Available online at www.sciencedirect.com
ScienceDirect
Molecular Phylogenetics and Evolution 47 (2008) 1–20
MOLECULAR PHYLOGENETICS AND EVOLUTION
www.elsevier.com/locate/ympev

A timescale and phylogeny for "Bandicoots" (Peramelemorphia: Marsupialia) based on sequences for five nuclear genes

Robert W. Meredith [a,*,1], Michael Westerman [b,1], Mark S. Springer [a,*]

[a] Department of Biology, University of California, 900 University Avenue, Riverside, CA 92521, USA
[b] Department of Genetics, La Trobe University, Bundoora, Vic. 3083, Australia

Received 26 October 2006; revised 31 December 2007; accepted 4 January 2008
Available online 12 January 2008

Abstract

Relationships among the living and recently extinct genera of bandicoots (Marsupialia: Peramelemorphia) have proven difficult to discern. Previous phylogenetic studies have used only morphology or mitochondrial DNA and have reported conflicting results in regards to their relationships. Most phylogenetic reconstructions recognize a basal split between the bilby *Macrotis* (Thylacomyidae) and the Peramelidae. The Peramelidae is composed of the Peramelinae (*Isoodon* and *Perameles*), Echymiperinae (*Echymipera* and *Microperoryctes*), and Peroryctinae (*Peroryctes*). Within Peramelidae, *Echymipera* and *Microperoryctes* usually group together to the exclusion of *Peroryctes*. This clade is sister to the Peramelinae. Placement of the recently extinct pig-footed bandicoot (*Chaeropus*: Chaeropodidae) has been ambiguous. We address the interrelationships and estimate times of divergence for the living bandicoot genera using a 6 kilobase concatenation consisting of protein-coding regions of five nuclear genes (ApoB, BRCA1, IRBP, Rag1, and vWF). We analyzed this concatenation using maximum parsimony, maximum likelihood, and Bayesian methods and estimated times of divergence using two Bayesian relaxed molecular clock methods. In all concatenated analyses, all nodes associated with the Peramelemorphia were robustly supported (bootstrap support percentages = 100; posterior probabilities = 1.00). *Macrotis* was recovered as basal to the remaining living bandicoots. Within the Peramelidae, *Echymipera* and *Microperoryctes* grouped to the exclusion of *Peroryctes* and this clade was sister to the Peramelinae. Only Rag1 amplified for *Chaeropus*; analyses based on this gene provide moderate support for an association of *Chaeropus* plus Peramelidae to the exclusion of *Macrotis*. Both relaxed clock Bayesian methods suggest that the living bandicoots are a relatively recent radiation originating sometime in the late Oligocene or early Miocene with subsequent radiations in the late Miocene to early Pliocene.

Keywords: Marsupial; Bandicoot; Peramelemorphia; Phylogeny; Relaxed molecular clock; Bilby; Fossil

1. Introduction

Taxonomic relationships of bandicoots (Order Peramelemorphia) to other Australasian marsupials are poorly understood. Bandicoots exhibit a mosaic of primitive and derived characters. They are unique among marsupials in having a complex allantoic placenta; they retain the plesiomorphic polyprotodont dentition, a condition that is shared with all South American species and the Australasian carnivorous species; and they share the derived condition of having syndactylous hind feet (fusion of 2nd and 3rd pedal digits) with all Australasian Diprotodontia. Furthermore, relationships within the order have proven difficult to ascertain.

Bensley (1903) argued for two distinct lineages of living bandicoots, one comprising the bilbies (*Macrotis*) and one comprising all other genera. This separation of bilbies from other living bandicoots has been supported by a number of workers (see Archer and Kirsch, 1977; Westerman et al., 1999). The bilbies (including the fossil form, *Ischnodon australis*) have been accorded either familial or subfamilial

* Corresponding authors. Fax: +1 909 787 4826.
E-mail addresses: robert.meredith@email.ucr.edu (R.W. Meredith), mark.springer@ucr.edu (M.S. Springer).
[1] These authors contributed equally to this work.

1055-7903/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2008.01.002

status (Thylacomyidae or Thylacomyinae). The six other living bandicoot genera are usually grouped into one family (Peramelidae) comprising three subfamilies—Peroryctinae (*Peroryctes*), Echymiperinae (*Echymipera*, *Microperoryctes*, and *Rhynchomeles*), and Peramelinae (*Isoodon and Perameles*). Peroryctines and echymiperines differ from peramelines in a number of derived cranial characters including a tube-like foramen rotundum and are essentially forest-dwelling animals either limited to New Guinea (*Microperoryctes*, *Peroryctes*, *Rhynchomeles*), or nearly so (*Echymipera*). Within this grouping, the Peroryctinae is regarded as being very divergent from the Echymiperinae (see Nowak and Dickman, 2005; Wilson and Reeder, 2005). The peramelines are mainly an Australian group whose members share few, if any, derived character states. Indeed, *Perameles* is often considered to have the most plesiomorphic dentition of any modern bandicoot.

There have been major difficulties in ascertaining the taxonomic relationships of the recently extinct pig-footed bandicoot, *Chaeropus ecaudatus*. Few specimens of this highly specialized, cursorial, semi-arid adapted herbivorous species were ever collected and few fossil remains have been described. It has several unique derived characters and Tate (1948) pointed out that *Chaeropus* was taxonomically remote from other genera. Groves and Flannery (1990) suggested that *Chaeropus* shares a number of derived character states with *Macrotis* that were often ignored or overlooked. For example, thylacomyids differ from peramelines and peroryctines in that although they show four principal cusps, the metacone has shifted lingually and the two principal external cusps are relatively larger (see Rich, 1991). *Chaeropus* also has lingual metaconids compared to other bandicoots (see Wright et al., 1991) and "the cusps of the buccal tier [metacone and paracone] and the crests associated with these, are major features of the tooth" (p. 232), suggesting that it may be related to thylacomyids.

Westerman et al. (1999) summarized the molecular studies on bandicoot relationships. Molecular studies have consistently demonstrated a large difference between *Macrotis* (Thylacomyidae) and other bandicoots. They have also consistently demonstrated not only a large difference between the Peramelinae (*Isoodon* + *Perameles*) and the mainly New Guinean Peroryctinae and Echymiperinae, but also that the Peroryctinae is very distinct from the Echymiperinae.

The mitochondrial DNA sequencing studies of Westerman et al. (1999) included sequences from a spirit-preserved museum specimen of the extinct *Chaeropus ecaudatus*. This study favored *Chaeropus* as the sister taxon to all other bandicoots but statistical tests failed to reject a *Chaeropus* + *Macrotis* clade. More recent DNA studies on bandicoots, whether of complete mitochondria (Nilsson et al., 2004; Phillips et al., 2006), of single nuclear genes (Baker et al., 2004), or multiple nuclear genes (Amrine-Madsen et al., 2003), have included only a limited number of bandicoot taxa. Furthermore, only Kirsch et al. (1997) and Westerman et al. (1999) have employed molecular dating methods to infer the evolutionary history of bandicoots (Table 1). These studies employed fossil-calibrated molecular clocks to either DNA hybridization data (Kirsch et al., 1997) or mitochondrial 12S rRNA gene sequences (Westerman et al., 1999). On average, the mitochondrial point estimates of divergence time for the bandicoot subfamily and family nodes were seven million years younger than the DNA hybridization estimates.

Parametric (e.g., Thorne et al., 1998; Kishino et al., 2001) and semi-parametric (e.g., Sanderson, 2002) dating methods are becoming increasingly common given that they allow for relaxation of the molecular clock and generally perform better than strict molecular clock methods (Yang and Rannala, 2006; Smith et al., 2006; Benton and Donoghue, 2007). Of considerable interest are reliable calibration points. Benton and Donoghue (2007) suggest that minimum constraints require (1) a fossil with well-supported apomorphies of the given group; (2) a correct phylogenetic tree; and a (3) well-dated fossil-bearing stratum. When these conditions are satisfied, the probability of a later divergence can be considered zero and the constraint can be treated as a "hard" bound (Benton and Donoghue, 2007). When these conditions are not satisfied, "soft" bounded constraints may be more appropriate. Maximum constraints are much more controversial and difficult to specify (Benton and Donoghue, 2007; Benton and Ayala, 2003; Hedges and Kumar, 2004; Reisz and Müller, 2004). Reisz and Müller (2004) and Müller and Reisz (2005) suggest phylogenetic bracketing. Benton and Donoghue (2007) suggest a pluralistic approach that uses both "phylogenetic bracketing" and considers "the absence of fossils from underlying deposits" (p. 28). In addition, maximum and minimum constraints should be "fully substantiated" so that any refinements in geological dates, the discovery of new fossils, or changes in diagnostic apomorphies can be incorporated into new analyses. Soft and hard bounded analyses will recover similar estimates if the molecular data and fossil constraints are not in "conflict" with one another (Yang and Rannala, 2006). Analyses employing soft bounded constraints will out perform hard bounded analyses when poor constraints are used. Soft bounded con-

Table 1
Previously estimated divergence dates in millions of years for the Peramelemorphia

	DNA hybridization[a]	12S rRNA Transversions[b]
Node		
Peramelinae	12.36	1.75
Echymiperinae	10.51	5.26
Peroryctinae	16.11	18.39
Peramelidae	25.07	20.15
Base of Peramelemorphia	36.36	19.65

[a] Kirsch et al. (1997).
[b] Westerman et al. (1999) unadjusted estimate.

straints can correct for this, but hard bounds are fixed and the addition of sequence data will have no effect.

In this paper we use a nuclear gene dataset to test hypotheses of bandicoot relationships as previously suggested by the single mitochondrial 12S rRNA gene, DNA hybridization, and morphological studies. We also employ the relaxed molecular clock approaches of Drummond et al. (2006) and Thorne and Kishino (2002) to this protein-coding concatenation to present a timescale for peramelemorphian evolution.

2. Materials and methods

2.1. Taxon sampling

Our study included all recognized bandicoot genera but *Rhynchomeles* and 15 outgroup taxa representing the Microbiotheria (*Dromiciops*), Diprotodontia (six genera sampling the Macropodiformes, Petauroidea, Phalangeroidea, and Vombatiformes), Dasyuridae (five genera sampling from the subtribes Planigalini, Phascogalini, and Dasyurini), and Notoryctemorphia (*Notoryctes*), all of which are indicated in Table 2. There is robust support for the inclusion of Peramelemorphia in the monophyletic cohort Australidelphia, which consists of all Australasian orders and the South American Microbiotheria (Amrine-Madsen et al., 2003; Phillips et al., 2006). However, the sister group to bandicoots remains unclear and as a result we chose representatives from all australidelphian orders. The inclusion of multiple outgroups also allowed for additional constraints that were incorporated into molecular dating analyses.

2.2. Gene sequences

DNA was extracted from tissue samples (organ or muscle) using the QUIAGEN DNeasy Tissue extraction kit or the methodologies of either Kirsch et al. (1990) or Westerman et al. (1999). Protein-coding portions of five nuclear genes were amplified as described elsewhere (Amrine-Madsen et al., 2003, and references therein) for the following genes: exon 26 of Apolipoprotein B (ApoB), exon 11 of breast and ovarian cancer susceptibility gene 1 (BRCA1), exon 1 of interphotoreceptor retinoid binding protein gene (IRBP), intronless recombination activating gene-1 (Rag1), and exon 28 of von Willebrand factor gene (vWF). Primers new to this study are given in the Supplementary Information.

QIAGEN QIAquick PCR purification kits were used to clean the PCR products. All PCR products were sequenced in both directions using the University of California Riverside's Core Genetics Institute ABI 3730xl automated DNA sequencer. Internal sequencing primers were designed when needed. Accession numbers for all sequences used in this analysis are given in Table 2, including 88 previously published sequences and 22 sequences new to this study.

2.3. DNA alignments and data compatibility

Alignment-ambiguous regions were identified using the program SOAP v1.2a4 (Löytynoja and Milinkovitch, 2001) with gap opening (13–17) and gap extension (5–9) penalties in steps of two. Nine different alignments for each gene segment resulted. These alignments were then manually re-aligned using the program SE-AL taking into account the amino acid residues (Rambaut, 1996). We identified three regions within BRCA1 that were not alignable totaling 39 base pairs. The aligned nexus file can be found in the Supplementary Information. We used the partition homogeneity test (Farris et al., 1994; Swofford, 2002) with 1000 replications and 100 taxon input orders per replicate and the bootstrap compatibility method (De Queiroz, 1993; Teeling et al., 2000) with 500 bootstrap replicates and a 90% bootstrap support criterion to test the appropriateness of combining the individual gene segments into one multigene concatenation. The bootstrap compatible method indicated that it was appropriate to combine the five gene segments into one multigene data set. The partition homogeneity test was significant ($p = 0.047$). However, several authors have noted that this test rejects the hypothesis of data compatibility too often (e.g., Cunningham, 1997; Barker and Lutzoni, 2002; Darlu and Lecointre, 2002). Cunningham (1997) suggested that a critical alpha value of somewhere between 0.01 and 0.001 should be used to prevent excessive type I errors. Given these results we elected to concatenate our individual gene segments into one combined data set. We were only able to amplify the Rag1 sequence for the extinct genus *Chaeropus* due to the highly degraded DNA sample, which was obtained from a spirit-preserved museum specimen.

