trees from the partitioned Bayesian analysis (7380 trees) with ten draws from the prior distribution. Analyses with all three sets of priors yielded similar results (posterior probabilities always within 0.0000–0.0173 (unordered) and 0.000–0.1089 (ordered)) and we only report the results from the analysis with the priors set to $\alpha = 3$ and $\beta = 2$.

Results

Figure 1 shows the maximum posterior probability (MPP) tree for the partitioned Bayesian analysis with the mean Bayesian Posterior Probability (BPP) based on two independent runs and the ML bootstrap support percentage (BSP) derived from PhyML analysis. The MP tree resulted in one island of ten trees at 5650 steps. The 50% majority-rule consensus tree based on the ten MP most parsimonious trees is shown in the in Fig. S1 in an Accessory Publication on the *Australian Journal of Zoology* website. The ML tree obtained from the PhyML analysis is given in Fig. S2 in an Accessory Publication on the *Australian Journal of Zoology* website. Bootstrap and posterior probabilities for the MP, ML and Bayesian phylogenetic analyses are summarised in Table 1. Figure 1 is rooted between *Dromiciops* and all other australidelphians based on recent phylogenetic studies (e.g. Amrine-Madsen *et al.* 2003; Phillips *et al.* 2006; Meredith *et al.* 2008, in press).

All phylogenetic studies recovered the Phalangeroidea (Phalangeridae + Burramyidae) as the sister-group of a monophyletic Macropodiformes. Support for Macropodiformes was robust. Within Macropodiformes, Hypsiprymnodontidae (*Hypsiprymnodon*) was recovered as the sister-group to a clade comprising Potoroidae + Macropodidae. Potoroidae and Macropodidae were both recovered with robust support. Within the Potoroidae, *Bettongia* always grouped with *Aepyprymnus* to the exclusion of *Potorous*.

Among macropodids, there was a basal split between *Lagostrophus* and all other taxa. *Dendrolagus*, *Peradorcas*, *Petrogale* and *Thylogale* formed a well-supported clade. Within this clade, *Peradorcas* and *Petrogale* grouped together and these two joined *Dendrolagus*. *Dorcopsulus* and *Dorcopsis* always grouped together with robust support. *Macropus* and *Wallabia* grouped together to the exclusion of other macropodids. The *Macropus* subgenera (*Notamacropus*), (*Osphranter*) and (*Macropus*) were recovered as monophyletic in all analyses. Support for *M.* (*Osphranter*) and *M.* (*Macropus*) was robust in all analyses; support for *M.* (*Notamacropus*) was moderate in bootstrap analyses and high in Bayesian analyses. However, the genus *Macropus* was always recovered as paraphyletic, with *Wallabia* as the sister-taxon to either *M.* (*Notamacropus*) (ML and partitioned Bayesian analyses) or a *M.* (*Notamacropus*) + *M.* (*Osphranter*) clade (non-partitioned Bayesian analyses). With the exception of *M.* (*Notamacropus*) + *Wallabia* + *M.* (*Osphranter*), which received robust support in partitioned Bayesian analyses, relationships among the major lineages in the *Wallabia–Macropus* clade (i.e., *M.* (*Notamacropus*), *M.* (*Osphranter*), *M.* (*Macropus*), *Wallabia*) were not well supported. Within *M.* (*Osphranter*), *M. antilopinus* and *M. robustus* always grouped to the exclusion of *M. rufus*. Within *M.* (*Notamacropus*), *M. irma* was recovered as the sister-taxon to all other *M.* (*Notamacropus*) species; support for the latter clade was robust in bootstrap and Bayesian analyses. Also within *M.* (*Notamacropus*), *M. agilis* grouped with *M. eugenii*, *M. parma* grouped with *M. parryi* and these two clades grouped together to the exclusion of *M. rufogriseus*.

Table 1. Posterior probabilities and bootstrap summaries

Nodes supported by 100% bootstrap support and 1.00 posterior probabilities are not shown. Partitioned = each gene was modeled to have its own model of molecular evolution; Non-partitioned = *ApoB*, *BRCA1*, *IRBP*, *Rag1* and *vWF* were treated as a single gene; MP = maximum parsimony; ML = maximum likelihood

Phylogenetic hypothesis	MP	ML	Bayesian analyses	
			Partitioned	Non-partitioned
Macropodiformes + Phalangeroidea	36	65	0.95	0.92
Macropodidae monophyly (including *Lagostrophus*)	96	99	1.00	1.00
Dendrolagus + *Peradorcas* + *Petrogale*	94	97	1.00	1.00
Thylogale + *Dendrolagus* + *Peradorcas* + *Petrogale*	97	99	1.00	1.00
Dorcopsis + *Dorcopsulus* + *Thylogale* + *Dendrolagus* + *Peradorcas* + *Petrogale*	38	41	0.78	0.80
Lagorchestes + *Macropus* + *Wallabia*	33	36	0.94	0.92
Setonix + *Lagorchestes* + *Macropus* + *Wallabia*	57	57	1.00	1.00
Setonix + *Lagorchestes* + *Macropus* + *Wallabia* + *Dorcopsis* + *Dorcopsulus* + *Thylogale* + *Dendrolagus* + *Peradorcas* + *Petrogale*	50	38	0.67	66
M. (*Notamacropus*) monophyly (*sensu* Dawson and Flannery 1985) (*M. irma*, *M. parma*, *M. agilis*, *M. rufogriseus*, *M. eugenii*, *M. parryi*)	80	83	0.98	1.00
Macropus + *Wallabia*	52	58	0.97	0.96
M. (*Osphranter*) + *Wallabia* + *M.* (*Notamacropus*)	35	42	0.98	0.90
Wallabia + *M.* (*Notamacropus*)	49	61	0.65	0.48
M. parryi + *M. parma*	95	99	1.00	1.00
M. parryi + *M. parma* + *M. agilis* + *M. eugenii*	71	62	0.97	0.96
M. rufogriseus + *M. parryi* + *M. parma* + *M. agilis* + *M. eugenii*	97	98	1.00	1.00

Tests of prior hypotheses

Results of the AU statistical tests are summarised in Table 2 and results of the KH and SH tests are given in Table S3 in an Accessory Publication on the *Australian Journal of Zoology* website. Five hypotheses were compared for the sister-group of the Macropodiformes. The AU test rejected a sister-group relationship between Macropodiformes and both the Phalangeridae and Vombatiformes, but all of the other test results were insignificant. The AU test favored a sister-group relationship of *Hypsiprymnodon* to all other macropodiforms. Of the five different hypotheses for the phylogenetic position of *Lagostrophus*, the AU test favoured a sister-group relationship between *Lagostrophus* and all other macropodids. Three hypotheses were compared for the placement of *Wallabia*. The

Table 2. Approximately unbiased (AU) test results

Phylogenetic hypothesis	−ln likelihood	Δ	*P*
AU			
(1) Macropodiformes (sister-group to)			
(a) Petauroidea[1, 21]	38977.32018	2.15051	0.434
(b) Phalangeriformes[9, 10, 17, 23]	38981.29327	6.12359	0.061
(c) Phalangeroidea (best)[10, 16, 20]	38975.16967		0.769
(d) Phalangeridae[6, 14, 18, 21]	38986.09205	10.92238	0.046*
(e) Vombatiformes	39051.59242	76.42275	2e-06*
(2) *Hypsiprymnodon* (sister-group to)			
(a) Macropodidae + Potoroidae (best)[7–9, 11–13, 21, 22, 24, 26]	38975.16967		1.000
(b) Potoroidae[2, 5, 6, 11, 19, 25]	39108.01044	132.84077	1e-43*
(3) *Lagostrophus* (sister group to)			
(a) *Lagorchestes*[4]	39096.55947	121.38979	2e-06*
(b) All other macropodids (best)[9, 15, 26]	38975.16967		0.970
(c) Potoroidae[5, 11]	38993.86712	18.69745	0.031*
(d) *Petrogale* + *Onychogalea* + *Lagorchestes* + *Peradorcas*[25]	39165.23317	190.06349	2e-33*
(e) *Petrogale* + *Peradorcas* + *Thylogale* + *Setonix* + *Lagorchestes* + *Onychogalea*[2, 3, 14]	39167.13122	191.96154	3e-06*
(4) *Wallabia* (sister group to)			
(a) *Macropus*[2, 8, 9, 11, 26]	38983.54295	8.37328	0.134
(b) *M. (Notomacropus)*	38975.65395	0.48428	0.511
(c) *M. (Notomacropus)* + *M.* (*Osphranter*) (best)	38975.16967		0.669

*$P < 0.05$; [1]Amrine-Madsen *et al*. 2003; [2]Archer 1984; [3]Asher *et al*. 2004; [4]Bensley 1903; [5]Baverstock *et al*. 1989; [6]Baverstock *et al*. 1990; [7]Burk *et al*. 1998; [8]Burk and Springer 2000; [9]Cardillo *et al*. 2004; [10]Flannery 1987; [11]Flannery 1989; [12]Kavanagh *et al*. 2004; [13]Kear *et al*. 2007; [14]Kirsch 1977; [15]Kirsch *et al*. 1997; [16]Meredith *et al*. 2008; [17]Marshall *et al*. 1990; [18]Munemasa *et al*. 2006; [19]Pearson 1950*a* (as summarised in Case 1984: fig. 3); [20]Phillips and Pratt 2008; [21]Osborne *et al*. 2002; [22]Raven and Gregory 1946; [23]Springer and Kirsch 1991; [24]Szalay 1994; [25]Tate 1948; [26]Westerman *et al*. 2002.

