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RELATIONSHIPS AND DIVERGENCE TIMES AMONG THE ORDERS AND FAMILIES OF MARSUPIALIA

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ABSTRACT--The approximately 330 living species of marsupials are currently divided into three American (Didelphimorphia, Microbiotheria, and Paucituberculata) and four Australasian (Dasyuromorphia, Diprotodontia, Notoryctemorphia, and Peramelemorphia) orders. Studies of interordinal relationships generally support the monophyly of Australidelphia, which includes the four Australian orders and the South American Microbiotheria. Within Australidelphia, monophyly of the Australasian orders (Eomarsupialia), relationships between the Australasian orders, and diprotodontian interfamilial relationships are still disputed. We analyzed protein-coding portions of five nuclear genes (ApoB, BRCA1, IRBP, Rag1, and vWF) from representatives of all extant marsupial families using maximum parsimony, maximum likelihood, and Bayesian methods. Two relaxed molecular clock methods (*Multidivtime*, IRDIVTIME) were employed to estimate divergence times. Likelihood and Bayesian analyses favored rooting the tree between Didelphimorphia and all other marsupials, but alternate positions for the root (Paucituberculata versus other marsupials, Ameridelphia versus Australidelphia) could not be rejected. Australidelphia was supported in all analyses, but interordinal relationships within this clade were not strongly supported. Diprotodontian monophyly was recovered in all analyses. Within this order there was a basal split between Vombatiformes (koalas, wombats) and Phalangerida (kangaroos, possums). Within Phalangerida, Macropodiformes grouped with Phalangeroidea to the exclusion of Petauroidea. Within Petauroidea, there was a basal split between Acrobatidae and all other petauroids. Among the remaining petauroids, Pseudocheiridae grouped with Petauridae to the exclusion of Tarsipedidae. *Multidivtime* divergence time estimates suggest a Late Cretaceous common ancestor for Marsupialia (80.4-78.1 Ma), interordinal divergences that range into the early Paleocene (60.7-59.5 Ma for Dasyuromorphia to Peramelemorphia), and mostly Paleogene interfamilial diversification. IRDIVTIME analyses resulted in slightly older dates for the most recent common ancestor of Marsupialia (83.9-80.6 Ma), but also showed increased stemminess (i.e., proportion of overall tree length comprised of internal branches) and a longer time window (~ 36 million years) for interordinal cladogenesis than *Multidivtime* analyses. *Multidivtime* dates for the last common ancestor of Australidelphia (65.0-64.8 Ma) allow for overland dispersal to Australia prior to the submergence of the South Tasman Rise at 64 Ma (Woodburne and Case, 1996). By contrast, IRDIVTIME dates for the last common ancestor of Australidelphia (62.2-58.2 Ma) are slightly younger than dates for the submergence of the South Tasman Rise and imply over water dispersal.

INTRODUCTION

Marsupialia is a diverse group of mammals that includes more than 330 extant species that occur in North America, South America, and Australasia (Wilson and Reeder, 2005). Simpson's (1945) classification of mammals lumped all marsupials into a single order with six superfamilies. Recent classifications typically recognize seven orders and 18–22 families of living marsupials (Marshall et al., 1990; Aplin and Archer, 1987; Kirsch et al., 1997; Springer et al., 1997). Among the seven orders, Didelphimorphia (New World opossums), Microbiotheria (the Chilean monito del monte), and Paucituberculata (South American caenolestids) are American whereas Dasyuromorphia (dasyurids, numbats, thylacines), Diprotodontia (wombats, koalas, kangaroos, Old World possums),

Notoryctemorphia (the Australian marsupial moles), and Peramelemorphia (bandicoots and bilbies) are Australasian. Hypotheses of phylogenetic relationships among marsupial orders and families have emerged from morphological, molecular, and combined data. Among the more taxonomically comprehensive studies are those based on serology (Kirsch, 1977), morphology (Szalay, 1982, 1994; Horovitz and Sánchez-Villagra, 2003; Sánchez-Villagra et al., 2007; Beck et al., 2008), single copy DNA-DNA hybridization (Kirsch et al., 1997), concatenations of nuclear gene sequences (Amrine-Madsen et al., 2003; Meredith et al., 2008a), mitochondrial genome sequences (Nilsson et al., 2004), concatenations of mitochondrial and nuclear sequences (Phillips et al., 2006; Phillips and Pratt, 2008; Beck, 2008), and combined molecular/morphological data (Asher et al., 2004; Beck et al., 2008).

Szalay (1982) proposed that marsupials grouped into two distinct cohorts, Ameridelphia and Australidelphia, based on features of the tarsus. Ameridelphia includes Didelphimorphia and Paucituberculata; members of this group are characterized by a separate lower ankle joint pattern (SLAJP). Australidelphia, in turn, comprises Microbiotheria and all of the Australasian orders. Australidelphians are characterized by a continuous lower ankle joint pattern (CLAJP). Numerous lines of evidence including nuclear gene sequences (Amrine-Madsen et al., 2003; Meredith et al., 2008a), mitogenomic sequences (Nilsson et al., 2003, 2004), combined mitochondrial and nuclear gene sequences (Phillips et al., 2006; Beck, 2008; Phillips and Pratt, 2008), morphology (Asher et al., 2004; Beck et al., 2008), and mixed molecular-morphological data sets (Asher et al., 2004; Beck et al., 2008) corroborate the monophyly of Australidelphia. In contrast, Ameridelphia has received little support and is usually recovered as paraphyletic at the base of Marsupialia. Within Australidelphia, it remains unclear if Archer's (1984) Eomarsupialia (i.e., the four Australasian orders) is monophyletic or if microbiotheres are nested inside of this group. Several molecular studies have recovered a clade containing Peramelemorphia, Dasyuromorphia, and Notoryctemorphia (Amrine-Madsen et al., 2003; Phillips et al., 2006; Phillips and Pratt, 2008; Beck, 2008; Meredith et al., 2008a), but only with marginal support.

Most marsupial orders contain one, two, or at most three extant families. The single exception is Diprotodontia, which includes 11 extant families (*sensu* Table 1). Diprotodontians are characterized by diprotodonty, a state in which the first pair of lower medial incisors are enlarged and procumbent. Diprotodontian monophyly is also supported by the presence of a fasciculus aberrans, which connects the two cerebral hemispheres of the brain (Abbie, 1937), albeit with the caveat that the occurrence of this feature remains to be investigated in burramyids and tarsipedids (Aplin and Archer, 1987; Luckett, 1994). The monophyly of Diprotodontia has been corroborated by numerous molecular studies (e.g., Springer and Kirsch, 1991; Kirsch et al., 1997; Amrine-Madsen et al., 2003; Kavanagh et al., 2004; Nilsson et al., 2004; Munemasa et al., 2006; Phillips et al., 2006; Phillips and Pratt, 2008; Beck, 2008; Meredith et al., 2008a). Molecular studies (Phillips and Pratt, 2008; Beck, 2008; Meredith et al., 2008a) also support the subdivision of Diprotodontia into the suborders Vombatiformes, which includes koalas and wombats, and Phalangerida, which includes three families of kangaroos and six families of possums. Within Phalangerida, Phillips and Pratt (2008) suggest a fundamental split between petauroid possums (Acrobatidae, Tarsipedidae, Petauridae, Pseudocheiridae) and a clade comprising kangaroo families, Burramyidae, and Phalangeridae based on analyses of mitochondrial and nuclear gene sequences. Meredith et al.'s (2008a) analysis of sequences for five nuclear genes and Beck's (2008) analysis of seven nuclear genes and fifteen mitochondrial loci are compatible with this arrangement, but both of these analyses did not include sequences for Hypsiprymnodontidae, Acrobatidae, and Tarsipedidae. The association of burramyids and phalangerids with kangaroos, rather than with other possum families, contradicts possum monophyly (i.e., Phalangeriformes, *sensu* Kirsch et al., 1997), which has previously been supported by single-copy DNA hybridization (Springer and Kirsch, 1991; Kirsch et al., 1997)

Here, we extend the nuclear gene concatenation of Amrine-Madsen et al. (2003) and Meredith et al. (2008a) to include representatives of all recent marsupial families except for the recently extinct Thylacinidae and Chaeropodidae. Our data set bolsters the taxonomic sampling of Meredith et al. (2008a) by adding the marsupial families Acrobatidae, Hypsiprymnodontidae, Myrmecobiidae, Tarsipedidae, and Thylacomyidae. We examine phylogenetic relationships using Bayesian, maximum parsimony, and maximum likelihood methods and develop a timeline for marsupial evolution using relaxed clock dating methods.

METHODS

Taxon Sampling

We included 28 marsupial taxa that are representative of all marsupial orders and all recent marsupial families except for Thylacinidae and Chaeropodidae. We also included four placental outgroups, one from each of the four superordinal groups of Murphy et al. (2001), as follows: Afrotheria (*Elephas*/*Loxodonta* chimeric); Xenarthra (*Bradypus*); Euarchontoglires (*Homo*); and Laurasiatheria (*Lama*). Ordinal and familial representation is depicted in Table 1.

Gene Sampling

Genomic DNA was extracted using DNeasy Tissue extraction kits (QIAGEN) or the methodology of Kirsch et al. (1990). Portions of five nuclear genes were amplified with *Taq* DNA polymerase (Invitrogen) or Platinum *Taq* DNA polymerase (Invitrogen) using a PCR temperature regime that included initial denaturation at 94^{o} for two minutes; 35 cycles of one minute at 94^{o} (denaturation), one minute at 50^{o} (annealing), and one minute at 72^{o} (extension); and a final extension for ten minutes at 72^{o}. The amplified nuclear gene segments were from exon 26 of ApoB (Apolipoprotein B gene), exon 11 of BRCA1 (breast and ovarian cancer susceptibility gene 1), exon 1 of IRBP (interphotoreceptor retinoid binding protein gene), intronless RAG1 (recombination activating gene-1), and exon 28 of vWF (von Willebrand factor gene). External forward and reverse primers new to this study are given in Supplementary Information. These genes were chosen because of their previously demonstrated utility in resolving higher-level marsupial relationships (e.g., Amrine-Madsen et al., 2003; Meredith et al., 2008a, b).

PCR products were cleaned using QIAquick Gel Extraction Kits (QIAGEN) or AccuPrep™ Gel Purification Kits (Bioneer Cooperation). The PCR products were then sequenced in both directions using an automated DNA sequencer (ABI3730xl) at the University of California Riverside's Core Instrumentation Facility. When necessary, internal sequencing primers were designed. Accession numbers for previously published sequences and 28 new sequences are given in Supplementary Information.

Alignments

Sequences for each gene segment were aligned using the program SOAP v1.2a4 (Löytynoja and Milinkovitch, 2001) after translating DNA sequences into amino acid sequences. Amino acid sequences were aligned using 25 different combinations of gap opening and gap extension settings [gap opening (11–19) and gap extension (3–11) penalties were in steps of two]. These alignments were then translated back into DNA sequences and manually realigned using the program SE-AL (Rambaut, 1996). A total of 5869 aligned nucleotide sites were retained for phylogenetic analyses (ApoB = 768 sites; BRCA1 = 2343 sites; IRBP = 1241 sites; Rag1 = 543 sites; vWF = 974 sites). This is fewer than the number of aligned sites that were analyzed by both Amrine-Madsen et al., (2003) and Meredith et al., (2008a), but in those studies SOAP v1.2a4 was not used to identify alignment-ambiguous regions.