2.4. Phylogenetic analyses

PhyML (Guindon and Gascuel, 2003) and Paup 4.0b10 (Swofford, 2002) were used to perform the maximum-likelihood (ML) and maximum parsimony (MP) analyses, respectively, on the concatenated alignment with all five genes and the Rag1 only analysis (*Chaeropus* included). We also coded phylogenetically informative indels as present or absent and analyzed this data set using MP. Gaps were treated as missing data in all analyses. For the ML analyses, Modeltest 3.06 (Posada and Crandall, 1998) was used to determine the best fit model of nuclear substitution and associated model parameters under the Akaike Information Criterion (AIC). Models chosen were GTR+Γ (ApoB, BRCA1, concatenation), K81uf+Γ (IRBP), TIM+Γ+I (Rag1), GTR+Γ+I (Rag1 with *Chaeropus*), and TVMef+Γ (vWF). We employed heuristic searches using 1000 randomized addition orders with tree-bisection and reconnection (TBR) branch swapping for MP analyses and started the ML analyses from a neighbor-joining tree. Five hundred (ML) and 1000 (MP) bootstrap replicates were performed for each analysis.

Table 2
Classification and Genbank accession numbers used in this study[a]

TAXON	ApoB	Rag1	IRBP	vWF	BRCA1
Peramelemorphia					
Peramelidae					
Echymiperinae					
Echymipera kalubu	AY243420	AY243386	AF025383	AY243405	AF355796
Microperoryctes longicauda	EU369355*	EU369368*	EU369365*	EU369372*	EU369360*
Peroryctinae					
Peroryctes raffrayana	EU369356*	EU369369*	EU369366*	EU369373*	EU369361*
Peramelinae					
Isoodon macrourus	EU160446	EU160453	EU160450	EU160456	EU160441
Perameles nasuta/gunnii	AY243426	AY243394	AY243437	AY243411	AY243450
Thylacomyidae					
Macrotis lagotis	EU369354*	AY125024	EU369364*	EU369371*	EU369359*
Chaeropodidae					
Chaeropus ecaudatus	N/A	EU369367*	N/A	N/A	N/A
Notoryctemorphia					
Notoryctidae					
Notoryctes typhlops	AY243424	AY243391	AF025385	AY243408	AY243447
Dasyuromorphia					
Dasyuridae					
Dasyurini					
Dasyurus albopunctatus	AY243430	AY243398	AY532681	AY243414	AY243452
Phascolosorex dorsalis	EU369357*	EU372017*	AY686532	EU369374*	EU369362*
Phascogalini					
Phascogale tapoatafa	AY243427	AY243395	AF025382	AY243412	AF355795
Antechinus stuartii/flavipes/swainsonii	EU160447	AY125023	AY532666	EU160457	EU160442
Planigalini					
Planigale sp./maculata	EU369358*	EU369370*	AY243438	EU369375*	EU369363*
Diprotodontia					
Macropodiformes					
Macropodidae					
Macropus/Dendrolagus	AY243422	AY243388	AY243435	AJ224670	AF284033
Potoroidae					
Aepyprymnus rufescens	EU160444	EU160451	EU160448	EU160454	EU160439
Phalangeriformes					
Phalangeroidea					
Phalangeridae					
Phalanger orientalis	AF548431	AY243393	AY243436	AY243410	AY243449
Burramyidae					
Cercartetus nanus	EU160445	EU169452	EU160449	EU160455	EU160440
Petauroidea					
Petauridae					
Petaurus breviceps	AY243433	AY243400	AY243441	AY243417	EU160443
Pseudocheiridae					
Pseudocheirus/Pseudochirops	AY243425	AY243392	AY243440	AY243416	AY243448
Vombatiformes					
Vombatidae					
Vombatus ursinus	AY243429	AY243397	AF284031	AF497260	AF284031
Phascolarctidae					
Phascolarctos cinereus	AY243421	AY243387	AY243434	AY243406	AY243445
Microbiotheria					
Dromiciops gliroides	AY243423	AY243389	AF025384	AY243407	AY243446

[a] New sequences indicated by *. Chimeric genera are indicated with slashes. Marsupial taxonomy is after Wilson and Reeder (2005). We recognize that Phalangeriformes is most likely a paraphyletic group based on recent molecular evidence (Meredith et al., 2007; Phillips and Pratt, 2008).

Bayesian posterior probabilities were calculated for all data sets using MrBayes v3.1.1 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003), which carries out Metropolis-coupled Markov chain Monte Carlo sampling. In addition, we performed two types of Bayesian analyses for each concatenated data set. In the first type of analysis one model of sequence evolution was used and in the second analysis each gene was allowed to have its own model of sequence evolution. Models of sequence evolution were chosen as indicated above and in cases where the chosen model was not available in MrBayes the next most general model was chosen. In all Bayesian analyses we employed four Markov chains (three hot and one cold) with random starting trees, default prior settings, and chain sampling every 1000 generations. When the average standard deviation of split frequencies for the simultaneous

analyses fell below 0.01 the analyses were terminated (more than 4.5 million generations).

2.5. Statistical tests of tree topologies

We applied the Kishino–Hasegawa (KH), Shimodaira–Hasegawa (SH), and approximately unbiased (AU) statistical tests to access confidence in our tree selection for both the concatenated data set and the Rag1 only data set (Kishino and Hasegawa, 1989; Shimodaira and Hasegawa, 1999; Shimodaira, 2002). CONSEL (Shimodaira, 2002) was used to perform the KH, SH, and AU tests. Each of these methods can be biased in tree selection. If multiple tree topologies are simultaneously compared the KH test can fail and place over confidence in the wrong tree especially if the ML tree is chosen. SH tests correct for comparing multiple tree topologies but tend to keep a larger number of tree topologies and may be over conservative. The AU test can be the least biased of these methods but if selection bias is extreme it may fail (Shimodaira, 2002). For the concatenated data set we compared four different tree topologies for the base of Peramelemorphia, three topologies for the position of Peroryctinae, and four topologies for the position of *Macrotis*. For the Rag1 data set we compared seven tree topologies for the position of *Chaeropus*.

2.6. Molecular dating analyses

The molecular clock hypothesis was tested using the likelihood ratio statistic. Significant *P*-values ($P < 0.001$) were found for ApoB, BRCA1, IRBP, vWF and the concatenation. The molecular clock could not be rejected for Rag1 ($P > 0.01$). As a result we employed *Multidivtime* (version 9-25-03) (Thorne et al., 1998; Kishino et al., 2001; Thorne and Kishino, 2002) and BEAST v.1.4 (Drummond and Rambaut, 2003; Drummond et al., 2006) to estimate divergence times. Both are Bayesian methods that employ a relaxed molecular clock and allow the usage of multiple constraints from the fossil record. However, they are different in that BEAST does not require a rooted tree topology with a designated outgroup because it simultaneously co-estimates both the phylogeny and divergence times for all taxa. Furthermore, BEAST does not assume autocorrelation of molecular rates among lineages and allows for both hard and soft constraints. To determine if more data would increase the precision of our divergence estimates for the Peramelemorphia, we plotted the confidence interval (CI) range verses the mean estimate (Yang and Rannala, 2006).

2.6.1. Multidivtime

For the *Multidivtime* analyses, we used the phylogeny shown in Fig. 1 to estimate divergence times. The program *estbranches* was used to estimate branch lengths (Thorne et al., 1998; Kishino et al., 2001; Thorne and Kishino, 2002) and *Multidivtime* was used to estimate divergence times. Two different analyses were performed on the five gene nuclear data set. In the first analysis, the data set was partitioned to allow each gene over time to have its own rate trajectory. This is in contrast to the second analysis, in which the genes were not partitioned, which then forces all of the genes to change rate by a common factor. We used the F84 model of sequence evolution (Swofford et al., 1996) with four discrete categories for the Γ distribution of rates in both analyses. This is the most complicated model implemented by *Multidivtime*. We chose to use the most complicated model because Modeltest indicated that the best fit model of sequence evolution for both the individual genes and the concatenation were at least as complicated as the F84 + Γ model (see Section 2.4). The transition/transversion parameter and the estimates of the rate categories of the gamma distribution were calculated in PAUP 4.0b10 (Swofford, 2002). The mean prior distribution for the root of Australidelphia was set to 70 million. This date is ten million years older than the oldest described australidelphian (*Khasia*), which has been dated to 60.4–59.2 MYA (Marshall and De Muizon, 1988; Marshall et al., 1997; Gradstein et al., 2004). In addition, previous molecular dating studies have suggested a mean of 65.2 million years for the base of Australidelphia (e.g. Drummond et al., 2006). We set the mean of the prior distribution for the rate of nucleotide substitution at the ingroup root node (Australasian taxa) equal to the median amount of evolution from the Australasian taxa root to the Australasian taxa tips. This was divided by the mean of the prior root distribution for Australasian taxa. Markov chain Monte Carlo analyses were run for one million generations with a burnin of 100,000 generations and were sampled every 100 generations.

Estimated dates of divergence are strongly influenced by the constraints. The Australasian early Tertiary terrestrial vertebrate fossil record is relatively poorly known with a complete absence of deposits from the early Eocene to the late Oligocene. As a result, molecular dating analyses were performed with different maxima to gage the effect of these constraints on the estimated dates of divergence. Constraints on divergence times were as follows and node numbers are shown on Fig. 2:

a. Node 1. The Murgon deposits in southeastern Queensland have produced the oldest terrestrial marsupial fossils from Australia (54.6 MYA; Godthelp et al., 1999). Despite intensive collecting, crown group diprotodontians have not been recovered. However, this is the only terrestrial Eocene mammal bearing deposit in Australia and the absence of diprotodontians from Murgon does not constitute proof that they were absent. Unlike the Dasyuridae and Peramelidae, the late Oligocene contains several families of Diprotodontia (e.g. pseudocheirids, burramyids, and phascolarctids) that are very derived and morphologically similar to the living members of these families. In light of this evidence, we used two different maxima for the base of Diprotodontia;

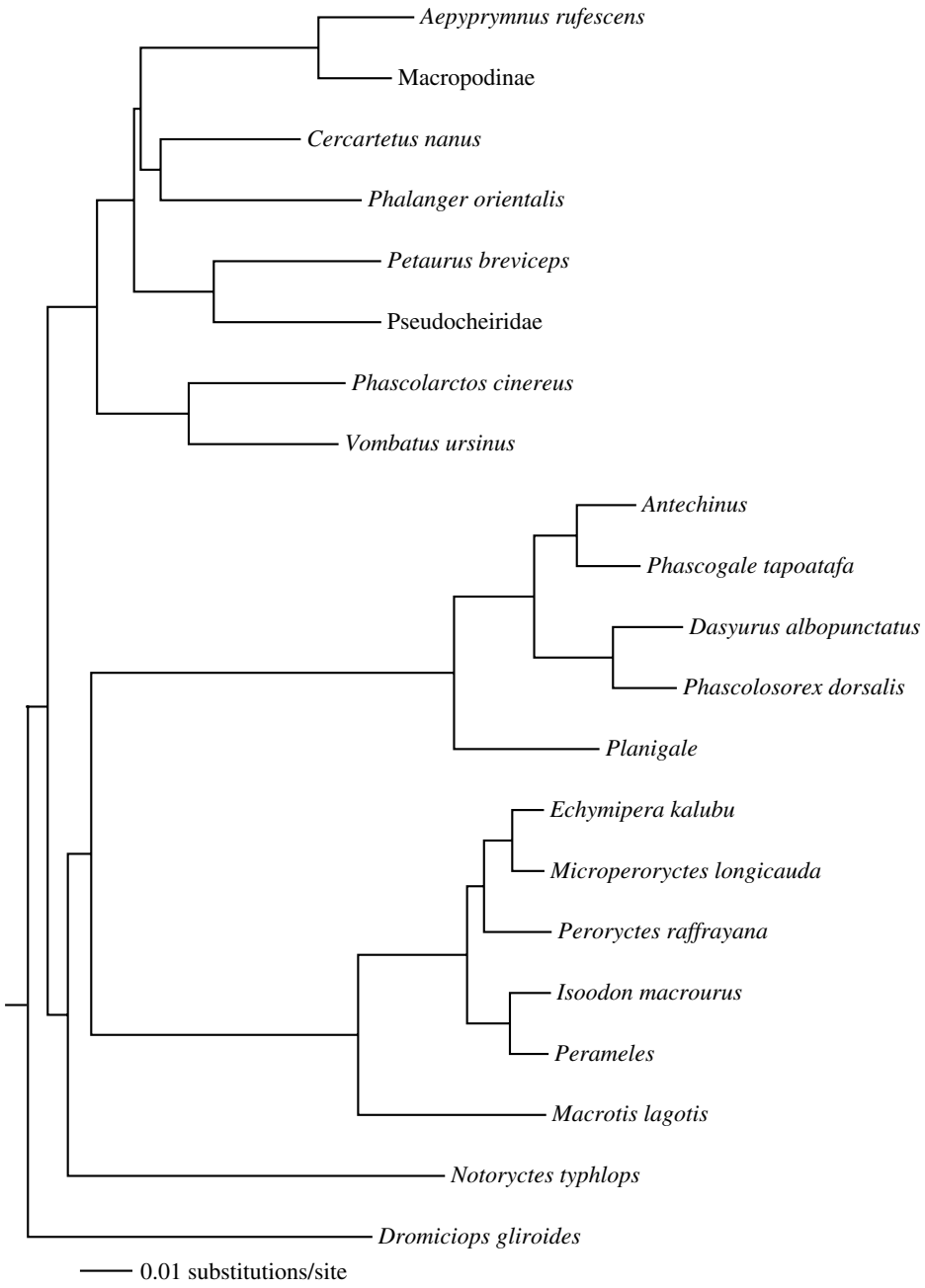

Fig. 1. ML phylogram obtained using the 6 kb concatenation consisting of protein-coding regions of five nuclear genes under the GTR+Γ model of sequence evolution.