AU test could not distinguish between these or any of the remaining hypotheses.

Molecular dating

Figure 2 shows a timescale for kangaroo divergence based on the partitioned *Multidivtime* analysis. Point estimates along with 95% credibility intervals (*Multidivtime*) and 95% highest posterior densities (HPDs; BEAST) for all divergence date analyses are given in Table S4 in an Accessory Publication on the *Australian Journal of Zoology* website. BEAST statistics are summarised in Table S5 in an Accessory Publication on the *Australian Journal of Zoology* website. The mean estimate of the covariance statistic indicated that there was virtually no autocorrelation of rates for the non-partitioned (0.13–0.083) and partitioned (0.018–0.090) analyses. The coefficient of variation statistic suggests that *Rag1* (0.31–0.32) is evolving more clocklike than the other four genes followed by *BRCA1* (0.34), *vWF* (0.52–0.53), *IRBP* (0.65) and *ApoB* (0.70–0.77). On average, dates for the non-partitioned *Multidivtime* analysis were 1.65 million years older than dates for the *Multidivtime* partitioned analysis. Dates for the hard-bounded BEAST non-partitioned analysis were on average 0.18 million years older than the hard-bounded BEAST partitioned analysis. Dates for the soft-bounded BEAST partitioned analysis were on average 1.0 million years older than dates for the soft-bounded BEAST non-partitioned analysis. Dates for the partitioned soft-bounded BEAST analyses were on average 0.81 million years older than dates for the partitioned hard-bounded BEAST analyses. The mean date

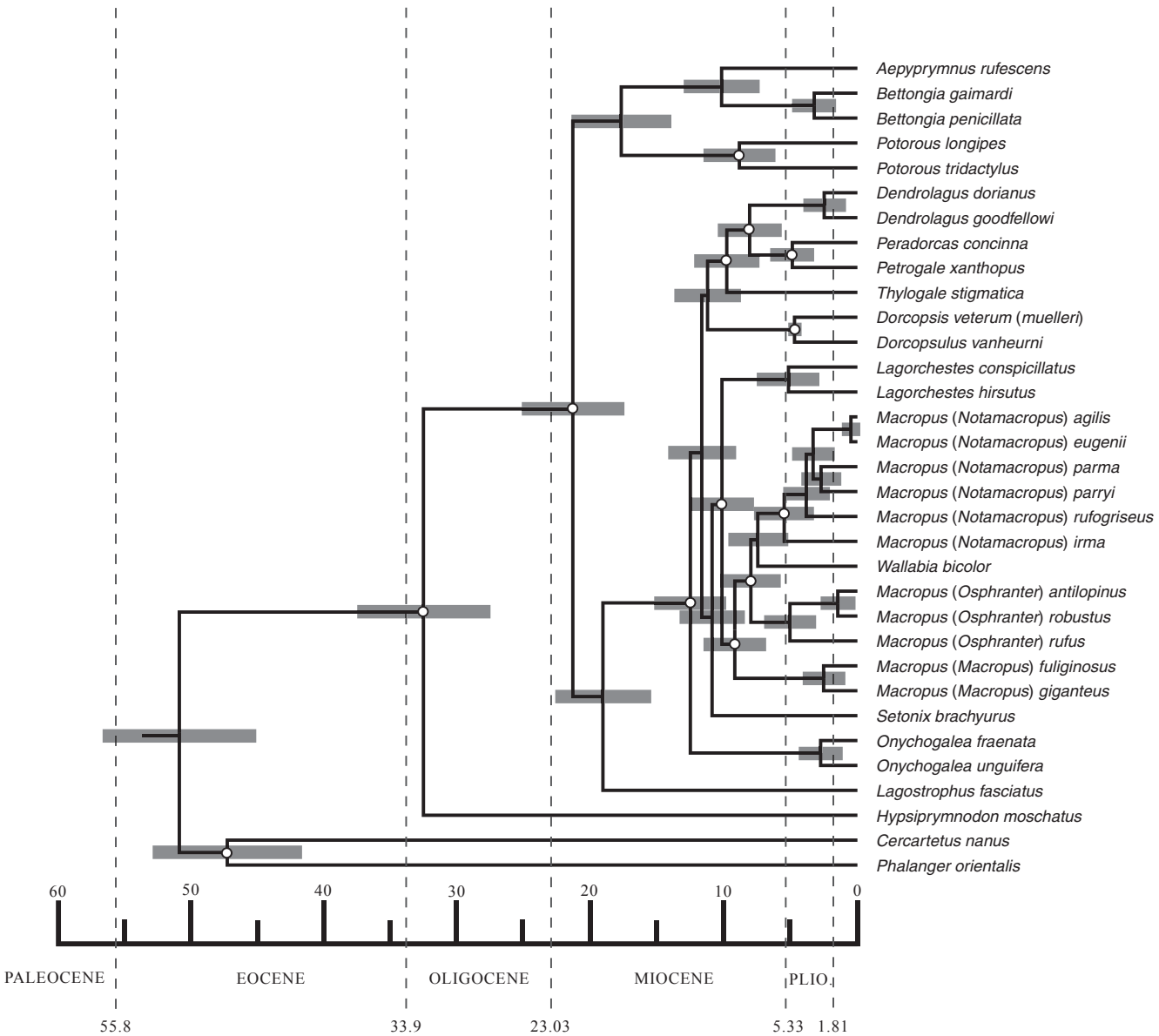

Fig. 2. Timeline in millions of years before the present for kangaroo diversification based on the *Multidivtime* partitioned analysis. Gray bars denote 95% credibility intervals; fossil constrained nodes are indicated with open circles. Plio. = Pliocene.

for the non-partitioned, hard-bounded BEAST analyses was 0.006 million years older than the mean date for the non-partitioned soft-bounded BEAST analyses.

All point estimates suggest Hypsiprymnodontidae diverged from Potoroidae and Macropodidae in the early Oligocene or latest Eocene. Potoroidae and Macropodidae split from one another in the early Miocene or latest Oligocene. Potoroids last shared a common ancestor in the Miocene with point estimates from different analyses ranging from 15.7 to 19.7 million years ago. The split between *Aepyprymnus* and *Bettongia* is also Miocene in age with point estimates ranging from 8.5 to 12.1 million years ago. The split between *Lagostrophus* and other macropodids is early Miocene in age (range of point estimates = 16.9–21.8 million years ago). The remaining macropodid genera diverged from each other in the middle Miocene, late Miocene and early Pliocene. Divergence times among the fourteen extant species of *Macropus* (*M.* (*Notamacropus*) (8 species), *M.* (*Osphranter*) (4 species) and *M.* (*Macropus*) (2 species)) are Plio-Pleistocene in age.

