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A phylogeny of Diprotodontia (Marsupialia) based on sequences for five nuclear genes

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ABSTRACT

Even though the marsupial order Diprotodontia is one of the most heavily studied groups of Australasian marsupials, phylogenetic relationships within this group remain contentious. The more than 125 living species of Diprotodontia can be divided into two main groups: Vombatiformes (wombats and koalas) and Phalangerida. Phalangerida is composed of the kangaroos (Macropodidae, Potoroidae, and Hypsiprymnodontidae) and possums (Phalangeridae, Burramyidae, Petauridae, Pseudocheiridae, Tarsipedidae, and Acrobatidae). Much of the debate has focused on relationships among the families of possums and whether possums are monophyletic or paraphyletic. A limitation of previous investigations is that no study to date has investigated diprotodontian relationships using all genera. Here, we examine diprotodontian interrelationships using a nuclear multigene molecular data set representing all recognized extant diprotodontian genera. Maximum parsimony, maximum likelihood, and Bayesian methods were used to analyze sequence data obtained from protein-coding portions of ApoB, BRCA1, IRBP, Rag1, and vWF. We also applied a Bayesian relaxed molecular clock method to estimate times of divergence. Diprotodontia was rooted between Vombatiformes and Phalangerida. Within Phalangerida, the model-based methods strongly support possum paraphyly with Phalangeroidea (Burramyidae + Phalangeridae) grouping with the kangaroos (Macropodiformes) to the exclusion of Petauroidea (Tarsipedidae, Acrobatidae, Pseudocheiridae, and Petauridae). Within Petauroidea, Tarsipedidae grouped with both Petauridae and Pseudocheiridae to the exclusion of Acrobatidae. Our analyses also suggest that the diprotodontian genera *Pseudochirops* and *Strigocuscus* are paraphyletic and diphyletic, respectively, as currently recognized. Dating analyses suggest Diprotodontia diverged from other australidelphians in the late Paleocene to early Eocene with all interfamilial divergences occurring prior to the early Miocene except for the split between the Potoroidae and Macropodidae, which occurred sometime in the mid-Miocene. Ancestral state reconstructions using a Bayesian method suggest that the patagium evolved independently in the Acrobatidae, Petauridae, and Pseudocheiridae. Ancestral state reconstructions of ecological venue suggest that the ancestor of Diprotodontia was arboreal. Within Diprotodontia, the common ancestor of Macropodidae was reconstructed as terrestrial, suggesting that tree kangaroos (*Dendrolagus*) are secondarily arboreal.

1. Introduction

Of the seven extant marsupial orders, Diprotodontia is the largest and most ecologically diverse. More than 125 living diprotodontian species are currently recognized. Most diprotodontians are herbivorous, but there are also forms specialized for nectarivory, folivory, and insect-omnivory (Aplin and Archer, 1987). Locomotory specializations include arboreality, bipedal locomotion, fossoriality, and gliding. When fossil taxa are considered, Diprotodontia is even more diverse and includes the carnivorous thylacoleonids, commonly known as marsupial lions, and herbivorous diprotodontids, among which are the largest known marsupials from any time period (Long et al., 2002).

The name Diprotodontia was coined by Owen in 1866 for Australian diprotodont marsupials (Aplin and Archer, 1987). Diprotodonty, a state in which the lower medial incisors are enlarged and procumbent, occurs in all diprotodontians and is widely considered a diagnostic morphological synapomorphy for the group (Kirsch, 1977; Aplin and Archer, 1987). A superficial cervical thymus gland has also been reported as a diagnostic diprotodontian character by Yadav (1973), who examined 51 diprotodontian taxa, including representatives of all diprotodontian families (*sensu* Wilson and Reeder, 2005) except for Hypsiprymnodontidae, and found that this feature was present in all

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diprotodontians but not in other marsupials. However, Yadav (1973) did not include *Dromiciops gliroides* in his study. The presence or absence of the superficial cervical thymus in *D. gliroides* is of special interest given Kirsch et al.'s (1991) hypothesis that *D. gliroides* and Diprotodontia are sister taxa. A Diprotodontia + *Dromiciops* clade was also suggested by Drummond et al. (2006). More recently, Haynes (2001) found evidence for a deep cervical thymus in two species of *Isoodon* and suggested that bandicoots may be intermediate between other polyprotodont marsupials and Diprotodontia. The presence of the fasciculus aberrans, which connects the cerebral hemispheres, has also been suggested as a diagnostic diprotodontian character (Abbie, 1937). The fasciculus aberrans occurs in all diprotodontians that were investigated by Abbie (1937), who as noted by Aplin and Archer (1987), even coined the name Duplicicommissurala to replace Diprotodontia based on this putative diagnostic character. However, there remain important gaps in taxonomic sampling for this character including *Tarsipes* and burramyids (Aplin and Archer, 1987; Luckett, 1994). The intermediate placement of bandicoots between polyprotodont and diprotodontian marsupials has also been suggested based on the presence of the syndactylous condition exhibited by both Diprotodontia and Peramelemorphia (= Syndactyla) (e.g. Jones, 1923; Szalay, 1994; Weisbecker and Nilsson, 2008). However, Syndactyla has been rejected in almost all recent molecular, morphological, and combined analyses although Szalay and Sargis (2001, 2006) still favor this grouping.

Even with only a few diagnostic morphological characters that occur broadly across Diprotodontia, morphological cladistic studies almost always recover this order as monophyletic (e.g. Archer, 1984; Aplin and Archer, 1987; Luckett, 1994; Marshall et al., 1990; Springer et al., 1997; Horovitz and Sánchez-Villagra, 2003). Molecular studies are in solid agreement with morphology in supporting diprotodontian monophyly (Baverstock, 1984; Baverstock et al., 1987, 1990; Springer and Kirsch, 1991; Kirsch et al., 1997; Colgan, 1999; Amrine-Madsen et al., 2003; Baker et al., 2004; Kavanagh et al., 2004; Nilsson et al., 2004; Munemasa et al., 2006; Phillips et al., 2006; Phillips and Pratt, 2008; Beck, 2008; Beck et al., 2008; Meredith et al., 2008a). Combined morphological and molecular analyses have likewise supported diprotodontian monophyly (Asher et al., 2004; Beck et al., 2008).

Simpson (1945) included all marsupials in Order Marsupialia with six superfamilies; Simpson's (1945) Phalangeroidea is synonymous with Diprotodontia (Table 1) and included three extant families: Phalangeridae, Phascolomidae (= Vombatidae), and Macropodidae. Subsequent to Simpson's classic work, other workers elevated Marsupialia and its superfamilies to higher taxonomic ranks. Kirsch (1977) recognized three extant superfamilies (Vombatoidea, Phalangeroidea, and Tarsipedoidea) in the Order Diprotodonta (different spelling following Ride, 1964). Kirsch's (1977) Phalangeroidea contained the kangaroos and all of the possums except for *Tarsipes*, which was placed in its own superfamily; Vombatoidea consisted of Phascolarctidae and Vombatidae. Woodburne (1984) placed *Tarsipes*, along with the other possums and kangaroos, in the new Suborder Phalangeriformes and elevated Vombatoidea to Suborder Vombatiformes. Aplin and Archer (1987) retained the same arrangement as Woodburne (1984), but replaced Phalangeriformes with Phalangerida as a *nomen novem* given that Phalangeriformes was considered to be unavailable. Aplin and Archer's (1987) classification was the first to recognize the families Pseudocheiridae and Acrobatidae. Marshall et al. (1990) retained the same suborders as Woodburne (1984), but recognized all of the families in Aplin and Archer (1987). Szalay (1994) also used the suborders of Marshall et al. (1990), but proposed a different classification within Phalangeriformes, which has not been followed since (Table 1). Kirsch et al. (1997) removed the kangaroos from Phalangeriformes and elevated them to Suborder Macropodiformes. As a result, Phalangeriformes consisted of only the Australasian possums. Within Phalangeriformes, Kirsch et al. (1997) grouped Burramyidae and Phalangeridae in Superfamily Phalangeroidea and the remaining Australasian possums (Pseudocheiridae, Petauridae, Acrobatidae, Tarsipedidae) in Superfamily Petauroidea. Wilson and Reeder (2005) provide one of the most recent marsupial classifications and recognize the three suborders of Kirsch et al. (1997). Kear and Cooke (2001) and Wilson and Reeder (2005) also recognize Macropodidae, Potoroidae, and Hypsiprymnodontidae as distinct families within Macropodiformes.

Resolving higher-level relationships within Diprotodontia has proved difficult. Aplin and Archer (1987), Flannery (1987), and Springer and Woodburne (1989) found morphological support for a Macropodiformes + Phalangeriformes (*sensu* Kirsch et al., 1997) clade. Horovitz and Sánchez-Villagra (2003) recovered a sister group relationship between Vombatiformes and Macropodiformes (*sensu* Kirsch et al., 1997) with this collective group nested inside of a paraphyletic Phalangeriformes. Nilsson et al. (2004), Asher et al. (2004), and Beck et al. (2008) also found support for a Vombatiformes + Macropodiformes clade. In contrast, Szalay and Sargis (2006), Weisbecker and Sánchez-Villagra (2006), and Weisbecker and Archer (2008) suggest that this association is spurious and results from convergent/parallel changes associated with the terrestrial plantigrade locomotion exhibited by both groups. Single-copy DNA–DNA hybridization studies (e.g. Springer and Kirsch, 1991; Springer et al., 1997; Kirsch et al., 1997) provided some support for the monophyly of Phalangeriformes, but were unable to resolve relationships between Phalangeriformes, Macropodiformes, and Vombatiformes. Analyses of mitochondrial rRNA gene sequences (Kavanagh et al., 2004) consistently failed to recover Phalangeriformes. Osborne et al.'s (2002) analysis of mitochondrial ND2 and ND2 + 12S rRNA gene sequences returned inconclusive results for both Phalangeriformes and Phalangeriformes + Macropodiformes. Nilsson et al. (2004) analyzed sequences from complete mitochondrial genomes and obtained strong support for a basal diprotodontian split between *Tarsipes* and all other taxa including two phalangeriforms (*Pseudocheirus*, *Trichosurus*), one vombatiform (*Vombatus*), and two macropodiforms (*Macropus*, *Potorous*). In contrast, concatenated nuclear DNA sequences (Amrine-Madsen et al., 2003; Meredith et al., 2008a, in press-a; Springer et al., 2009) and combined mitochondrial and nuclear DNA sequences (Phillips and Pratt, 2008; Beck, 2008; Beck et al., 2008) provide robust support for a basal split between Vombatiformes and Phalangerida (i.e. Macropodiformes + Phalangeriformes). There is also emerging support from several molecular studies (Beck, 2008; Beck et al., 2008; Phillips and Pratt, 2008; Meredith et al., 2008a, in press-a; Springer et al., 2009) for the paraphyly of Phalangeriformes, with phalangeroid possums (Burramyidae, Phalangeridae) more closely related to Macropodiformes than to other petauroid possums (Pseudocheiridae, Petauridae, Acrobatidae, Tarsipedidae). A possible morphological synapomorphy for this group is the presence of an enlarged plagiualacoid premolar (Phillips and Pratt, 2008; Beck, 2008).

In the present paper, we examine evolutionary relationships of all recognized diprotodontian families and genera (*sensu* Wilson and Reeder, 2005) using a molecular data set consisting of five nuclear protein-coding gene segments. Outgroup representation includes representatives of all recognized marsupial orders and families (*sensu* Springer et al., 2009; Meredith et al., in press-a) and representatives of the four major clades of placental mammals (i.e. Afrotheria, Euarchontoglires, Laurasiatheria, and Xenarthra; Murphy et al., 2001). We also use the Bayesian relaxed molecular clock method of Thorne and Kishino (2002) to construct a timescale for Marsupialia. Finally, we use a Bayesian approach to estimate ancestral states and the deployment of character state changes for gliding and ecological venue within Marsupialia.

Table 1
Previous diprotodontian classifications.