54.6 and 65 million years. As a minimum we used 25.5 million years based on the oldest described fossil diprotodontians from Zone A of the Etadunna Formation (see nodes 3 and 4).

b. Node 2. Several genera of Oligo-Miocene kangaroos are known from both central Australia and Riversleigh. However, there is no consensus over which subfamily (or family) they belong to and which ones give rise to the lophodont kangaroos (i.e. macropodids including the sthenurines). For example, Case (1984) treats *Purtia mosaicus* as a potoroid and *Nambaroo* species A and B as a macropodids. Both genera come from Zone C of the Etadunna Formation (Ngapakaldi Local Fauna; 25.0–25.5 MYA; Woodburne et al., 1993). However, *Purtia* is sometimes considered a macropodoid *incertae sedis* (Long et al., 2002) or a bulungamayine (Prideaux, 2004) and *Nambaroo* is treated as a balbarine by some authors (e.g. Cooke, 2006).

Possible candidates ancestral to the lophodont kangaroos include the Balbarinae and the Bulungamayinae (e.g. Long et al., 2002; Cooke, 2006). Cooke (1999) and Prideaux (2004) favor the Bulungamayinae but suggest that this subfamily might be a paraphyletic

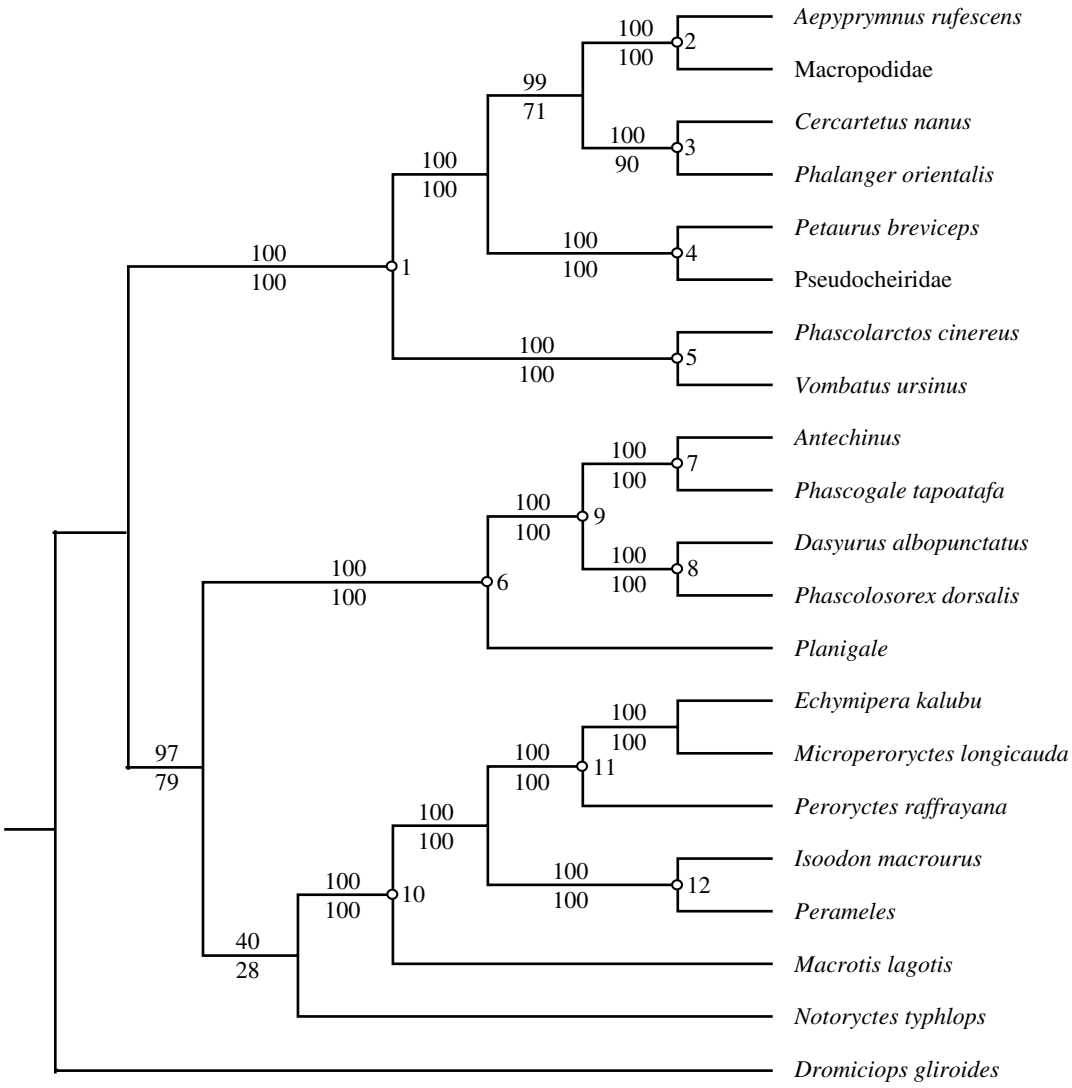

Fig. 2. Bayesian tree obtained using the 6 kb concatenation consisting of protein-coding regions of five nuclear genes under the GTR+Γ model of sequence evolution. Values above and below branches correspond to the mean percentage Bayesian posterior probabilities based on two independent runs and the bootstrap support percentages, respectively. Constrained nodes for dating analyses are indicated by number and open circles.

stem group. In contrast, Flannery (1989) favored the balbarines as ancestral to the lophodont kangaroos. Given the uncertainty in both the assignment of fossil taxa and the placement of the extinct (sub)families within the Macropodiformes we used a conservative minimum of 12 million years for the potoroid-macropodid split. This minimum is based on the middle Miocene Bullock Creek Local Fauna from the Camfield Beds in the Northern Territory (~12 MYA; Woodburne et al., 1985; Murray and Megirian, 1992; Long et al., 2002). This local fauna possibly contains the oldest macropodiforms referable to the Macropodidae (Macropodinae and Sthenurinae). The material includes sthenurine like postcranial material (Prideaux, 2004) and an unnamed small high crowned molar tooth (Murray and Megirian, 1992).

Both the Balbarinae and Bulungamayinae have fossil records extending back to the late Oligocene (Cooke, 2006). Systems A and B of Riversleigh have produced both bulungamayines and balbarines (Long et al., 2002). Myers and Archer (1997) have correlated these systems with the Ngama Local Fauna (Zone D of the Etadunna Formation), which has been dated at 24.7–25.0 million years (Woodburne et al., 1993). Zone A of the Etadunna Formation has produced "*Kyeema mahoneyi*", sp. nov. a highly plesiomorphic kangaroo that is difficult to assign to any subfamily. In view of this, we used the Eocene-Oligocene boundary (33.9 millions; Gradstein et al., 2004) as the maximum for the potoroid-macropodid split.

c. Node 3. The known Burramyidae fossil record extends back to late Oligocene. *Burramys wakefieldi* comes from Zone D of the Etadunna Formation, which has been dated to approximately 24.7–25.0 million years using magnetostratigraphy (Woodburne et al., 1993). The late Oligocene Geilston Bay deposits

of Tasmania are at least 23 million years old and have produced the burramyid genus *Cercartetus* and a specimen referred to Phalangeridae *incertae sedis* (Tedford and Kemp, 1998). Middle Miocene phalangerids have been described from Riversleigh but their phylogenetic affinities are debated. We used 24.7 million as a minimum and either 54.6 or 65 million years as the maximum for Node 3.

d. Node 4. *Paljara* sp. A from Zone A of the Etadunna Formation is the oldest described pseudocheirid. This zone has been dated at approximately 25.5 million years (Woodburne et al., 1993). *Djaludjangi yadjana* from the middle Miocene of Riversleigh was originally described as the oldest known petaurid (Brammall, 1998). However, Crosby et al. (2004) consider it Petauroidea *incertae sedis*. We used 25.5 million years as the minimum and either 54.6 or 65 million years as the maximum for Node 4.

e. Node 5. *Perikoala robusta* is the oldest known phascolarctid. These specimens come from Zone A of the Etadunna Formation, which has been dated to approximately 25.5 million years (Woodburne et al., 1993). *Rhizophascolonus crowcrofti* is the oldest described vombatid and specimens are known from the Miocene Wipajiri Formation. Older undescribed vombatids are known from the late Oligocene of Riversleigh (Archer and Hand, 2006). We used 25.5 million years as the minimum and either 54.6 or 65 million years as the maximum for Node 5.

f. Node 6. *Barinya wangala* is the oldest definitive stem dasyurid and has been placed in its own subfamily (Wroe, 1998). Specimens of *B. wangala* come from the early to middle Miocene of Riversleigh (~11.61–23.03 MYA; Gradstein et al., 2004; Wroe, 1998). The oldest fossil dasyurids referable to the living subfamilies are from the 4.46 million year old Hamilton Local Fauna (Turnbull et al., 2003; Wroe, 2003; Archer and Hand, 2006). Additional "dasyurids" have been described from the late Oligocene/early Miocene, but these taxa are now considered Dasyuromorphia *incertae sedis* (e.g., *Mayigriphus*) or even Marsupialia *incertae sedis* (e.g. *Wakamatha, Keeuna*, *Ankotarinja, Djarthia*; Godthelp et al., 1999; Wroe, 2003). It should be noted that the majority of Oligo-Miocene specimens are only known from isolated elements, and as a result some of these species could still be "archaic" members of the crown group Dasyuridae. In light of this evidence, we use 4.46 million years as the minimum and the base of the Oligocene (33.9 MYA; Gradstein et al., 2004) as the maximum for Node 6.

g. Node 7. An *Antechinus* sp. is known from the Hamilton Local Fauna (4.46 MYA; Archer, 1982; Turnbull et al., 2003). We used 4.46 million years as the minimum and the base of the Miocene (23.03 MYA; Gradstein et al., 2004) as the maximum for the split between *Antechinus* and *Phascogale*.

h. Node 8. The extinct *Dasyurus dunmalli* is known from the Bluff Downs Local Fauna (Allingham Formation; Bartholomai, 1971). The Allingham Formation is overlain by a basalt flow dated at 3.62 million years (Mackness et al., 2000). We used 3.62 million years as a minimum and the base of the Miocene (23.03 MYA; Gradstein et al., 2004) as the maximum for the split between *Dasyurus* and *Phascolosorex*.

i. Node 9. The oldest described member of either the Dasyurini or Phascogalini is *Antechinus* sp. from the Hamilton Local Fauna. We used 4.46 million years as the minimum and the base of the Miocene (23.03 MYA; Gradstein et al., 2004) as the maximum for the split between Dasyurini and Phascogalini.

j. Node 10. The oldest fossils referable to the crown group Peramelemorphia are from Pliocene local faunas. *Perameles allinghamensis* is from the Bluff Downs Local Fauna (Allingham Formation; Archer and Wade, 1976), *Perameles bowensis* is from the Bow Local Fauna (Muirhead et al., 1997), *cf. Peroryctes tedfordi* is from the Pliocene Hamilton Local Fauna (Turnbull et al., 2003), and two unnamed Peramelinae (?*Perameles* sp. 1 and 2) species are known from Rackham's Roost Site Local Fauna of Riversleigh (Archer et al., 2001). The Riversleigh site cannot be dated directly and the Bow Local Fauna has not been dated but has been biocorrelated to the Bluff Downs Local Fauna (Archer et al., 1999), which is overlain by basalt dated at 3.62 million years (Mackness et al., 2000). The Hamilton Local Fauna has been dated to 4.46 million years (Turnbull et al., 2003). We used 4.46 million years as a minimum for the base of Peramelemorphia. The late Oligocene Kangaroo Well Local Fauna has produced the oldest described peramelemorph (*Yarala kida*; ~23.8 MYA; Schwartz, 2006), but members of this monotypic family do not appear to be part of the crown group radiation (Archer et al., 1999; Archer and Hand, 2006). There is one tooth of a putative stem perameloid from Murgon (54.6 MYA; Godthelp et al., 1999; Archer and Kirsch, 2006; Archer and Hand, 2006), but it has yet to be formally described. The Oligo-Miocene Etadunna Formation and the Miocene Wipijiri Formation have also produced bandicoots, although these taxa remain to be formally described (Case, 2001). Case (2001) provides evidence for a faunal turnover of bandicoots in the Etadunna and Wipijiri formations. The lower zones A–B of the Etadunna Formation are characterized by "archaic" bandicoots that have tribosphenic molars. These are probably closely related to the yaralids (Schwartz, 2006). The bandicoots from zone D of the Etadunna Formation (24.7–25.0 MYA; Woodburne et al., 1993) and the Wipijiri Formation