Ancestral state reconstruction of grades of dental organisation

Ancestral state reconstructions for grades of dental organisation are given in Table S6 in an Accessory Publication on the *Australian Journal of Zoology* website and shown in Fig. 3. Our ancestral state reconstructions for both the ordered and unordered analyses suggest that the potoroid and basal macropodoid grade is the ancestral condition for Macropodiformes, Potoroidae and Macropodidae + Potoroidae. The potoroid and basal macropodoid grade is retained in the potoroid lineages. However, in the ordered analyses, the potoroid and basal macropodoid grade was less likely (0.7404). In the unordered analyses, a browser appears to be the most likely ancestor to Macropodidae (0.7070), all macropodids exempting *Lagostrophus* (0.9585) and the macropodid node that additionally excludes *Onychogalea* (0.9994). In contrast, the ordered analyses suggest an intermediate browser/grazer was the most likely ancestor of all macropodids (0.5695) and all macropodids exempting *Lagostrophus* (0.7371). The browser grade is also reconstructed for the *Dorcopsis* + *Dorcopsulus* + *Thylogale* + *Petrogale* + *Peradorcas* + *Dendrolagus* clade in both the ordered and unordered analyses. In the latter clade, the browser grade is maintained throughout all of the constituent lineages. The ancestor of the *Setonix* + *Lagorchestes* + *Macropus* + *Wallabia* clade was most likely a browser (0.6557), but a grazer is also a possibility (0.3144) in the unordered analyses. In the ordered analyses, the former clade was almost equally likely to be either a browser (0.4915) or intermediate browser/grazer (0.5034). The ancestor of the *Lagorchestes* + *Macropus* + *Wallabia* clade was reconstructed as an intermediate browser/grazer (0.6001), but may have also been a grazer (0.3993) in the unordered analyses. The ordered analyses suggest the most likely ancestor of the *Lagorchestes* + *Macropus* + *Wallabia* clade was an intermediate browser/grazer (0.9976). The ancestors of *Macropus* + *Wallabia*, *M.* (*Osphranter*) + *Wallabia* + *Macropus* (*Notamacropus*), *M.* (*Osphranter*) and *M.* (*Macropus*) were most likely grazers in the unordered analyses. The ordered analyses give an almost equal probability that the ancestor of *Macropus* + *Wallabia* clade and the *M.* (*Osphranter*) + *Wallabia* + *Macropus* (*Notamacropus*) clade were either an intermediate browser/grazer or grazer. The ancestor of both the *Wallabia* + *Macropus* (*Notamacropus*) and *M.* (*Notamacropus*) clades was most likely an intermediate browser/grazer in both the ordered and unordered analyses.

Discussion

Phylogenetic relationships

Hypsiprymnodontidae

Our analysis corroborates Hypsiprymnodontidae as the sister-group to all other living macropodiforms. These results are consistent with previous mitochondrial and mitochondrial plus nuclear gene analyses (Burk *et al.* 1998; Burk and Springer 2000; Osborne *et al.* 2002; Westerman *et al.* 2002) and the morphological study of Szalay (1994). As noted earlier, Raven and Gregory (1946) also hypothesised that the basal split among kangaroos was between *Hypsiprymnodon* and other taxa. The placement of *Hypsiprymnodon* as the sister-group to all other living macropodiforms strongly supports the conclusions of Burk *et al.* (1998) that the sacculated stomach, reduction of litter size to one and bipedal hopping evolved only once after the divergence of *Hypsiprymnodon* from the other macropodiforms. Furthermore, morphological characters shared between *Hypsiprymnodon* and potoroids are likely plesiomorphic for macropodiforms or are the result of convergent evolution. Examples include the frontal squamosal contact, the deeply enlarged masseteric canal that is confluent with the dental canal, convexing of the dentary below the middle of the molar row, a steep molar gradient, an I_1 with ventrolateral enamel restriction and a ventral enamel flange, a finely serrated P_3, absence of the metaconid on M_2, a proximoventral process on the fifth metatarsal and vaginal and urinary bladder attributes (Burk *et al.* 1998 and references therein). As a corollary, features shared by other macropodiforms including all postcranial modifications (e.g. pedal and tarsal) necessary for bipedal hopping are in all likelihood shared derived features.

Potoroidae

Numerous molecular and morphological studies have suggested an association of *Bettongia* and *Aepyprymnus* to the exclusion of *Potorous* (e.g. Raven and Gregory 1946; Tate 1948; Flannery 1984, 1989; Bensley 1903; Szalay 1994; Burk *et al.* 1998; Burk and Springer 2000; Westerman *et al.* 2002). Morphological synapomorphies uniting *Aepyprymnus* and *Bettongia* include a post-glenoid process and a discrete ectotympanic process (Flannery 1989). Among previous molecular studies, Westerman *et al.* (2002) recovered robust bootstrap support for *Aepyprymnus* and *Bettongia* based on mitochondrial rRNA genes. Our study is the first molecular study based on nuclear genes to provide robust support for *Aepyprymnus* + *Bettongia*

Macropodidae

Lagostrophus

Westerman *et al.* (2002) also found that *Lagostrophus* was a distinct kangaroo lineage based on analyses of mitochondrial rRNA and nuclear protamine gene sequences. Nilsson's (2006)

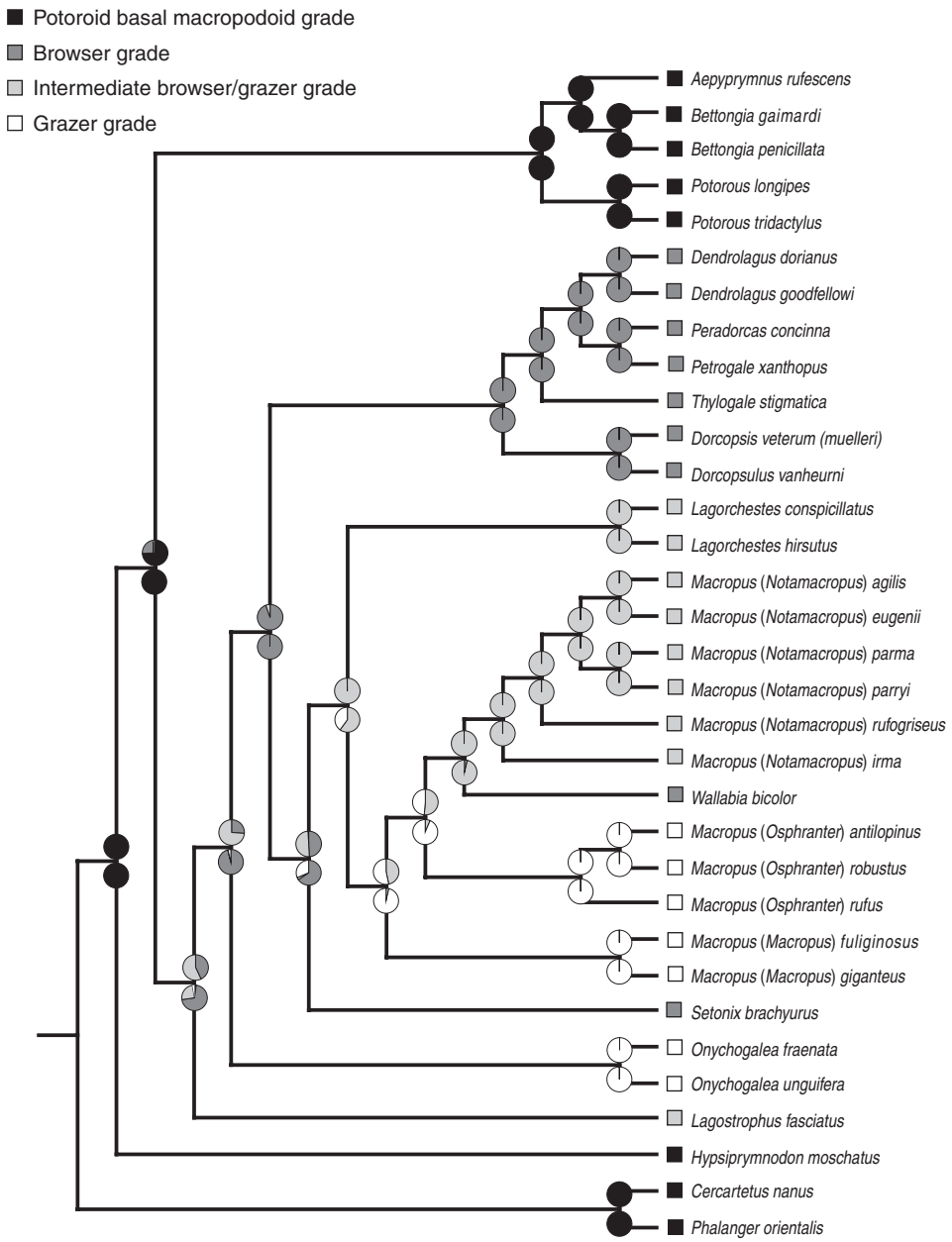

Fig. 3. Ancestral reconstructions of Sanson's (1989) grades of dental organisation obtained with SIMMAP. Posterior probability distributions for grades of dental organisation at each ancestral node are shown with pie graphs. Morphological priors used in this analysis were $\alpha = 3$ and $\beta = 2$; (also see text and in Table S6 in an Accessory Publication on the *Australian Journal of Zoology* website). Pie graphs above and below nodes refer to the ordered and unordered analyses, respectively. Posterior probabilities are conditional on having trees with the applicable clade.

analysis of complete mitochondrial genomes for three kangaroos found strong support for an association between *Lagostrophus* and the macropodid *Macropus robustus*. Our results are consistent with both Westerman *et al.* (2002) and Nilsson (2006), but unlike these studies, we found statistical support firmly establishing *Lagostrophus* as the sister-taxon to all other macropodids. These results are consistent with Flannery's (1983, 1989) hypothesis that *Lagostrophus* is a surviving sthenurine, but determining whether *Lagostrophus* is a sthenurine macropodid or is more closely related to macropodine macropodids must await isolation and sequencing of subfossil ancient sthenurine DNA.