Data Compatibility

Bootstrap compatibility tests (de Queiroz, 1993; Teeling et al., 2000) were performed using RAxML (Stamatakis, 2006). Five hundred replications were performed for each gene using the GTR + Γ model of sequence evolution. Analyses were started from randomized MP starting trees and employed the fast hill-climbing algorithm; all free model parameters were estimated. There were no conflicting nodes at the 90% bootstrap support level. We also employed the partition homogeneity test (one test with five partitions that corresponded to each gene segment) using PAUP 4.0b10 (1000 replicates, 10 taxon input orders per replicate; Farris et al., 1994; Swofford, 2002). The partition homogeneity test was not significant ($p = 0.398$). Given these results we elected to combine all of the gene sequences into a concatenated data set for phylogenetic analyses.

Phylogenetic Analyses

Maximum parsimony (MP) analyses were performed on the concatenated alignment set using

PAUP 4.0b10 (1000 replicates, 10 taxon input orders per replicate; Swofford, 2002). Maximum-likelihood (ML) analyses were performed using RAxML-VI-HPC (Stamatakis, 2006) with the GTR + Γ model of sequence evolution. We performed partitioned analyses that allowed each gene segment in the concatenation to have its own parameter estimates for the GTR + Γ model of sequence evolution as well as non-partitioned analyses that used the same estimated parameters for the GTR + Γ model for the entire concatenation. The ML analyses were started from randomized MP starting trees, employed the fast hill-climbing algorithm, and estimated all free model parameters. Gaps were treated as missing data in all analyses. Bootstrap analyses employed the aforementioned options with 500 replicates (ML and MP). The best ML tree was determined using the GTRMIX model of sequence evolution, the fast hill-climbing algorithm, with 100 inferences using 100 distinct randomized maximum parsimony trees as implemented in RAxML-VI-HPC (Stamatakis, 2006).

Metropolis-coupled Markov chain Monte Carlo sampling as implemented in MrBayes v3.1.1 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck, 2003) was used to calculate Bayesian posterior probabilities. For each data set we performed two different Bayesian analyses: (1) each gene segment in the concatenation was allowed to have its own model of sequence evolution; (2) one model of sequence evolution was used for the entire concatenation. Modeltest 3.06 (Posada and Crandall, 1998) was used to select the best fit model of molecular evolution for the Bayesian analyses (Posada and Crandall, 1998). We chose AIC criterion rather than the likelihood ratio test based on arguments advanced by Posada and Buckley (2004). Models chosen were TrN + I + Γ (ApoB); GTR + I + Γ (BRCA1, Rag1, and concatenation); and TVM + I + Γ (vWF, IRBP). In cases where the model of sequence evolution suggested by Modeltest was not available in MrBayes 3.1.1 (e.g., TVM + I + Γ), we implemented the more general model (e.g., GTR + I + Γ). In all analyses we used default settings for priors, random starting trees, and eight Markov chains (seven hot and one cold); chains were sampled every 1000 generations. Analyses were terminated after the average standard deviation of split frequencies for the simultaneous analyses fell below 0.001, which was always in excess of 5 million generations. Burn-in was set at 25% of the total chain length.

SH tests (Shimodaira and Hasegawa, 1999) were performed with PAUP 4.10b (Swofford, 2002) on the five trees with the highest likelihood scores from the non-partitioned Bayesian analysis. Model parameter estimates for SH tests were taken from the Modeltest 3.06 results with the AIC criterion. The SH test was performed with RELL optimization and 1000 replications.

Molecular Dating Analyses

The likelihood ratio statistic was used to evaluate the molecular clock hypothesis for each of the five genes and the concatenation. All data sets strongly rejected the molecular clock hypothesis ($P < 0.001$). As a result, we elected to estimate divergence times using two methods that employ a relaxed molecular clock and permit the incorporation of multiple constraints from the fossil record.

First, we used *Multidivtime* (version 9-25-03) (Thorne et al., 1998; Kishino et al., 2001; Thorne and Kishino, 2002), which provides Bayesian estimates of divergence times. *Multidivtime* assumes autocorrelation of molecular rates among lineages, requires a rooted tree topology, and allows for fixed minimum and maximum constraints on selected divergence times. We used the Bayesian tree shown in Figure 1. Branch lengths were estimated using the program *estbranches* (Thorne et al., 1998; Kishino et al., 2001; Thorne and Kishino, 2002); *Multidivtime* (Thorne et al., 1998; Kishino et al., 2001; Thorne and Kishino, 2002) was used to estimate divergence times. The five-gene concatenation was analyzed using two different approaches: (1) all of the genes were assumed to change rate by a common factor on each branch, i.e., the concatenation was treated as a single gene, and (2) each gene was allowed gene-specific rate trajectories over time (Thorne and Kishino, 2002). In both *Multidivtime* analyses we used the F84 + Γ model of sequence evolution with four rate categories for the Γ distribution. The F84 + Γ model is the most complex model implemented in *Multidivtime*. We used PAUP 4.0b10 (Swofford, 2002) to estimate both the transition/transversion parameter and the rate categories of the Γ distribution for the topology shown in Figure 1. We used an age of 75 million years for the mean of the prior distribution for the root of Marsupialia. This date is 6–10 million years older than the oldest putative crown-group metatherian

fossils, which belong to the herpetotheriid genus *Nortedelphys* (Case et al., 2005). *Nortedelphys* also emerged as a crown-group metatherian in the cladistic analysis of Goin et al. (2006), although other cladistic studies place Herpetotheriidae outside of crown-group Metatheria (Sánchez-Villagra et al., 2007). We set the mean of the prior distribution for the rate of molecular evolution at the ingroup root node equal to the median amount of evolution from the ingroup root to the ingroup tips divided by the mean of the prior distribution for the root of Marsupialia following Springer et al. (2003). Analyses were run for one million generations with a burnin of 100,000 generations to allow Markov chains to reach stationarity before sampling the chains every 100 generations.

We employed 32 hard constraints based on both the fossil record and previous phylogenetic analyses for taxa that were included in our analysis. Most constraints were taken from Meredith et al. (2008a, b). Node numbers refer to Figure 3:

a. Node 1: Maximum of 65 million years and minimum of 25.5 million years (Meredith et al., 2008a).
b. Node 2. Maximum of 33.9 million years and minimum of 12 million years (Meredith et al., 2008a).
c. Node 3. Maximum of 65 million years and minimum of 24.7 million years (Meredith et al., 2008a).
d. Node 4. Maximum of 65 million years and minimum of 25.5 million years (Meredith et al., 2008a).
e. Node 5. Maximum of 65 million years and minimum of 25.5 million years (Meredith et al., 2008a).
f. Node 6. Maximum of 33.9 million years and minimum of 4.46 million years (Meredith et al., 2008a).
g. Node 7. Maximum of 23.03 million years and minimum of 4.46 million years (Meredith et al., 2008a).
h. Node 8. The oldest described member of the Dasyurini is *Dasyurus dunmalli* from the Bluff Downs Local Fauna (Bartholomai, 1971). Mackness et al. (2000) dated this fauna minimally at 3.62 million years. No dasyurine dasyurids are known from any of the Australasian Oligo-Miocene deposits (see Node 6). We used 3.62 million years as the minimum and the base of the Miocene (23.03 MYA; Gradstein et al., 2004) as the maximum for the base of Dasyurini (i.e., split between *Dasyurus* and *Phascolosorex*).
i. Node 9. Maximum of 23.03 million years and minimum of 4.46 million years (Meredith et al., 2008a).
j. Node 10. The oldest fossil representatives of the Dasyuromorphia are thylacinid specimens from the Oligo-Miocene deposits of Riversleigh (Queensland) and the Etadunna Formation (South Australia). Recent molecular work suggests that thylacinids group with dasyurids to the exclusion of myrmecobiids (e.g., Krajewski et al., 2000). *Badjcinus turnbulli* is known from a Riversleigh System A deposit (White Hunter Site; Wroe, 2003). This site has been biocorrelated to the Ngama Local Fauna (25-24.7 Ma; Woodburne et al., 1993), which is derived from the Etadunna Formation (Myers and Archer, 1997). As a result we used 24.7 million years as the minimum for the Myrmecobiidae + Dasyuridae split. Given the uncertain taxonomic assignment of the "dasyurid" like Murgon specimens and the Oligo-Miocene specimens (see Node 6) we chose a cautious maximum of 65 million years.
k. Node 11. Maximum of 33.9 million years and minimum of 4.46 million years (Meredith et al., 2008b).
l. Node 12. Maximum of 23.03 million years and minimum of 3.62 million years (Meredith et al. 2008a, b).
m. Node 13. Maximum of 23.03 million years and minimum of 4.46 million years (Meredith et al., 2008a).
n. Node 14. Maximum of 70.6 million years and minimum of 54.6 million years (Meredith et al., 2008a).

o. Node 15. Maximum of 55.8 million years and minimum of 12.2 million years (Meredith et al., 2008a).
p. Node 16. Maximum of 55.8 million years and a minimum of 6.8 million years (Meredith et al., 2008a).

The second method that was used to estimate divergence times employed a matrix of amino acid distances, a multidimensional vector space (MVS) procedure to detect and remove biases in models of molecular evolution caused by unrecognized convergent evolution, and a procedure for estimating divergence times that is robust to abrupt changes in the rate of molecular evolution (Kitazoe et al., 2005, 2007). Kitazoe et al. (2007) provide a link to all of the programs that are necessary to perform these operations. All positions in the amino acid alignment that contained gaps or missing data were excluded because Kitazoe et al.'s (2005) MVS approach is based on distances rather than characters. This resulted in 1387 aligned amino acid sites that were included in the final analysis. The program AMINODIST(Kitazoe et al., 2007) was used to calculate an amino acid distance matrix under the JTT (Jones et al., 1992) + Γ model with $\alpha = 0.5$ following Kitazoe et al. (2007). The MVS-A and MVS-B programs (Kitazoe et al., 2007) were used to improve the additivity of the distance matrix. NEIGHBOR was used to construct neighbor-joining trees based on MVS-corrected distances. Because NEIGHBOR