(10.5–11.5 MYA; Langford et al., 1995) are more derived and may be ancestral to the living species (Case, 2001). The zone D species has an enlarged metaconule (more quadritubercular) and two of the three Wipijiri species have tooth morphologies that are very similar to the living species. The third Wipijiri species is more similar to the zone D species. However, it is still possible that these "archaic" bandicoots are crown group bandicoots given that they are only known from teeth. Given this evidence, we use the base of the Oligocene (33.9 MYA; Gradstein et al., 2004) as a maximum for the base of Peramelemorphia.

k. Node 11. *cf. Peroryctes tedfordi* is the oldest described member of either the Peroryctinae or Echymiperinae. These specimens come from the Pliocene Hamilton Local Fauna (4.46 MYA; Turnbull et al., 2003). We used 4.46 million years as the minimum and the base of the Miocene (23.03 MYA; Gradstein et al., 2004) as the maximum for the split between the Peroryctinae and Echymiperinae.

l. Node 12. *Perameles allinghamensis* (Bluff Downs Local Fauna; Allingham Formation; Archer and Wade, 1976) and *Perameles bowensis* (Bow Local Fauna; 3.62 MYA; Muirhead et al., 1997; Mackness et al., 2000) are the oldest members of the Peramelinae. 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 split between the *Perameles* and *Isoodon*.

2.6.2. BEAST analyses

Similar to the *Multidivtime* analyses, multiple BEAST v1.4 analyses were performed on the concatenated data set. In the first set of analyses the genes were partitioned and in the second set of analyses the genes were not partitioned. The mean prior distribution for the rate of molecular evolution at the root node used in the *Multidivtime* non-partitioned analysis was used as the mean substitution rate per year in the non-partitioned BEAST analysis. For the partitioned BEAST analysis, the mean prior distribution for the rate of molecular evolution at the root node was estimated for each gene and these values were then used as the mean substitution rate per year. We used Modeltest 3.06 (Posada and Crandall, 1998) (see Section 2.4) as a guide for choosing the model of molecular evolution implemented in the BEAST analyses. If the suggested Modeltest 3.06 model contained more than two substitution rates, the GTR model was used. Otherwise, the HKY model was used. We employed the uncorrelated lognormal rate variation model with a Yule prior distribution for branching rates and the starting tree indicated in Fig. 1. All of the Markov chain Monte Carlo analyses were run for 30 million generations with a burnin of three million generations and sampled every 1000 generations. Each of the different analyses was performed two times to test for convergence among chains and the runs were combined to obtain an estimate of the posterior probability distributions. Stationarity/mixing was visually checked using Tracer 1.2 (Rambaut and Drummond, 2003) to ensure that the chains were run long enough (the estimated sample size was greater than 200 for all posterior estimates). We used the same constraints as employed in the *Multidivtime* analyses. We also explored the affect of using soft bounds. In these analyses, the node constraint followed a standard normal distribution. 95% of the distribution was set to be between the upper and lower bounds and 2.5% in each of the tails.

3. Results

3.1. Phylogenetic analyses and alignments

Figs. 1 and 3 show the ML phylograms for the combined and Rag1 data sets, respectively, that resulted from PhyML analyses. Figs. 2 and 4 show the Maximum Posterior Probability (MPP) trees for the combined and Rag1 data sets, respectively, that resulted from MrBayes analyses with partitioned data. Following Waddell and Shelley (2003, p. 203), the "MPP tree is found as a "combinable component" consensus of all sampled trees, while the Bayesian Posterior Probability (BPP) of a clade is found as the proportion of all sampled trees with that clade." Figs. 2 and 4 also show mean Bayesian Posterior Probabilities (BPP) based on two independent runs and ML Bootstrap Support Percentages (BSP) derived from PhyML for each clade. BPPs for partitioned and non-partitioned analyses and BSPs for MP and ML analyses are summarized in Table 3. Figs. 1–4 were rooted between *Dromiciops* and all other australidelphians following several recent studies that support the monophyly of Australasian marsupials (e.g. Amrine-Madsen et al., 2003; Phillips et al., 2006).

The ML (Fig. 1) and MPP-partitioned (Fig. 2) trees for the combined data set were identical except for the placement of *Notoryctes*, which was the sister to Peramelemorphia + Dasyuromorphia on the ML tree (Fig. 1) and Peramelemorphia on the MPP-partitioned tree (Fig. 2). The MPP tree for non-partitioned data was identical to the ML tree. MP analysis resulted in three trees at 4898 steps (Supplementary Information). Relationships among bandicoots on the MP trees were identical to those that were recovered in ML and Bayesian analyses. Bootstrap and Bayesian analyses supported the monophyly of individual marsupial orders (100% BSP and 1.00 BPP). Within both Peramelemorphia and Dasyuridae all nodes were strongly supported (100% BSP and 1.00 BPP). Within Peramelemorphia, *Macrotis* was the sister taxon to a monophyletic Peramelidae. Within the Peramelidae, *Microperoryctes* and *Echymipera* (Echymiperinae) grouped to the exclusion of *Peroryctes* (Peroryctinae). This clade was the sister group to a monophyletic Peramelinae (*Isoodon* and *Perameles*). Within the Dasyuridae, Phascogalini (*Phascogale* + *Antechinus*) and Dasyurini (*Dasyu-*

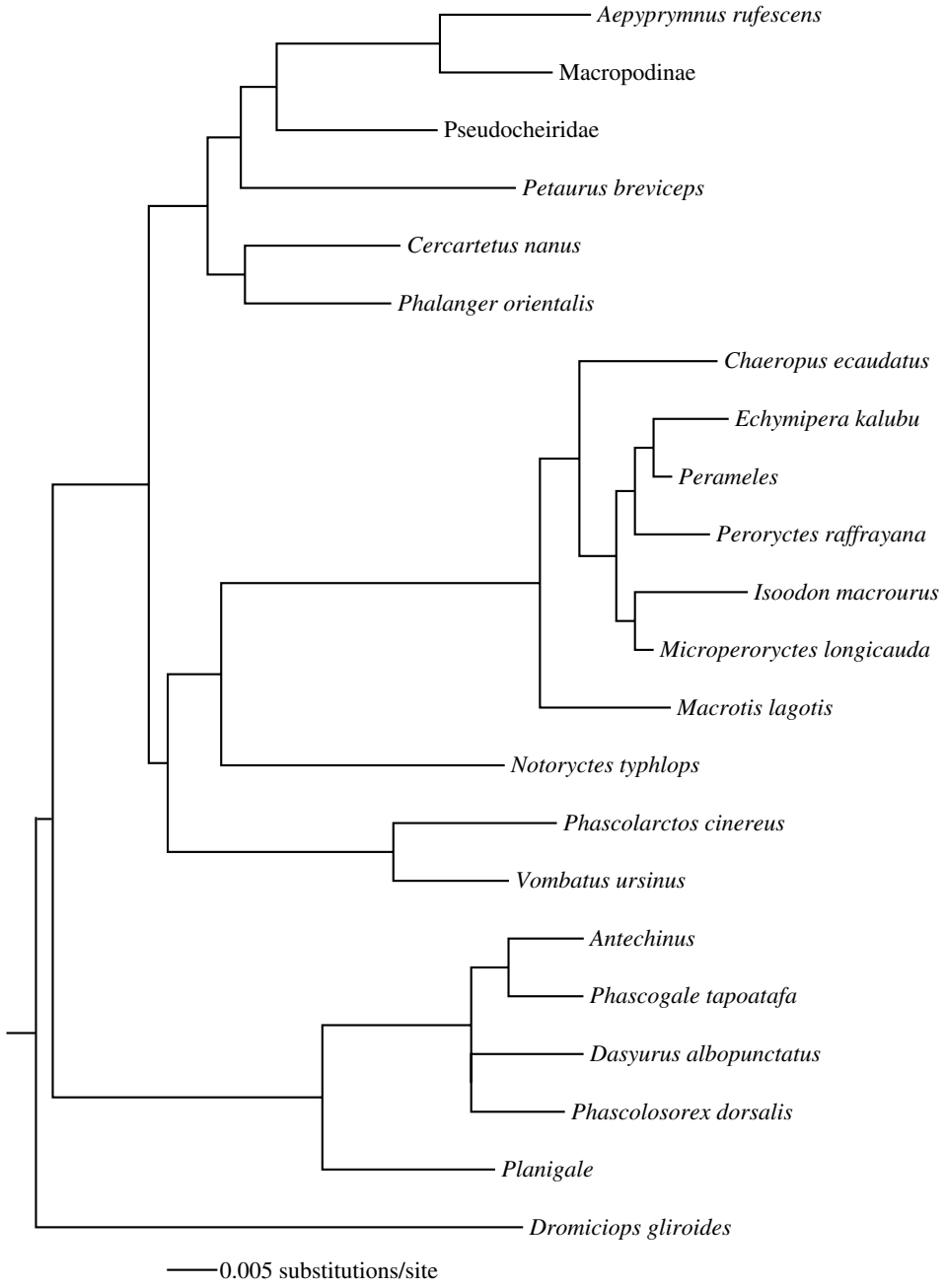

Fig. 3. ML phylogram obtained using Rag1 under the GTR+Γ+I model of sequence evolution.

rus + *Phascolosorex*) grouped to the exclusion of Planigale (Planigalini).

The ML (Fig. 3), MPP (Fig. 4), and MP (Supplementary Information) trees for the Rag1 data set exhibited topological differences among the outgroup taxa, but were identical for relationships within Peramelemorphia. In all cases *Chaeropus* was the sister taxon to Peramelidae and *Macrotis* was the sister taxon to *Chaeropus* + Peramelidae. Relationships within Peramelidae differed from analyses based on the combined data set and instead recovered *Peroryctes* as the sister taxon to *Echymipera* + *Perameles* and this clade as the sister taxon to *Microperoryctes* plus *Isoodon*. Bootstrap and Bayesian analyses supported Peramelidae and *Chaeropus* + Peramelidae with varying degrees of support (Table 3). Relationships within the Peramelidae were only moderately supported by the Rag1 data.

3.2. Indels

Synapomorphic indels (insertions and deletions) can serve as an important secondary source of phylogenetic information as has been demonstrated in didelphid marsupials (Steiner et al., 2005), xenarthrans (van Dijk et al., 1999), bats (Teeling et al., 2002), and interordinal studies