Dorcopsis + Dorcopsulus *clade*

Almost all phylogenetic studies that have included *Dorcopsis* and *Dorcopsulus* have suggested that these two genera are sister-taxa (e.g. Flannery 1984, 1989; Baverstock *et al.* 1989, 1990; Szalay 1994; Burk *et al.* 1998; Burk and Springer 2000;

Westerman *et al.* 2002). Mitochondrial data (Burk and Springer 2000; Westerman *et al.* 2002) provide robust support for this conclusion. Our study adds robust support from a nuclear gene dataset in support of this hypothesis.

Dendrolagus + Peradorcas + Petrogale + Thylogale *clade*

Our study finds robust support for the association of *Peradorcas* and *Petrogale*, these two with *Dendrolagus* and these three with *Thylogale*. The grouping of these four genera was previously recovered in the MC'F studies of Baverstock *et al.* (1989, 1990), but this was considered surprising. Serology has suggested a close association between *Thylogale* and *Petrogale*, but only a distant relationship to *Dendrolagus* (Kirsch 1977). Kirsch *et al.* (1995, 1997) found a close association between *Thylogale*, *Petrogale* and *Dendrolagus* based on DNA hybridisation data. Kirsch *et al.* (1995) suggested that *Thylogale* and *Petrogale* were more closely related to one another based on the unique morphology of *Dendrolagus*, whereas Kirsch *et al.* (1997) recovered *Petrogale* and *Dendrolagus* as sister-taxa to the exclusion of *Thylogale*. Ziegler (1977) suggested that *Dendrolagus* evolved from rock inhabiting *Petrogale*-like species in New Guinea. Mitochondrial and protamine P_1 gene studies provide some support for *Petrogale* + *Peradorcas* and for a clade comprising these two and *Dendrolagus*, but not for an association of *Thylogale* with these three genera (Burk *et al.* 1998; Burk and Springer 2000; and Westerman *et al.* 2002).

Macropus *and* Wallabia

Unlike previous DNA studies (Burk *et al.* 1998; Burk and Springer 2000; Osborne *et al.* 2002; Westerman *et al.* 2002), we included most (11 of 13) of the extant *Macropus* species and representatives of all three subgenera (*M.* (*Macropus*), *M.* (*Osphranter*), *M.* (*Notamacropus*)). In the context of this expanded taxonomic sampling, we find strong support for the monophyly of each of the three *Macropus* subgenera.

Our results support the monophyly of *Macropus* + *Wallabia*. Previous studies supporting the monophyly of this group include whole sera immunology (Kirsch 1977), allozyme data (Richardson and McDermid 1978); morphology (Flannery 1984, 1989) and mitochondrial and combined mitochondrial plus protamine P_1 DNA sequences (Burk and Springer 2000; Westerman *et al.* 2002).

Flannery (1989) favoured a sister-group relationship between *Wallabia* and a monophyletic *Macropus*. Our results favour paraphyly of the genus *Macropus*, with *Wallabia* nested inside of this genus, possibly as the sister-taxon to *M.* (*Notamacropus*). Paraphyly of *Macropus* with *Wallabia* is consistent with the observation that *Wallabia* is known to hybridise with *M.* (*Notamacropus*) *agilis* (Van Gelder 1977). It is also interesting that Tate (1948) suggested a sister-group relationship between *Wallabia bicolor* and *Macropus* (*Notamacropus*) *agilis*, both of which were formerly included in the genus *Protemnodon* by Tate. Given that the majority of evidence favours *Macropus* paraphyly, with *Wallabia* as a distinct lineage within this clade, we recommend subsuming the genus *Wallabia* within *Macropus* as a new subgenus, i.e., *Macropus* (*Wallabia*).

Timeline for Macropodiformes evolution

Numerous kangaroo fossils are known from the late Oligocene and thereafter, but there are no fossils prior to the late Oligocene. Some genera (e.g., *Setonix*) do not have a fossil record prior to the Pleistocene. Given the limitations of the kangaroo fossil record, molecular estimates of divergence times are essential for understanding the evolution, timing and possible causes of the kangaroo radiation. All of our dating analyses, including the soft- and hard-bounded analyses, yielded similar results, suggesting that fossils are not in conflict with either themselves or the molecular data (Yang and Rannala 2006).

There has only been one previous study on kangaroos that estimated divergence times and included all genera (Westerman *et al.* 2002). Their point estimates suggested an age of 31–34 million years for the base of Macropodiformes, 24 million years for the potoroid–macropodid split, 21–22 million years for the base of Potoroidae, 19–20 million years for the separation of *Lagostrophus* and other macropodids and 10–11 million years for the last common ancestor of all macropodids, exclusive of *Lagostrophus*. We obtained similar point estimates for the base of Macropodiformes (28–35 million years), the potoroid–macropodid split (20–24 million years), the base of Potoroidae (16–20 million years), the separation of *Lagostrophus* and other macropodids (17–22 million years) and the last common ancestor of all macropodids exclusive of *Lagostrophus* (11–15 million years).

Case (1989) suggested that the replacement of podocarp-dominated forests by more diverse *Nothofagus*-dominated forests during the late Eocene led to the diversification of the arboreal possums. Furthermore, this would also have been an impetus for the diversification of the terrestrial marsupial fauna. It can also be surmised that the hallmark characteristic of kangaroos, the bipedal hop, did not evolve until there was a suitable habitat that favoured the evolution of this mode of locomotion. This habitat emerged some time during the Oligocene with the disappearance of the rainforests in central Australia. Our molecular data are consistent with this hypothesis. We obtained point estimates in the range of 41–54 million years for the split between Macropodiformes and Phalangeroidea. Hypsiprymnodontidae subsequently diverged from other kangaroos approximately 28 to 35 million years ago, coinciding with the opening up of the rainforest canopy. The oldest post-Eocene Australasian marsupial bearing deposits are late Oligocene or early Miocene in age. The oldest purported crown-group kangaroo is "*Kyeema*" from Zone A of the Etadunna Formation (~25.5 million years; Woodburne *et al.* 1993). Case (pers. comm. in Woodburne *et al.* 1993) suggests potoroid affinities for *Kyeema*. Our point estimates for the macropodid–potoroid divergence are younger than *Kyeema* and suggest that this taxon is more likely a stem taxon on the branch leading to Macropodidae + Potoroidae rather than a potoroid.

Australia continued to "dry out" through the mid Miocene into the late Pliocene. Potoroidae and Macropodidae split from one another in the early Miocene (20–24 million years ago). By this time rainforests were no longer the predominant vegetation over most of Australia (Megirian *et al.* 2004; Martin 2006). Diversification among crown taxa in both Potoroidae and Macropodidae commenced in the range of 16–22 million years ago. This time interval is characterised by the first signs of

aridification and the establishment of a dry season (Martin 2006). The most spectacular radiation occurred within Macropodidae starting about 12 million years ago. This time period coincides with a cooler and drier environment and a major contraction of the rainforests (Martin 2006). By about 5 million years ago, all lineages leading to the living macropodid genera and *Macropus* subgenera were established. This period of diversification among macropodids was concurrent with the spread of grasslands throughout Australia (Martin 2006). Within *Macropus*, the latest divergences within the *M.* (*Notamacropus*) and *M.* (*Osphranter*) species can be correlated to the establishment of the modern climatic regime (Martin 2006).