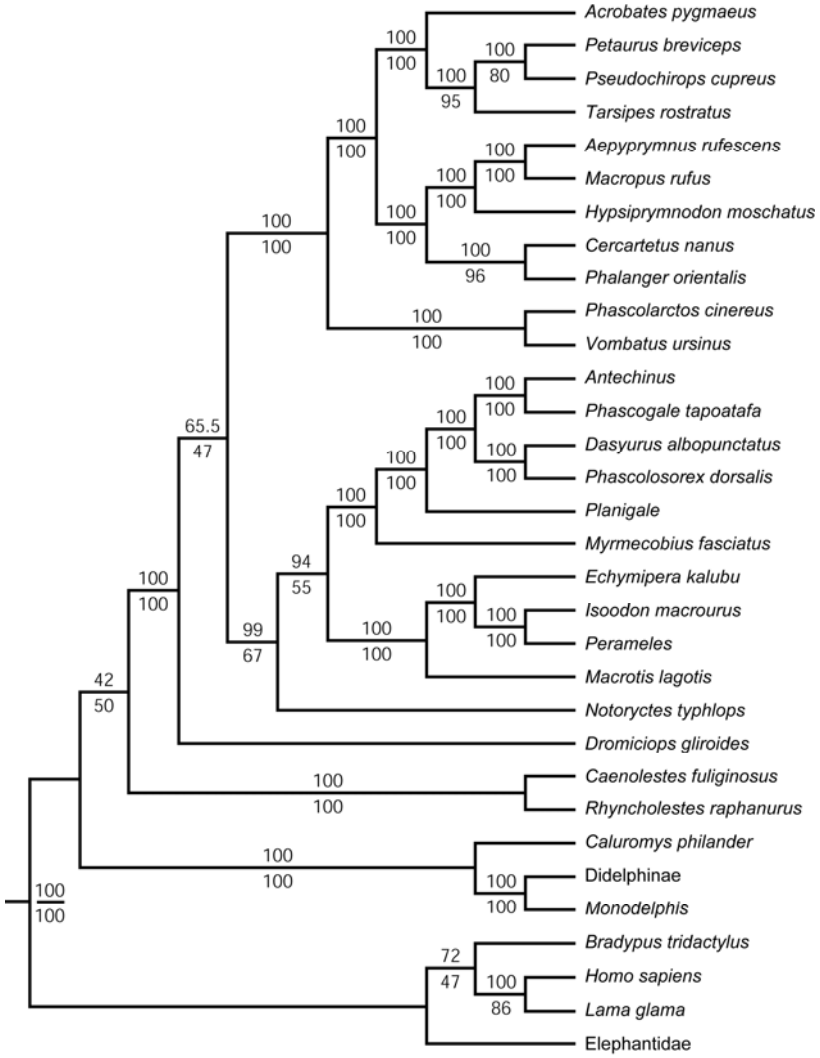


Figure 1. Bayesian tree obtained from partitioned analysis in which each of the five gene regions were modeled separately. Values above branches correspond to mean Bayesian Posterior Probabilities, expressed as percentages, based on the two simultaneous runs. Values below branches are ML bootstrap support percentages from the RAxML analysis with partitioned data.

only allows for the inclusion of a single outgroup, we performed separate analyses using each of the four outgroups (*Lama*, *Homo*, Elephantidae, *Bradypus*). The MVS trees with *Lama* and *Homo* as outgroups were topologically identical to the Bayesian tree shown in Figure 1. When Elephantidae and *Bradypus* were used as outgroups, the MVS trees were topologically identical to the Bayesian tree (Figure 1) except that the root shifted to a position between Paucituberculata and other marsupials instead of between Didelphimorphia and other marsupials. IRDIVTIME analyses were performed with the F-IR cost function (Kitazoe et al., 2007), which places a smaller penalty on abrupt rate changes than other models.

RESULTS

Phylogenetic Analyses

Figure 1 shows the Maximum Posterior Probability (MPP) tree that resulted from the MrBayes analysis with partitioned data. The MPP tree is a combinable component consensus of all sampled trees (Waddell and Shelley, 2003). Bayesian Posterior Probabilities (BPP) and ML bootstrap support percentages based on RAxML with partitioned data are also shown in Figure 1. Figure 2 shows the ML phylogram obtained from the partitioned ML analysis with RAxML. The MP tree is shown in Supplementary Information. Table 2 summarizes BPP, ML bootstrap support percentages, and MP bootstrap support percentages.

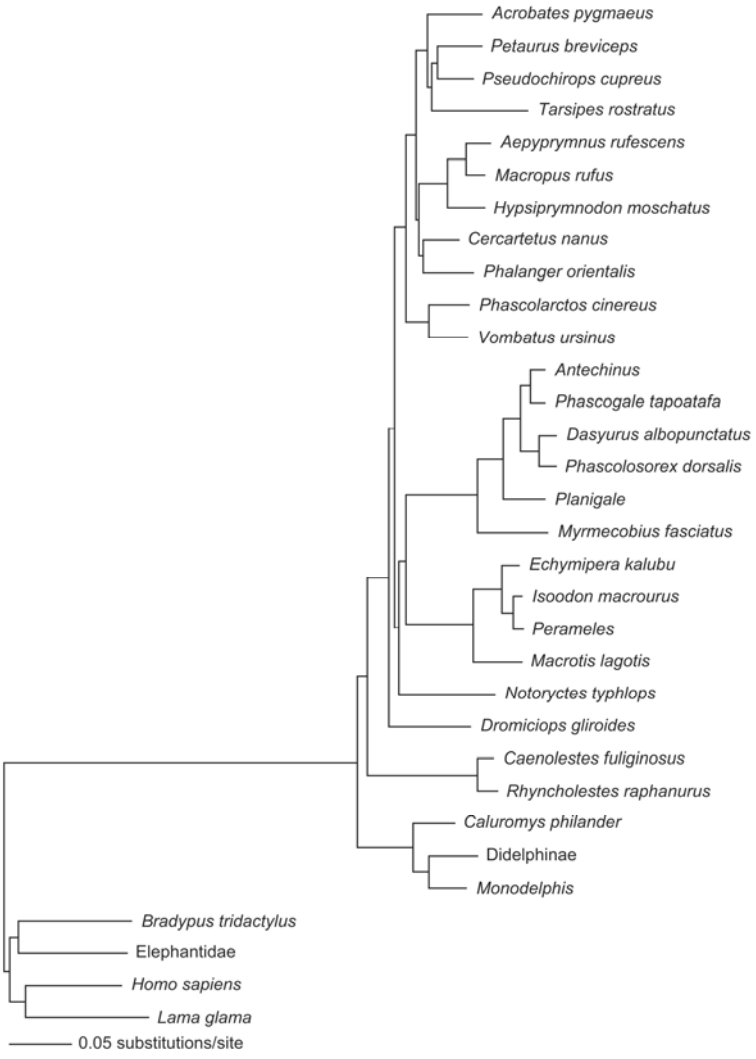


Figure 2. ML phylogram obtained from the partitioned analysis in which each of the five gene regions was modeled separately.

Marsupial orders were recovered as monophyletic with posterior probabilities of 1.00 and bootstrap values of 100%. However, with the exception of Australidelphia, interordinal relationships were not well resolved. Bayesian and ML analyses favored rooting the tree between Didelphimorphia and other marsupials, but rooting the tree between Paucituberculata and other marsupials or between Ameridelphia and Australidelphia also received support. MP bootstrap analyses favored rooting the tree between Paucituberculata and other marsupials (99% bootstrap support for Didelphimorphia + Australidelphia).

Australidelphia was supported in all analyses (posterior probabilities =1.00; ML and MP bootstrap support = 100%). Within Australidelphia, the monophyly of an Australasian clade (i.e., Eomarsupialia, Archer, 1984) was only weakly supported (posterior probabilities = 0.65–0.74; ML bootstrap support = 47–48%; MP bootstrap support = 65%). Within Eomarsupialia, the orders Dasyuromorphia, Peramelemorphia, and Notoryctemorphia grouped to the exclusion of Diprotodontia. Bayesian support for this association was high (posterior probabilities = 0.99-1.00), but bootstrap support was lower (67-73%). Dasyuromorphia grouped with Peramelemorphia to the exclusion of the Notoryctemorphia with mixed support (BPP = 0.94-0.97; ML bootstrap support = 55-64%; MP bootstrap support = 40%).

Within Didelphimorphia, Didelphinae grouped with *Monodelphis* to the exclusion of *Caluromys* (BPP = 1.00; ML and MP bootstrap support = 100%). Within Dasyuromorphia, all nodes were resolved (BPP = 1.00; ML and MP bootstrap support = 100%). The basal split in Dasyuromorphia is between Myrmecobiidae and Dasyuridae. Within Dasyuridae, Phascogalini (*Antechinus* + *Phascogale*) joined Dasyurini (*Dasyurus* + *Phascolosorex*) to the exclusion of Planigalini (*Planigale*). All nodes within Peramelemorphia were firmly resolved (BPP = 1.00; ML and MP bootstrap support = 100%).

Taxon sampling for Diprotodontia included representatives of all extant families and all nodes were well supported in Bayesian and ML bootstrap analyses (BPP = 0.96–1.00; ML bootstrap support = 80–100). The basal split in Diprotodontia is between the Vombatiformes (Vombatidae + Phascolarctidae) and all other diprotodontians (Phalangerida). Within Phalangerida, Phalangeroidea (Phalangeridae + Burramyidae) grouped with Macropodiformes (Potoroidae, Macropodidae, and Hypsiprymnodontidae) to the exclusion of Petauroidea (Acrobatidae, Tarsipedidae, Petauridae, and Pseudocheiridae). Within Macropodiformes, Potoroidae grouped with Macropodidae to the exclusion of Hypsiprymnodontidae. In the petauroid clade, there was a basal split between Acrobatidae and all other petauroids. Among the remaining petauroids, Petauridae grouped with Pseudocheiridae to the exclusion of Tarsipedidae.

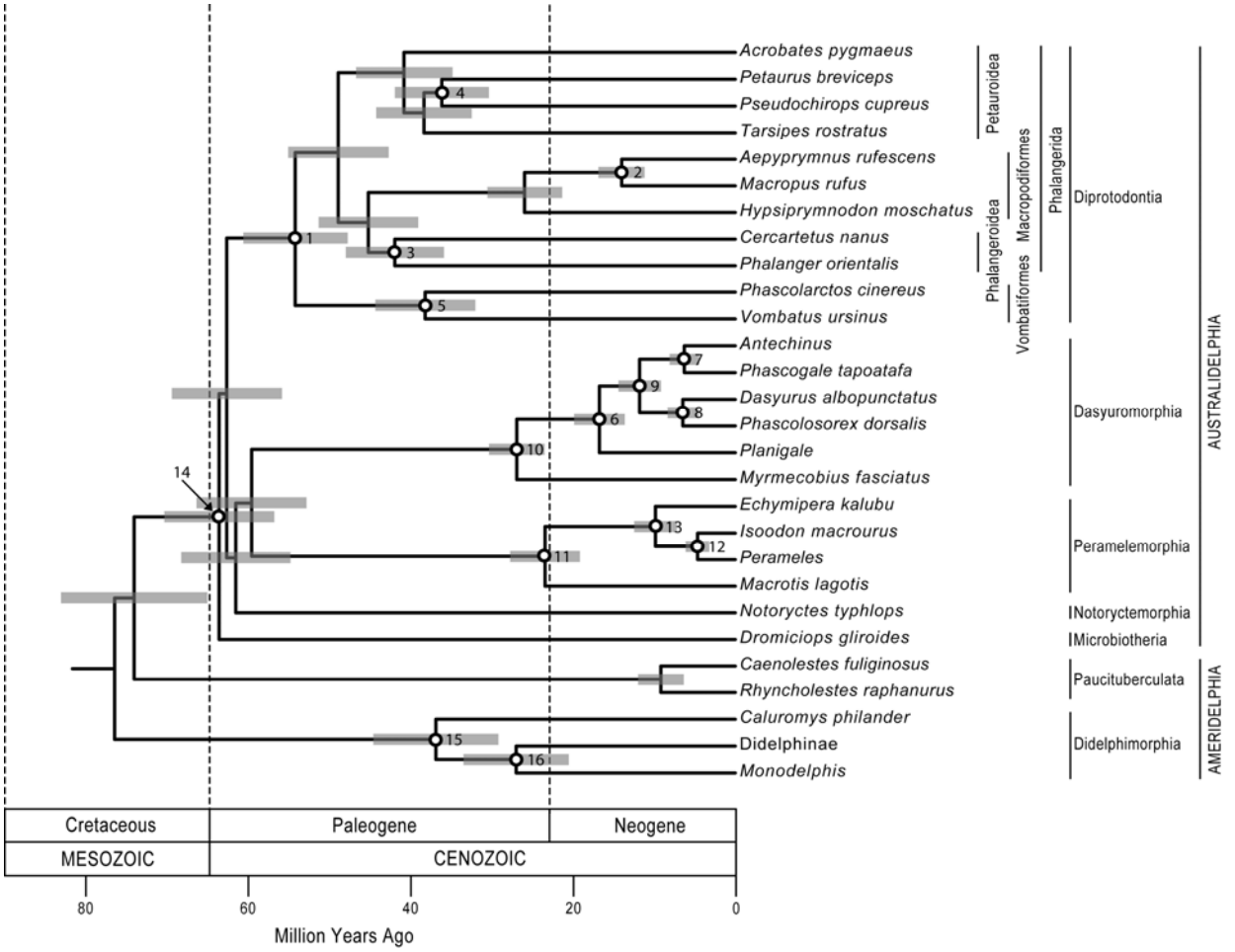
Among the trees that were sampled in the non-partitioned Bayesian analysis, the five most frequent topologies included the tree shown in Figure 1 (16.9%) and trees which showed the following rearrangements relative to this tree: (1) sister group relationship between *Dromiciops* and Diprotodontia (14.0%); (2) marsupial root between Ameridelphia and Australidelphia (12.2%); (3) marsupial root between Paucituberculata and other marsupials (9.3%); and (4) placental root between Atlantogenata and Boreoeutheria (7.4%). The SH test indicated that there were no statistically significant differences between any of these trees.