Simpson (1945)	Kirsch (1977)	Woodburne (1984)
Superfamily Phalangeroidea (Diprotodontia) Family Phalangeridae (included Acrobatidae, Petauridae, *Cercartetus*) Subfamily Tarsipedinae Subfamily Phascolarctinae (included Pseudocheiridae and Phascolarctidae) Subfamily Burramyinae (*Burramys* only) Family Phascolomidae (included Vombatidae) Family Macropodidae Subfamily Potoroinae (included Hypsiprymnodontidae)	Order Diprotodontia Superfamily Vombatoidea Family Phascolarctidae Family Vombatidae Superfamily Phalangeroidea Family Phalangeridae Family Petauridae Subfamily Petaurinae Subfamily Pseudocheirinae Family Burramyidae (included Acrobatidae) Family Macropodidae Subfamily Macropodinae Subfamily Potoroinae Superfamily Tarsipedoidea Family Tarsipedidae	Order Diprotodontia Suborder Vombatiformes Superfamily Vombatoidea Family Vombatidae Superfamily Phascolarctoidea Family Phascolarctidae Suborder Phalangeriformes Superfamily Phalangeroidea Family Phalangeridae Family Petauridae Subfamily Petaurinae Subfamily Pseudocheirinae Family Burramyidae (included Acrobatidae) Family Macropodidae Subfamily Potoroinae Tribe Hypsiprymnodontini Tribe Potoroini Subfamily Macropodinae Superfamily Tarsipedoidea Family Tarsipedidae
Aplin and Archer (1987)	**Marshall et al. (1990)**	**Szalay (1994)**
Order Diprotodontia Suborder Vombatiformes Infraorder Phascolarctomorphia Family Phascolarctidae Infraorder Vombatomorphia Family Vombatidae Suborder Phalangerida Superfamily Phalangeroidea Family Phalangeridae Superfamily Macropodoidea Family Macropodidae Family Potoroidae Superfamily Burramyoidea Family Burramyidae Superfamily Petauroidea Family Pseudocheiridae Family Petauridae Superfamily Tarsipedoidea Family Tarsipedidae Family Acrobatidae	Order Diprotodontia Suborder Vombatiformes Superfamily Phascolarctoidea Family Phascolarctidae Superfamily Vombatoidea Family Vombatidae Suborder Phalangeriformes Superfamily Macropodoidea Family Macropodidae Subfamily Macropodinae Subfamily Potoroinae Tribe Hypsiprymnodontini Tribe Potoroini Superfamily Phalangeroidea Family Phalangeridae Superfamily Petauroidea Family Burramyidae Family Pseudocheiridae Family Petauridae Family Acrobatidae Family Tarsipedidae	Order Diprotodontia Suborder Phalangeriformes Superfamily Phalangeroidea Family Phalangeridae Superfamily Petauroidea Family Petauridae Subfamily Petaurinae Tribe Pseudocheirini Subfamily Burramyinae Tribe Burramyini Tribe Acrobatini Family Tarsipedidae Superfamily Macropodoidea Family Hypsiprymnodontidae Family Macropodidae Subfamily Potoroinae Subfamily Macropodinae Suborder Vombatiformes Superfamily Phascolarctoidea Family Phascolarctidae Superfamily Vombatoidea Family Vombatidae
Kirsch et al. (1997)	**McKenna and Bell (1997)**	**Wilson and Reeder (2005)**
Order Diprotodontia Suborder Vombatiformes Superfamily Vombatoidea Family Vombatidae Superfamily Phascolarctoidea Family Phascolarctidae Suborder Phalangeriformes Superfamily Phalangeroidea Family Phalangeridae Subfamily Phalangerinae Tribe Phalangerini Tribe Trichosurini Subfamily Ailuropinae Family Burramyidae Superfamily Petauroidea Family Petauridae Family Pseudocheiridae Family Acrobatidae Family Tarsipedidae Suborder Macropodiformes Superfamily Macropodoidea Family Macropodidae Subfamily Macropodinae Tribe Macropodini Tribe Sthenurini Subfamily Potoroinae Tribe Potoroini Family Hypsiprymnodontidae Subfamily Hypsiprymnodontinae	Order Diprotodontia Family Tarsipedidae Superfamily Vombatoidea Family Vombatidae Superfamily Phalangeroidea Family Phalangeridae Family Burramyidae Family Macropodidae Subfamily Potoroinae Subfamily Macropodinae Family Petauridae Subfamily Petaurinae Subfamily Pseudocheirinae Family Phascolarctidae Family Acrobatidae	Order Diprotodontia Suborder Vombatiformes Family Vombatidae Family Phascolarctidae Suborder Phalangeriformes Superfamily Phalangeroidea Family Burramyidae Family Phalangeridae Superfamily Petauroidea Family Petauridae Family Pseudocheiridae Family Acrobatidae Family Tarsipedidae Suborder Macropodiformes Family Macropodidae Family Hypsiprymnodontidae Family Potoroidae

2. Materials and methods

2.1. Taxon sampling

Our study included representatives of all recognized diprotodontian genera (*sensu* Wilson and Reeder, 2005) and all extant marsupial families (*sensu* Springer et al., 2009; Meredith et al., in press-a). The 81 mammalian species are indicated in Supplementary Information. Marsupial classification follows Wilson and Reeder (2005), although we recognize two families within Didelphimorphia (Didelphidae and Caluromyidae) following Kirsch and Palma (1995). We chose one representative from each of the four major placental clades (i.e. Afrotheria, Euarchontoglires, Laurasiatheria, and Xenarthra; Murphy et al., 2001) as outgroups to Marsupialia.

2.2. Gene sequences

Genomic DNA was extracted following the protocols outlined in Kirsch et al. (1990) or Meredith et al. (2008a). Given the resolution obtained in the previous marsupial studies of Amrine-Madsen et al. (2003) and Meredith et al. (2008a,b, in press-a,b), we added sequences to these data sets. The genes used include portions of exon 26 of ApoB (Apolipoprotein B), 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). Primers used to amplify the five gene regions of all of the taxa in this study can be found in the primer fasta files in Supplementary Information. There is one fasta file for each gene and the primers are aligned to *Monodelphis domestica* sequences downloaded from Ensembl 48. All gene regions were amplified in one segment except for BRCA1, which was amplified in three or four overlapping segments. All PCRs were carried out with Taq DNA polymerase (Invitrogen) using the following protocol: initial denaturation at 94 °C for 2 min; 35 cycles of 1 min at 94 °C (denaturation), 1 min at 50 °C (annealing), and 1 min at 72 °C (extension), and a final extension at 72 °C for 10 min.

If the primary PCR did not yield a product, nested primer pair sets for full nested and half nested PCRs were carried out using the aforementioned protocols. For example, the primer pair set F60 and R820 was usually used to amplify the ApoB segment. For the fully nested or half nested 50 μl PCRs we used 1ul of the PCR product obtained from the original PCR as the template DNA. Examples of fully nested and half nested PCRs for ApoB are the primers pairs Mars-F90 and Per820 for the former and F60 and Per820 for the latter. PCR products were then run out on a 1% agarose gel and the product of interest was excised and cleaned using the QIAquick Gel Extraction Kit (QIAGEN) or the AccuPrep™ Gel Purification kit (Bioneer Corporation). Cleaned products were sequenced using an automated DNA sequencer (ABI 3730xl) at the Core Genomics Instrumentation Facility at the University of California, Riverside. Sequencing primers were designed as necessary and all PCR products were sequenced in both directions. Contigs were assembled using Sequencher 4.1.

Accession numbers of new and previously published sequences are given in Supplementary Information Table S1. The data set is complete, i.e. all 81 taxa have been amplified for protein-coding portions of the five nuclear genes used in this study.

2.3. DNA alignments and data compatibility

We implemented SOAP v1.2a4 (Löytynoja and Milinkovitch, 2001) with gap opening (11–19) and gap extension (3–11) penalties in steps of two to identify alignment-ambiguous regions. This resulted in 25 different alignments for each gene, which were then manually re-aligned using SE-AL (Rambaut, 1996) taking amino acid residues into account. This resulted in the identification of nine alignment-ambiguous regions in BRCA1 (237 bp) and one region in IRBP (36 bp). The appropriateness of combining the individual gene segments into a multigene data set was tested using both the partition homogeneity test (one test with five partitions for each gene, 100 replicates, and 10 taxon input orders per replicate; Farris et al., 1994; Swofford, 2002) and the bootstrap compatibility method (500 bootstrap replicates, and a 90% bootstrap support criterion; De Queiroz, 1993; Teeling et al., 2002). For the ML bootstrap compatibility tests each gene segment had its own model of sequence evolution as suggested by the Akaike Information Criterion following the suggestion of Posada and Buckley (2004) (implemented in Modeltest 3.06; Posada and Crandall, 1998). Modeltest chose the following models: GTR+I+Γ (ApoB, BRCA1, Rag1) and TVM+I+Γ (IRBP, vWF). The partition homogeneity test was significant ($P = 0.01$), but the bootstrap compatibility method indicated that it was appropriate to combine the data set. It should be noted that the partition homogeneity test can be too conservative, which leads to excessive type I errors (e.g. Cunningham, 1997; Barker and Lutzoni, 2002; Darlu and Lecointre, 2002); Cunningham (1997) suggests a critical alpha value of between 0.01 and 0.001. As a result, we elected to concatenate the individual gene segments into a multigene data set (5894 bp without alignment-ambiguous regions).

2.4. Phylogenetic analyses

We employed RAxML-VI-HPC (v2.2.3; Stamatakis, 2006) to perform partitioned and non-partitioned maximum likelihood (ML) analyses and PAUP 4.0b10 (Swofford, 2002) to perform maximum parsimony (MP) analyses. Modeltest 3.06 (Posada and Crandall, 1998) under the Akaike Information Criterion (AIC) was implemented to identify the best fit model of sequence evolution for the non-partitioned data (Posada and Buckley, 2004). The chosen model was GTR+I+Γ, but we used the GTR+Γ model of sequence evolution as v2.2.3 of RAxML-VI-HPC did not allow for invariant sites. For both gene partitioned and codon positioned analyses, each gene or codon, respectively, was given its own model of molecular evolution as suggested by Modeltest 3.06 (Posada and Crandall, 1998) [see above for gene models; codon position 1 (GTR+Γ), codon position 2 (GTR+Γ), codon position 3 (GTR+Γ)]. In the MP analyses, we implemented 1000 randomized addition orders with tree-bisection and reconnection (TBR) branch swapping and 1000 random input orders. The RAxML-VI-HPC partitioned and non-partitioned ML analyses were started from randomized MP starting trees employing the fast hill-climbing algorithm with all other free model parameters estimated. In both the MP and ML analyses gaps were treated as missing. Bootstrap analyses employed either 1000 (MP) or 500 (ML) replicates.

Bayesian posterior probabilities were calculated using the parallel version of MrBayes v3.1.1 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003), which carries out Metropolis-coupled Markov chain Monte Carlo sampling. Models selected by Modeltest (see above) were used in the Bayesian analyses. If Modeltest chose a model not implemented in MrBayes, we used the next most general model. We performed three different analyses on the multigene data set. In the non-partitioned analyses a single model of sequence evolution was implemented; in the partitioned gene analyses each gene was given its own model of sequence evolution; and in the codon partitioned analyses each codon position was given its own model of sequence evolution as suggested by Modeltest (see above). All Bayesian analyses used default priors, random starting trees, eight chains (one cold, seven hot), and were terminated once the average standard deviation of split frequen-

cies for the simultaneous analyses fell below 0.01 (~20 million generations).

2.5. Statistical tests of tree topologies

We evaluated alternative phylogenetic hypotheses using the approximately unbiased (AU) (Shimodaira, 2002), Kishino–Hasegawa (KH) (Kishino and Hasegawa, 1989), and Shimodaira–Hasegawa (SH) (Shimodaira and Hasegawa, 1999) statistical tests. The AU test attempts to overcome tree selection biases associated with the KH (Kishino and Hasegawa, 1989) and SH (Shimodaira and Hasegawa, 1999) tests, but is only approximately unbiased (Shimodaira, 2002). AU tests were performed with Consel (Shimodaira, 2002). Constrained tree searches were performed on the non-partitioned data set using RAxML-VI-HPC (v2.2.3; Stamatakis, 2006) under the GTR+Γ model of sequence evolution and the fast hill-climbing algorithm for ten runs with all of the free model parameters estimated. This resulted in ten different ML trees obtained from ten different randomized MP starting trees. The tree with the highest likelihood score was then imported into PAUP 4.0b10 (Swofford, 2002) to perform a full likelihood evaluation for each site. Eleven categories of *a priori* hypotheses were evaluated and are summarized with literature sources in Supplementary Information Table 2. Results of the AU tests are given in Table 3 and both the SH and KH test results can be found in Supplementary Information Table 3.