Grades of dental organisation

Sanson (1989) hypothesised that the four grades of dental organisation ((1) potoroid and basal macropodoid grade; (2) browser grade; (3) intermediate browser/grazer grade; and (4) grazer grade) represent a progression in complexity associated with the evolution of dental features that effectively process browse and then grass. Sanson (1989) also suggested that the character complexes describing these grades of dental organisation evolved convergently in different lineages. If we tie together our estimated dates of divergence and ancestral-state reconstructions for grades of dental organisation, we arrive at an improved understanding of when key character state changes took place as well as possible reasons for these changes. It is clear that the potoroid and basal macropodoid grade was the ancestral condition for macropodiforms in the early Oligocene. This grade has persisted in living Potoroidae and Hypsiprymnodontidae, presumably because these taxa occupy niches that are similar to those that were occupied by their ancestors. About 11–15 million years ago, browsers appeared. The majority of Australia at this time period was probably covered in sclerophyll woodlands and sedgelands (Martin 2006). The intermediate browser/grazer and grazer grades appear in conjunction with the spread of grasslands.

We recovered *Onychogalea* (a grazer) as the sister-group to all other macropodids (excepting *Lagostrophus*, which branched off earlier). Grazing appears to have evolved independently in *Onychogalea* and at the base of the *Macropus* + *Wallabia* clade, with a reversion back to the intermediate browser/grazer grade occurring in the *Wallabia* + *Macropus* (*Notamacropus*) clade. Our data also suggest that the intermediate browser/grazer grade evolved on three separate occasions: in the *Lagostrophus* lineage; in the ancestor of the *Lagorchestes* + *Wallabia* + *Macropus* clade; and in the *Wallabia* + *Macropus* (*Notamacropus*) clade. Therefore, grades of dental organisation appear to be pliable within Macropodiformes. However, statistical tests were unable to distinguish between the hypotheses that included our best-partitioned Bayesian tree and the monophyly of both browsers and grazer. Our results need to be verified with expanded taxon sampling (i.e., more kangaroo species – especially the remaining *Macropus* species) and more robust molecular phylogenies. The addition of fossil taxa may also help to resolve the evolution of the grades of dental organisation.

Character evolution

Burk *et al.* (1998) and Burk and Springer (2000) reviewed morphological character evolution within Macropodiformes in the context of molecular phylogenies that associated potoroids and macropodids to the exclusion of *Hypsiprymnodon*. These authors concluded that morphological features associated with bipedal hopping (e.g. loss of hallux, loss of prehensile tail, enlarged hindlimbs), reduction of litter size to one and a sacculated forestomach evolved only once – in the common ancestor of potoroids and macropodids. The molecular phylogenies presented here, which are based on an independent set of five nuclear genes, also support a sister-group relationship between potoroids and macropodids and thereby provide additional support for the patterns of character evolution discussed by Burk *et al.* (1998) and Burk and Springer (2000). In addition, our relaxed clock timescale and recent fossil discoveries (Kear *et al.* 2007) provide additional information for understanding the evolution of morphological and life history characters in kangaroo evolution.

Kear *et al.* (2007) described a new species of Balbaridae (*Nambaroo gillespieae*) from late Oligocene deposits of Riversleigh. Specimens from this species include the first definitive postcranial material assignable to this (sub)family. Kear *et al.*'s (2007) phylogenetic analyses suggest the balbarids are the sister-group (along with *Ekaltadeta*) to all other kangaroos with *N. gillespieae* sister to all other balbarids. Kear *et al.*'s (2007) morphological cladistic results agree with our molecular results insofar as they support a sister-group relationship between living potoroids and macropodids to the exclusion of *Hypsiprymnodon*. Kear *et al.*'s (2007) analysis of postcranial material suggests that *N. gillespieae* may have had adaptations towards hopping, but probably used the tail in a quadrupedal gait with bounding or pentapedal locomotion. This type of locomotion is used by taxa such as *Dorcopsis*. However, *Dorcopsis* uses the bipedal hop as its fast gait and *N. gillespieae* probably did not (Kear *et al.* 2007). Kear *et al.* (2007) also suggest that *N. gillespieae* may even have been partly arboreal, given the similarities in postcranial morphology shared with the arboreal *Dendrolagus*. One would expect stem kangaroos to be at least partly arboreal given their shared ancestry with arboreal phalangeroid possums. Kear *et al.*'s (2007) finding that the Balbaridae are the sister-group to all other kangaroos also supports the hypothesis that true bipedal hopping evolved only once on the lineage leading to the common ancestor potoroids and macropodids.

Hypsiprymnodon shares with other kangaroos some morphological characters associated with the transition to terrestrial locomotion. These include a stepped calcaneocuboid joint, an elongated hind foot and aspects of the transverse tarsal joint. Our results suggest that these features evolved after macropodiforms diverged from phalangeroids, but before *Hypsiprymnodon* split from other kangaroos. The length of the time interval during which these features were acquired ranges from 13 to 20 million years in different analyses that we performed. The hind foot of *Nambaroo gillespieae* is potentially informative for elucidating the sequence of character transformations on the stem macropodiform branch. *N. gillespieae* shares the primitive condition of retaining the hallux with *Hypsiprymnodon* and phalangeroid outgroups. *N. gillespieae* is more primitive than *Hypsiprymnodon* in retaining a short pes. Finally, *N. gillespieae* and *Hypsiprymnodon* are both more derived than outgroup phalangeroids in possessing a stepped calcaneocuboid joint, which is a presumed adaptation to restrict movement along the

Table 3. Morphological characters related to locomotion (modified from Burk and Springer 2000)

Character	Taxa				References
	Nambaroo gillespieae	*Hypsiprymnodon*	Macropodidae	*Dendrolagus*	
(1) Absence of hallux	No	No	Yes	Yes	Szalay 1994; Kear *et al*. 2007
(2) Absence of digital pads	?	No	Yes	Yes	Flannery 1989; Kear *et al*. 2007
(3) Reduced digits on 2nd and 3rd digits of foot	No	No	Yes	Yes	Szalay 1994; Kear *et al*. 2007
(4) Absence of prehensile tail	No	No	Yes	Yes	Szalay 1994; Flannery *et al*. 1995; Kear *et al*. 2007
(5) Hindlimbs much longer than forelimbs	No	No	Yes	No	Johnson and Strahan 1982; Flannery 1989; Szalay 1994; Kear *et al*. 2007
(6) Tibial–fibular facet lengthened	No	No	Yes	No	Flannery and Szalay 1982; Kear *et al*. 2007
(7) Strongly stepped calcaneocuboid facet	Yes	Yes	Yes	No	Flannery and Szalay 1982; Szalay 1994; Kear *et al*. 2007
(8) Elongated foot	No	Yes	Yes	No	Szalay 1994; Kear *et al*. 2007
(9) Flexion–extension of transverse lateral joint	Possible lateral movement in distal tarsal elements	Yes	Yes	No: medio-lateral articulation	Szalay 1994; Kear *et al*. 2007

longitudinal axis of the foot (Bishop 1997). Thus, the stepped calcaneocuboid joint was probably acquired before the pes was lengthened on the branch leading to crown-group kangaroos.

Subsequent to the separation of *Hypsiprymnodon* from the macropodid–potoroid lineage, features associated with bipedal hopping, reduction of litter size to one and a sacculated forestomach evolved over a period of 9–11 million years. As Australia began to become more and more arid, there was an increase in low nutrient foods as the grasslands spread over Australia. *Hypsiprymnodon* is unique among extant kangaroos in having a simple stomach that is more similar to the phalangerids. All other kangaroos have a more complex stomach that can be divided into three parts: sacciform portion of forestomach; tubiform portion of forestomach; and hindstomach (from anterior to posterior; Hume 1982). The forestomach is the fermentation chamber (Dawson 1989). In large macropodids the sacciform forestomach is reduced and the tubiform forestomach is greatly enlarged. Freudenberger *et al.* (1989) suggest that this modification may be a further adaptation to grassland and arid-zone herbivory in larger kangaroos.