Molecular Dating

Figures 3 and 4 show timescales for Marsupialia based on the partitioned *Multidivtime* analysis and the IRDIVTIME analysis with *Homo* as the outgroup, respectively. Point estimates of divergence times and 95% credibility intervals (*Multidivtime* only) for the *Multidivtime* and IRDIVTIME analyses are given in Table 3. For the *Multidivtime* analyses, dates in the non-partitioned analysis were, on average, slightly older (0.2 million years) than in the partitioned analysis. On average, *Multidivtime* dates were 5.7 million years older than IRDIVTIME dates.

Point estimates for the base of Marsupialia were 78.1 and 80.4 Ma in partitioned and non-partitioned *Multidivtime* analyses, respectively. Slightly older dates for the base of Marsupialia (81.7, 83.9, 81.3, and 80.6 Ma with *Bradypus*, Elephantidae, *Homo*, and *Lama* as outgroups, respectively) were obtained in IRDIVTIME analyses. The window of interordinal diversification ranged from 80.4-59.5 Ma and 78.1-60.7 Ma in partitioned and non-partitioned *Multidivtime* analyses, respectively. IRDIVTIME analyses suggest that interordinal cladogenesis was deployed over a much broader time window that

Figure 3. Timeline in millions of years before the present for Marsupialia based on the *Multidivtime* analysis in which each gene region was allowed to have its own model of sequence evolution. Fossil constrained nodes are indicated by open circles. 95% credibility intervals are indicated by gray bars.



began 83.9-80.6 Ma and extended until 47.8-45.7 Ma. *Multidivtime* estimates for the base of Australidelphia and interordinal splits within this clade were Paleocene in age and ranged from 65.0 to 59.5 million years ago. IRDIVTIME estimates for these nodes were Paleocene and Eocene in age and ranged from 62.2-58.2 Ma (base of Australidelphia) to 47.8-45.7 Ma (Peramelemorphia to Dasyuromorphia).

Intraordinal divergences were in the Cenozoic with the deepest splits occurring within Diprotodontia. The base of Diprotodontia was estimated at 55.5-55.3 Ma (*Multidivtime*) or 47.2-44.9 Ma (IRDIVTIME). *Multidivtime* estimates of divergence times suggest that all extant diprotodontian families were separate from each other by the end of the Eocene except for Macropodidae and Potoroidae, which diverged in the Miocene. IRDIVTIME dates for diprotodontian families suggest that phalangeroid and petauroid families were distinct by the end of the Oligocene and that the three families of kangaroos (Hypsiprymnodontidae, Potoroidae, Macropodidae) separated from each other in the Miocene. Estimates for the last common ancestor of Vombatiformes were similar and ranged from 38.7-37.7 Ma with *Multidivtime* and 37.1-35.4 Ma with IRDIVTIME.

The proportion of total tree length that is comprised of internal branches is known as stemminess (Phillips, 2008). Calculations of stemminess were performed for the initial phylograms (estbranches, NEIGHBOR), intermediate trees that were corrected for non-additivity (MVS-NEIGHBOR), and chronograms, i.e., dated phylogenies obtained with *Multidivtime* and IRDIVTIME (Table 4). Stemminess

was similar for the initial phylograms (*estbranches* = 36.0%; NEIGHBOR = 35.3%). The MVS-NEIGHBOR trees showed a slight increase in stemminess (36.8-37.0%) relative to the initial trees. All of the chronograms showed a decrease in stemminess relative to the phylograms (*Multidivtime* non-partitioned = 31.7%; IRDIVTIME = 32.7-34.8%).

DISCUSSION

Marsupial Cohorts and the Root of Marsupialia

Szalay (1982) proposed a fundamental split between the cohorts Australidelphia and Ameridelphia based on ankle morphology. Our results confirm the monophyly of Australidelphia. Support for Australidelphia agrees with previous morphological (Luckett, 1994; Szalay, 1994; Szalay and Sargis, 2001; Horovitz and Sánchez-Villagra, 2003), molecular (Kirsch et al., 1991, 1997; Springer et al., 1998; Phillips et al., 2001, 2006; Amrine-Madsen et al., 2003; Meredith et al., 2008a; Beck, 2008), and mixed data sets (Asher et al., 2004; Beck et al., 2008). Our analyses rooted the marsupial tree between Didelphimorphia and Paucituberculata + Australidelphia, which renders Ameridelphia paraphyletic, but this arrangement only received moderate support in Bayesian and ML analyses. Further, the SH test indicated that there are no statistically significant differences for three different root positions (Didelphimorphia versus other marsupials, Ameridelphia versus Australidelphia, Paucituberculata versus other marsupials). Nevertheless, Bayesian and ML support for this hypothesis agrees with complete mitochondrial genomes (Nilsson et al., 2003, 2004), concatenated nuclear genes with fewer taxa (Amrine-Madsen et al., 2003; Meredith et al., 2008a), concatenated nuclear + mitochondrial data sets (Beck, 2008), morphological data (Horovitz and Sánchez-Villagra, 2003), and mixed data sets (Asher et al., 2004; Beck et al., 2008). Rare genomic changes such as chromosomal rearrangements, transposon insertions, and indels are needed to resolve the root of Marsupialia with confidence.

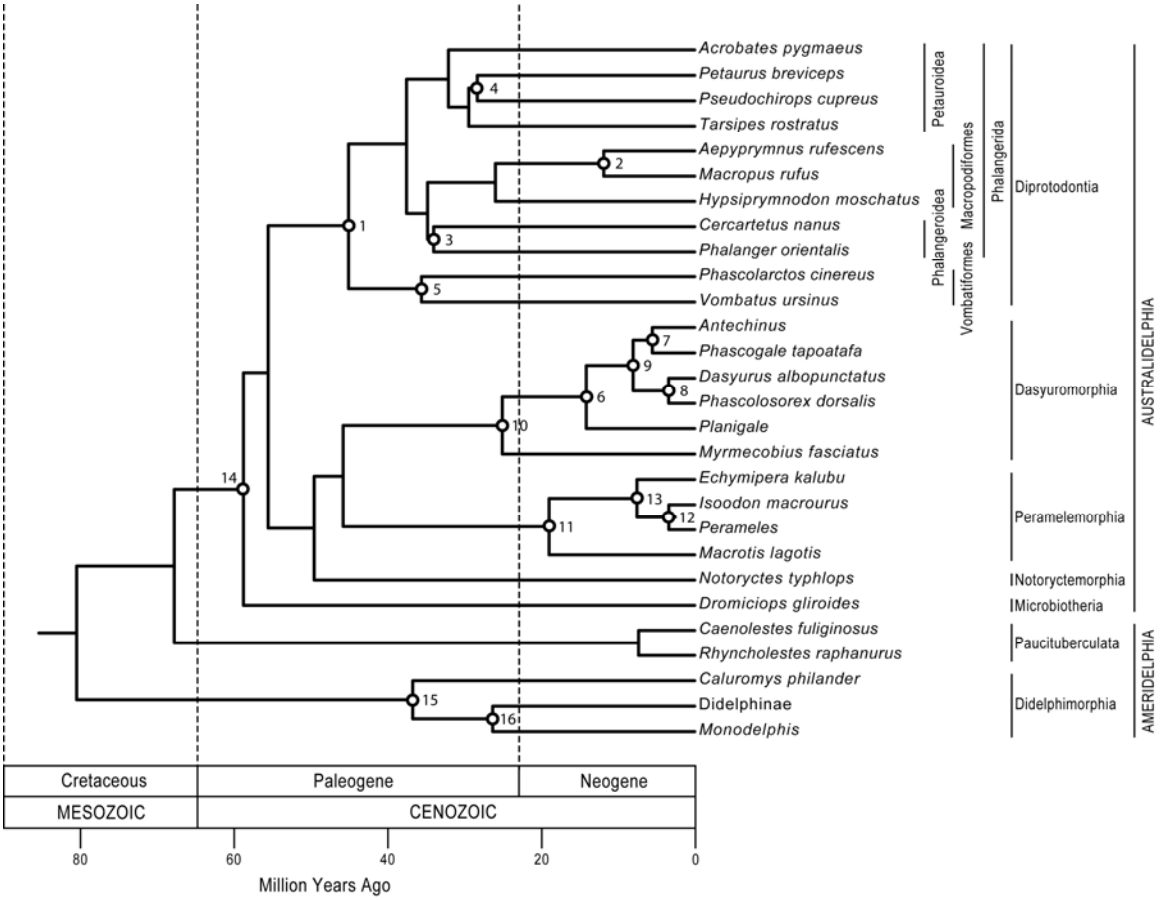


Figure 4. Timeline in millions of years before the present for Marsupialia based on the IRDIVTIME analysis using *Homo* as the outgroup. Fossil constrained nodes are indicated by open circles.

Didelphimorphia

Our study included only three didelphimorphs. All of our analyses indicate that *Caluromys* is the sister taxon to a Didelphinae + Marmosinae (*Monodelphis*) clade. This finding is consistent with morphological (Reig et al., 1987) and molecular (Kirsch et al., 1997; Amrine-Madsen et al., 2003; Steiner et al., 2005; Jansa and Voss, 2005; Meredith et al., 2008a) studies.

Australidelphia

Like previous phylogenetic analyses we failed to find robust support for the basal split within Australidelphia. Bayesian and bootstrap analyses suggest that Australasian marsupials form a monophyletic clade to the exclusion of the South American order Microbiotheria, but SH tests did not find a statistically significant difference between topologies with (1) a monophyletic Eomarsupialia and (2) nesting of *Dromiciops* within Australidelphia as the sister taxon to Diprotodontia. Previous molecular studies provide some support for a monophyletic Eomarsupialia (Amrine-Madsen et al., 2003; Meredith et al., 2008a; Phillips et al., 2006; Phillips and Pratt, 2008), but other molecular (e.g., Kirsch et al., 1991, 1997; Burk et al., 1999; Nilsson et al., 2003, 2004; Munemasa et al., 2006) and morphological (e.g., Szalay and Sargis, 2001; Horovitz and Sánchez-Villagra, 2003) studies have recovered Microbiotheria nested somewhere within the Australasian radiation.