2.6. Molecular dating analyses

We tested the molecular clock hypothesis using the likelihood ratio statistic. The test rejected the molecular clock hypothesis for ApoB, BRCA1, IRBP, Rag1, and vWF ($P < 0.01$). As a result, we employed the Bayesian relaxed molecular clock method implemented by *Multidivtime* (version 9-25-03) to estimate posterior probabilities of divergence times (Kishino et al., 2001; Thorne and Kishino, 2002). *Multidivtime* requires a rooted tree topology, only allows for fixed minimum and maximum constraints, assumes autocorrelated rates of molecular evolution among lineages, and estimates branch lengths using the program *estbranches* (Thorne et al., 1998; Kishino et al., 2001; Thorne and Kishino, 2002). We used the Bayesian phylogeny shown in Fig. 1 and allowed each gene to have its own rate trajectory over time. We implemented the F84 model of sequence evolution (Swofford et al., 1996) with four discrete categories for the Γ distribution. This is the most complicated model implemented by *Multidivtime* and this model was chosen because Modeltest (see Phylogenetic Analyses) suggested models that were at least this complicated. PAUP 4.0b10 (Swofford, 2002) was used to estimate the transition/transversion parameter and the rate categories of the gamma distribution for the tree shown in Fig. 1. We used 75 million years as the mean prior distribution for the root of Marsupialia. This date is 6–10 million years older than the oldest described crown-group metatherian fossils, i.e. the herpetotheriid genus *Nortedelphys* (Case et al., 2005). We recognize that the interpretation of these fossils as crown-group metatherians is not agreed on by all workers (e.g. the cladistic analysis of Sánchez-Villagra et al., 2007). However, the cladistic analysis of Goin et al. (2006) recovered *Nortedelphys* as a crown-group metatherian. Following the recommendations of Thorne and Kishino (2002), the prior distribution for the rate of molecular evolution at the ingroup root node was set equal to the median amount of evolution from the ingroup root node to the ingroup tips. This value was then divided by the mean of the prior distribution for the root of Marsupialia. The analysis was run for 1 million generations with chain sampling every 100 generations and a burnin of 100,000 generations to allow the Markov chains to reach stationarity. We implemented the same 32 constraints found in Meredith et al. (in press-a).

2.7. Ancestral state reconstructions

We used Version 1.0 B2.3.2 of SIMMAP (Bollback, 2006) to estimate ancestral states for gliding [(0) absent; (1) flaps of skin in inguinal and axillary areas; (2) present] and ecological venue [(0) arboreal; (1) terrestrial; (2) semi-fossorial; (3) fossorial]. Ecological venue, although a continuous character, was partitioned into discrete character states based on the predominant habitat occupied by the genus. For example, a genus was treated as arboreal if its members are known to spend most of their time in the trees. SIMMAP calculates the posterior probability distribution of the rate of change and the total number of character state changes needed to explain the distribution of character states in the terminal taxa. SIMMAP implements the simulation procedure of Nielsen (2002) to stochastically map discrete characters onto multiple trees thereby taking phylogenetic uncertainty into account. The posterior distribution is sampled by averaging over multiple trees with the number of changes proportional to branch length. During the analyses, character states remain constant at tips. The gamma distributed rate prior must be specified and is described by the parameters α and β: α and β describe the mean (α/β) and variance (α/β^2). The gamma distribution was approximated with 50 discrete categories; branch lengths were rescaled before applying the prior to maintain branch length proportionality. Ranges of morphological priors were used to investigate the robustness of our estimates. Priors used were $\alpha = 1$ and $\beta = 1$; $\alpha = 3$ and $\beta = 2$; and $\alpha = 5$ and $\beta = 5$. For the inferences referring to the ancestral state of gliding, we used an equal prior on the bias prior. Venue characters were treated as both ordered and unordered characters. We used 2% of the post-burnin Bayesian trees obtained from the partitioned Bayesian analysis. Trees were sampled at equal intervals starting from the post-burnin trees resulting in 451 trees, each of which was used in the SIMMAP analyses (342 different tree topologies). We employed ten draws from the prior distribution. We report results only from those analyses using the priors set to $\alpha = 3$ and $\beta = 2$ given that similar results were obtained from all three sets of priors [posterior probabilities always within 0.0000–0.0704 (unordered) and 0.000–0.1101 (ordered)].

3. Results

3.1. Phylogenetic analyses

Fig. 1 shows the Maximum Posterior Probability (MPP) tree for the gene partitioned Bayesian analysis with mean Bayesian Posterior Probabilities (BPP) based on two independent runs and ML Bootstrap Support Percentages (BSP) derived from the RaxML gene partitioned analysis. The MPP tree depicts the most often encountered post-burnin tree and is found as a combinable component consensus of all sampled trees (Waddell and Shelley, 2003). Fig. 2 shows the ML phylogram obtained from the RAxML gene partitioned analysis. The MP analysis resulted in one island of trees (36 trees at 13,356 steps; 50% majority-rule consensus tree given in Supplementary Information). BSPs for the ML and MP analyses and BPPs for the partitioned and non-partitioned analyses are summarized in Table 2.

The MPP gene partitioned and non-partitioned trees were identical except for the placement of Paucituberculata. In the partitioned analyses, Didelphimorphia was recovered as the sister group to Australidelphia whereas in the non-partitioned analyses Paucituberculata was the sister group to Australidelphia. The MPP and ML analyses, in which the data was partitioned by codon

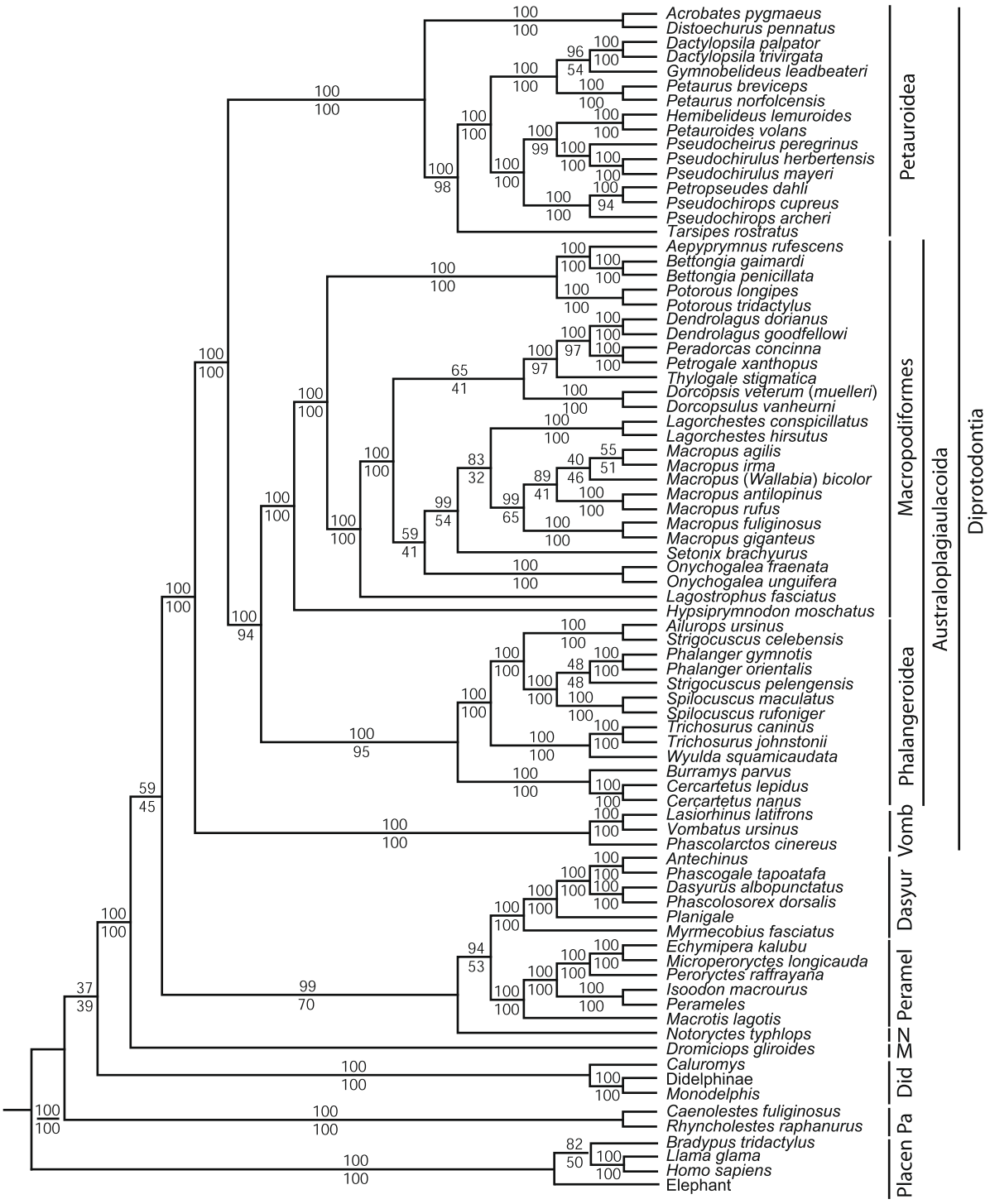

Fig. 1. Bayesian tree obtained from the gene partitioned Bayesian analysis. Values above and below branches correspond to the mean percentage Bayesian posterior probabilities based on the two simultaneous runs and the ML bootstrap support percentages for the RAxML gene partitioned analyses, respectively. Placen = Placentalia; Did = Didelphidae; Pa = Paucituberculata; M = Microbiotheria; N = Notoryctemorphia; Peramel = Peramelemorphia; Dasyur = Dasyuromorphia; Vomb = Vombatiformes.

position, were identical except for within Macropodidae. The MPP tree grouped *Lagorchestes* and *Setonix* to the exclusion of the *Macropus* + *Wallabia* clade whereas the ML analysis grouped *Lagorchestes* and *Macropus* + *Wallabia* to the exclusion of *Setonix*.

The cohort Australidelphia was recovered as monophyletic in all analyses, but none of the analyses recovered a monophyletic Ameridelphia. Within Australidelphia, the four Australasian orders grouped together with weak support (42–55% BSP and 0.54 BPP) in all but the codon partitioned analyses. The basal split among Australasian taxa was between Diprotodontia (100% BSP and 1.00 BPP) and a clade comprising *Notoryctes*, Dasyuromorphia, and Peramelemorphia (58% MP BSP, 70–77% ML BSP, 1.00 BPP). The two codon position analyses recovered *Dromiciops* as the sister group of Diprotodontia (45% BSP and 0.47–0.56 BPP). All marsupial orders and families were recovered as monophyletic. Bootstrap and Bayesian analyses supported monophyly of all marsupial genera (100% BSP and 1.00 BPP) except for *Strigocuscus* and *Pseudochirops*.