Whereas we recovered robust statistical support for an association of *Dendrolagus* with *Petrogale* (and *Peradorcas*) and of this group with *Thylogale*, Kear *et al.* (2007) recovered *Dendrolagus* as the basal living taxon among macropodids. *Petrogale*, in turn, grouped with *Macropus* and *Wallabia*. The basal placement of *Dendrolagus* among living macropodids may be a consequence of character reversals in this genus given that tree kangaroos have readapted to an arboreal or semi-arboreal lifestyle, possibly occupying a niche that resembles that which was occupied by the common ancestor of the Macropodiformes. Morphological features of *Dendrolagus* that may be secondarily primitive include enlargement of the forelimbs, the lengthening of the tibial–fibular contact and reduction in the elongation of the foot (Table 3). Given the possibility that all of these features may be correlated with secondary adaptation to an arboreal habitat, it is not surprising that Kear *et al.*'s (2007) analysis recovered *Dendrolagus* as the most primitive member of the living Macropodiformes. Vestibular labyrinth morphology also suggests that *Dendrolagus* is secondarily adapted for arboreality (Schmelzle *et al.* 2007). This finding also highlights the problem of separating homology from homoplasy in morphological cladistic analyses (Springer *et al.* 2007).

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References

Amrine-Madsen, H., Scally, M., Westerman, M., Stanhope, M. J., Krajewski, C., and Springer, M. S. (2003). Nuclear gene sequences provide evidence for the monophyly of australidelphian marsupials. *Molecular Phylogenetics and Evolution* **28**, 186–196. doi: 10.1016/S1055-7903(03)00122-2

Aplin, K., and Archer, M. (1987). Recent advances in marsupial systematics with a new syncretic classification. In 'Possums and Opossums: Studies in Evolution'. (Ed. M. Archer.) pp. xv–lxxii. (Surrey Beatty & Sons: Chipping Norton.)

Archer, M. (1984). The Australian marsupial radiation. In 'Vertebrate Zoogeography and Evolution in Australasia'. (Eds M. Archer and G. Clayton.) pp. 633–808. (Hesperian Press: Perth.)

Archer, M., Arena, R., Bassarova, M., Black, K., and Brammall, J., *et al.* (1999). The evolutionary history and diversity of Australian mammals. *Australian Mammalogy* **21**, 1–45.

Archer, M., Arena, D. A., Bassarova, M., Beck, R., and Black, K. *et al.* (2006). Current status of species-level representation in faunas from selected fossil localities in the Riversleigh World Heritage Area, northwestern Queensland. *Alcheringa Special Issue* **1**, 1–17.

Asher, R. J., Horovitz, I., and Sanchez-Villagra, M. R. (2004). First combined cladistic analysis of marsupial interrelationships. *Molecular Phylogenetics and Evolution* **33**, 240–250. doi: 10.1016/j.ympev.2004.05.004

Baker, M. L., Wares, J. P., Harrison, G. A., and Miller, R. D. (2004). Relationships among the families and orders of marsupials and the major mammalian lineages based on recombination activating gene-1. *Journal of Mammalian Evolution* **11**, 1–16. doi: 10.1023/B:JOMM.0000029143.39776.ec

Baverstock, P. R., Richardson, B. J., Birrell, J., and Krieg, M. (1989). Albumin immunologic relationships of the Macropodidae (Marsupialia). *Systematic Biology* **38**, 38–50.

Baverstock, P. R., Krieg, M., and Birrell, J. (1990). Evolutionary relationships of Australian marsupials as assessed by albumin immunology. In 'Mammals from Pouches and Eggs: Genetics, Breeding and Evolution of Marsupials and Monotremes'. (Eds J. A. M. Graves, R. M. Hope and D. Cooper.) pp. 131–145. (CSIRO: Melbourne.)

Beck, R., Godthelp, H., Weisbecker, V., Archer, M., and Hand, S. J. (2008). Australia's oldest marsupial fossils and their biogeographical implications. *PLoS ONE* **3**, e1858. doi: 10.1371/journal.pone.0001858

Bensley, B. A. (1903). On the evolution of the Australian Marsupialia: with remarks on the relationships of marsupials in general. *Transactions of the Linnean Society London* **9**, 83–217.

Benton, M. J., and Donoghue, P. C. J. (2007). Paleontological evidence to date the tree of life. *Molecular Biology and Evolution* **24**, 26–53. doi: 10.1093/molbev/msl150

Bishop, N. (1997). Functional anatomy of the macropodid pes. *Proceedings of the Linnean Society of New South Wales* **117**, 17–50.

Bollback, J. P. (2006). SIMMAP: stochastic character mapping of discrete traits on phylogenies. *BMC Bioinformatics* **7**, 88. doi: 10.1186/1471-2105-7-88

Burk, A., and Springer, M. S. (2000). Intergeneric relationships among Macropodoidea (Metatheria: Diprotodontia) and the chronicle of kangaroo evolution. *Journal of Mammalian Evolution* **7**, 213–237. doi: 10.1023/A:1009488431055

Burk, A., Westerman, M., and Springer, M. S. (1998). The phylogenetic position of the musky rat-kangaroo and the evolution of bipedal hopping in kangaroos (Macropodidae: Diprotodontia). *Systematic Biology* **47**, 457–474. doi: 10.1080/106351598260824

Cardillo, M., Bininda-Edmonds, O. R. P., Boakes, E., and Purvis, A. (2004). A species-level phylogenetic supertree of marsupials. *Journal of Zoology* **264**, 11–31. doi: 10.1017/S0952836904005539

Case, J. A. (1984). A new genus of Potoroinae (Marsupialia: Macropodidae) from the Miocene Ngapakaldi Local Fauna, South Australia, and a definition of the Potoroinae. *Journal of Paleontology* **58**, 1074–1086.

Case, J. A. (1989). Antarctica: the effect of high latitude heterochroneity on the origin of the Australian marsupials. In 'Origins and Evolution of the Antarctic Biota'. (Ed. J. A. Crame.) pp. 217–226. Geological Society of America Special Publication 47.

Dawson, T. J. (1989). Diets of macropodoid marsupials: general patterns and environmental influences. In 'Kangaroos, Wallabies and Rat-kangaroos'. (Eds. G. Grigg, P. Jarman and I. Hume.) pp. 129–142. (Surrey Beatty & Sons: Chipping Norton.)

Dawson, L., and Flannery, T. (1985). Taxonomic and phylogenetic status of living and fossil kangaroos and wallabies of the genus *Macropus* Shaw (Macropodidae: Marsupialia), with a new subgeneric name for the larger wallabies. *Australian Journal of Zoology* **33**, 473–498. doi: 10.1071/ZO9850473

De Queiroz, A. (1993). For consensus sometimes. *Systematic Biology* **42**, 368–372. doi: 10.2307/2992470

Drummond, A. J., and Rambaut, A. (2007). BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* **7**, 214. doi: 10.1186/1471-2148-7-214

Drummond, A. J., Ho, S. Y. W., Phillips, M. J., and Rambaut, A. (2006). Relaxed phylogenetics and dating with confidence. *PLoS Biology* **4**, e88. doi: 10.1371/journal.pbio.0040088

Eisenberg, J. F. (1981). 'The Mammalian Radiations.' (University of Chicago Press: Chicago, IL.)

Farris, J. S., Kallersjo, M., Kluge, A. G., and Bult, C. (1994). Testing significance of incongruence. *Cladistics* **10**, 315–319. doi: 10.1111/j.1096-0031.1994.tb00181.x

Flannery, T. F. (1983). Revision in the subfamily Sthenurinae (Marsupialia: Macropodoidea) and the relationships of the species of *Troposodon* and *Lagostrophus*. *Australian Mammalogy* **6**, 15–28.

Flannery, T. F. (1984). Kangaroos: 15 million years of Australian bounders. In 'Vertebrate Zoogeography and Evolution in Australasia'. (Eds M. Archer and G. Clayton.) pp. 817–836. (Hesperian Press: Carlisle.)

Flannery, T. F. (1987). The relationships of the macropodoids (Marsupialia) and the polarity of some morphological features within Phalangeriformes. In 'Possums and Opossums: Studies in Evolution'. (Ed. M. Archer) pp. 741–747. (Surrey Beatty & Sons: Chipping Norton.)

Flannery, T. F. (1989). Phylogeny of the Macropodoidea: a study in convergence. In 'Kangaroos, Wallabies and Rat-kangaroos'. (Eds G. Grigg, P. Jarman and I. Hume.) pp. 1–46. (Surrey Beatty & Sons: Chipping Norton.)

Flannery, T. F., and Rich, T. H. R. (1986). Macropodoids of the middle Miocene Namba Formation, South Australia, and the homology of some dental structures in kangaroos. *Journal of Paleontology* **60**, 418–447.

Flannery, T. F., and Szalay, F. S. (1982). *Bohra paulae*, a new giant fossil tree-kangaroo (Marsupialia: Macropodidae) from New South Wales, Australia. *Australian Mammalogy* **5**, 38–54.