Our results provide marginal support for a clade comprised of Peramelemorphia, Dasyuromorphia, and Notoryctemorphia. Within the latter clade, Peramelemorphia groups with Dasyuromorphia to the exclusion of Notoryctemorphia. Some support for an association of Peramelemorphia, Dasyuromorphia, and Notoryctemorphia has emerged from previous analyses of nuclear genes (Amrine-Madsen et al., 2003; Meredith et al., 2008a) and combined mitochondrial and nuclear DNA genes (Phillips et al., 2006; Beck, 2008; Phillips and Pratt, 2008).

Dasyuromorphia

Within Dasyuromorphia, we found robust support for all nodes. Our results indicate a basal split between Dasyuridae and Myrmecobiidae, a result that is consistent with analyses of nuclear and mitochondrial DNA sequences (Krajewski et al., 2000). Within the Dasyuridae, our results are in agreement with DNA hybridization results (Kirsch et al., 1990, 1997) and gene sequencing studies (Krajewski et al., 2000) that recovered a basal split between Dasyurini + Phascogalini and Planigalini.

Peramelemorphia

Within Peramelemorphia, our results support a basal split between Thylacomyidae and Peramelidae. This result is consistent with analyses of mitochondrial (Westerman et al., 1999, 2001) and nuclear (Meredith et al., 2008b) DNA. Within Peramelidae, we find support for an association of the perameline genera *Isoodon* and *Perameles* together to the exclusion of Echymiperinae (*Echymipera*). This result agrees with DNA hybridization studies (Kirsch et al., 1997), nuclear DNA (Meredith et al., 2008b), and mitochondrial DNA (Westerman et al., 1999, 2001).

Diprotodontia

Among the Australasian orders, Diprotodontia is taxonomically and ecologically the most diverse. Morphological synapomorphies uniting Diprotodontia include diprotodonty, a superficial thymus, a fasciculus aberrans connecting the two hemispheres of the brain (Abbie, 1937), and additional shared derived characters identified by Horovitz and Sánchez-Villagra (2003).

Our study is the first analysis to include multiple nuclear genes for representatives of all diprotodontian families. In the context of this complete family level taxon sampling for Diprotodontia, we recovered a basal split between Vombatiformes (Phascolarctidae and Vombatidae) and all other diprotodontians (Phalangerida). Vombatiformes monophyly is consistent with sperm head morphology (Hughes, 1965; Harding, 1987), molecular (Kirsch, 1968, 1977; Springer and Kirsch, 1991; Kirsch et al., 1997; Springer et al., 1997; Burk et al., 1999; Amrine-Madsen et al., 2003; Kavanagh et al., 2004; Munemasa et al., 2006; Phillips and Pratt, 2008; Meredith et al., 2008a), morphology (Horovitz and Sánchez-Villagra, 2003), and combined molecular and morphological (Asher et al., 2004; Beck et al.,

2008) studies. Within Phalangerida, we failed to recover Phalangeriformes (possum monophyly). In contrast, our results support an association of Phalangeroidea (Burramyidae and Phalangeridae) and Macropodiformes to the exclusion of Petauroidea (Acrobatidae, Tarsipedidae, Petauridae, and Pseudocheiridae). This finding is consistent with the nuclear DNA studies of Meredith et al., (2008a) and Beck (2008), which included fewer diprotodontian families, and the combined mitochondrial and nuclear DNA study of Phillips and Pratt (2008). Amrine-Madsen et al. (2003) also failed to recover possum monophyly, but recovered Petauridae + Pseudocheiridae as the sister group to Macropodiformes. Amrine-Madsen et al. (2003) used the same nuclear genes that were employed in our analyses, but lacked exemplars of Acrobatidae, Burramyidae, Hypsiprymnodontidae, and Tarsipedidae. The apparent payoff for the denser taxon sampling employed here is additional support for several nodes in Diprotodontia and a more informative hypothesis of marsupial relationships. Phalangeriform paraphyly, which is strongly supported by our results, is in contrast to previous phylogenetic studies and classifications that favor the monophyly of this group (Kirsch et al., 1997; Wilson and Reeder, 2005).

The grouping of Phalangeridae and Burramyidae (Phalangeroidea) is consistent with DNA hybridization (Springer and Kirsch, 1991; Kirsch et al., 1997), nuclear DNA (Baker et al., 2004; Meredith et al., 2008a), some mitochondrial DNA sequence analyses (Osborne et al., 2002), and an analysis of combined mitochondrial and nuclear sequences (Beck, 2008) studies. Other studies have suggested a sister-group relationship of burramyids to Vombatiformes (Osborne et al., 2002; Kavanagh et al., 2004), all other possums (Edwards and Westerman, 1995), or Acrobatidae (Gunson et al., 1968; Szalay, 1994).

Within Macropodiformes, we recovered robust support for the grouping of Potoroidae and Macropodidae to the exclusion of Hypsiprymnodontidae. This result agrees with mitochondrial (Burk et al., 1998; Burk and Springer, 2000; Osborne et al., 2002; Kavanagh et al., 2004), nuclear DNA (Meredith et al., 2008a), combined nuclear and mitochondrial DNA (Westerman et al., 2002), pedal morphology (Szalay, 1994), and morphology (Kear et al., 2007) studies. In contrast, the MC'F studies of Baverstock et al. (1989, 1990) and the morphological studies of Archer (1984) and Flannery (1989) suggest a Potoroidae + Hypsiprymnodontidae clade.

We found robust support for Petauroidea. Petauroidea monophyly is consistent with analyses of DNA hybridization (Springer and Kirsch, 1991; Kirsch et al., 1997, Springer et al., 1997), mitochondrial DNA (Osborne et al., 2002; Kavanagh et al., 2004), nuclear DNA (Baker et al., 2004; Meredith et al., 2008a), and combined mitochondrial and nuclear DNA (Phillips and Pratt, 2007; Beck, 2008) data. Within Petauroidea, we found strong support for all recovered relationships. We recovered a basal split between Acrobatidae and all other petauroids. Within the remaining petauroids, Petauridae grouped with Pseudocheiridae to the exclusion of Tarsipedidae. These relationships within the petauroids are consistent with mitochondrial (Kavanagh et al., 2004) and combined mitochondrial and nuclear DNA (Phillips and Pratt, 2008) studies. Previous morphological (Aplin and Archer, 1987), MC'F (Baverstock et al., 1990), and Rag1 DNA studies (Baker et al., 2004) recovered Tarsipedoidea (Tarsipedidae + Acrobatidae).

Multidivtime Versus IRDIVTIME

The present study is the first to employ two different relaxed molecular clock methods (*Multidivtime* and IRDIVTIME) to a nuclear data set consisting of all recognized marsupial families. *Multidivtime* was employed to estimate dates based on DNA sequences whereas IRDIVTIME was used with amino acid sequences. Our results demonstrate that IRDIVTIME dates for the root of Marsupialia are similar, albeit slightly older, than *Multidivtime* dates. An important difference between IRDIVTIME and *Multidivtime* results is that interordinal divergences are deployed over a broader time window in the former (83.9-80.6 Ma to 47.8-45.7 Ma) than the latter (80.4-78.1 Ma to 60.7-59.5 Ma). Similarly, interordinal cladogenesis within Australidelphia occurred over a much broader time window in IRDIVTIME analyses (62.1-58.2 Ma to 47.8-45.7 Ma) than *Multidivtime* analyses (65.0-64.8 Ma to 60.7-59.5 Ma). These observations are consistent with higher stemminess on the IRDIVTIME chronograms than the *Multidivtime* chronograms. Clearly, assumptions of different models of sequence evolution and different models of changing rates of evolution along different branches of a topology have important ramifications for estimating divergence times. As shown by Phillips (2008), model misspecification can result in tree compression (underestimation of hidden substitutions) or tree extension (overestimation of

hidden substitutions). Phillips (2008) further showed that model misspecification will be most problematic in molecular dating analyses when only deep or shallow fossil calibrations are used. For example, if deeper nodes are used for calibration, the inferred divergence times for shallower nodes will be too young under tree extension and too old under tree compression. The inclusion of both shallow and deep calibrations, when available, helps to mitigate the effects of model misspecification (Phillips, 2008).

We employed 32 constraints (16 minima, 16 maxima) including relatively shallow and deep divergences within Marsupialia. Even with this relatively large number of constraints, there remain significant differences between IRDIVTIME and *Multidivtime* results. These differences can be evaluated using additional fossil calibrations and/or other historical information such as paleogeographic reconstructions. In the present case, IRDIVTIME dates for marsupial divergences are closer to a literal reading of the fossil record than are *Multidivtime* results. However, the dearth of fossils from the Australasian Paleogene argues against a literal reading of the fossil record, which is simply too incomplete to adjudicate between some of the differences between *Multidivtime* and IRDIVTIME, e.g., interordinal splits in Australidelphia and interfamilial splits in Diprotodontia. *Djarthia murgonensis* from the early Eocene Tingamarra Local Fauna in southeastern Queensland is the oldest putative australidelphian with a minimum age of 54.6 +/- 0.05 million years (Beck et al., 2008). The age of this fossil is slightly younger than both *Multidivtime* and IRDIVTIME dates for the base of Australidelphia. The Tingamarra Local Fauna has also produced putative bandicoot fossils at 54.6 million years that are compatible with and younger than *Multidivtime* dates for the Peramelemorphia-Dasyuromorphia split (60.7-59-5 Ma), but incompatible with and older than IRDIVTIME dates for the split between Peramelemorphia and Dasyuromorphia (47.8-45.9 Ma). If the referred material (Woodburne and Case, 1996; Archer et al., 1999) is truly peramelemorphian, then the IRDIVTIME dates for the split between Peramelemorphia and Dasyuromorphia are as much as 8.7 million years too young. However, as noted by Beck (2008), putative peramelemorphians from the Tingamarra lack unequivocal apomorphies of crown-group bandicoots that distinguish Peramelemorphia from other marsupial orders. Perhaps more importantly for judging *Multidivtime* versus IRDIVTIME results, *Multidivtime* dates for the base of Australidelphia (65.0-64.8 mya) allow for overland dispersal of stem eomarsupials to Australia prior to the submergence of the South Tasman Rise at 64 mya (Woodburne and Case, 1996). By contrast, IRDIVTIME dates for the last common ancestor of Australidelphia (62.2-58.2 mya) are slightly younger than dates for the submergence of the South Tasman Rise, which implies over water dispersal of stem eomarsupials to Australia. The increased likelihood of overland versus over water dispersal argues in favor of the older dates. Finally, we note that it has proved difficult to resolve relationships among australidelphian orders, even with multigene data sets. This difficulty is expected if interordinal cladogenesis was deployed over a narrow time window as suggested by *Multidivtime* (i.e., 4.1-5.5 million year window in the early and middle Paleocene). By contrast, the window for australidelphian interordinal diversification suggested by IRDIVTIME is much broader (i.e., 12.5-14.4 million year window extending from the early-middle Paleocene to the middle Eocene). For these reasons we have more confidence in *Multidivtime* than IRDIVTIME dates.