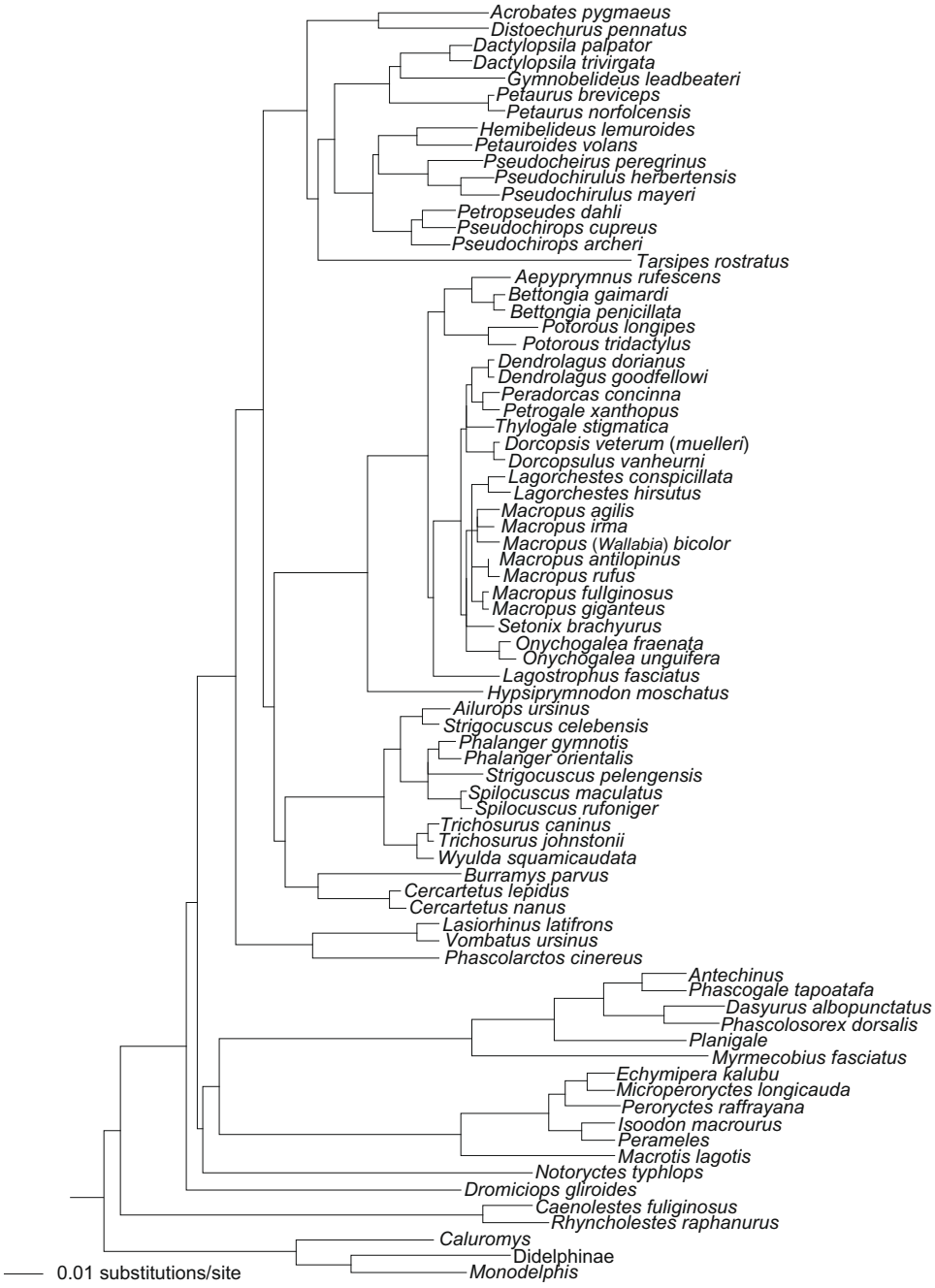

Fig. 2. Maximum likelihood phylogram obtained from the RAxML gene partitioned analysis (GTR+Γ model of sequence evolution) using protein-coding portions of ApoB, BRCA1, IRBP, Rag1, and vWF. Placental outgroups are not shown.

Within Diprotodontia, Phalangerida and Vombatiformes were recovered with strong support (100% BSP and 1.00 BPP). Within Phalangerida, Phalangeroidea grouped with Macropodiformes to the exclusion of Petauroidea (71% MP BSP, 65–94% ML BSP, 1.00 BPP). Within Petauroidea, there was robust support for a basal split between Acrobatidae and all other petauroids (54% MP BSP, 94–98% ML BSP, 1.00 BPP). Among the remaining petauroids, Petauridae grouped with Pseudocheiridae to the exclusion of Tarsipedidae (95–100% BSP, 1.00 BPP). Within the Petauridae, *Dactylopsila* grouped with *Gymnobelideus* to the exclusion of *Petaurus* (54–59% BSP, 0.95–0.97BPP). Within Pseudocheiridae, *Hemibelideus* joined *Petauroides* (100% BSP, 1.00 BPP), *Pseudocheirus* joined *Pseudochirulus* (100% BSP, 1.00 BPP), and *Pseudochirops* was paraphyletic with *P. cupreus* grouping with *Petropseudes* to the exclusion of *P. archeri* (88–94% BSP, 1.00 BPP). Deeper in the pseudocheirid tree, the *Hemibelideus* + *Petauroides* clade grouped with the *Pseudocheirus* + *Pseudochirulus* clade to the exclusion of the *Pseudochirops* + *Petropseudes* clade (95–99% BSP, 1.00 BPP). Within Macropodiformes, Potoroidae grouped with Macropodidae (100% BSP, 1.00 BPP) to the exclusion of Hypsiprymnodontidae. Within Potoroidae, *Aepyprymnus* grouped with *Bettongia* (100% BSP, 1.00 BPP) to the exclusion of *Potorous*. Within Macropodidae, *Lagostrophus* was the sister taxon to all other macropodids (100% BSP, 1.00 BPP). *Peradorcas* joined *Petrogale* (100% BSP, 1.00 BPP), these two grouped with *Dendrolagus* (94–99% BSP, 1.00 BPP), and this collective group joined *Thylogale* (99% BSP, 1.00 BPP). *Dorcopsis*

Table 2
Posterior probabilities and bootstrap summaries. All clades that are supported at the 100% bootstrap and 1.00 posterior probability levels are not shown. Bayesian results report the average of the two simultaneous runs. Partitioned = each gene was given its own model of molecular evolution; non-partitioned = ApoB, BRCA1, IRBP, Rag1, and vWF were treated as a single gene; codon = each codon position was given its own model of molecular evolution; MP = maximum parsimony; ML = maximum likelihood.

Phylogenetic hypothesis	MP	ML			Bayesian analyses		
		RAxML			MrBayes		
		Partitioned	Non-partitioned	Codon	Partitioned	Non-partitioned	Codon
Ameridelphia	0.2	19	21	23	0.30	0.27	0.28
Paucituberculata + Australidelphia	0	42	42	58	0.34	0.42	0.63
Didelphimorphia + Australidelphia	97	39	37	19	0.37	0.32	0.09
Australasian taxa	55	45	42	40	0.59	0.54	0.44
Dromiciops + Diprotodontia	0	36	44	45	0.41	0.47	0.56
Notoryctemorphia + Peramelemorphia + Dasyuromorphia	58	70	77	75	0.99	1.00	1.00
Peramelemorphia + Dasyuromorphia	37	53	62	69	0.94	0.97	0.99
Macropodiformes + Petauroidea	0	0	0	26	0.00	0.00	0.05
Macropodiformes + Vombatiformes	0	0	0	0	0.00	0.00	0.00
Macropodiformes + Phalangeroidea	71	94	94	65	1.00	1.00	0.95
Phalangeroidea	100	95	95	93	1.00	1.00	1.00
Phalanger + *Strigocuscus pelengensis*	0	48	49	47	0.48	0.57	0.70
Phalanger + *Spilocuscus*	44	34	35	25	0.40	0.33	0.21
Strigocuscus monophyly	0	0	0	0	0.00	0.00	0.00
Tarsipedidae + Petauridae + Pseudocheiridae	54	98	97	94	1.00	1.00	1.00
Petauridae + Pseudocheiridae	95	100	100	100	1.00	1.00	1.00
Dactylopsila + *Gymnobelideus*	59	54	55	57	0.96	0.97	0.96
Hemibelideus + *Petauroides* + *Pseudocheirus* + *Pseudochirulus*	95	99	99	99	1.00	1.00	1.00
Petropseudes + *Pseudochirops cupreus*	88	94	92	87	1.00	1.00	1.00
Pseudochirops monophyly	0	0	0	0	0.00	0.00	0.00
Macropodidae monophyly	93	98	98	95	1.00	1.00	1.00
Hypsiprymnodon sister to the potoroines	0	0	0	0	0.00	0.00	0.00
Lagostrophus sister to the Potoroidae	6.7	3.6	4.0	5.0	0.00	0.00	0.00
Dendrolagus + *Peradorcas* + *Petrogale*	94	97	97	98	1.00	1.00	1.00
Thylogale + *Dendrolagus* + *Peradorcas* + *Petrogale*	97	97	97	99	1.00	1.00	1.00
Dorcopsis + *Dorcopsulus* + *Thylogale* + Dendrolagus + *Peradorcas* + *Petrogale*	36	41	39	47	0.65	0.67	0.57
Setonix + *Lagorchestes* + *Macropus* [including *Macropus* (*Wallabia*)]	54	54	55	57	0.99	0.99	0.96
Lagorchestes + *Macropus* [including *Macropus* (*Wallabia*)]	31	32	34	37	0.83	0.65	0.41
Setonix + *Lagorchestes*	22	23	22	21	0.14	0.32	0.53
Onychogalea + *Setonix* + *Lagorchestes* + *Macropus* [including *Macropus* (*Wallabia*)]	31	41	40	37	0.59	0.56	0.78
Macropus monophyly [including *Macropus* (*Wallabia*)]	58	65	63	64	0.99	0.96	0.96
Subgenus *Macropus* + *Macropus* (*Wallabia*) + Subgenus *Notamacropus*	34	41	39	49	0.11	0.40	0.78
Subgenus *Osphranter* + *Macropus* (*Wallabia*) + Subgenus *Notamacropus*	33	41	39	40	0.89	0.59	0.68
Macropus (*Wallabia*) + Subgenus *Notamacropus*	47	46	46	49	0.40	0.57	0.44
Macropus monophyly [*Macropus* (*Wallabia*) not included]	7	7	8	8	0.00	0.00	0.00
Macropus Subgenus *Notamacropus* monophyly (*sensu* Dawson and Flannery, 1985) (*M. irma*, *M. agilis*)	62	51	52	57	0.55	0.76	0.93

and *Dorcopsulus* joined together with robust support (100% BSP and 1.00 BPP). *Macropus* monophyly, inclusive of *Macropus* (*Wallabia*) *bicolor*, was supported (58–65% BSP, 0.96–0.99 BPP). Within Phalangeridae, there was a basal split between *Wyulda* + *Trichosurus* (100% BSP, 1.00 BPP) and all other phalangerids (100% BSP, 1.00 BPP). In the latter group there was a division between *Phalanger*, *Strigocuscus pelengensis*, and *Spilocuscus* (100% BSP, 1.00 BPP) versus *Ailurops* and *Strigocuscus celebensis* (100% BSP, 1.00 BPP).

3.2. Indels

Several synapomorphic indels are present in our BRCA1 alignment. Phalangeridae is supported by two unique deletions (753–755; 771–782). Petauridae monophyly is supported by a unique insertion (357–368). Within Pseudocheiridae, the grouping of *Petropseudes* and *Pseudochirops* is supported by a unique deletion (783–785). The species of *Petaurus* share a unique deletion (1347–1349) and insertion (1638–1640). The species of *Dactylopsila* share a unique deletion (2307–2321). Macropodidae and Potoroidae share two unique deletions (171–179 and 1902–1905). The species of *Dendrolagus* (474–477) share a unique insertion and *Dorcopsulus* and *Dorcopsis* share a unique deletion (1713–1715). *Aepyprymnus* and *Bettongia* share a unique deletion (783–803).

3.3. Statistical tests

Results of the AU tests are reported in Table 3 and both the KH and SH statistical test results are given in Supplementary Information Table 3. Five hypotheses were compared for the sister group of Diprotodontia. The AU test could not discriminate between any of the different hypotheses. We compared four different hypotheses for basal relationships within Diprotodontia. The AU test indicated statistical significance for a sister group relationship of Vombatiformes to Phalangerida. Three hypotheses were compared for the phylogenetic position of the Burramyidae. The AU test rejected Burramyidae + Acrobatidae but could not discriminate between Burramyidae + (Phalangeridae + Macropodiformes) and Phalangeridae + Burramyidae. Five hypotheses were compared for the phylogenetic position of the Phalangeridae. The AU test rejected three hypotheses but could not discriminate between Phalangeridae + Burramyidae and Phalangeridae + Macropodiformes. Three different phylogenetic hypotheses were compared for the interfamilial relationships within the Petauroidea. The AU test indicated statistical significance for Acrobatidae + ((Tarsipedidae + (Petauridae + Pseudocheiridae)). We compared three different hypotheses for the placement of pseudocheirid genera. The AU test indicated statistical significance for the *Pseudocheirus* + *Pseudochirulus* clade as the sister group to the *Petauroides* + *Hemibelideus* clade. Within

Table 3
Approximately unbiased (AU) test results.