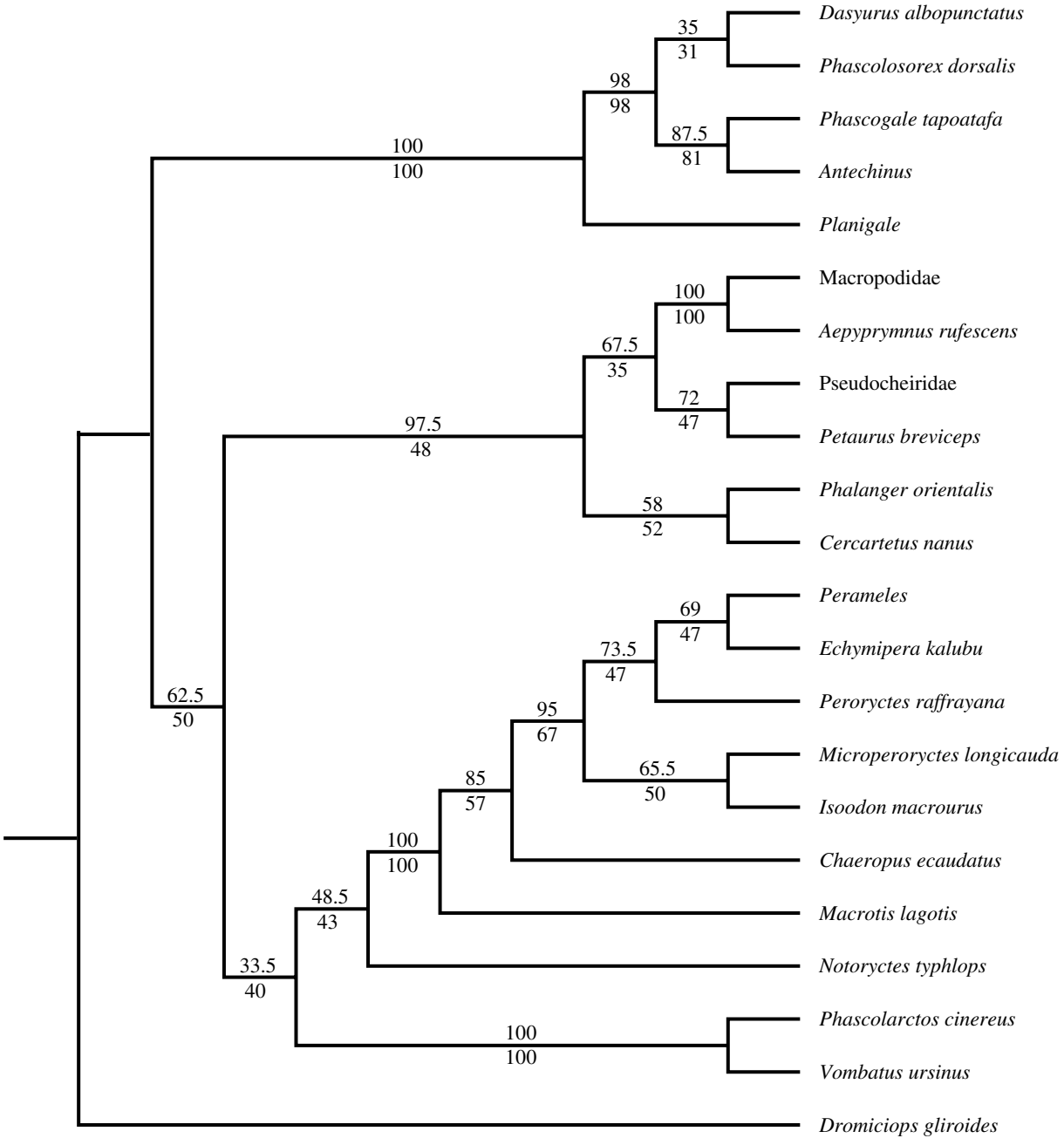

Fig. 4. The Rag1 Bayesian tree (543 bp) obtained using the GTR+Γ+I model of sequence evolution. Values above and below branches correspond to the mean percentage Bayesian posterior probabilities based on two independent runs and the bootstrap support percentages, respectively.

of placental mammal relationships (de Jong et al., 2003; Madsen et al., 2001; Poux et al., 2002; Thomas et al., 2003). Peramelemorphia monophyly is supported by five unique deletions (positions 483–497; 1390–1392; 1697–1695; 1999–2001; 2219–2221; and 2235–2237 in our BRCA1 alignment. Within Peramelemorphia, *Isoodon* + *Perameles* share a unique insertion in BRCA1 (position 2142–2147). The MP analyses of the modified concatenated alignment with all five genes and indels coded as present or absent yielded bootstrap support values identical to the non-modified concatenated alignment with all five genes (not shown).

3.3. Statistical tests

Results of KH, SH, and AU tests are given in Table 4 for both the multigene data set and the Rag1 only data set. These tests were used on the multigene data set to compare four different hypotheses for the root of Peramelemorphia. Rooting on Peramelinae, Echymiperinae, and Peroryctinae were all rejected in favor of rooting on *Macrotis* for all three tests. Within Peramelemorphia, we compared the position of Peroryctinae and *Macrotis*. For the position of Peroryctinae, a sister group relationship to all other bandicoots and Peramelinae were both rejected in favor of Peroryctinae + Echymi-

Table 3
Summary of bootstrap and posterior probabilities for the Bayesian, maximum likelihood (ML), and maximum parsimony (MP) analyses

Hypothesis	Analyses[a]									
	Multigene						Rag1 only			
	MP	ML	Bayesian				MP	ML	Bayesian	
			Partitioned		Non-partitioned				Run 1	Run 2
			Run 1	Run 2	Run 1	Run 2				
Notoryctes + Dasyuridae	17.6	30.8	0.00	0.00	0.00	0.00	0.08	0.07	0.07	0.07
Notoryctes + Peramelemorphia	61	28.2	0.40	0.41	0.22	0.22	50	43	0.48	0.49
Peramelemorphia + *Notoryctes* + Dasyuridae	15	78.8	0.97	0.97	0.99	0.99	0.08	0.17	0.07	0.07
Dasyuridae + Peramelemorphia	20.9	38.6	0.35	0.33	0.48	0.49	0.06	0.09	0.04	0.04
Peramelemorphia + *Notoryctes* + Diprotodontia	61	6.2	0.01	0.01	0.00	0.00	0.71	0.50	0.62	0.63
Dasyuridae	100	100	1.00	1.00	1.00	1.00	100	100	1.00	1.00
Dasyurini	100	100	1.00	1.00	1.00	1.00	22	31	0.35	0.35
Phascogalini	100	100	1.00	1.00	1.00	1.00	67	81	0.87	0.88
All Dasyuridae but Planigalini	100	100	1.00	1.00	1.00	1.00	89	66	0.98	0.98
Peramelemorphia	100	100	1.00	1.00	1.00	1.00	100	100	1.00	1.00
Microperoryctes + *Echymipera*	100	100	1.00	1.00	1.00	1.00	0.00	0.00	<0.01	<0.01
Microperoryctes + *Isoodon*	0.00	0.00	0.00	0.00	0.00	0.00	44	50	0.66	0.65
Echymipera + *Perameles*	0.00	0.00	0.00	0.00	0.00	0.00	30	47	0.69	0.69
Echymiperinae + Peroryctinae	100	100	1.00	1.00	1.00	1.00	0.00	0.00	<0.01	<0.01
Peramelinae	100	100	1.00	1.00	1.00	1.00	<0.01	0.02	0.03	0.03
Peramelidae	100	100	1.00	1.00	1.00	1.00	52.5	67	0.95	0.95
Peramelidae + Chaeropodidae	N/A	N/A	N/A	N/A	N/A	N/A	57	57	0.85	0.85
Peramelidae + Thylacomyidae	N/A	N/A	N/A	N/A	N/A	N/A	0.11	0.12	0.00	0.00
Chaeropus + *Macrotis*	N/A	N/A	N/A	N/A	N/A	N/A	0.21	0.21	0.07	0.08

[a] Partitioned = each gene was partitioned to have its own model of molecular evolution; Non-partitioned = concatenation was treated as a single gene. In both the ML and MP analyses the concatenation was treated as a single gene.

Table 4
Results of the Kishino–Hasegawa (KH), Shimodaira–Hasegawa (SH), and approximately unbiased (AU) tests

Phylogenetic hypotheses	−ln likelihood	Δ	*P*		
			KH	SH	AU
1. Base of Peramelemorphia					
Between *Macrotis* and remaining bandicoots (best)	33718.09746	—	1.00	1.00	1.00
Between Peramelinae and remaining bandicoots	33855.27799	137.18053	0.00*	0.00*	0.00*
Between Peroryctinae and remaining bandicoots	33883.85623	165.75877	0.00*	0.00*	0.00*
Between Echymiperinae and remaining bandicoots	33883.85623	165.75877	0.00*	0.00*	0.00*
2. Position of Peroryctinae (sister group to)					
Echymiperinae (best)	33718.09746	—	0.990	0.995	0.998
Peramelinae	33741.55492	23.45747	0.010*	0.153	0.002*
All remaining peramelemorphians	33883.85623	165.75877	0.000*	0.00*	0.000*
3. Position of Macrotis (sister group to)					
Peramelinae	33855.82779	137.73034	0.00*	0.00*	0.00*
Peroryctinae	33882.30843	164.21097	0.00*	0.00*	0.00*
Echymiperinae	33882.55208	164.45462	0.00*	0.00*	0.00*
Peramelidae (best)	33718.09746	—	1.00	1.00	1.00*
4. Position of Chaeropus (sister group to) (Rag1 data only)					
Macrotis	2126.07621	1.79651	0.079	0.118	0.041
All crown group peramelemorphians	2126.07621	1.79651	0.133	0.370	0.127
Peroryctinae	2129.54744	5.26774	0.060	0.109	0.022*
Echymiperinae	2134.06159	9.78189	0.236	0.643	0.193
Peramelinae	2134.10656	9.82686	0.072	0.108	0.032*
Peramelidae (best)	2124.27970	—	0.764	0.962	0.967
Groves and Flannery (1990) placement	2139.06942	14.78972	0.047*	0.059	0.007*

* $P = <0.05$.

perinae clade. There were significant differences in the pairwise comparisons between the four competing hypotheses for the placement of *Macrotis*. These tests favored a sister group relationship to the peramelids.

For the Rag1 only data set we tested seven hypotheses for the position of *Chaeropus*. The SH test could not discriminate between any of the different hypotheses. The KH test rejected the Groves and Flannery (1990) topology in which the *Chaeropus* + *Macrotis* clade grouped with the Peramelinae to the exclusion of the Peroryctinae + Echymiperinae clade. In addition to rejecting the Groves and Flannery (1990) topology, the AU test rejected grouping *Chaeropus* with either the Peroryctinae or Peramelinae but failed to discriminate between the remaining hypotheses.

3.4. Molecular dating

Fig. 5 shows a timescale for bandicoot divergences based on the partitioned *Multidivtime* dating analysis with the diprotodontian maximum set to 65 million years. *Multidivtime* and BEAST v1.4 molecular divergence date estimates using hard bounds with the diprotodontian maximum set to 65 million years are reported in Table 5 with 95% credibility intervals and highest posterior densities (HPD), respectively. Results of all other dating analyses can be found in Supplementary Information.

Point estimates of divergence times with partitioned data (Table 5) indicate that Peramelemorphia diverged from *Notoryctes* 55–65 million years ago. Thylacomyidae (*Macrotis*) diverged from the Peramelidae 20–29 million years ago (Table 5; Supplementary Information). The base of Peramelidae was estimated at 9–16 million years. Within Peramelidae, the base of Echymiperinae (*Microperoryctes* and *Echymiperinae*) was estimated at 4–6 million years. The base of Peroryctinae + Echymiperinae was estimated at 7–13 million years. The base of Peramelinae (*Isoodon* and *Perameles*) was estimated at 4–8 million years.

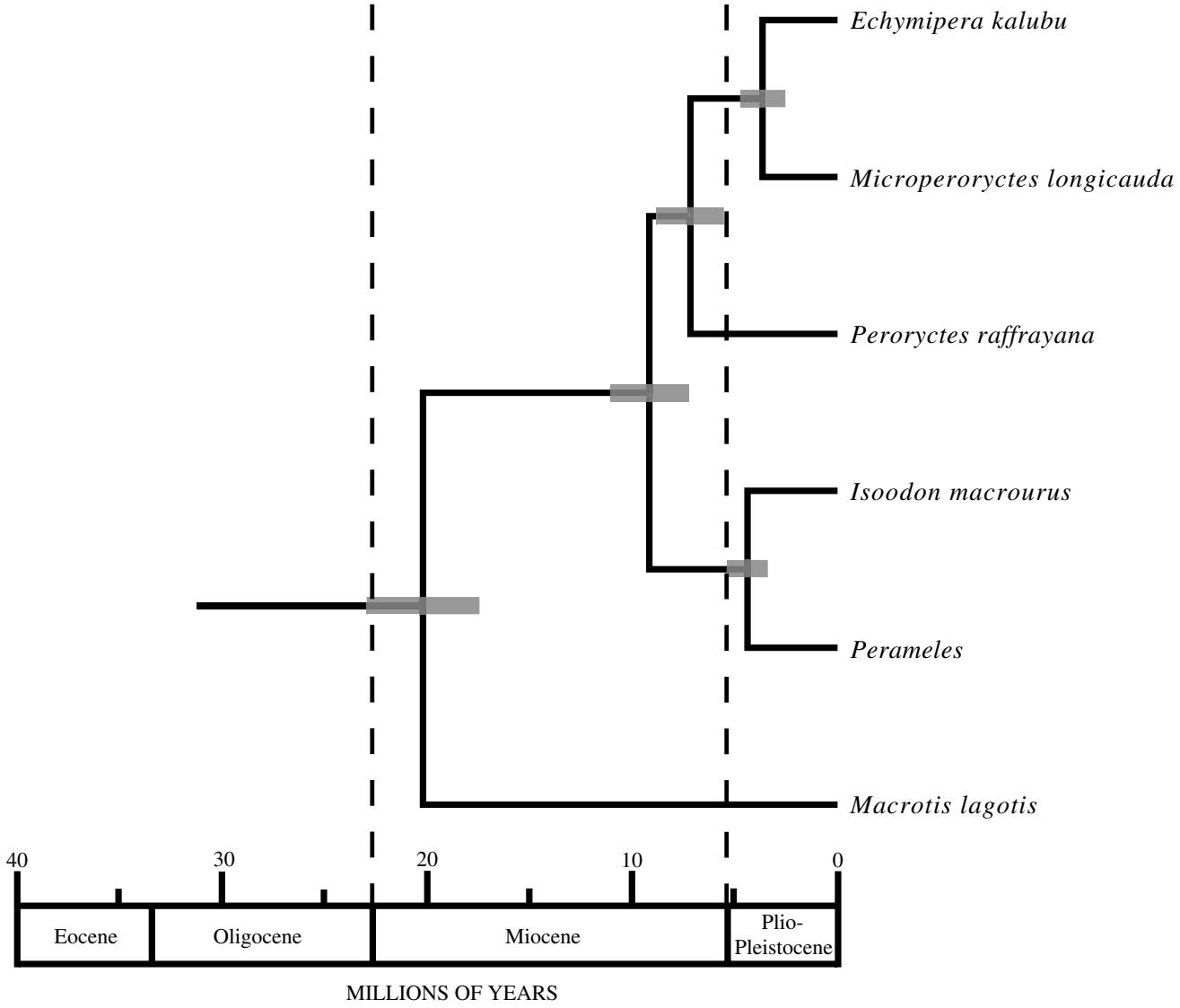

Fig. 5. Molecular time scale in millions of years with associated confidence intervals for Peramelemorphia using the 6 kb concatenation consisting of protein-coding regions of five nuclear genes. Divergence estimates are based on the partitioned *Multidivtime* analysis in which the diprotodontian maximum was set to 65 MYA.