Kitazoe et al's (2007) application of IRDIVTIME to placental mitochondrial protein sequences also resulted in estimates of divergence times that were consistently younger than those that were obtained with a variety of other molecular dating approaches, e.g., Kitazoe et al.'s (2007) date for the base of Placentalia was 84 million years whereas other studies were in the range of 140-100 million years for this same node. Kitazoe et al.'s (2007) analysis was limited to mitochondrial amino acid sequences and direct comparisons with dates that were obtained with nuclear sequences have not been reported. Analyses with translated amino acid sequences from Janecka et al.'s (2007) nuclear DNA data set returned dates that were slightly younger than *Multidivtime* dates based on analyses of DNA data for these same loci (Springer, unpublished), e.g., Janecka et al. (2007) reported 88.8 million years for the base of Euarchontoglires whereas the equivalent date based on IRDIVTIME was 87.8 million years. To date, Kitazoe et al.'s (2005, 2007) methods for phylogeny reconstruction and molecular dating have received minimal attention, possibly because they are distance-based methods rather than character-based methods.

A Timeline for Marsupial Evolution

Meredith et al. (2008a) and Springer et al. (2009) summarized previous molecular dating studies, most of which employed strict molecular clocks, report only one or a few cladogenic events, failed to include all marsupial order/families, and/or used phylogenies that are incongruent with our proposed phylogeny. Dating analyses that relax the molecular clock assumption generally outperform strict molecular clock methods (Yang and Rannala, 2006; Smith et al., 2006; Benton and Donoghue, 2007).

We place the base of Marsupialia at 84-78 million years. Previous estimates for the last common ancestor of marsupials range from 100-64 million years (Springer, 1997; Hasegawa et al., 2003; Nilsson et al., 2003, 2004; Woodburne et al., 2003). All other interordinal divergences were placed in the Upper Cretaceous/Paleocene (*Multidivtime* analyses) or Upper Cretaceous/Paleocene/Eocene (IRDIVTIME). Within Australidelphia, the basal split between the Microbiotheria and Eomarsupialia was placed at 65-58 million years, which is compatible with the hypothesis of a single dispersal event from South America to Australia via Antarctica. As discussed above, this dispersal event would have been overland if it occurred before the complete submergence of South Tasman Rise at approximately 64 Ma (Woodburne and Case 1996).

Multidivtime and two of the four IRDIVTIME divergence estimates suggest a middle to late Eocene (40.2-36.9) age for the last common ancestor of living didelphimorphs. Steiner et al., (2005) obtained a similar date for the base of Didelphimorphia (~40 mya) and noted that this date coincides with the first unequivocal phases of Andean uplift. In contrast, the other two IRDIVTIME dates for the base of Didelphimorphia (24.8 and 25.7 mya) are late Oligocene in age. These dates are associated with topologies for which the basal split in Marsupialia is between Paucituberculata and other marsupials rather than Didelphimorphia and other marsupials.

The basal split in Peramelemorphia occurred in the late Oligocene/early Miocene (24.2-19.1 Ma). Mitogenomic estimates suggest 25 Ma (Nilsson et al., 2004) and a five gene nuclear concatenation suggested a basal split anywhere between 29-20 Ma (Meredith et al., 2008b). The base of Dasyuromorphia was dated at 27.6-25.2 Ma (late Oligocene). Krajewski et al. (2000) obtained a similar estimate of 28-25 Ma.

The base of Diprotodontia was estimated at 55.5-44.9 Ma. Mitochondrial genomes and nuclear concatenations have suggested an age of approximately 46 Ma (Nilsson et al., 2004; Drummond et al., 2006) for this split. Our estimates for the base of Vombatiformes (39.1-35.4) are in good agreement with Meredith et al. (2008a), who recovered a date of 37-35 Ma for this clade. Dates for Phalangerida, Phalangeroidea, and Petauroidea were estimated at 49.9-37.4, 43.3-33.9, and 41.6-32.0 Ma, respectively. Our estimates for the base of Macropodiformes (27.0-21.6 Ma) are much younger than those obtained by Burk et al. (1998) and Burk and Springer (2000), who used fossil-calibrated molecular clocks (mitochondrial transversions) to date the base of Macropodiformes at 45-38 Ma.

Case (1989) proposed that the vombatiform, macropodiform, and possum lineages were all present in the Eocene. The Australian Paleocene was dominated by gymnosperms (Hill, 2004). Angiosperms became much more diverse in the Eocene, including the large-scale replacement of podocarp-dominated forests by *Nothofagus*-dominated forests (Case, 1989; Hill, 2004; Martin, 2006). Case (1989) hypothesized that the major lineages of possums became established in the Eocene in response to floristic changes that occurred during this time period. Fundamental changes in oceanic and atmospheric circulation occurred during the latest Eocene/earliest Oligocene in conjunction with the strengthening of the Circum-Antarctic current (Martin, 2006). *Nothofagus*-dominated forests decreased in prominence during the Oligocene and other types of plant communities, including open forest sclerophyll woodlands, increased in prominence (Martin, 2006). Case (1989) hypothesized that diversification of terrestrial vombatoids, including vombatids plus several families that are now extinct, occurred after the diversification of arboreal possums and was directly in response to opening of the forest canopy and replacement of *Nothofagus*-dominated forests by drier, more open forests and woodlands by the end of the early Oligocene. Our *Multidivtime* estimates are in general agreement with Case's (1989) hypothesis: basal splits in Phalangerida are older than the basal split in Vombatiformes and all possum families are distinct prior to the end of the Eocene. IRDIVTIME dates agree with *Multidivtime* dates in suggesting that possum diversification commenced in the Eocene, but differ from *Multidivtime* dates in suggesting that

most possum families separated from each other after the basal vombatiform split, including several interfamilial divergences (i.e., petauroids) that occurred in the Oligocene. Corroboration of Case's (1989) hypothesis and dates for the origin of possum families must await the discovery of Paleogene Australasian marsupial sites.

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LITERATURE CITED

Abbie, A. A . 1937. Some observations on the major subdivisions of the Marsupialia, with special reference to the position of the Peramelidae and Caenolestidae. Journal of Anatomy 71:424–436.

Amrine-Madsen, H., M. Scally, M. Westerman, M. J. Stanhope, C. Krajewski, and M. S. Springer. 2003. Nuclear gene sequences provide evidence for the monophyly of australidelphian marsupials. Molecular Phylogenetics and Evolution 28:186–196.

Aplin, K., and M. Archer. 1987. Recent advances in marsupial systematics with a new syncretic classification; pp. XV–LXXII in M. Archer (ed.), Possums and Opossums: Studies in Evolution. Surrey Beatty and Sons, Chipping Norton, New South Wales.

Archer, M. 1984. The Australian marsupial radiation; pp. 633–808 in M. Archer, G. Clayton (eds.), Vertebrate zoogeography and evolution in Australasia. Hesperian Press, Perth, Australia.

Archer, M., R. Arena, M. Bassarova, K. Black, J. Brammall, B. Cooke, P. Creaser, K. Crosby, A. Gillespie, H. Godthelp, M. Gott, S. J. Hand, B. Kear, A. Krikmann, B. Mackness, J. Muirhead, A. Musser, T. Myers, N. Pledge, Y. Wang, and S. Wroe. 1999. The evolutionary history and diversity of Australian mammals. Australian Mammalogy 21:1–45.

Asher, R. J., I. Horovitz, and M. R. Sánchez-Villagra. 2004. First combined cladistic analysis of marsupial mammal interrelationships. Molecular Phylogenetics and Evolution 33:240–250.

Baker, M. L., J. P. Wares, G. A. Harrison, and R. D. Miller. 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.

Bartholomai, A. 1971. *Dasyurus dunmalli*, a new species of fossil marsupial (Dasyuridae) in the upper Cainozoic deposits of Queensland. Memoirs of the Queensland Museum 16:19-26.

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

Baverstock, P. R., M. Krieg, and J. Birrell. 1990. Evolutionary relationships of Australian marsupials as assessed by albumin immunology; pp. 131–145 in J. A. M. Graves, R. M. Hope, D. Cooper (eds.), Mammals from Pouches and Eggs: Genetics, Breeding and Evolution of Marsupials and Monotremes. C. S. I. R. O., Australia.

Bartholomai, A. 1971. *Dasyurus dunmalli,* a new species of fossil marsupial (Dasyuridae) in the upper Cainozoic deposits of Queensland. Memoirs of the Queensland Museum 16:19–26.

Beck, R. M., 2008. A dated phylogeny of marsupials using a molecular supermatrix and multiple fossil constraints. Journal of Mammalogy 89:175–189.

Beck, R. M., H. Godthelp, V. Weisbecker, M. Archer, and S. J. Hand. 2008. Australia's oldest marsupial fossils and their biogeographical implications. PlosOne 3:e1858.

Benton, M. J., and P. C. J. Donoghue. 2007. Paleontological evidence to date the tree of life. Molecular Biology and Evolution 24:26–53.

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

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

Burk, A., M. Westerman, D. J. Kao, J. R. Kavanagh, and M. S. Springer. 1999. An analysis of marsupial interordinal relationships based on 12S rRNA, tRNA valine, 16S rRNA, and cytochrome b sequences. Journal of Mammalian Evolution 6:317–334.

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

Case, J. A., F. J. Goin, and M. O. Woodburne. 2005. "South American" marsupials from the Late Cretaceous of North America and the origin of marsupial cohorts. Journal of Mammalian Evolution 12:461–494.

de Queiroz, A. 1993. For consensus (sometimes). Systematic Biology. 42:368–372.

Drummond, A. J., S. Y. W. Ho, M. J. Phillips, and A. Rambaut. 2006. Relaxed phylogenetics and dating with confidence. PLoS Biology 4:699–710.

Edwards, D., and M. Westerman. 1995. The molecular relationships of possum and glider families as revealed by DNA-DNA hybridisations. Australian Journal of Zoology 43:231–240.

Farris, J. S, M. Kallersjo, A. G. Kluge, and C. Bult. 1994. Testing significance of incongruence. Cladistics 10:315–319.

Flannery, T. F. 1989. Phylogeny of the Macropodoidea: A study in convergence; pp. 1–46 in G. Grigg, P. Jarman, I. Hume (eds.), Kangaroos, Wallabies and Rat-kangaroos. Surrey Beatty & Sons, Chipping Norton, Australia.

Goin, F. J., R. Pascual, M. F. Tejedor, J. N. Gelfo, M. O. Woodburne, J. A. Case, M. A. Reguero, M. Bond, G. M. Lopez, A. L. Cione, D. Udrizar Sauthier, L. Balarino, R. A. Scasso, F. A. Medina, and M. C. Ubaldon. 2006. The earliest Tertiary therian mammal from South America. Journal of Vertebrate Paleontology 26:505–510.

Gradstein, F. M., J. G. Ogg, A. G. Smith, and 39 co-authors. 2004. A Geologic Timescale. Cambridge University Press, Cambridge.

Gunson, M. M., G. B. Sharman, and J. A. Thomson. 1968. The affinities of *Burramys* (Marsupialia: Phalangeroidea) as revealed by its chromosomes. Australian Journal of Science 31:40–41.

Harding, H. R. 1987. Interrelationships of the families of the Diprotodontia – a view based on spermatozoan ultrastructure; pp. 195–216 in M. Archer (ed.), Possums and Opossums: Studies in Evolution. Surrey Beatty and Sons, Chipping Norton, New South Wales.

Hasegawa, M., Thorne J. L., and H. Kishino. 2003. Time scale of eutherian evolution estimated without assuming a constant rate of molecular evolution. Genes Genetics and Systematics 78:267–283.