	−ln likelihood	Δ	P AU
1. Diprotodontia (sister group to)			
(a) Notoryctemorphia	76292.44676	6.78062	0.129
(b) Peramelemorphia	76297.61259	11.94644	0.071
(c) *Microbiotheria* (best)	76285.66614		0.687
(d) Peramelemorphia + Notoryctemorphia	76298.23641	12.57027	0.040
(e) Peramelemorphia + Notoryctemorphia + Dasyuromorphia	76286.06802	0.40188	0.642
2. Basal relationships within Diprotodontia			
(a) ((Macropodiformes, Vombatiformes), Phalangeriformes)	76374.67044	89.00430	3e−08*
(b) ((Macropodiformes, Phalangeriformes), Vombatiformes)	76304.46577	18.79963	0.013*
(c) ((Vombatiformes, Phalangeriformes), Macropodiformes)	76374.65047	88.98433	3e−08*
(d) (Vombatiformes, Phalangerida) (best)	76285.66614		0.987
3. Burramyidae (sister group to)			
(a) Acrobatidae	76471.94784	186.28169	2E−04*
(b) Phalangeridae + Macropodiformes	76301.66006	15.99392	0.058
(c) Phalangeridae (best)	76285.66614		0.960
4. Phalangeridae (sister group to)			
(a) Vombatiformes + Macropodiformes	76413.30532	127.63918	3E−06*
(b) All other diprotodontians but Macropodiformes	76401.04120	115.37506	2E−05*
(c) All diprotodontians but Burramyidae	76397.55965	111.89350	1E−9*
(d) Burramyidae (best)	76285.66614		0.960
(e) Macropodiformes	76301.66006	15.99392	0.053
5. Petauroidea (interfamilial relationships)			
(a) (((Tarsipedidae, Acrobatidae), Petauridae), Pseudocheiridae)	76359.64151	73.97537	2E−6*
(b) (((Tarsipedidae, Acrobatidae), Pseudocheiridae), Petauridae)	76507.93903	222.27288	2E−32*
(c) (Acrobatidae, (Tarsipedidae, (Petauridae, Pseudocheiridae))) (best)	76285.66614		0.965
6. Pseudocheiridae (intergeneric relationships)			
(a) (((*Petropseudes*, *Pseudochirops*), (*Petauroides*, *Hemibelideus*)), (*Pseudocheirus*, *Pseudochirulus*))	76295.68927	10.02313	0.022*
(b) (((*Pseudocheirus*, *Pseudochirulus*), (*Petauroides*, *Hemibelideus*)), (*Petropseudes*, *Pseudochirops*)) (best)	76285.66614		0.978
(c) (*Pseudochirulus*, (*Pseudocheirus*, ((*Petropseudes*, *Pseudochirops*), (*Petauroides*, *Hemibelideus*))))	76476.69632	191.03018	2E−56*
7. *Pseudochirops*			
(a) *Pseudochirops* monophyly	76303.10503	17.43889	0.006*
(b) *Pseudochirops cupreus* + *Petropseudes* (best)	76285.66614		0.994
8. *Strigocuscus pelengensis* (sister group to)			
(a) *Spilocuscus* + *Phalanger* (excluding *P. gymnotis*)	76328.12638	42.46023	5E−4*
(b) *P. gymnotis* + *Spilocuscus* + *Ailurops* + *Strigocuscus celebensis* + *Trichosurus* + *Wyulda*	76494.89802	209.23187	6E−6*
(c) *Phalanger gymnotis*	76323.79434	38.12819	0.001*
(d) *Strigocuscus celebensis*	76540.69337	255.02723	1E−51*
(e) *Phalanger* (best)	76285.66614		1.000
9. *Ailurops* (sister group to)			
(a) *Strigocuscus celebensis* (best)	76285.66614	142.04068	1.000
(b) All other phalangerids	76427.70682	328.42975	2E−57*
(c) *Phalanger* + *Strigocuscus celebensis*	76614.09589		2E−35*
10. *Hypsiprymnodon* (sister group to)			
(a) Macropodidae + Potoroidae (best)	76285.66614		1.000
(b) Potoroidae	76431.25629	145.59015	7E−62*
11. *Lagostrophus* (sister group to)			
(a) *Lagorchestes*	76409.88965	124.22351	4E−6*
(b) All other macropodids (best)	76285.66614		0.963
(c) Potoroidae	76301.30503	15.63889	0.038*
(d) *Petrogale* + *Onychogalea* + *Lagorchestes* + *Peradorcas*	76484.39588	198.72974	3E−6*

* $P < 0.05$.

Pseudocheiridae, the AU test indicated statistical significance for *Pseudochirops cupreus* grouping with *Petropseudes dahli* over *Pseudochirops* monophyly. We compared five hypotheses for the phylogenetic placement of *Strigocuscus pelengensis*. The AU test indicated statistical significance for a sister group relationship of *S. pelengensis* and *Phalanger*, although we note that the list of *a priori* hypotheses did not include a sister group relationship of *S. pelengenesis* to *Phalanger* + *Spilocuscus*. We compared three different hypotheses for the phylogenetic position of *Ailurops*. The AU test indicated statistical significance for a sister group relationship to *S. celebensis*. We compared two different phylogenetic hypotheses for the placement of *Hypsiprymnodon*. The AU test indicated statistical significance for a sister group relationship to a Potoroidae + Macropodidae clade. Four hypotheses were compared for the phylogenetic position of *Lagostrophus*. The AU test indicated statistical significance for a sister group relationship to all other macropodids.

3.4. Molecular dating

Fig. 3 shows the *Multidivtime* timescale for Marsupialia. Supplementary Information Table 4 gives the point estimates of divergence times along with 95% credibility intervals. Our analyses suggest that crown-group marsupials last shared a common ancestor in the Late Cretaceous (~76 million years ago). Didelphimorphia diverged from Australidelphia ~73 million years ago. The base of Australidelphia (~63 million years ago) and all of the interordinal splits (61–73 million years ago) were placed in the early

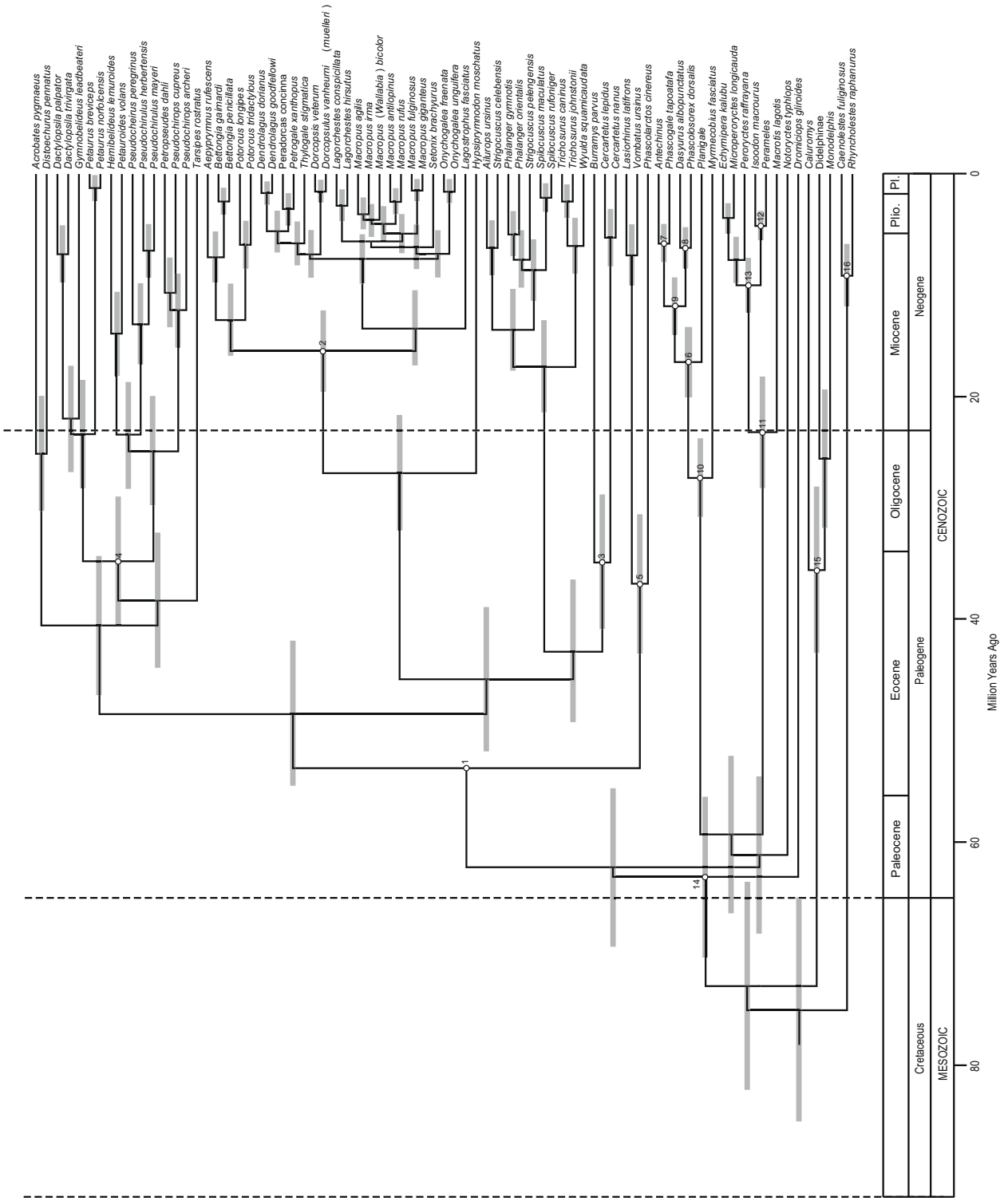

Fig. 3. Timeline in millions of years before present for diprotodontian evolution based on the *Multidivtime* partitioned analysis. Grey bars indicate 95% credibility intervals. Numbers by open circled nodes indicate calibrated nodes. Plio. = Pliocene; Pl. = Pleistocene.

Paleocene or Late Cretaceous. All intraordinal divergences were placed in the Cenozoic with the deepest divergences occurring within the Diprotodontia. The base of Diprotodontia was estimated at ~53 million years. The last common ancestor of Phalangerida was estimated at ~48 million years. All families within Diprotodontia were established before the end of the Paleogene except for Potoroidae and Macropodidae, which diverged from each other in the early Neogene (~16 million years ago).

3.5. Ancestral state reconstructions

Supplementary Information Tables 5 and 6 show the results of the ancestral state reconstructions when the morphological priors were set to $\alpha = 3$ and $\beta = 2$. Fig. 4 and Supplementary Fig. 2 show the posterior probability of the reconstructed ancestral states for the gliding membrane for the ordered and unordered analyses, respectively. Fig. 5 and Supplementary Fig. 3 show the ancestral

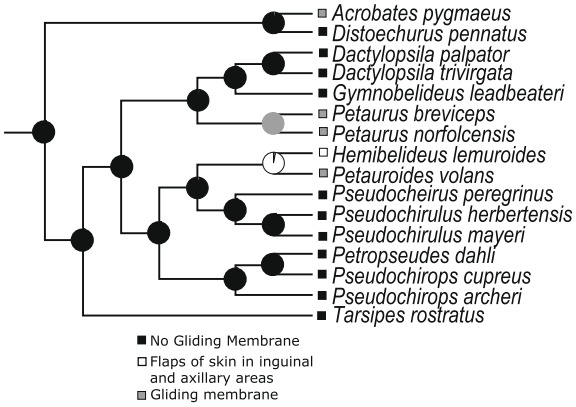

Fig. 4. SIMMAP posterior probability distributions for gliding membrane ancestral state reconstructions for ordered analyses. Pie graphs at nodes represent the posterior probability of the given ancestral state reconstruction.

state reconstructions for ecological venue for the unordered and ordered analyses, respectively. Our results for the ordered analyses indicate that the gliding membrane evolved on three separate occasions: once in Petauridae (*Petaurus* clade); once in Pseudocheiridae (*Petauroides*); and once in Acrobatidae (*Acrobates*). Furthermore, the ordered analyses suggest the common ancestor of the *Petauroides* and *Hemibelideus* clade possessed flaps of skin in the inguinal and axillary areas (0.9831) and was retained in the lineage leading to *Hemibelideus*. In contrast, the unordered analyses suggest the common ancestor of the *Petauroides* and *Hemibelideus* clade possessed a gliding membrane (0.5379) that was subsequently lost in *Hemibelideus*.