Table 5
Partitioned molecular divergence estimates in millions of years

Node	Divergence estimates					
	Multidivtime		BEAST			
			Hard bounded		Soft bounded	
	Diprotodontia maximum					
	54.6 MYA	65 MYA	54.6 MYA	65 MYA	54.6 MYA	65 MYA
Echymiperinae	3.5 (2.5–4.7)	3.6 (2.6–4.8)	6.1 (2.5–10.6)	6.0 (2.4–10.4)	6.2 (2.7–10.6)	6.3 (2.6–10.5)
Peroryctinae	7.0 (5.5–8.8)	7.10 (5.6–8.9)	12.1 (7.1–18.0)	12.1 (7.0–18.1)	12.7 (7.80–17.7)	12.4 (8.0–17.4)
Peramelinae	4.3 (3.6–5.5)	4.3 (3.6–5.6)	7.2 (3.6–11.5)	7.2 (3.6–11.7)	8.3 (4.1–13.1)	7.9 (3.7–12.589)
Peramelidae	9.0 (7.3–10.9)	9.1(7.3–11.1)	15.0 (10.0–21.0)	15.1 (10.0–21.2)	15.6 (10.6–21.2	15.2 (10.3–20.1)
Base of Peramelemorphia	20.4 (17.3–22.8)	20.7 (17.4–22.9)	29.0 (23.2–33.9)	29.0 (23.1–33.9)	28.6 (21.1–36.1)	28.2 (21.2–36.6)
Peramelemorphia + Notoryctes	54.6 (47.2–60.9)	56.2 (47.7–65.8)	64.6 (47.7–79.9)	64.8 (46.9–80.4)	63.2 (46.4–78.6)	64.0 (46.6–78.1)

Point estimates of divergence times based on non-partitioned data (Supplementary Information) were more similar for BEAST analyses than for *Multidivtime* analyses. Differences between *Multidivtime* dates with partitioned and non-partitioned data ranged from 0.3 to 5 million years. All BEAST dates with non-partitioned data were younger than corresponding dates with partitioned data. Differences were most apparent for the deepest nodes, e.g., the base of Peramelemorphia was dated at 54 million years with non-partitioned data and 65 million years with partitioned data.

Alternate maxima for the base of Diprotodontia (54.6 versus 65.0 million years) had almost no effect on point estimates of divergence times obtained with BEAST (maximum difference = 0.2 million years). *Multidivtime* estimates were more sensitive to the Diprotodontia maximum, especially for the base of Peramelemorphia and the Peramelemorphia + *Notoryctes* clade. The estimated date for the Peramelemorphia + *Notoryctes* clade was approximately six million years older with the 65.0 million years maximum than with the 54.6 million years maximum.

Analyses employing soft bounds were similar to their corresponding hard bounded analyses. When the diprotodontian maximum was set to 54.6 million years, point estimates differed on average by 0.7 (partitioned) and 0.22 (non-partitioned) million years. When the diprotodontian maximum was set to 65.0 million years point estimates differed on average by 0.05 (partitioned) and 0.18 (non-partitioned) million years.

Fig. 6 shows the results of plotting the 95% confidence intervals against the mean divergence times for the hard bounded *Multidivtime* partitioned analyses in which the Diprotodontia maximum was set to 65 million years (plots of other analyses not shown). In all *Multidivtime* analyses, the regression line suggests that the addition of more data will have a minor effect on improving the precision of our estimates. BEAST estimates as compared to *Multidivtime* estimates will require more data to reach linearity in all of our analyses (not shown).

BEAST statistics for all analyses are given in the Supplementary Information. The BEAST non-partitioned analyses recovered 4–7 different tree topologies with the maximum a posteriori (MAP) tree (Fig. 1) accounting for 86–87% of the posterior probability (minus the burnin). There was virtually no indication of autocorrelation of rates as indicated by the covariance statistic. The sum of the total number of different tree topologies recovered for each of the individual genes in the BEAST partitioned analyses ranged from 36 (BRCA1) to 2660 (Rag1) with the MAP tree accounting for anywhere between 5.9% (Rag1) to 41% (vWF) of the posterior probability (Supplementary Information). None of the MAP trees obtained in the partitioned analyses were identical to the tree shown in Fig. 1. A small degree of autocorrelation of rates was observed for BRCA1 and IRBP. Of the five genes analyzed, Rag1 appears to be more clocklike as indicated by the coefficient of variation statistic followed by BRCA1, vWF, IRBP, and then ApoB. These results are consistent with our likelihood ratio tests, which rejected the molecular clock for the concatenation and for all of the individual genes except for Rag 1. However, the apparent clocklike behavior of Rag1 may be a consequence of less statistical power owing to the shorter sequence length of Rag 1 relative to the other gene segments.

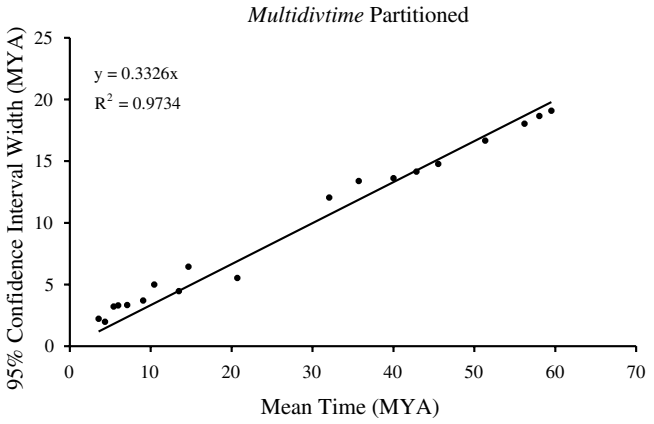

Fig. 6. 95% confidence intervals in millions of years (MYA) verses the mean estimates in million of years (MYA) for the partitioned *Multidivtime* hard bounded analyses in which the diprotodontian maximum was set to 65 million years. Regression line was forced through the origin.

4. Discussion

4.1. Phylogeny

The combined nuclear gene data set gave a well resolved tree in which the bilby, *Macrotis lagotis*, was sister to all other living bandicoots consistent with the morphological studies of Tate (1948) and of prior molecular studies (Kirsch, 1968, 1977; Kirsch et al., 1990, 1997; Baverstock et al., 1990; Close et al., 1990; Westerman et al., 1999, 2001; Westerman and Krajewski, 2000). The remaining genera form two well-resolved clades comprising: 1) *Isoodon* and *Perameles* (Peramelinae) and 2) Echymiperinae (*Echymipera* + *Microperoryctes*) + Peroryctinae (*Peroryctes*).

MP, ML, and Bayesian analyses of the Rag1 data set were generally similar to the 5-gene concatenation, though the nodes were less well resolved. They differed in that Echymiperinae and Peramelinae were paraphyletic (Fig. 4). Analyses with the Rag1 data set demonstrate that *Chaeropus* is a distinct bandicoot lineage and further suggest an association of *Chaeropus* + Peramelidae to the exclusion of *Macrotis*. We recovered a non-conventional grouping of the Vombatiformes and Peramelemorphia using the Rag1 only data set. However, this particular grouping is poorly supported. The genetic distinctness of the pig-footed bandicoot based on Rag1 sequences confirms earlier 12S rRNA gene sequence results (Westerman et al., 1999). However, 12S rRNA gene sequences favor an association of *Macrotis* + Peramelidae to the exclusion of *Chaeropus*. The genetic distinctness of the extinct, herbivorous, pig-footed bandicoot suggested by both nuclear and mitochondrial DNA sequences suggests the need for a reappraisal of character polarity in bandicoots. Since both nuclear and mitochondrial 12S rRNA genes resolve *Macrotis* and *Chaeropus* as divergent from the remaining living bandicoots, then the cylindrical skulls of the Peroryctinae and Echymiperinae must either be the derived condition for 'modern' bandicoots, or the 'excessively flattened peramelid-type of skulls' seen as "unusual in marsupials" (Groves and Flannery, 1990, p. 3), must have evolved three times independently in the Thylacomyidae, Chaeropodidae, and Peramelinae or twice if the Chaeropodidae and Thylacomyidae are sister taxa.

Our studies support Tate's (1948) contention that *Chaeropus*, with its extensive morphological modifications, is quite different from other bandicoots. Although our statistical tests were unable to discriminate between several of the competing hypotheses for the position of *Chaeropus*, the statistical tests suggest *Chaeropus* is sister to the peramelids (Table 4). Groves and Flannery (1990) pointed out that *Chaeropus* and *Macrotis* share a number of derived character states that have often been ignored or overlooked (see Tables 1a and 3a, Groves and Flannery, 1990). Thus, thylacomyids differ from peramelines and peroryctines in that although they show four principal cusps, the metacone has shifted lingually and the two principal external cusps are relatively larger (see Rich, 1991). *Chaeropus* also has lingual metaconids compared to other bandicoots (see Wright et al., 1991) and "the cusps of the buccal tier [metacone and paracone] and the crests associated with these, are major features of the tooth" (p. 232), suggesting that it may be related to thylacomyids. Both bilbies and pig-footed bandicoots are semi-arid adapted animals but both lineages may have originated well before the habitats with which they living genera are associated (see Section 4).

The remaining bandicoot genera fall into one or other of two well resolved lineages, Peramelinae (*Isoodon* and *Perameles*) and Peroryctinae (*Peroryctes*) + Echymiperinae (*Echymipera* and *Microperoryctes*). Within this latter group, the six kb, five nuclear gene data set showed a well-resolved sister relationship between *Peroryctes* and the Echymiperinae, which had not been well delineated by the earlier 12S RNA study. Unfortunately, we were unable to amplify any nuclear gene sequences from the Seram bandicoot, *Rhynchomeles prattorum*, but complete 12S rRNA gene data (Westerman, unpublished) show that this animal is closely related to the genus *Echymipera* as suggested on morphological grounds. The sister grouping of *Peroryctes* with *Echymipera* + *Microperoryctes* (and presumably, *Rhynchomeles*) is also supported by the microcomplement fixation (MC'F) study of Baverstock et al. (1990) and the DNA-DNA hybridization studies of Kirsch et al. (1990, 1997). However, we note that *Peroryctes* is genetically divergent from *Echymipera* + *Microperoryctes*, consistent with the recognition of two subfamilies. Thus, the molecular data all support a primary dichotomy between the four primarily forest dwelling New Guinean genera and the two more open-country-adapted Australian forms.

4.2. Timeline for Peramelemorphia evolution

We have estimated the divergence times of the major bandicoot lineages using relaxed molecular clock methods. Our estimates remain somewhat problematic in that their accuracy depends, in least in part, on fossil calibration dates. For bandicoots, the fossil record is scant before the Pliocene and many of our divergences appear to predate this epoch. Furthermore, the first appearance of a fossil taxon belonging to a given lineage will always underestimate the true time of divergence given accurate taxonomic assignment of the fossil. This is further exacerbated for the Australasian groups due to the paucity of early Tertiary terrestrial vertebrate bearing fossiliferous formations. In addition, these early Tertiary formations all sample similar environments and provide only a meager glimpse into the evolution of the Australasian fauna by sampling only a very restricted area and a short interval of time. We estimate the date of the split between bandicoots and their sister taxon as being at least 54 million years ago (MYA), with crown group bandicoots appearing at least 20 MYA. The earliest probable peramelemorphian remains comprise a tooth from the Eocene Tingamarra

deposits from Murgon in Queensland (Archer, 1982; Archer and Kirsch, 2006), but it has not yet been ascribed to any known family or genus and appears to represent a stem peramelemorphian. If this is indeed a bandicoot, then it is consistent with our estimated dates of divergence.