Horovitz, I., and M. R. Sánchez-Villagra. 2003. A morphological analysis of marsupial mammal higher-level phylogenetic relationships. Cladistics 19:181–212.

Hughes, R. L. 1965. Comparative morphology of spermatozoa from five families Australian Journal of Zoology 13:533–543.

Hill, R. S. 2004. Origins of the southeastern Australian vegetation. Philosophical Transactions of the Royal Society of London 359:1537–1549.

Huelsenbeck, J. P., and F. Ronquist. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17:754–755.

Janecka, J. E., W. Miller, T. H. Pringle, F. Wiens, A. Zitzmann, K. M. Helgen, M. S. Springer, and W. J. Murphy. 2007. Molecular and genomic data identify the closest living relative of primates. Science 318:792-794.

Jansa, S. A., and R. S. Voss. 2005. Phylogenetic relationships of the marsupial genus *Hyladelphys* based on nuclear gene sequences and morphology. Journal of Mammalogy 86:853–865.

Jones, D. T., W. R. Taylor, and J. M. Thornton. 1992. The rapid generation of mutation data matrices from protein sequences. Computational and Applied Biosciences 8:275–282.

Kavanagh, J. R., A. Burk-Herrick, M. Westerman, and M. S. Springer. 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.

Kear, B. P., B. N. Cooke, M. Archer, and T. F. Flannery. 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.

Kirsch, J. A. 1968. Prodromus of the comparative serology of Marsupialia. Nature 217:418–420.

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., F.-J. Lapointe, and M. S. Springer. 1997. DNA-hybridisation studies of marsupials and their implications for metatherian classification. Australian Journal of Zoology 45:211–280.

Kirsch, J. A. W., C. Krajewski, M. S. Springer, and M. Archer. 1990. DNA-DNA hybridization studies of carnivorous marsupials. II. Relationships among dasyurids (Marsupialia: Dasyuridae). Australian Journal of Zoology 38:673–696.

Kirsch, J. A. W., A. W. Dickerman, O. A. Reig, and M. S. Springer. 1991. DNA hybridization evidence for the Australasian affinity of the American marsupial *Dromiciops australis.* Proceedings of the National Academy of Sciences USA 88:10465–10469.

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

Kitazoe, Y., H. Kishino, T. Okabayashi, T. Watabe, N. Nakajima, et al. 2005. Multidimensional vector space representation for convergent evolution and molecular phylogeny. Molecular Biology and Evolution 22:704–715.

Kitazoe, Y., H. Kishino, P. J. Waddell, N. Nakajima, T. Okabayashi, T. Watabe, and Y. Okuhara. 2007. Robust time estimation reconciles views of the antiquity of placental mammals. PLoS ONE 2: e384.

Krajewski, C., S. Wroe, and M. Westerman. 2000. Molecular evidence for the pattern and timing of cladogenesis in dasyurid marsupials. Zoological Journal of the Linnaean Society 130:375–404.

Luckett, W. P. 1994. Suprafamilial relationships within Marsupialia: resolution and discordance from multidisciplinary data. Journal of Mammalian Evolution 2:255–283.

Löytynoja, A., and M. C. Milinkovitch. 2001. SOAP, cleaning multiple alignments from unstable blocks. Bioinformatics 17:573–574.

Mackness, B. S., P. W. Whitehead, and G. C. McNamara. 2000. New potassium-argon basalt date in relation to the Pliocene Bluff Downs local fauna, northern Australia. Australian Journal of Earth Sciences 47:807–811.

Marshall, L. G., J. A. Case, and M. O. Woodburne. 1990. Phylogenetic relationships of the families of marsupials; pp. 433-505 in H. H. Genoways (ed.), Current Mammalogy. 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.

Meredith, R. W., M. Westerman, J. A. Case, and M. S. Springer. 2008a. A phylogeny and timescale for marsupial evolution based on sequences for five nuclear genes. Journal of Mammalian Evolution 15:1–36.

Meredith, R. W., M. Westerman, and M. S. Springer. 2008b. A timescale and phylogeny for "Bandicoots" (Peramelemorphia: Marsupialia) based on sequences for five nuclear genes. Molecular Phylogenetics and Evolution 47:1-20.

Munemasa, M., M. Nikaido, S. Donnellan, C. C. Austin, N. Okada, and M. Hasegawa 2006. Phylogenetic analysis of diprotodontian marsupials based on complete mitochondrial genomes. Genes Genetics and Systematics 81:181–191.

Murphy, W. J., E. Eizirik, S. J. O'Brien, O. Madsen, M. Scally, C. J. Douady, E. Teeling, O. A. Ryder, M. J. Stanhope, W. W. de Jong, and M. S. Springer. 2001. Resolution of the early placental mammal radiation using Bayesian phylogenetics. Science 294:2348–2351.

Myers, T. J., and M. Archer. 1997. *Kuterintja ngama* (Marsupialia, Ilariidae): A revised systematic analysis based on material from the late Oligocene of Riversleigh, northwestern Queensland. Memoirs of the Queensland Museum 41:379–392.

Nilsson, M. A., A. Gullberg, A. E. Spotorno, U. Arnason, and A. Janke. 2003. Radiation of extant marsupials after the K/T boundary: evidence from complete mitochondrial genomes. Journal of Molecular Evolution 57:S3–S12.

Nilsson, M. A., U. Arnason, P. B. Spencer, and A. Janke. 2004. Marsupial relationships and a timeline for marsupial radiation in South Gondwana. Gene 13:189–196.

Osborne, M. J., and L. Christidis. 2002. Molecular relationships of the cuscuses, brushtail and scaly-tailed possums (Phalangerinae). Australian Journal of Zoology 50:135–149.

Phillips, M. J. 2008. Branch-length estimation bias misleads molecular dating for a vertebrate mitochondrial phylogeny Gene doi:10.1016/j.gene.2008.08.017

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

Phillips, M. J., Y.-H. Lin, G. L. Harrison, and D. Penny. 2001. Mitochondrial genomes of a bandicoot and a brushtail possum confirm the monophyly of australidelphian marsupials. Proceedings of the Royal Society of London Series B 268:1533–1538.

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

Posada, D., and T. Buckley. 2004. Model selection and model averaging in phylogenetics: Advantages of Akaike Information Criterion and Bayesian approaches over likelihood ratio tests. Systematic Biology 53:793-808.

Posada, D., and K. A. Crandall. 1998. MODELTEST: Testing the model of DNA substitution. Bioinformatics 14:817–818.

Rambaut, A. 1996. Se-Al: sequence alignment editor. Available at http://evolvezoooxacuk/.

Reig, O. A., J. A. W. Kirsch, and L. G. Marshall. 1987. Systematic relationships of the living and Neocenozoic American "opossum-like" marsupials (Suborder Didelphimorphia) with comments on the classification of these and of the Cretaceous and Paleogene New World and European metatherians; pp. 1–90 in M. Archer (ed.), Possums and Opossums: Studies in Evolution. Surrey Beatty and Sons, Chipping Norton, New South Wales.

Ronquist, F., and J. P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574.

Sánchez-Villagra, M. R., S. Ladevèze, I. Horovitz, C. Argot, J. J. Hooker, T. E. Macrini, T. Martin, S. Moore-Fay, C. de Muizon, T. Schmelzle, and R. J. Asher. 2007. Exceptionally preserved North American Paleogene metatherians: adaptations and discovery of a major gap in the opossum fossil record. Biology Letters 3:318–322.

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

Simpson, G. G. 1945. The principles of classification and a classification of mammals. Bulletin of the American Museum of Natural History 91:1–232.

Smith, A. B., D. Pisani, J. A. Mackenzie-Dodds, B. Stockley, B. L. Webster, and T. J. Littlewood. 2006. Testing the molecular clock: molecular and paleontological estimates of divergence times in the Echinoidea (Echinodermata). Molecular Biology and Evolution 23:1832–1851.

Springer, M. S. 1997. Molecular clocks and the timing of the placental and marsupial radiations in relation to the Cretaceous–Tertiary boundary. Journal of Mammalian Evolution. 4:285–302.

Springer, M. S., and J. A. W. Kirsch. 1991. DNA hybridization, the compression effect, and the radiation of diprotodontian marsupials. Systematic Zoology 40:131–151.

Springer, M. S., J. A. W. Kirsch, and J. A. Case. 1997. The chronicle of marsupial evolution; pp. 129–161 in T. Givinish, K. Sytsma (eds.), Molecular Evolution and Adaptive Radiations. Cambridge University Press.

Springer, M. S., C. Krajewski, and R. W. Meredith. 2009. Metatheria; pp. 466-470 in S. B. Hedges, S. Kumar (eds.), The Timetree of Life. Oxford University Press, New York.

Springer, M. S., W. J. Murphy, E. Eizirik, S. J. O'Brien. 2003. Placental mammal diversification and the Cretaceous–Tertiary boundary. Proceedings of the National Academy of Sciences 100:1056–1061.

Springer, M. S., M. Westerman, J. R. Kavanagh, A. Burk, M. O. Woodburne, D. J. Kao, and C. Krajewski. 1998. The origin of the Australasian marsupial fauna and the phylogenetic affinities of the enigmatic monito del monte and marsupial mole. Proceedings of the Royal Society of London Series B 265:2381–2386.

Stamatakis, A. 2006. RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22:2688–2690.

Steiner, C., M. Tilak, J. P. Douzery, and F. M. Catzeflis. 2005. New DNA data from a transthyretin nuclear intron suggest an Oligocene to Miocene diversification of living South America opossums (Marsupialia: Didelphidae). Molecular Phylogenetics and Evolution 35:363–379.

Swofford, D. L. 2002. PAUP 4.0b10*. Phylogenetic Analysis Using Parsimony (* and other Methods), Version 4.0. Sinauer Associates, Massachusetts.

Szalay, F. S. 1982. A new appraisal of marsupial phylogeny and classification; pp. 621–640 in M. Archer (ed.), Carnivorous Marsupials. Royal Zoological Society of New South Wales, Mossman, New South Wales, Australia.

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

Szalay, F. S., and E. J. Sargis. 2001. Model-based analysis of postcranial osteology of marsupials from the Palaeocene of Itaborai (Brazil) and the phylogenetics and biogeography of Metatheria. Geodiversitas 23:139–302.

Teeling, E. C., M. Scally, D. J. Kao, M. L. Romagnoli, M. S. Springer, and M. J. Stanhope. 2000. Molecular evidence regarding the origin of echolocation and flight in bats. Nature 403:188–192.

Thorne, J. L, and H. Kishino. 2002. Divergence time and evolutionary rate estimation with multilocus data. Systematic Biology 51:689–702.

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

Waddell, P. J. and S. Shelley. 2003. Evaluating placental inter-ordinal phylogenies with novel sequences including RAG1, g-fibrinogen, ND6, and mt-tRNA, plus MCMC-driven nucleotide, amino acid, and codon models. Molecular Phylogenetics and Evolution 28:197–224.

Westerman, M., M. S. Springer, and C. Krajewski. 2001. Molecular relationships of the New Guinean bandicoot genera *Microperoryctes* and *Echymipera* (Marsupialia: Peramelina). Journal of Mammalian Evolution 8:93–105.

Westerman, M., M. S. Springer, J. Dixon, and C. Krajewski. 1999. Molecular relationships of the extinct pig-footed bandicoot *Chaeropus ecaudatus* (Marsupialia: Perameloidea) using 12S rRNA sequences. Journal of Mammalian Evolution 6:271–288.