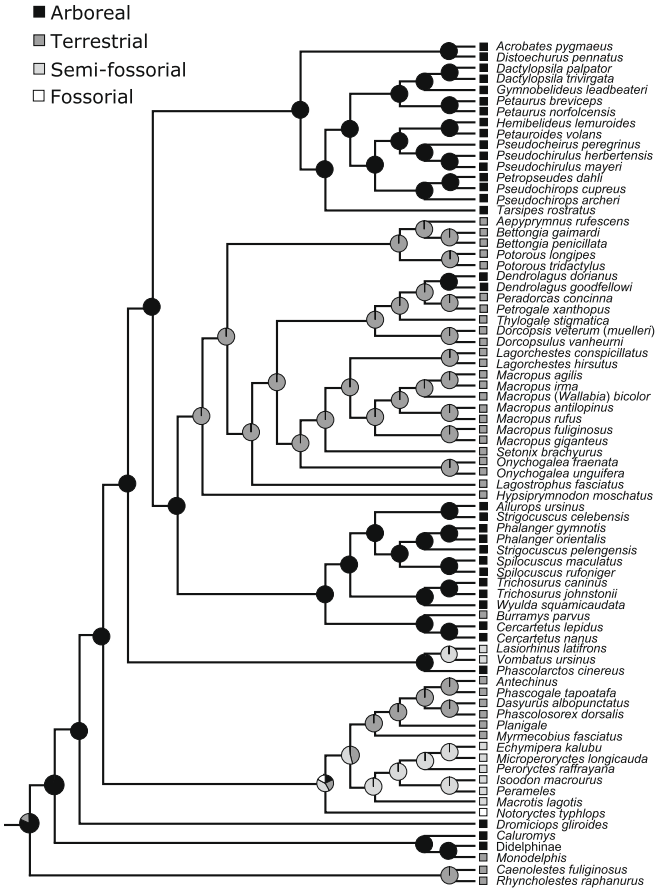

Fig. 5. SIMMAP unordered posterior probability distributions for venue ancestral state reconstructions. Pie graphs at nodes represent the posterior probability of the given ancestral state reconstruction.

The unordered ancestral state reconstructions for ecological venue suggest that the ancestors of Marsupialia, Australidelphia + Didelphimorphia, Australidelphia, all Australasian taxa, Diprotodontia, Phalangerida, Macropodiformes + Phalangeroidea, Vombatiformes, Petauroidea, and Phalangeroidea were arboreal. All nodes within Petauroidea and Phalangeroidea were also reconstructed as arboreal. The basal node of Macropodiformes was reconstructed as terrestrial as were all internal nodes except for the node uniting the two *Dendrolagus* species. The base of Peramelemorphia was reconstructed as semi-fossorial as were all of its internal nodes. The base of Dasyuromorphia and all of its internal nodes were reconstructed as terrestrial. The Peramelemorphia + Dasyuromorphia node was reconstructed as either semi-fossorial (0.5257) or terrestrial (0.4603). The reconstruction for the ancestor of Peramelemorphia + Dasyuromorphia + Notoryctemorphia was ambiguous although semi-fossoriality (0.4139) had a higher posterior probability than any of the other character states. The ordered analysis reconstructed the same ancestral states for the majority of the nodes (Supplementary Information Table 6 and Fig. 3). The ordered analysis differed from the unordered analysis in suggesting terrestriality for the australidelphian ancestor (0.5924), the ancestor of Didelphimorphia + Australidelphia (0.9361), and Marsupialia (0.7717). Other differences include less support for arboreality in the ancestor of Diprotodontia (0.8079) and more support for semi-fossoriality in the ancestors of Peramelemorphia + Dasyuromorphia (0.9579) and Peramelemorphia + Dasyuromorphia + Notoryctemorphia (0.7112).

4. Discussion

4.1. Marsupialia cohorts and the root of Marsupialia

Szalay (1982) proposed the marsupial cohorts Ameridelphia and Australidelphia based on ankle joint morphology. Our results provide additional support for Australidelphia and add to a wealth of other molecular (Kirsch et al., 1991, 1997; Springer et al., 1998; Phillips et al., 2001, 2006; Amrine-Madsen et al., 2003; Meredith et al., 2008a, in press-a), morphological (Luckett, 1994; Szalay, 1994; Szalay and Sargis, 2001; Horovitz and Sánchez-Villagra, 2003), and mixed data set studies (Asher et al., 2004; Beck et al., 2008) supporting this clade. Like other previous studies, our results suggest that Ameridelphia is paraphyletic but could not discriminate between rooting Marsupialia on Didelphimorphia or Paucituberculata. Complete mitochondrial genomes (Nilsson et al., 2003, 2004), concatenated nuclear genes with fewer taxa (Amrine-Madsen et al., 2003; Meredith et al., 2008a, in press-a), morphological data (Horovitz and Sánchez-Villagra, 2003), and mixed data sets (Asher et al., 2004) have found weak support for rooting between Didelphimorphia and all other marsupials. In contrast, tarsal morphology has suggested rooting between Paucituberculata and all other marsupials (Szalay, 1994; Szalay and Sargis, 2001, 2006). Larger molecular data sets and additional types of genomic and molecular data (e.g. chromosomal rearrangements, transposon insertions, indels) will be required to resolve the root of Marsupialia.

Within Australidelphia we find only minimal support for an association of the Australasian orders Diprotodontia, Peramelemorphia, Dasyuromorphia, and Notoryctemorphia to the exclusion of the South American Microbiotheria. These results agree with Phillips et al.'s (2006) analysis of combined mitochondrial and nuclear sequences for a smaller set of taxa. As for the root of Marsupialia, additional molecular and genomic data will be required to

resolve australidelphian relationships and identify the sister taxon to Diprotodontia.

The monophyly of Australasian taxa requires only a single dispersal event from South America to Australia (presumably via Antarctica) to account for the phylogenetic apposition of American and Australasian marsupials (Amrine-Madsen et al., 2003). In contrast, nesting of microbiotheres within Australidelphia rather than as a sister taxon to all other australidelphians requires a more complicated biogeographic scenario, either with multiple dispersals to Australia or back-dispersal from Australia to South America (Kirsch et al., 1991). Beck et al. (2008) suggest that *Djarthia*, from the early Eocene Tingamarra Local Fauna in Australia, is the oldest and most primitive member of Australidelphia and that South American microbiotheres may be the result of back-dispersal from eastern Gondwana. Although Beck et al. (2008: e1858) suggest that the phylogenetic affinities of *Djarthia* are "confidently resolved", the decay index associating crown-group australidelphians to the exclusion of *Djarthia* is only one step in a parsimony analysis with morphological data and a combined analysis with molecular and morphological data yielded an unresolved trichotomy for *Djarthia*, *Dromiciops*, and living Australasian taxa.

4.2. Diprotodontia

Diprotodontia is taxonomically the largest and most diverse Australasian marsupial order. Putative synapomorphies for this group include diprotodonty, a superficial thymus gland (Yadav, 1973), a fasciculus aberrans (Abbie, 1937), and numerous additional morphological apomorphies identified by Horovitz and Sánchez-Villagra (2003), although only diprotodonty has been confirmed for all genera. Mitochondrial DNA sequences (Kavanagh et al., 2004; Nilsson et al., 2004; Munemasa et al., 2006), single-copy DNA–DNA hybridization (Springer and Kirsch, 1991; Kirsch et al., 1997), nuclear DNA sequences (Colgan, 1999; Amrine-Madsen et al., 2003; Baker et al., 2004; Meredith et al., 2008a), microcomplement fixation (MC'F) studies (Baverstock, 1984; Baverstock et al., 1987), combined mitochondrial and nuclear DNA (Phillips et al., 2006; Phillips and Pratt, 2008; Beck, 2008), cladistic analyses of morphological data (Archer, 1984; Aplin and Archer, 1987; Luckett, 1994; Marshall et al., 1990; Springer et al., 1997; Horovitz and Sánchez-Villagra, 2003), and combined morphological and molecular analyses (Asher et al., 2004) also support diprotodontian monophyly. Our results provide robust support for diprotodontian monophyly in the context of complete taxonomic sampling for living diprotodontian genera.

Some authors (e.g. Kirsch et al., 1997; Wilson and Reeder, 2005) divide Diprotodontia into three suborders: Macropodiformes (kangaroos and kin), Vombatiformes (wombats and koalas), and Phalangeriformes (possums and kin). Other classifications recognize only two suborders: Vombatiformes and Phalangerida (Macropodiformes + Phalangeriformes; Aplin and Archer, 1987). Our results support a basal split between Vombatiformes and Phalangerida and are consistent with Aplin and Archer (1987). On the other hand, our results are incompatible with classifications that recognize three monophyletic suborders (Kirsch et al., 1997; Wilson and Reeder, 2005) owing to the paraphyly of Phalangeriformes (see below).

4.3. Phalangerida

We found robust statistical support for Phalangerida. This result is consistent with single-copy DNA–DNA hybridization (Kirsch et al., 1997), complete mitochondrial genomes (Munemasa et al., 2006 but see Nilsson et al., 2004), nuclear genes (Amrine-Madsen et al., 2003; Meredith et al., 2008a, in press-a), combined mitochondrial and nuclear DNA data sets (Phillips and Pratt, 2008; Beck, 2008), and combined nuclear DNA and morphology (Asher et al., 2004), although many of these earlier studies are missing key phalangeridan families (e.g. Acrobatidae, Tarsipedidae, and Burramyidae are missing from Amrine-Madsen et al., 2003). Morphological evidence in support of Phalangerida includes several basicranial characters such as the posterior expansion of the alisphenoid tympanic wing (Winge, 1941; Aplin and Archer, 1987; Springer and Woodburne, 1989).

4.4. Vombatiformes

All of our analyses supported a monophyletic Vombatiformes with strong support. Robust support for Vombatiformes also comes in the form of sperm head morphology (Hughes, 1965), sperm ultrastructure (Harding, 1987; Harding et al., 1987), thymus morphology (Yadav, 1973), serological data (Kirsch, 1968, 1977), morphology (Horovitz and Sánchez-Villagra, 2003), single-copy DNA hybridization (Springer and Kirsch, 1991; Kirsch et al., 1997; Springer et al., 1997), mitochondrial DNA (Burk et al., 1999; Kavanagh et al., 2004; Munemasa et al., 2006), nuclear DNA (Amrine-Madsen et al., 2003), combined nuclear and mitochondrial studies (Phillips and Pratt, 2008; Beck, 2008), and combined molecular and morphological studies (Asher et al., 2004).

4.5. Macropodiformes

Wilson and Reeder (2005) currently recognize three living families of kangaroos (Hypsiprymnodontidae, Potoroidae, and Macropodidae). MC'F (Baverstock et al., 1990) and some morphological studies (Archer, 1984; Flannery, 1989) have previously suggested a closer association of Hypsiprymnodontidae to Potoroidae than to Macropodidae. In contrast, our results are consistent with mitochondrial DNA (Burk et al., 1998; Burk et al., 2000; Osborne et al., 2002; Kavanagh et al., 2004), nuclear DNA (Meredith et al., 2008a, in press-a,b), combined nuclear and mitochondrial DNA (Westerman et al., 2002), and some morphological (Kear et al., 2007) analyses that group Potoroidae and Macropodidae to the exclusion of the Hypsiprymnodontidae. As discussed elsewhere (Burk et al., 1998; Burk and Springer, 2000; Meredith et al., in press-b), this result implies that bipedal hopping and reduction of litter size to one evolved only once in kangaroos – in the common ancestor of Macropodidae and Potoroidae.

Potoroidae (potoroos and bettongs) is composed of three extant genera. Potoroid monophyly is generally supported by morphology (Flannery, 1989) and molecular data (e.g. Westerman et al., 2002; Meredith et al., in press-b). Our results strongly support potoroid monophyly in the context of a sampling scheme that includes representatives of all diprotodontian genera. Within Potoroidae, mitochondrial DNA (Burk et al., 1998), nuclear DNA (Meredith et al., in press-b), combined mitochondrial and nuclear DNA (Burk and Springer, 2000; Westerman et al., 2002), morphology (Flannery, 1989) and single-copy DNA–DNA hybridization (Kirsch et al., 1997) studies have recovered *Bettongia* grouping with *Aepyprymnus* to the exclusion of *Potorous*. Our results are in solid agreement with these earlier studies. Earlier MC'F studies failed to resolve this trichotomy (Baverstock et al., 1990).

Macropodidae includes 12 extant genera. Traditionally, the monotypic genus *Lagostrophus* has been allied with *Lagorchestes* (Bensley, 1903), with the hypsodont macropodids (Raven and Gregory, 1946), or within Macropodidae (Tate, 1948). Our results reject these hypotheses and instead provide robust support for a basal split between *Lagostrophus* and all other macropodids. This result is consistent with mitogenomic (Nilsson, 2006) and combined nuclear and mitochondrial DNA studies (Westerman et al., 2002). More recent morphological studies have suggested that *Lagostrophus* is a sister group to Sthenurinae (Flannery, 1989).

Sthenurines are an extinct group of kangaroos that first appeared in the late Miocene (Murray, 1991; Kear, 2002) and went extinct by the end of the Pleistocene (Flannery, 1989; Prideaux, 2004). Putative synapomorphies uniting *Lagostrophus* and sthenurines together have been rejected by other authors as primitive and/or convergent characters (Murray, 1991, 1995; Prideaux, 2004). Our results demonstrate that *Lagostrophus* is the sole survivor of an ancient kangaroo lineage that has been separated from other macropodids for many millions of years and allows for the possibility that *Lagostrophus* is a sthenurine. However, testing of this hypothesis in a molecular phylogenetic framework will require gene and/or protein sequences from Pleistocene sthenurine specimens.