The oldest formally described bandicoot fossil is *Yarala kidi* from the late Oligocene Kangaroo Well Local Fauna of the Northern Territory (Schwartz, 2006). The early Miocene Systems B and C faunas of Riversleigh have also produced a species of the same genus (*Yarala burchfieldi*; Muirhead, 2000). These fossils, along with other, as yet undescribed material from the Oligo-Miocene deposits of Riversleigh and Central Australia, appear to be unrelated to any modern bandicoot genera and represent an extinct superfamily of 'archaic' bandicoots—Yaraloidea (Case, 2001; Schwartz, 2006; Archer and Hand, 2006). Members of this superfamily are distinguished from all other peramelemorphians by a combination of mostly plesiomorphic features (Muirhead, 2000), though as Turnbull et al. (2003, p. 536) point out, 'the monophyly of taxa currently anticipated to be yaraloids has yet to be demonstrated'. Members of this superfamily are the most plesiomorphic of any known bandicoot taxa, and appear to be at, or near, the base of the Order Peramelemorphia.

Yaralids were relatively diverse in the Australian early-mid Miocene rainforest faunas of Riversleigh but subsequently became less common and are unknown in deposits after the late Miocene. These 'archaic' forms appear to have been replaced by 'modern' bandicoots at some time in the mid-late Miocene as part of a major marsupial faunal turnover coincident with the climatic and floral changes associated with the collision of the Australian and Pacific plates and the uplift of New Guinea in the middle Miocene. For example, although there were numerous thylacinid dasyuromorphs present in early Miocene faunas of Australia (Long et al., 2002), an explosion of diversity occurred in the middle to late Miocene which gave rise to the extant dasyurid subfamilies. A further radiation of dasyurids and other marsupials took place in the Pliocene and Pleistocene.

The earliest fossil remains of described 'modern' bandicoots are known only from deposits post-dating our molecular estimates of origin. The earliest known fossil thylacomyid (*Ischnodon australis*) is from the early-mid Pliocene Palankarinna Fauna (Stirton, 1955); the earliest fossil peroryctine is from the Pliocene Hamilton Local Fauna (4.46 MYA; Turnbull et al., 2003); and the earliest fossil peramelines (*Perameles allinghamensis*, *P. bowensis* and *P. sobbei*) are from the early Pliocene Bluff Downs and Bow local faunas (Muirhead, 2000) or the Pleistocene (Price, 2004). More fossils may yet be found to fill in these gaps, but the dearth of fossil modern bandicoots in Australia is intriguing. However, it should be noted that Case (2001) suggests that within the Etadunna Formation and Wipijiri Formations there is a faunal turnover in which the archaic species from zones A-B (late Oligocene; 25.7–25.0 MYA) are replaced by more modern forms in zones D-F (late Oligocene to late Miocene; 24.7–10.5 MYA) that might be "ancestral to the modern taxa." Our estimated dates of divergence strongly support this hypothesis. Therefore, we suggest that 'modern' bandicoot lineages may, in fact, have been a part of the Australian marsupial fauna for considerably longer than suggested by the named taxa.

If our molecular divergence date estimates are accurate, then the separation of 'modern' bandicoots (thylacomyids, chaeropodids, peramelids) from 'archaic' bandicoot lineages such as yaraloids must have begun in the late Oligocene or early Miocene. In contrast, Muirhead (2000) argued for a post middle Miocene–pre Pliocene bottleneck for bandicoots followed by a loss of 'archaic' forms and a divergence of modern perameloids resulting from the collisions of the Australian and Pacific Plates. This collision, together with the uplifting of New Guinea, the establishment of the Southern Ice Cap and the later major drying associated with full development of this Southern Ice Cap in the terminal Miocene led to profound changes in Australian climates and habitats. Yet our estimate for the divergence of the two major modern bandicoot lineages Thylacomyidae and the ancestors of Peramelidae are more consistent with a late Oligocene origin as hinted by Case (2001).

The first 300,000 years of the Oligocene is characterized by dramatic drop in global temperatures that is concomitant with the glaciation of Antarctica (Coxall et al., 2005). For example, there is aridification in central Asia (Dupont-Nivet et al., 2007) and in North America average temperatures dropped (Zanazzi et al., 2007). By the start of the Oligocene, Australia was already an island continent moving northwards following its mid-late Eocene separation from Antarctica. During the middle Oligocene the northern edge of the Australian Plate had already begun to collide with the Pacific Plate in the region of New Guinea. Mountain building in this region accelerated and by the late Oligocene the Sepik Terrane had docked (Head, 2002). All this led to drainage pattern changes in Central Australia and the introduction of climates with at least seasonal aridity. The Oligocene climates of Australia were cooler and drier than had previously been experienced and there were vegetation changes as drier forest types replaced more complex rainforests in many areas (White, 1994; Megirian et al., 2004). Such changes would be a major impetus for faunal changes including the primary divergence of peramelemorphs.

Clearly the presence of albeit undescribed peramelemorphian fossil in the early Eocene deposits at Tingamarra (Archer, 1982), as well as others, suggests a very early radiation of peramelemorphs in Australia, which is not in conflict with either the old date suggested by Woodburne and Case (1996) or our molecular estimate for the split with other australidelphians (54–65 MYA). What is surprising is the lack of fossils earlier than the Pliocene identifiable as thylacomyids, chaeropodids, and peramelids, especially of the former group, which, if the molecular dates are correct, must have

had an origin in the Oligocene. Yet neither *Yarala kida* nor any of the other, undescribed, Oligo-Miocene taxa can be placed within a clade comprising modern bandicoot genera (Muirhead, 1994; Case, 2001). The earliest recognized/described thylacomyid fossil (*Ischnodon australis*) dates only to the early-mid Pliocene Palankarinna Fauna of South Australia (Stirton, 1955) and the earliest *Chaeropus* fossils are all late Pleistocene (see Muirhead and Godthelp, 1995 for references).

The late Oligocene divergence of *Macrotis*, and presumably *Chaeropus*, from other bandicoots, considerably predates the suggested origin (in the late-Pleistocene) of the semi-arid environments and habitats of Australia with which these two genera were associated and to which they are adapted. This may suggest the Oligo-Miocene Etadunna bandicoots are thylacomyids/chaeropodids, and/or the identification of some of the "stem" peramelemorphians are incorrect and they are actually crown peramelemorphs. These questions will only be answered with discovery of more complete specimens and/or new early Tertiary deposits.

The nuclear DNA sequences also suggest a relatively early divergence—in the medial Miocene—of the three subfamilies Peroryctinae, Echymiperinae, and Peramelinae as well as for subsequent radiations within them in the late Miocene and early Pliocene. These dates are consistent with an effect on bandicoots of the onset of further cooling and drying (Miocene Oscillation), which led to the break-up of closed rainforest habitats across inland Northern Territory, South Australia and NW Queensland (Megirian et al., 2004). By the late Miocene, seasonal dryness and winter rainfall patterns were replacing predominantly summer rainfall.

Peroryctines and echymiperines seem to have radiated following their vicariant dispersal into the rainforests of the emergent New Guinea in the middle Miocene. Both groups seem to have subsequently remained associated with rainforest habitats even following the very recent back migration of a single species (*Echymipera rufescens*) into northern Australia just prior to the severing of the latest Ice-Age land bridge by rising seawaters. The mid-Miocene was a time of complex events associated with plate collisions including the docking of the East Papuan and other terranes to the emerging New Guinea about 15 MYA, followed by further mountain building in the Central Cordillera, docking of the Irian Jaya and central Island Arc terranes and the Sunda Arc formation about 10 MYA (White, 1994; Head, 2002).

Initial radiation within Echymiperinae into its component genera seems to have occurred in the late Miocene. Prolonged major cooling occurred in the late Pliocene (3–2 Mya), coincident with the beginning of further rapid uplifts of the central cordilleras of New Guinea. Subsequent radiations in *Echymipera* and *Microperoryctes* seem also to have been mid-late Pliocene events (see Westerman et al., 2001). This was also the time of subsequent radiation within Peroryctinae and Echymiperinae, which gave rise to the giant and Raffrey's bandicoots (Westerman, in preparation).

Our results suggest that the peramelemorph radiation in Australia occurred during the mid to late Miocene as bandicoots, and other taxa, adapted to the more mesic and xeric habitats that replaced inland forests. These more open habitats were associated with altered rainfall patterns and vegetation of Australia caused by the uplift of the New Guinean Central Cordillera (see above). Subsequent speciation within the genera *Isoodon* and *Perameles* took place in the Pliocene (see Westerman and Krajewski, 2000).

Acknowledgments

We thank two anonymous reviewers for comments on an earlier version of this manuscript. This work was partially supported by NSF (to M.S.S.).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2008.01.002.

References

Amrine-Madsen, H., Scally, M., Westerman, M., Stanhope, M.J., Krajewski, C., Springer, M.S., 2003. Nuclear gene sequences provide evidence for the monophyly of australidelphian marsupials. Mol. Phylogenet. Evol. 28, 186–196.

Archer, M., 1982. Review of the dasyurid (Marsupialia) fossil record, integration of data bearing on phylogenetic interpretation, and suprageneric classification. In: Archer, M. (Ed.), Carnivorous Marsupials. Surrey Beatty & Sons, Chipping Norton, New South Wales, pp. 397–443.

Archer, M., Kirsch, J.A.W., 1977. The case for Thylacomyidae and Myrmecobiidae, Gill, 1872, or why are marsupial families so extended? Proc. Linn. Soc. New South Wales. 102, 18–25.

Archer, M., Kirsch, J.A.W., 2006. The evolution and classification of marsupials. In: Armati, P.J., Dickman, C.R., Hume, I.D. (Eds.), Marsupials. Cambridge Univ. Press, New York, pp. 1–21.

Archer, M., Wade, M., 1976. Results of the Ray E. Lemley Expeditions Part 1 the Allingham Formation and a New Pliocene Vertebrate Fauna from Northern Queensland Australia. Mem. Qld. Mus. 17, 379–398.

Archer, M., Hand, S.J., Godthelp, H., 2001. Australia's Lost World: Prehistoric Animals of Riversleigh. Indiana Univ. Press, IN.

Archer, M., Hand, S.J., 2006. The Australian marsupial radiation. In: Merrick, J.R., Archer, M., Hickey, G.M., Lee, M.S.Y. (Eds.), Evolution and Biogeography of Australasian Vertebrates. Auscipub, New South Wales, pp. 575–646.

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

Baker, M.L., Wares, J.P., Harrison, G.A., Miller, R.D., 2004. Relationships among the families and orders of marsupials and the major mammalian lineages based on recombination activating gene-1. J. Mammal. Evol. 11, 1–16.

Barker, F.K., Lutzoni, F.M., 2002. The utility of the incongruence length difference test. Syst. Biol. 51, 625–637.

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

Baverstock, K.P.R., Flannery, T., Aplin, K., Birrell, J., Krieg, M., 1990. Albumin immunologic relationships of the bandicoots (Perameloidea Marsupialia) a preliminary report. In: Seebeck, J.H., Brown, P.R., Wallis, R.L., Kemper, C.M. (Eds.), Bandicoots and Bilbies. Surrey Beatty & Sons, Chipping Norton, New South Wales, pp. 13–18.

Bensley, B.A., 1903. On the evolution of the Australian Marsupialia: with remarks on the relationships of marsupials in general. Trans. Linn. Soc. Lond. 9, 83–217.

Benton, M.J., Ayala, F.J., 2003. Dating the tree of life. Science 300, 1698–1700.

Benton, M.J., Donoghue, P.C.J., 2007. Paleontological evidence to date the tree of life. Mol. Biol. Evol. 24, 26–53.

Brammall, J.R., 1998. A new petauroid possum from the Oligo-Miocene of Riversleigh, northwestern Queensland. Alcheringa 23, 31–50.

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

Case, J.A., 2001. Turnover of bandicoots in the Oligo-Miocene of South Australia. J. Vertebr. Paleontol. Supp. 21, 39.

Close, R.L., Murray, J.D., Briscoe, D.A., 1990. Electrophoretic and chromosome surveys of the taxa of short-nosed bandicoots within the genus *Isoodon*. In: Seebeck, J.H., Brown, P.R., Wallis, R.L., Kemper, C.M. (Eds.), Bandicoots and Bilbies. Surrey Beatty & Sons, Chipping Norton, New South Wales, pp. 19–27.

Cooke, B.N., 2006. Kangaroos. In: Merrick, J.R., Archer, M., Hickey, G.M., Lee, M.S.Y. (Eds.), Evolution and Biogeography of Australasian Vertebrates. Auscipub, New South Wales, pp. 647–672.

Cooke, B., 1999. *Wanburoo hilarus* gen. et sp. nov., a lophodont bulungamayine kangaroo (Marsupialia: Macropodoidea: Bulungamayinae) from the Miocene deposits of Riversleigh, northwestern Queensland. Rec. W. Aus. Mus. Supp. No 57, 239–253.

Coxall, H.K., Wilson, P.A., Pälike, H., Lear, C.H., 2005. Rapid stepwise onset of Antarctic glaciation and deeper calcite compensation in the Pacific Ocean. Nature 433, 553–557.

Crosby, K., Bassarova, M., Archer, M., Carbery, K., 2004. Fossil possums in Australasia: discovery, diversity and evolution. In: Goldingay, R.L., Jackson, S.M. (Eds.), The Biology of Australian Possums and Gliders. Surrey Beatty & Sons, Chipping Norton, New South Wales, pp. 161–176.