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

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

Woodburne, M. O., and J. A. Case. 1996. Dispersal, vicariance, and the Late Cretaceous to early Tertiary land mammal biogeography from South America to Australia. Journal of Mammalian Evolution 3:121–161.

Woodburne, M. O., T. H. Rich, and M. S. Springer. 2003. The evolution of tribospheny and the antiquity of mammalian clades. Molecular Phylogenetics and Evolution 28:360–385.

Woodburne, M. O., B. J. MacFadden, J. A. Case, M. S. Springer, N. S. Pledge, J. D. Power, J. M. Woodburne, and K. B. Springer. 1993. Land mammal biostratigraphy and magnetostratigraphy of

the Etadunna Formation (late Oligocene) of South Australia. Journal of Vertebrate Paleontology 13:483–515.

Wroe, S. 2003. Australian marsupial carnivores: recent advances in palaeontology; pp. 102–123 in M. Jones, C. Dickerman, M. Archer (eds.), Predators with Pouches: The Biology of Marsupial Carnivores. CSIRO Publishing, Collingwood, Australia.

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

TABLES

Table 1. Ordinal and familial representation of genera included in this study[a].

- Marsupialia (Infraclass Metatheria)
  - Order Didelphimorphia
    - Family Didelphidae
      - Subfamily Didelphinae (*Didelphis*/*Lutreolina*)
      - Subfamily Marmosinae (*Monodelphis*)
    - Family Caluromyidae (*Caluromys*)
  - Order Paucituberculata
    - Family Caenolestidae (*Caenolestes*, *Rhyncholestes*)
  - Order Microbiotheria
    - Family Microbiotheriidae (*Dromiciops*)
  - Order Dasyuromorphia
    - Family Dasyuridae
      - Subfamily Sminthopsinae (*Planigale*)
      - Subfamily Dasyurinae
        - Tribe Phascogalini (*Phascogale*, *Antechinus*)
        - Tribe Dasyurini (*Dasyurus, Phascolosorex*)
    - Family Myrmecobiidae (*Myrmecobius*)
  - Order Peramelemorphia
    - Family Peramelidae
      - Subfamily Peramelinae (*Perameles*, *Isoodon*)
      - Subfamily Echymiperinae (*Echymipera*)
    - Family Thylacomidae (*Macrotis*)
  - Order Notoryctemorphia
    - Family Notoryctidae (*Notoryctes*)
  - Order Diprotodontia
    - Suborder Vombatiformes
      - Family Vombatidae (*Vombatus*)
      - Family Phascolarctidae (*Phascolarctos*)
    - Suborder Phalangerida
      - Family Potoroidae (*Aepyprymnus*)
      - Family Macropodidae (*Macropus*)
      - Family Hypsiprymnodontidae (*Hypsiprymnodon*)
      - Family Phalangeridae (*Phalanger*)
      - Family Burramyidae (*Cercartetus*)
      - Family Petauridae (*Petaurus*)
      - Family Pseudocheiridae (*Pseudochirops*)
      - Family Acrobatidae (*Acrobates*)
      - Family Tarsipedidae (*Tarsipes*)
- Placentalia (Infraclass Eutheria)
  - Order Primates (*Homo*)
  - Order Artiodactyla (*Lama*)
  - Order Proboscidea (*Elephas*/*Loxodonta*)
  - Order Xenarthra (*Bradypus*)

[a]Commas separate taxa that correspond to distinct terminals in phylogenetic analyses; slashes indicate chimeric taxa that correspond to a single terminal in phylogenetic analyses.

Table 2. Summary of bootstrap support percentages and posterior probabilities[a]

Hypothesis	Maximum Parsimony	Maximum Likelihood		Bayesian Analyses			
		Partitioned	Non-Partitioned	Partitioned		Non-Partitioned	
				Run 1	Run 2	Run 1	Run 2
Diprotodontia	100	100	100	1.00	1.00	1.00	1.00
Petauroidea	100	100	100	1.00	1.00	1.00	1.00
Petauridae + Pseudocheiridae	73	80	85	1.00	1.00	1.00	1.00
Tarsipedidae + Petauridae + Pseudocheiridae	26	95	90	1.00	1.00	1.00	1.00
Vombatiformes	100	100	100	1.00	1.00	1.00	1.00
Macropodiformes	100	100	100	1.00	1.00	1.00	1.00
Macropodidae + Potoroidae	100	100	100	1.00	1.00	1.00	1.00
Phalangeroidea	97	96	96	1.00	1.00	1.00	1.00
Macropodiformes + Phalangeroidea	78	100	93	1.00	1.00	1.00	1.00
Phalangerida	100	100	100	1.00	1.00	1.00	1.00
Dasyuromorphia	100	100	100	1.00	1.00	1.00	1.00
Dasyuridae	100	100	100	1.00	1.00	1.00	1.00
Phascogalini	100	100	100	1.00	1.00	1.00	1.00
Dasyurini	100	100	100	1.00	1.00	1.00	1.00
Dasyurini + Phascogalini	100	100	100	1.00	1.00	1.00	1.00
Peramelemorphia	100	100	100	1.00	1.00	1.00	1.00
Peramelidae	100	100	100	1.00	1.00	1.00	1.00
Peramelinae	100	100	100	1.00	1.00	1.00	1.00
Peramelemorphia + Dasyuromorphia	40	55	64	0.94	0.94	0.97	0.97
Notoryctemorphia + Peramelemorphia + Dasyuromorphia	67	67	73	0.99	0.99	100	100
Eomarsupialia	65	47	48	0.66	0.65	0.73	0.74
Australidelphia	100	100	100	1.00	1.00	1.00	1.00
Paucituberculata	100	100	100	1.00	1.00	1.00	1.00
All marsupials except Didelphimorphia	<1	50	51	0.42	0.42	0.50	0.51
All marsupials except Paucituberculata	99	29	26	0.28	0.28	0.22	0.22
Ameridelphia	<1	21	23	0.29	0.31	0.27	0.28
Didelphimorphia	100	100	100	1.00	1.00	1.00	1.00
Didelphinae + Marmosinae	100	100	100	1.00	1.00	1.00	1.00
Marsupialia	100	100	100	1.00	1.00	1.00	1.00

[a]Partitioned = each gene was partitioned to have its own model of molecular evolution; Non-partitioned = concatenation was treated as a single gene

Table 3. *Multidivtime* and IRDIVTIME divergence estimates.

Divergence Estimates[a]						
	Multidivtime		IRDIVTIME			
Clade	Partitioned	Non-partitioned	*Bradypus* outgroup	Elephantidae outgroup	*Homo* outgroup	*Lama* outgroup
Diprotodontia	55.3 (48.4-61.1)	55.5 (48.4–61.2)	47.2	45.7	44.9	45.2
Petauroidea	41.6 (35.8-47.5)	40.9 (34.7–46.9)	33.3	32.2	32.0	32.2
Petauridae + Pseudocheiridae	36.8 (31.2-42.6)	34.9 (29.0–40.8)	29.3	28.3	28.2	28.4
Tarsipedidae + Petauridae + Pseudocheiridae	39.1 (33.2-44.9)	38.2 (32.0–44.1)	30.5	29.6	29.4	29.6
Vombatiformes	39.0 (32.9-45.0)	37.7 (31.0–44.3)	37.1	35.9	35.4	35.7
Macropodiformes	26.4 (22.1-31.4)	27.0 (22.4–32.4)	22.0	21.6	21.6	21.7
Macropodidae + Potoroidae	14.2 (12.1-17.7)	14.6 (12.2–18.8)	12.0	12.0	12.0	12
Phalangeroidea	42.8 (36.8-48.8)	43.3 (37.0–49.3)	35.3	34.2	33.9	34.1
Macropodiformes + Phalangeroidea	46.1 (39.9-52.1)	47.3 (40.7–53.2)	36.1	35.0	34.8	34.9
Phalangerida	49.9 (43.6-55.8)	49.5 (42.8–55.4)	39.1	37.8	37.4	37.7
Dasyuromorphia	27.3 (24.8-31.4)	27.6 (24.8–32.5)	25.2	25.2	25.2	25.2
Dasyuridae	17.0 (14.3-20.4)	18.0 (14.9–22.5)	14.7	14.6	14.2	14.3
Phascogalini	6.4 (4.9-8.3)	6.8 (5.0–9.4)	5.8	5.8	5.6	5.7
Dasyurini	6.5 (4.9-8.5)	5.5 (4.0–7. 6)	3.6	3.6	3.6	3.6
Dasyurini + Phascogalini	11.9 (9.7-14.8)	11.9 (9.4–15.4)	8.4	8.4	8.1	8.2
Peramelemorphia	23.8 (19.9-28.4)	24.2 (19.6–29.8)	19.6	19.2	19.1	19.1
Peramelidae	10.0 (7.8-12.8)	10.4 (7. 7–14.1)	7.8	7.7	7.7	7.7
Peramelinae	4.7 (3.7-6.4)	5.0 (3.7–7.3)	3.6	3.6	3.6	3.6
Peramelemorphia + Dasyuromorphia	60.7 (53.5-66.7)	59.5 (52.0-65.3)	47.8	46.4	45.7	45.9
Peramelemorphia + Dasyuromorphia + Notoryctemorphia	62.7 (55.4-68.4)	61.9 (53.9-67.5)	51.9	50.3	49.3	49.7
Eomarsupialia	63.9 (56.3-69.5)	63.5 (55.4-69.0)	58.5	56.5	55.1	55.7
Australidelphia	64.8 (57.1-70.3)	65.0 (56.7-70.4)	62.2	60.1	58.2	58.9
Paucituberculata	9.3 (6.8-12.2)	10.5 (7.3-14.7)	10.1	11.1	7.4	7.5
All marsupials except Paucituberculata	NA	NA	72.4	69.9	NA	NA
All marsupials except Didelphimorphia	75.6 (66.0-83.8)	77.2 (66.5-86.1)	NA	NA	70.0	67.9
Didelphimorphia	37.4 (30.3-45.3)	40.2 (31.2-49.6)	25.7	24.8	39.1	36.9
Didelphinae + Marmosinae	27.4 (21.4-34.2)	28.9 (21.2-37.2)	18.5	17.9		26.5
Marsupialia	78.1 (68.0-87.2)	80.4 (69.1-90.9)	81.7	83.9	81.3	80.6

[a]Divergence times for the most recent common ancestor of each clade are in millions of years.

Table 4. Percentage stemminess in phylograms and chronograms[a]

Analysis	Percentage Stemmines		
	Estbranches tree/neighbor joining tree before MVS correction	Neighbor-joining tree after MVS correction	*Multidivtime*/IRDIVTIME chronograms
Multidivtime Partitioned	NC	NA	31.47
Multidivtime Non-Partitioned	35.97	NA	31.71
IRDIVTIME w/ *Bradypus*	35.29[b]	36.80	34.51
IRDIVTIME w/ Elephantidae	35.29[b]	36.83	34.83
IRDIVTIME w/ *Homo*	35.29[b]	36.83	32.74
IRDIVTIME w/ *Lama*	35.29[b]	36.99	32.99

[a]All stemminess calculations were performed after excluding all branches between placentals and the branch connecting Placentalia to Marsupialia. Abbreviations as follows: NC = not calculated because *Multidivtime* analyses with partitioned data produce a different phylogram for each data partition; NA = not applicable.

[b]Stemminess calculations were based on the neighbor joining tree for 28 marsupials and four placental outgroups.