Single-copy DNA–DNA hybridization (Springer and Kirsch, 1991; Kirsch et al., 1995), mitochondrial DNA sequences (Burk et al., 1998; Burk and Springer, 2000; Westerman et al., 2002), and morphology (Woodburne, 1967; Flannery, 1984) have suggested that *Dorcopsulus* and *Dorcopsis* are each other's closest relatives and comprise the sister group to all other macropodids excepting *Lagostrophus*. Our analyses confirmed the evolutionary affinities of *Dorcopsulus* and *Dorcopsis* with each other, but leave open the position of these taxa relative to other macropodids. Our results provide robust support for the association of *Petrogale* with *Peradorcas*, these two with *Dendrolagus*, and these three with *Thylogale*. One or more of these clades have appeared in other published analyses, although usually not all in the same analysis. For example, the *Thylogale* + *Petrogale* + *Dendrolagus* + *Peradorcas* clade was recovered with MC'F data (Baverstock et al., 1990), but with *Dendrolagus* rather than *Thylogale* as the sister taxon to the other three. In contrast, some morphological studies have suggested an association between *Dendrolagus* and the *Dorcopsis* + *Dorcopsulus* clade (Bensley, 1903; Raven and Gregory, 1946; Tate, 1948). An association of *Thylogale* and *Petrogale* clade has been suggested based on chromosomal (Hayman and Martin, 1974) and immunological studies (Kirsch, 1977), but *Dendrolagus* and *Peradorcas* were not present in all of these studies. Our results support the monophyly of *Macropus* provided that *Wallabia* is recognized as a subgenus of *Macropus* following the recommendation of Meredith et al. (in press-b).

4.6. Phalangeriformes

We found no support for the monophyly of the Phalangeriformes (possum monophyly), although our results support the monophyly of two distinct possum clades: Petauroidea (Acrobatidae, Tarsipedidae, Petauridae, and Pseudocheiridae) and Phalangeroidea (Burramyidae and Phalangeridae). Phalangeroidea (Burramyidae and Phalangeridae) group with Macropodiformes (kangaroos and kin) and this collective group is sister to Petauroidea (Acrobatidae, Tarsipedidae, Petauridae, and Pseudocheiridae; Figs. 1 and 2). This result is consistent with findings based on nuclear DNA (Meredith et al., in press-a), partial mitochondrial genomes (Osborne et al., 2002), complete mitochondrial genomes (Munemasa et al., 2006) and combined nuclear and mitochondrial DNA studies (Phillips and Pratt, 2008), but in contrast to single-copy DNA–DNA hybridization studies (Springer and Kirsch, 1991; Kirsch et al., 1997), which support Australasian possum monophyly. The finding that Phalangeriformes is a paraphyletic taxon suggests that morphological characters supporting possum monophyly, such as a tube-like ectotympanic that is fused to other bones of the skull (Flannery, 1987; Springer and Woodburne, 1989), are convergent in phalangeroids and petauroids or were lost in macropodiforms. We propose the name Australoplagiaulacoida for the clade containing macropodiforms, phalangerids, and burramyids based on the occurrence of serrated premolars in presumed primitive members of each of these constituent groups (Archer et al., 1999; Long et al., 2002; Archer and Hand, 2006; Beck, 2008; Phillips and Pratt, 2008).

4.7. Petauroidea

We found robust statistical support for the monophyly of Petauroidea and its constituent families. Petauroidea monophyly is consistent with nuclear DNA studies (Baker et al., 2004; Meredith et al., in press-a) and combined mitochondrial and nuclear DNA studies (Phillips and Pratt, 2008). Within Petauroidea we found robust support for all relationships recovered except for the placement of *Gymnobelideus* within the Petauridae. Our analyses support a basal split between the Acrobatidae and all other petauroids. Tarsipedidae, in turn, is the sister taxon to Pseudocheiridae + Petauridae (Figs. 1 and 2). This arrangement of petauroid families is consistent with previous mitochondrial (Kavanagh et al., 2004), nuclear (Meredith et al., in press-a), and combined mitochondrial and nuclear DNA studies (Phillips and Pratt, 2008). However, unlike the previous studies we find robust support for all interfamilial relationships within Petauroidea (Table 2). Petauroid relationships recovered in our study are in contrast to hypotheses that have been proposed based on cranial and reproductive characters (Aplin and Archer, 1987), MC'F data (Baverstock et al., 1990), and a single nuclear gene study (Baker et al., 2004) that find an association between Tarsipedidae and Acrobatidae (= Tarsipedoidea of Aplin and Archer, 1987). However, the strong bootstrap support found for this clade in the Rag1 analysis of Baker et al. (2004) may have been misreported (see Phillips and Pratt, 2008, p. 602–603). The association of *Tarsipes* with Pseudocheiridae + Petauridae, rather than Acrobatidae, implies that numerous presumed synapomorphies for Tarsipedoidea evolved in the common ancestor of Petauroidea and were lost in the common ancestor of Pseudocheiridae + Petauridae, or evolved in parallel in acrobatids and *Tarsipes*.

4.8. Petauridae

The family Petauridae is composed of three extant genera. Our results favor an association of *Gymnobelideus* with *Dactylopsila* although bootstrap support for this association is weak. Morphological (Smith, 1984; Aplin and Archer, 1987; Archer, 1984; Flannery, 1994), chromosomal characters (McKay, 1984), brain encephalization (Nelson and Stephan, 1982), serological data (Kirsch and Calaby, 1977), and single-copy DNA–DNA hybridization studies (Springer et al., 1994) suggest a close relationship between *Petaurus* and *Gymnobelideus*. However, cytological data are ambiguous because *Gymnobelideus* has the ancestral chromosome number (2n=22) like the other petaurids, but its chromosomal morphology is different (Murray et al., 1990). Baverstock et al.'s (1990) MC'F analyses suggest unity of *Gymnobelideus* with *Dactylopsila* rather than with *Petaurus*. Edwards and Westerman's (1995) single-copy DNA–DNA hybridization studies provide weak corroboration for MC'F results. Mitochondrial DNA sequences also support an association of *Gymnobelideus* with *Dactylopsila* (Osborne et al., 2002).

4.9. Pseudocheiridae

Pseudocheiridae includes six extant genera (*Pseudochirulus*, *Pseudocheirus*, *Hemibelideus*, *Petauroides*, *Pseudochirops*, *Petropseudes*). Previous studies suggest that these genera group into three main lineages. First, karyological studies (Hayman and Martin, 1974; McKay, 1984; McQuade, 1984), MC'F studies (Baverstock1984; Baverstock et al., 1987, 1990), craniodental studies (Archer, 1984; Springer, 1993), and single-copy DNA–DNA hybridization studies (Springer et al., 1992) support a sister group relationship between *Hemibelideus* and *Petauroides*. Second, low chromosome number (Hayman and Martin, 1974; Murray et al., 1990), dental characters (Archer, 1984; Springer, 1993), and MC'F analyses (Baverstock et al., 1987, 1990) suggest that *Petropseudes*

and *Pseudochirops* comprise a monophyletic group. Finally, an association of *Pseudocheirus* and *Pseudochirulus* is supported by single-copy DNA–DNA hybridization studies (Springer et al., 1992; Kirsch et al., 1997). Our results corroborate the monophyly of these three lineages and provide additional support for a basal split between the *Pseudochirops* + *Petropseudes* clade and all other pseudocheirids. Within the *Pseudochirops* + *Petropseudes* group, we find strong statistical support (Table 3) for the paraphyly of *Pseudochirops* in that *Petropseudes* groups with *Pseudochirops cupreus* to the exclusion of *P. archeri*. MC'F results (Baverstock et al., 1990) and cladistic analyses of craniodental characters (Springer, 1993) support *Pseudochirops* monophyly, but Tate's (1945) suggestion that *Petropseudes* is more closely related to *P. cupreus* than to *P. archeri* agrees with our results. Even though *Petropseudes* is ecomorphologically different from *Pseudochirops*, we recommend that *Petropseudes dahli* now be recognized as *Pseudochirops* (*Petropseudes*) *dahli* given both the paraphyly problem and taxonomic priority of the genus *Pseudochirops* as currently recognized. Our suggestion makes *Pseudochirops* monophyletic but still recognizes the uniqueness of *Petropseudes*.

4.10. Acrobatidae

Aplin and Archer (1987) removed *Distoechurus* and *Acrobates* from Burramyidae and placed them in the new family Acrobatidae based on the distinctiveness of these genera. Our results provide robust support for the monophyly of Acrobatidae (Table 2).

4.11. Phalangeroidea

Phalangeroidea includes Phalangeridae and Burramyidae and was recovered in all of our analyses (Figs. 1 and 2). Our results are in agreement with single-copy DNA–DNA hybridization (Springer and Kirsch, 1989; Springer and Kirsch, 1991; Kirsch et al., 1997), some mitochondrial DNA sequence analyses (Osborne et al., 2002), and nuclear DNA sequences (Baker et al., 2004; Meredith et al., 2008a, in press-a; Springer et al., 2009). Other morphological and molecular studies have supported alternate hypotheses to Phalangeroidea. Analyses of mitochondrial gene sequences recovered weak support for a burramyid + Vombatiformes clade (Kavanagh et al., 2004) or even a basal position of burramyids within Diprotodontia (Osborne et al., 2002). Chromosome number has suggested an association of burramyids with Acrobatidae (Gunson et al., 1968).

Morphological studies have recovered a multitude of different phylogenetic hypotheses for the placement of the burramyids. Szalay (1994) using pedal morphology placed the burramyids in Petauridae and both Springer and Woodburne (1989) and Marshall et al. (1990) suggested a possible association with petauroids based on craniodental data, although the latter authors favored an association of phalangerids and burramyids based on all available evidence. In contrast, morphology in combination with nuclear DNA has suggested a basal position for Burramyidae within Diprotodontia (Asher et al., 2004) and morphology alone has suggested that burramyids are basal within Phalangerida (Aplin and Archer, 1987). Other morphological studies have suggested a sister group relationship between Phalangeridae and Macropodiformes (Archer, 1984; Flannery, 1987) or group the burramyids as the sister group to a clade comprising pseudocheirids, phalangerids, Vombatiformes, and Macropodiformes based on 230 morphological characters (Horovitz and Sánchez-Villagra, 2003).

4.12. Phalangeridae

Phalangeridae (*sensu* Wilson and Reeder, 2005) includes six living genera (*Spilocuscus*, *Phalanger*, *Ailurops*, *Strigocuscus*, *Trichosurus*, *Wyulda*). Our finding that the basal phalangerid split is between *Trichosurus* + *Wyulda* and all other phalangerids agrees with single-copy DNA–DNA hybridization (Kirsch and Wolman, 2001), mitochondrial sequences (Ruedas and Morales, 2005; Osborne and Christidis, 2002) and nuclear BRCA1 analyses (Raterman et al., 2006). However, these studies were missing one or both of *Ailurops* and *Strigocuscus pelengensis*. In contrast, previous morphological studies have suggested that *Ailurops* is basal to all other phalangerids (Flannery et al., 1987; Crosby and Norris, 2003; Crosby et al., 2004; Crosby, 2007) and that *Strigocuscus celebensis* and the *Trichosurus* + *Wyulda* clade are sister taxa (Trichosurini) (George, 1987; Flannery et al., 1987; Crosby and Norris, 2003; Crosby et al., 2004; Crosby, 2007). An association of *Trichosurus*, *Wyulda*, and *S. celebensis* together based on morphology suggests that the periotic morphology exhibited by these taxa is homologous. However, our finding that Trichosurini is diphyletic implies convergent evolution in periotic morphology between *Trichosurus* + *Wyulda* and *S. celebensis*.

Previous molecular studies have not included *Strigocuscus pelengensis* (*sensu* Flannery, 1994; Wilson and Reeder, 2005). Groves (1987) considered this taxon a close relative of *S. celebensis*, but retained both species in the genus *Phalanger*. George (1987) recognized *Strigocuscus celebensis* as the only extant species in *Strigocuscus* and placed *pelengensis* in *Phalanger*. Flannery et al. (1987, p. 494) suggested that *pelengensis* is basal to a clade containing both *Phalanger* and *Spilocuscus*, or alternatively belongs within *Phalanger*. Our finding that *S. pelengensis* is associated in a clade with *Phalanger* and *Spilocuscus* is generally consistent with Flannery et al. (1987) and George (1987), although with the caveat that Flannery et al.'s (1987) *Strigocuscus gymnotis* is instead *Phalanger gymnotis* (Springer et al., 1990).