Cunningham, C.W., 1997. Is congruence between data partitions a reliable predictor of phylogenetic accuracy? Empirically testing an iterative procedure for choosing among phylogenetic methods. Syst. Biol. 46, 464–478.

Darlu, P., Lecointre, G., 2002. When does the incongruence length difference test fail? Mol. Biol. Evol. 19, 432–437.

de Jong, W.W., van Dijk, M.A.M., Poux, C., Kappe, G., van Rheede, T., Madsen, O., 2003. Indels in protein-coding sequences of Euarchontoglires constrain the rooting of the eutherian tree. Mol. Phylogenet. Evol. 28, 328–340.

De Queiroz, A., 1993. For consensus sometimes. Syst. Biol. 42, 368–372.

Drummond, A.J., Rambaut, A., 2003. BEAST version 1.4.3 [computer program]. Available: http://evolve.zoo.ox.ac.uk/beast. Accessed August 2007.

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

Dupont-Nivet, G., Krijgsman, W., Langereis, C.G., Abels, H.A., Dai, S., Fang, X., 2007. Tibetan plateau aridification linked to global cooling at the Eocene–Oligocene transition. Nature 445, 635–638.

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

Flannery, T.F., 1989. Phylogeny of the Macropodoidea; a case study in convergence. In: Grigg, G.C., Jarman, P.J., Hume, I.D. (Eds.), Kangaroos, Wallabies and Rat-kangaroos. Surrey Beatty & Sons, Chipping Norton, New South Wales, pp. 1–46.

Godthelp, H., Wroe, S., Archer, M., 1999. A new marsupial from the early Eocene Tingamarra Local Fauna of Murgon, southeastern Queensland: a prototypical Australian marsupial? J. Mammal. Evol. 6, 289–313.

Gradstein, F.M., Ogg, J.G., Smith, A.G., 39 co-authors, 2004. A geologic timescale. Cambridge Univ. Press, Cambridge.

Groves, C.P., Flannery, T.F., 1990. Revision of the families and genera of bandicoots. In: Seebeck, J.H., Brown, P.R., Wallis, R.L., Kemper, C.M. (Eds.), Bandicoots and Bilbies. Surrey Beatty & Sons, Chipping Norton, New South Wales, pp. 1–11.

Guindon, S., Gascuel, O., 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. 52, 696–704.

Head, M., 2002. Birds of Paradise, vicariance biogeography and terrane tectonics in New Guinea. J. Biogeog. 29, 261–283.

Hedges, S.B., Kumar, S., 2004. Precision of molecular time estimates. Trends Gen. 20, 242–247.

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

Kirsch, J.A.W., 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. Aust. J. Zool. Supp. Ser., 1–152.

Kirsch, J.A.W., Lapointe, F.-J., Springer, M.S., 1997. DNA-hybridisation studies of marsupials and their implications for metatherian classification. Aust. J. Zool. 45, 211–280.

Kirsch, J.A.W., Springer, M.S., Krajewski, C., Archer, M., Aplin, K., Dickerman, A.W., 1990. DNA-DNA hybridization studies of the carnivorous marsupials I. The intergeneric relationships of bandicoots (Marsupialia Perameloidea). J. Mol. Evol. 30, 434–448.

Kishino, H., Hasegawa, M., 1989. Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data and the branching order in Hominoidea. J. Mol. Evol. 29, 170–179.

Kishino, H., Thorne, J.L., Bruno, W.J., 2001. Performance of a divergence time estimation method under a probabilistic model of rate evolution. Mol. Biol. Evol. 18, 352–361.

Langford, R.P., Wilford, G.E., Truswell, E.M., Isern, A.R., 1995. Palaeogeographic Atlas of Australia. Volume 10. Cainozoic. Australian Geological Survey Organization, Canberra.

Long, J., Archer, M., Flannery, T.F., Hand, S.J., 2002. Prehistoric Mammals of Australia and New Guinea: One Hundred Million Years of Evolution. Univ. of New South Wales Press, Sydney.

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

Mackness, B.S., Whitehead, P.W., McNamara, G.C., 2000. New potassium-argon basalt date in relation to the Pliocene Bluff Downs Local Fauna, northern Australia. Aust. J. Earth Sci. 47, 807–811.

Madsen, O., Scally, M., Douady, C.J., Kao, D.J., DeBry, R.W., Adkins, R., Amrine, H.M., Stanhope, M.J., de Jong, W.W., Springer, M.S., 2001. Parallel adaptive radiations in two major clades of placental mammals. Nature 409, 610–614.

Marshall, L.G., De Muizon, C., 1988. The dawn of the age of mammals in South America. Nat. Geograph. Res. 4, 23–55.

Marshall, L.G., Sempere, T., Butler, R.F., 1997. Chronostratigraphy of the mammal-bearing Paleocene of South America. J. South Am. Earth Sci. 10, 49–70.

Megirian, D., Murray, P., Schwartz, L., Von Der Borch, C., 2004. Late Oligocene Kangaroo Well Local Fauna from Ulta Limestone (new name), and climate of Miocene oscillation across central Australia. Aust. J. Earth Sci. 51, 701–741.

Meredith, R.W., Westerman, M., Case, J.A., Springer, M.S., 2007. A phylogeny and timescale for marsupial evolution based on sequences for five nuclear genes. J. Mammal. Evol. doi:10.1007/s10914-007-9062-6.

Muirhead, J., 1994. Systematics, evolution and palaeobiology of recent and fossil bandicoots Peramelemorphia, Marsupialia. Unpublished PhD Thesis, University of New South Wales, Sydney.

Muirhead, J., 2000. Yaraloidea (Marsupialia, Peramelemorphia), a new superfamily of marsupial and a description and analysis of the cranium of the Miocene *Yarala burchfieldi*. J. Paleontol. 74, 512–523.

Muirhead, J., Godthelp, H., 1995. Fossil bandicoots from Chillagoe Northeastern Queensland and the first known specimen of the pig-footed bandicoot *Chaeropus* Olgilby 1838, from Queensland. Aust. Mammal. 19, 73–76.

Muirhead, J., Dawson, L., Archer, M., 1997. *Perameles bowensis*, a new species of *Perameles* (Peramelemorphia, Marsupialia) from Pliocene faunas of bow and Wellington Caves, New South Wales. Proc. Linn. Soc. New South Wales 117, 163–173.

Müller, J., Reisz, R.R., 2005. Four well-constrained calibration points from the vertebrate fossil record for molecular clock estimates. BioEssays 27, 1069–1075.

Murray, P.F., Megirian, D., 1992. Continuity and contrast in middle and late Miocene vertebrate communities from the Northern Territory. The Beagle 9, 195–218.

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

Nilsson, M.A., Arnason, U., Spenser, P.B.S., Janke, A., 2004. Marsupial relationships and a timeline for marsupial radiation in South Gondwana. Gene 340, 189–196.

Nowak, R.M., Dickman, C.R., 2005. Walker's Marsupials of the World. The Johns Hopkins Univ. Press, Maryland.

Phillips, M.J., Pratt, R.C., 2008. Family-level relationships among the Australasian marsupial "herbivores" (Diprotodontia: koala, wombats, kangaroos and possums). Mol. Phylogenet. Evol 46, 594–605.

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

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

Poux, C., van Rheede, T., Madsen, O., de Jong, W.W., 2002. Sequence gaps join mice and men: phylogenetic evidence from deletions in two proteins. Mol. Phylogenet. Evol. 19, 2035–2037.

Price, G.J., 2004. Fossil bandicoots (Marsupialia, Peramelidae) and environmental change during the Pleistocene on the Darling Downs, southwestern Queensland, Australia. J. Syst. Paleontol. 2, 347–356.

Prideaux, G., 2004. Systematics and evolution of the sthenurine kangaroos. Univ. Calif. Pub. Geol. Sci., 146.

Rambaut, A. 1996. Se-Al: Sequence Alignment Editor. Available at http://evolve.zoo.ox.ac.uk/.

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

Reisz, R.R., Müller, J., 2004. Molecular timescales and the fossil record: a paleontological perspective. Trends Gen. 20, 237–241.

Rich, T.H., 1991. Monotremes, placentals and marsupials: their record in Australia and its biases. In: Vickers-Rich, P., Monaghan, J.M., Baird, R.F., Rich, T.H. (Eds.), Vertebrate Paleontology of Australasia. Pioneer design Studios and Monash University Publications Committee, Melbourne, pp. 894–1070.

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

Sanderson, M.J., 2002. Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. Mol. Biol. Evol. 19, 101–109.

Schwartz, L., 2006. A new species of bandicoot from the Oligocene of Northern Australia and implications of bandicoots for correlating Australian Tertiary mammal faunas. Palaeontology 49, 991–998.

Shimodaira, H., 2002. An approximately unbiased test of phylogenetic tree selection. Syst. Biol. 51, 492–508.

Shimodaira, H., Hasegawa, M., 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. Mol. Biol. Evol. 16, 1114–1116.

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

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

Stirton, R.A., 1955. Late tertiary marsupials from South Australia. Rec. S. Aust. Mus. 11, 247–268.

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

Swofford, D.L., Olsen, G.J., Waddell, P.J., Hillis, D.M., 1996. Phylogenetic inference. In: Hillis, D.M., Moritz, C., Mable, B.K. (Eds.), Molecular Systematics, Second ed. Sinauer Associates, Inc MA, pp. 407–514.

Tate, G.H.H., 1948. Results of the Archibald Expeditions. No. 60. Studies in the Peramelidae (Marsupialia). Bull. Am. Mus. Nat. Hist. 92, 317–346.

Tedford, R.H., Kemp, N.R., 1998. Oligocene marsupials of the Geilston Bay Local Fauna, Tasmania. Am. Mus. Nov. 3244, 1–22.

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

Teeling, E.C., Madsen, O., Van Den Bussche, R.A., de Jong, W.W., Stanhope, M.J., Springer, M.S., 2002. Microbat paraphyly and the convergent evolution of a key innovation in Old World rhinolophoid microbats. Proc. Nat. Acad. Sci. U.S.A. 99, 1431–1436.

Thomas, J.W., Touchman, J.W., Blakesley, R.W., 68 co-authors, 2003. Comparative analyses of multi-species sequences from targeted genomic regions. Nature 424, 788–793.

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

Thorne, J.L., Kishino, H., Painter, I.S., 1998. Estimating the rate of evolution of the rate of molecular evolution. Mol. Biol. Evol. 15, 1647–1657.

Turnbull, W.D., Lundelius Jr., E.L., Archer, M., 2003. Dasyurids, perameloids, phalangeroids, and vombatoids from the early Pliocene Hamilton fauna, Victoria, Australia. Bull. Am. Mus. Nat. Hist. 279, 513–540.

van Dijk, M.A.M., Paradis, E., Catzeflis, F., de Jong, W.W., 1999. The virtues of gaps: Xenarthran (Edentate) monophyly supported by a unique deletion in alpha A-crystallin. Syst. Biol. 48, 94–106.

Waddell, P.J., Shelley, S., 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. Mol. Phylogenet. Evol. 28, 197–224.

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

Westerman, M., Krajewski, C., 2000. Molecular relationships of the Australian bandicoot genera *Isoodon* and *Perameles* Marsupialia: Peramelina. Aust. Mammal. 22, 1–8.

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

White, M.E., 1994. After the Greening: the Browning of Australia. Kangaroo Press, New South Wales.

Wilson, D.E., Reeder, D.M., 2005. Mammal Species of the World. Johns Hopkins Univ. Press, MD.

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

Woodburne, M.O., Tedford, R.H., Archer, M., Turnbull, W.D., Plane, M.D., Lundelius Jr., E.L., 1985. Biochronology of the continental mammal record of Australia and New Guinea. Spec. Publ. S. Aust. Dept. Mines Energy 5, 347–364.

Woodburne, M.O., MacFadden, B.J., Case, J.A., Springer, M.S., Pledge, N.S., Power, J.D., Woodburne, J.M., Springer, K.B., 1993. Land

mammal biostratigraphy and magnetostratigraphy of the Etadunna Formation (late Oligocene) of South Australia. J. Vertebr. Paleontol. 13, 483–515.

Wright, W., Sanson, C., McArthur, G.D., 1991. The diet of the extinct bandicoot *Chaeropus ecaudatus*. In: Vickers-Rich, P., Monaghan, J.M., Baird, R.F., Rich, T.H. (Eds.), Vertebrate Paleontology of Australasia. Pioneer design Studios and Monash University Publications Committee, Melbourne, pp. 229–247.

Wroe, S., 1998. The geologically oldest dasyurid, from the Miocene of Riversleigh, north-west Queensland. Palaeontology 42, 501–527.

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

Yang, Z., Rannala, B., 2006. Bayesian estimation of species divergence times under a molecular clock using multiple calibrations with soft bounds. Mol. Biol. Evol. 23, 212–226.

Zanazzi, A., Kohn, M.J., MacFadden, B.J., Terry Jr., D.O., 2007. Large temperature drop across the Eocene–Oligocene transition in central North America. Nature 445, 639–642.