Flannery, T. F., Boeadi, and Szalay, A. L. (1995). A new tree-kangaroo (*Dendrolagus*: Marsupialia) from Irian Jaya, Indonesia, with notes on ethnography and the evolution of tree-kangaroos. *Mammalia* **59**, 65–84.

Freudenberger, D. O., Wallis, I. R., and Hume, I. D. (1989). Phylogeny of the Macropodoidea: a study in convergence. In 'Kangaroos, Wallabies and Rat-kangaroos'. (Eds G. Grigg, P. Jarman and I. Hume.) pp. 1–46. (Surrey Beatty & Sons: Chipping Norton.)

Godthelp, H., Wroe, S., and Archer, M. (1999). A new marsupial from the early Eocene Tingamarra Local Fauna of Murgon, southeastern Queensland: A prototypical Australian marsupial? *Journal of Mammalian Evolution* **6**, 289–313. doi: 10.1023/A:1020517808869

Gould, J. (1842). On two new species of kangaroo (*Petrogale concinna* and *Halmaturus binog*). *Proceedings of the Zoological Society of London* **10**, 57–58.

Gradstein, F. M., Ogg, J. G., and Smith, A. G. (2004). 'A Geologic Timescale.' (Cambridge University Press: Cambridge.)

Guindon, S., and Gascuel, O. (2003). A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* **52**, 696–704. doi: 10.1080/10635150390235520

Hedges, S. B., and Kumar, S. (2004). Precision of molecular time estimates. *Trends in Genetics* **20**, 242–247. doi: 10.1016/j.tig.2004.03.004

Huelsenbeck, J. P., and Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**, 754–755. doi: 10.1093/bioinformatics/17.8.754

Hume, I. D. (1982). 'Digestive Physiology and Nutrition of Marsupials.' (Cambridge University Press: New York.)

Johnson, P. M., and Strahan, R. (1982). A further description of the musky rat-kangaroo, *Hypsiprymnodon moschatus* Ramsay, 1876 (Marsupialia: Potoroidae), with notes on its biology. *Australian Journal of Zoology* **21**, 28–46.

Kavanagh, J. R., Burk, A., Westerman, M., and Springer, M. S. (2004). Relationships among families of Diprotodontia (Marsupialia) and the phylogenetic position of the autapomorphic honey possum (*Tarsipes rostratus*). *Journal of Mammalian Evolution* **11**, 207–222. doi: 10.1023/B:JOMM.0000047338.85206.57

Kear, B. P., Cooke, B. N., Archer, M., and Flannery, T. F. (2007). Implications of a new species of the Oligo-Miocene kangaroo (Marsupialia: Macropodoidea) *Nambaroo*, from the Riversleigh World heritage Area, Queensland, Australia. *Journal of Paleontology* **81**, 1147–1167. doi: 10.1666/04-218.1

Kirsch, J. A. W. (1977). The comparative serology of Marsupialia and a classification of marsupials. *Australian Journal of Zoology Supplemental Series* **52**, 1–152.

Kirsch, J. A. W., Lapointe, F.-J., and Foeste, A. (1995). Resolution of portions of the kangaroo phylogeny (Marsupialia: Macropodidae) using DNA hybridisation. *Biological Journal of the Linnean Society. Linnean Society of London* **55**, 309–328. doi: 10.1111/j.1095-8312.1995.tb01068.x

Kirsch, J. A. W., Lapointe, F.-J., and Springer, M. S. (1997). DNA-hybridisation studies of marsupials and their implications for metatherian classification. *Australian Journal of Zoology* **45**, 211–280. doi: 10.1071/ZO96030

Kishino, H., and Hasegawa, M. (1989). Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data and the branching order in Hominoidea. *Journal of Molecular Evolution* **29**, 170–179. doi: 10.1007/BF02100115

Kishino, H., Thorne, J. L., and Bruno, W. J. (2001). Performance of a divergence time estimation method under a probabilistic model of rate evolution. *Molecular Biology and Evolution* **18**, 352–361.

Langer, P. (1979). Functional anatomy and ontogenetic development of the stomach in the macropodine species *Thylogale stigmatica* and *Thylogale thetis* (Mammalia: Marsupialia). *Zoomorphologie* **93**, 137–151. doi: 10.1007/BF00994127

Langer, P. (1980). Anatomy of the stomach in three specie s of Potoroinae (Marsupialia: Macropodidae). *Australian Journal of Zoology* **28**, 19–32. doi: 10.1071/ZO9800019

Löytynoja, A., and Milinkovitch, M. C. (2001). SOAP, cleaning multiple alignments from unstable blocks. *Bioinformatics* **17**, 573–574. doi: 10.1093/bioinformatics/17.6.573

Marshall, L. G., Case, J. A., and Woodburne, M. O. W. (1990). Phylogenetic relationships of the families of marsupials. In 'Current Mammalogy Vol. 2'. (Ed. H. Genoways.) pp. 433–506. (Plenum Press: New York.)

Martin, H. A. (2006). Cenozoic climatic change and the development of the arid vegetation in *Australia*. *Journal of Arid Environments* **66**, 533–563. doi: 10.1016/j.jaridenv.2006.01.009

Megirian, D., Murray, P., Schwartz, L., and Von Der Borch, C. (2004). Late Oligocene Kangaroo Well Local Fauna from Ulta Limestone (new name), and climate of Miocene oscillation across central Australia. *Australian Journal of Earth Sciences* **51**, 701–741. doi: 10.1111/j.1400-0952.2004.01085.x

Meredith, R. W., Westerman, M., Case, J. A., and Springer, M. S. (2008). A phylogeny and timescale for marsupial evolution based on sequences for five nuclear genes. *Journal of Mammalian Evolution* **15**, 1–36. doi: 10.1007/s10914-007-9062-6

Meredith, R. W., Krajewski, C., Westerman, M., and Springer, M. S. (In press). Relationships and divergence times among the orders and families of Marsupialia. *Museum of Northern Arizona Bulletin.*

Müller, J., and Reisz, R. R. (2005). Four well-constrained calibration points from the vertebrate fossil record for molecular clock estimates. *BioEssays* **27**, 1069–1075. doi: 10.1002/bies.20286

Munemasa, M., Nikaido, M., Donnellan, S., Austin, C. C., Okada, N., and Hasegawa, M. (2006). Phylogenetic analysis of diprotodontian marsupials based on complete mitochondrial genomes. *Genes & Genetic Systems* **81**, 181–191. doi: 10.1266/ggs.81.181

Murray, P. F. (1991). The sthenurine affinity of the late Miocene kangaroo, *Hadronomas puckridgi* Woodburne (Marsupialia, Macropodidae). *Alcheringa* **15**, 255–283. doi: 10.1080/03115519108619023

Murray, P. F. (1995). The postcranial skeleton of the Miocene kangaroo, *Hadronomas puckridgi* Woodburne (Marsupialia, Macropodidae). *Alcheringa* **19**, 119–170. doi: 10.1080/03115519508619271

Nielsen, R. (2002). Mapping mutations on phylogenies. *Systematic Biology* **51**, 729–739. doi: 10.1080/10635150290102393

Nilsson, M. A. (2006). Phylogenetic relationships of the banded hare wallaby (*Lagostrophus fasciatus*) and a map of the kangaroo mitochondrial control region. *Zoologica Scripta* **35**, 387–393. doi: 10.1111/j.1463-6409.2006.00237.x

Osborne, M. J., Christidis, L., and Norman, J. A. (2002). Molecular phylogenetics of the Diprotodontia (kangaroos, wombats, koala, possums, and allies). *Molecular Phylogenetics and Evolution* **25**, 219–228. doi: 10.1016/S1055-7903(02)00252-X

Pearson, J. (1946). The affinities of rat-kangaroos (Marsupialia) as revealed by a comparative study of female urogenital system. *Papers and Proceedings of the Royal Society of Tasmania* **1945**, 13–25.

Pearson, J. (1950*a*). A further note on the females urogenital system of *Hypsiprymnodon moschatus* (Marsupialia). *Papers and Proceedings of the Royal Society of Tasmania* **1949**, 203–209.

Pearson, J. (1950*b*). The relationships of the Potoroidae to the Macropodidae (Marsupialia). *Papers and Proceedings of the Royal Society of Tasmania* **1949**, 211–229.