4.13. Burramyidae

Burramyidae is composed of two genera (*sensu* Wilson and Reeder, 2005), *Burramys* and *Cercartetus*. All of our analyses provide robust support for the monophyly of this group. These two genera also comprise a monophyletic group based on analyses of mitochondrial sequences (Osborne et al., 2002).

4.14. Timescale for Diprotodontia in context of Australian climate changes

Reconstructing the paleobotany of Australasia is difficult given both the paucity of sites and limited geological coverage. As a result, corroboration of our hypotheses put forth here for the origin and timing of the Australasian marsupial radiation must await the discovery of Paleogene marsupial bearing sites.

Warm and wet closed rainforests were the predominant vegetation in Australia during the Paleocene (Martin, 2006). Gymnosperms were more common in southeastern Australia but angiosperms were the predominant vegetation in central Australia. By the early Eocene, angiosperms became more prevalent as the climate became warmer and humid. Temperatures decreased by the mid-late Eocene and more diverse angiosperm *Nothofagus* forests largely replaced podocarp forests. Although *Nothofagus* forests were the predominant vegetation in the mid-late Eocene, the understorey remained unchanged from the podocarp dominated forests and consisted mostly of non-angiosperm taxa (e.g. treeferns and ground ferns). In addition, sclerophyllous vegetation was present in central Australia by this time (Martin, 2006).

Due to the limited number of ecological niches available given the predominance of a single vegetation type (closed podocarp forests) and the proposed high latitudinal position of Australia, Case (1989) suggested there was little taxonomic diversity among Australasian marsupial taxa prior to the Eocene. Our divergence estimates are in general agreement with this hypothesis. The lineages

leading to the Dasyuromorphia, Peramelemorphia, and Diprotodontia were established prior to 55 million years ago, but none of their constituent extant families can be traced back to this time period. The spread of the more diverse *Nothofagus* forests in the early to mid Eocene brought about an increase in arboreal habitats and ecological niches and is hypothesized as the causal factor in the diversification of the arboreal marsupial families (Case, 1989). Our analyses support this hypothesis. During the early to mid Eocene, Vombatiformes split from Phalangerida and within Phalangerida the lineages leading to Petauroidea, Phalangeroidea, and Macropodiformes were established. Within the Petauroidea, Acrobatidae split from Tarsipedidae + Petauridae + Pseudocheiridae. Within Phalangeroidea, Burramyidae split from Phalangeridae.

The late Eocene to early Oligocene was characterized by a sudden drop in temperature that brought on the drying out of Australia (White, 1994; Martin, 2006). Ice sheets built up in Antarctica, which resulted in decreased rainfall in Australia (White, 1994). The Australian plate collided with the Asian plate, which resulted in the emergence of New Guinea and the uplift of the New Guinean Highlands. The resultant rain shadow covered a large portion of Australia. Once Australia was completely separated from Antarctica circumpolar currents were established south of Australia, which further changed the drainage patterns of central Australia. Dryer sclerophyll woodlands, sedgeland, and reed swamp communities then began to replace the *Nothofagus* dominated forests (MacPhail et al., 1994). By the end of the early Oligocene (28 million years ago), all terrestrial Australian marsupial families had become established as a result of the opening up of the rainforest canopy (Case, 1989).

Our divergence estimates suggest the terrestrial forms radiated later than the arboreal forms. The terrestrial forms did not radiate at the same time as the arboreal forms, possibly because the understorey of the *Nothofagus* dominated forests remained largely unchanged from the podocarp dominated forests (Case, 1989). It is only with the increase in floral diversity associated with the opening up of the forest that new ecological niches and habitats became available for terrestrial taxa (Case, 1989). Isolated rainforests persisted in central Australia to the early Miocene, which was characterized by an increase in temperature and humidity. The first signs of aridity are seen in the mid-Miocene and the once extensive rainforests of central Australia were severely truncated. By the late Miocene, it was cooler, drier, burning of the landscape became regular, and *Eucalyptus* vegetation was common (Martin, 2006). Our divergence estimates suggest that it is within the Miocene that the majority of the terrestrial forms within the Macropodiformes, Peramelemorphia, Vombatidae, and Dasyuromorphia radiated in response to these floral changes.

Central Australia continued to dry out through the Pliocene although during the early Pliocene there was a short time interval of warming and an increase in rainfall. Grasses became prevalent over most of Australia with rainforests contracting to the coasts and highland regions by the late Pliocene. However, in general the climate was wetter as compared to current climatic conditions. During the Pleistocene the current climatic conditions were established with drier periods correlating with glacial periods and wetter periods correlating with interglacial periods (Martin, 2006). Our divergence estimates for the radiation of the *Macropus* species around the Plio-Pleistocene boundary correspond with the aforementioned climatic events. The spread of the grasslands as a result of the climatic changes is thought to be the causal factor in the *Macropus* diversification (Flannery, 1989).

4.15. Ancestral state reconstructions

4.15.1. Gliding membrane

The possession of a gliding membrane or patagium has evolved multiple times in mammals. The gliding membrane allows the organism to move easily from tree to tree without having to descend to the ground, and therefore decreases the risk of predation. Gliding has arisen in three eutherian taxa: flying squirrels (Petauristinae); scaly-tailed flying squirrels (Anomaluridae); and colugos (Dermoptera). The presence of a gliding membrane in marsupials is restricted to Petauroidea. Springer et al. (1997) mapped the presence of a gliding membrane on a molecular tree using parsimony methods and concluded that gliding evolved on three separate occasions in petauroids. Our results with increased taxon sampling and better topological resolution confirm that gliding evolved three separate times within Petauroidea: once in Petauridae (*Petaurus*); once in Pseudocheiridae (*Petauroides*); and once in Acrobatidae (*Acrobates*). This is not surprising given that the morphology of the gliding membrane is not the same in these taxa (Johnson-Murray, 1987; Szalay, 1994; Tyndale-Biscoe, 2005): in *Petaurus* the patagium stretches from the hand (fifth ray) to the ankle; the patagium runs from the elbow to the ankle in *Petauroides*; and the patagium of *Acrobates* runs from the elbow to below the knee. In addition, *Acrobates*' tail is fringed by stiff hair, which is thought to aid in gliding. The sister taxon of *Petauroides* (i.e. *Hemibelideus*) is a leaper and appears to have an incipient gliding membrane that consists of flaps of skin in the inguinal and axillary areas (Szalay, 1994). Our unordered analyses suggest that it is a possibility that the gliding membrane was present in the ancestor of *Hemibelideus* and has subsequently been reduced in the living lineage. The petaurid *Gymnobelideus* is also a leaper but does not possess an incipient gliding membrane. The only other living acrobatid, *Distoechurus*, has the fringed tail and is a leaper but there is no hint of a gliding membrane. This suggests that the fringed tail evolved in the common ancestor of *Acrobates* and *Distoechurus*.

Open-forest habitat first developed in the Oligocene in conjunction with the drying out of Australia (Case, 1989). Our molecular dating results suggest that gliding evolved at the end of the Oligocene or thereafter in all three petauroid lineages: post 25.1 Ma in the Acrobatidae; post 14.4 Ma in the Pseudocheiridae if *Hemibelideus*' membrane is incipient or post 23.4 Ma in the Pseudocheiridae if the membrane evolved in the common ancestor of *Petauroides* and *Hemibelideus* and has subsequently been reduced in *Hemibelideus*; and between 23.3 and 1.3 Ma in the Petauridae. The transition from dense forest habitat to open-forest habitat may have been the impetus for the evolution of the gliding membrane in different possum lineages.

4.15.2. Ecological venue

The unordered analyses suggest that the ancestral australidelphian was arboreal (0.9951) as suggested by Szalay (1982). In addition, the oldest described Australian australidelphian (*Djarthia*) has mobile upper and lower ankle joints, which suggests arboreality (Beck et al., 2008). This makes sense given that our work has supported the placement of *Dromiciops* as the sister taxon to the Australasian taxa. It is thought that the ancestral Australidelphian emigrated from South America to Australia via Antarctica. The living microbiothere (*Dromiciops*) inhabits *Nothofagus* forests near the tip of South America. As mentioned above, *Nothofagus* forests once covered most of Australia and Antarctica and it is conceivable that the ancestral australidelphian followed the *Nothofagus* forests into Australia. However, if we treat the venue characters as ordered, the ancestor of Australidelphia was reconstructed as terrestrial (0.5924) and there was some support for a terrestrial ancestor of Australasian taxa (0.1916). This is in agreement with the findings of Springer et al. (1997), whose parsimony results on an unresolved and topologically different tree suggested the ancestor of the Australasian taxa was derived from a terrestrial ancestor. Both the ordered and unordered analyses suggest that the common ancestors of Diprotodontia, Petauroidea, Phalangeroidea, and Australoplagiaulacoida, respectively, were arboreal. All subsequent nodes

within Petauroidea and Phalangeridae were reconstructed as arboreal. The base of Macropodiformes and all nodes within Macropodiformes except for the common ancestor of *Dendrolagus* were reconstructed as terrestrial. This suggests species belonging to *Dendrolagus* are secondarily arboreal, a result supported by vestibular labyrinth morphology (Schmelzle et al., 2007). The transition from the arboreal macropodiform ancestor to the terrestrial macropodiform descendent occurred somewhere between ~45 and 27 million years ago.

Both unordered and ordered analyses suggest that the common ancestor of Vombatiformes was arboreal. Weisbecker and Archer (2008) also suggested that the ancestor of Vombatiformes was arboreal based on the (1) the occurrence of arboreality in phascolarctids, which are usually considered to represent the earliest diverging vombatiform family, and (2) carpal anatomy and digital proportions that suggest arboreality or at least frequent climbing in early diverging, extinct vombatiform taxa (*Ngapakaldia tedfordi*, *Nimbadon lavarackorum*, *Thylacoleo carnifex*). A caveat here is that phylogenetic relationships among extinct vombatiform families are poorly understood. As noted by Weisbecker and Archer (2008), only Munson (1992) used parsimony algorithms to address relationships among vombatiform families. In addition, the newly described vombatiform family Maradidae (Black, 2007) remains to be incorporated into a formal cladistic analysis.

Our divergence estimates, in conjunction with estimates for ecological venue, suggest that the semi-fossorial lifestyle of vombatids evolved after the separation of phascolarctids and vombatids at approximately 37 million years, but prior to the separation of the extant genera *Vombatus* and *Lasiorhinus* at approximately 7 million years ago. The oldest described vombatid is *Rhizophascolonus crowcrofti* from the Wipajiri Formation, which is late Oligocene/early Miocene in age (Woodburne et al., 1993; Brewer et al., 2008). *R. crowcrofti* is only known from cheekteeth, but the structure of these teeth suggests a highly abrasive diet that may have included bark and stems and ingested grit associated with masticating rhizomes and tubers (Brewer et al., 2008). Based on these inferences about diet, Brewer et al. (2008) predicted that the forelimbs of *R. crowcrofti* will show adaptations for scratch-digging regardless of whether this vombatid dug burrows. The oldest wombat fossils known from postcranial material are Plio-Pleistocene in age (e.g. Pledge, 1992; Brewer et al. (2007)) and are therefore younger than our inferred minimum age of ~7 million years for the evolution of semi-fossoriality in vombatids. Postcranial material from Oligocene and Miocene vombatids is essential for elucidating the origins of scratch-digging and semi-fossoriality in this taxonomic group.

The unordered analyses suggest the ancestors of Australidelphia and the Australasian taxa were arboreal. The ordered analyses suggest that the ancestor of Australidelphia (0.5924) and the Australasian taxa (0.8082) was terrestrial. The unordered and ordered analyses reconstruct the Perameleomorphia + Dasyuromorphia + Notoryctemorphia node as semi-fossorial (0.4139 and 0.7112, respectively). The unordered analysis reconstructed the Perameleomorphia + Dasyuromorphia node as either semi-fossorial (0.5257) or terrestrial (0.4603) and ordered analyses suggest the ancestor is semi-fossorial (0.9579). This might be a spurious finding resulting from the unresolved position of *Notoryctes*, arbitrary and crude scoring (e.g. there are many grades of arboreality and fossoriality), and not including fossil taxa. The inclusion of fossil taxa (if they existed for these nodes) would help resolve these issues in that they could be transitional forms.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2009.02.009.

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