Phillips, M. J., and Pratt, R. C. (2008). Family-level relationships among Australian marsupial "herbivores" (Diprotodontia: koala, wombats, kangaroos, and possums). *Molecular Phylogenetics and Evolution* **46**, 594–605. doi: 10.1016/j.ympev.2007.09.008

Phillips, M. J., McLenachan, P. A., Down, C., Gibb, G. C., and Penny, D. (2006). Combined mitochondrial and nuclear DNA sequences resolve the interrelations of the major Australasian marsupial radiations. *Systematic Biology* **55**, 122–137. doi: 10.1080/10635150500481614

Posada, D., and Crandall, K. A. (1998). MODELTEST: Testing the model of DNA substitution. *Bioinformatics* **14**, 817–818. doi: 10.1093/bioinformatics/14.9.817

Prideaux, G. (2004). Systematics and evolution of the sthenurine kangaroos. *University of California Publications in Geological Sciences* **146**.

Rambaut, A. (1996). Se-Al: Sequence Alignment Editor. Available at http://evolve.zoo.ox.ac.uk/ [Accessed August 2006].

Rambaut, A., and Drummond, A. J. (2003). Tracer version 1.2 [computer program]. Available: http://evolve.zoo.ox.ac.uk [Accessed August 2006].

Rambaut, A. J., and Drummond, A. (2007). BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* **7**, 214.

Raven, H. C., and Gregory, W. K. (1946). Adaptive branching of the kangaroo family in relation to habitat. *American Museum Novitates* **1309**, 1–33.

Reisz, R. R., and Müller, J. (2004). Molecular timescales and the fossil record: a paleontological perspective. *Trends in Genetics* **20**, 237–241. doi: 10.1016/j.tig.2004.03.007

Richardson, B. J., and McDermid, E. M. (1978). A comparison of genetic relationships within the Macropodidae as determined from allozyme, cytological and immunological data. *Australian Mammalogy* **2**, 43–52.

Ride, W. D. L. (1964). A review of Australian fossil marsupials. *Journal of the Royal Society of Western Australia* **47**, 97–131.

Ride, W. D. L. (1993). *Jackmahoneya* gen. nov. and the genesis of the macropodiform molar. *Memoirs of the Association of Australian Palaeontologists* **15**, 441–459.

Ronquist, F., and Huelsenbeck, J. P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**, 1572–1574. doi: 10.1093/bioinformatics/btg180

Sanson, G. D. (1989). Morphological adaptations of teeth to diets and feeding in the Macropodoidea. In 'Kangaroos, Wallabies and Rat-kangaroos'. (Eds G. Grigg, P. Jarman and I. Hume.) pp. 151–168. (Surrey Beatty & Sons: Chipping Norton, New South Wales.)

Schmelzle, T., Sánchez-Villagra, M. R., and Maier, W. (2007). Vestibular labyrinth diversity in diprotodontian marsupials. *Mammal Study* **32**, 83–97. doi: 10.3106/1348-6160(2007)32[83:VLDIDM]2.0.CO;2

Shimodaira, H. (2002). An approximately unbiased test of phylogenetic tree selection. *Systematic Biology* **51**, 492–508. doi: 10.1080/10635150290069913

Shimodaira, H., and Hasegawa, M. (1999). Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Molecular Biology and Evolution* **16**, 1114–1116.

Springer, M. S., and Kirsch, J. A. W. (1991). DNA hybridization, the compression effect and the radiation of diprotodontian mammals. *Systematic Zoology* **40**, 131–151. doi: 10.2307/2992253

Springer, M. S., Kirsch, J. A. W., and Case, J. A. (1997). The chronicle of marsupial evolution. In 'Molecular Evolution and Adaptive Radiation'. (Eds. T. J. Givnish, and K. J. Sytsma.) pp. 129–161. (Cambridge University Press: New York.)

Springer, M. S., Burk-Herrick, A., Meredith, R., Eizirik, E., Teeling, E., O'Brien, S. J., and Murphy, W. (2007). The adequacy of morphology for reconstructing the early history of placental mammals. *Systematic Biology* **56**, 673–684. doi: 10.1080/10635150701491149

Stamatakis, A. (2006). RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22**, 2688–2690. doi: 10.1093/bioinformatics/btl446

Swofford, D. L. (2002). 'PAUP*. Phylogenetic analysis using parsimony (*and other methods), Version 4.' (Sinauer Associates, Sunderland, MA.)

Szalay, F. S. (1994). 'Evolutionary History of the Marsupials and an Analysis of Osteological Characters.' (Cambridge University Press: New York.)

Tate, G. H. H. (1948). Results of the Archibald Expeditions. No. 59. Studies on the anatomy and phylogeny of the Macropodidae (Marsupialia). *Bulletin of the American Museum of Natural History* **91**, 233–251.

Teeling, E. C., Madsen, O., Van Den Bussche, R. A., de Jong, W. W., Stanhope, M. J., and Springer, M. S. (2002). Microbat paraphyly and the convergent evolution of a key innovation in Old World rhinolophoid microbats. *Proceedings of the National Academy of Sciences of the United States of America* **99**, 1431–1436. doi: 10.1073/pnas.022477199

Thomas, O. (1888). 'Catalogue of the Marsupialia and Monotremata in the Collection of the British Museum (Natural History)'. (British Museum of Natural History: London.)

Thorne, J. L., and Kishino, H. (2002). Divergence time and evolutionary rate estimation with multilocus data. *Systematic Biology* **51**, 689–702. doi: 10.1080/10635150290102456

Thorne, J. L., Kishino, H., and Painter, I. S. (1998). Estimating the rate of evolution of the rate of molecular evolution. *Molecular Biology and Evolution* **15**, 1647–1657.

Travouillon, K. J., Archer, M., Hand, S. J., and Godthelp, H. (2006). Multivariate analyses of Cenozoic mammalian faunas from Riversleigh, north-western Queensland. *Alcheringa Special Issue* **1**, 323–349.

Tyndale-Biscoe, C. H. (1964). The female urogenital system and reproduction of the marsupial *Lagostrophus fasciatus*. *Australian Journal of Zoology* **13**, 253–267.

Tyndale-Biscoe, H. (2005). 'Life of Marsupials.' (CSIRO Publishing: Melbourne.)

Van Gelder, R. G. (1977). Mammalian hybrids and generic limits. *American Museum Novitates* **2635**, 1–25.

Westerman, M., Burk, A., Amrine-Madsen, H., Prideaux, G. J., Case, J. A., and Springer, M. S. (2002). Molecular evidence for the last survivor of an ancient kangaroo lineage. *Journal of Mammalian Evolution* **9**, 209–223. doi: 10.1023/A:1022697300092

Wilson, D. E., and Reeder, D. M. (2005). 'Mammal Species of the World: a Taxonomic and Geographic Reference.' (The Johns Hopkins University Press: Baltimore, MD.)

Woodburne, M. O. (1984). Families of marsupials: relationships, evolution and biogeography. In 'Mammals: Notes for a Short Course'. (Ed. T. W. Broadhead.) pp. 48–71. (University of Tennessee, Department of Geological Sciences Studies in Geology 8.)

Woodburne, M. O., MacFadden, B. J., Case, J. A., Springer, M. S., Pledge, N. S., Power, J. D., Woodburne, J. M., and Springer, K. B. (1993). Land mammal biostratigraphy and magnetostratigraphy of the Etadunna Formation (late Oligocene) of South Australia. *Journal of Vertebrate Paleontology* **13**, 483–515.

Wroe, S., and Archer, M. (1995). Extraordinary diphyodonty-related change in dental function for a tooth of the extinct marsupial *Ekaltadelta ima* (Propleopinae, Hypsiprymnodontidae). *Archives of Oral Biology* **40**, 597–603. doi: 10.1016/0003-9969(95)00010-M

Wroe, S., Brammall, J., and Cooke, B. N. (1998). The skull of *Ekaltadeta ima* (Marsupialia, Hypsiprymnodontidae): an analysis of some marsupial cranial features and a re-investigation of propleopine phylogeny, with notes on the inference of carnivory in mammals. *Journal of Paleontology* **72**, 738–751.

Yang, Z., and Rannala, B. (2006). Bayesian estimation of species divergence times under a molecular clock using multiple calibrations with soft bounds. *Molecular Biology and Evolution* **23**, 212–226. doi: 10.1093/molbev/msj024

Ziegler, A. C. (1977). Evolution of New Guinea's marsupial fauna in response to forested environment. In 'The Biology of Marsupials'. (Eds B. Stonehouse and D. Gilmore.) pp. 117–138. (Macmillen: London.)

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