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A DATED PHYLOGENY OF MARSUPIALS USING A MOLECULAR SUPERMATRIX AND MULTIPLE FOSSIL CONSTRAINTS

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Phylogenetic relationships within marsupials were investigated based on a 20.1-kilobase molecular supermatrix comprising 7 nuclear and 15 mitochondrial genes analyzed using both maximum likelihood and Bayesian approaches and 3 different partitioning strategies. The study revealed that base composition bias in the 3rd codon positions of mitochondrial genes misled even the partitioned maximum-likelihood analyses, whereas Bayesian analyses were less affected. After correcting for base composition bias, monophyly of the currently recognized marsupial orders, of Australidelphia, and of a clade comprising Dasyuromorphia, *Notoryctes*, and Peramelemorphia, were supported strongly by both Bayesian posterior probabilities and maximum-likelihood bootstrap values. Monophyly of the Australasian marsupials, of *Notoryctes* + Dasyuromorphia, and of *Caenolestes* + Australidelphia were less well supported. Within Diprotodontia, Burramyidae + Phalangeridae received relatively strong support. Divergence dates calculated using a Bayesian relaxed molecular clock and multiple age constraints suggested at least 3 independent dispersals of marsupials from North to South America during the Late Cretaceous or early Paleocene. Within the Australasian clade, the macropodine radiation, the divergence of phascogaline and dasyurine dasyurids, and the divergence of perameline and peroryctine peramelemorphians all coincided with periods of significant environmental change during the Miocene. An analysis of ''unrepresented basal branch lengths'' suggests that the fossil record is particularly poor for didelphids and most groups within the Australasian radiation.

Key words: Ameridelphia, Australidelphia, Bayesian analysis, fossil record, marsupials, phylogenetic fuse, phylogeny, relaxed molecular clock, supermatrix

Molecular analyses of the higher-level phylogeny of marsupials have a long history (Nuttall 1904; Weymss 1953) and have been more common than equivalent morphological studies. Several of these molecular analyses have added considerably to our current understanding of marsupial phylogeny and classification (e.g., Kirsch 1968, 1977; Sarich et al. 1982). However, even with recent advances in sequencing and phylogenetic methods, different molecular data sets continue to support incongruent topologies (e.g., Amrine-Madsen et al. 2003; Asher et al. 2004; Baker et al. 2004; Nilsson et al. 2004); hence, the higher-level phylogeny of marsupials remains uncertain.

The affinities of several marsupial groups have proven particularly difficult to resolve. It is unclear whether the South American didelphid opossums (Didelphimorphia) and caenolestid shrew opossums (Paucituberculata) comprise a monophyletic group (Ameridelphia) or are a paraphyletic assemblage at the base of modern marsupials. Current evidence supports the monophyly of Australidelphia (Amrine-Madsen et al. 2003; Cardillo et al. 2004; Horovitz and Sanchez-Villagra 2003; Szalay 1982, 1994), a clade comprising the modern Australasian marsupial orders Peramelemorphia, Notoryctemorphia, Dasyuromorphia, and Diprotodontia and the South American monito del monte *Dromiciops* (Microbiotheria). The position of *Dromiciops* within Australidelphia remains unresolved and yet is crucial to our understanding of marsupial biogeography: if *Dromiciops* is the sister group to all Australasian marsupials (Amrine-Madsen et al. 2003; Phillips et al. 2006), then only a single dispersal of marsupials from South America to Australasia is implied; if *Dromiciops* is nested within the Australasian radiation (Asher et al. 2004; Cardillo et al. 2004; Nilsson et al. 2004), there must have been multiple dispersals of marsupials to Australasia or back-migration of microbiotheres from Australasia to South America. Within Australasian marsupials, the affinities of the highly autapomorphic, fossorial marsupial mole *Notoryctes*

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(Notoryctemorphia) are uncertain, as are those of the bandicoots (Peramelemorphia), with some studies (e.g., Kirsch et al. 1997) suggesting that the latter may not be members of Australidelphia.

Together with a robust phylogeny, divergence dates are essential for understanding the patterns, processes, and tempo of marsupial evolution and biogeography. For example, they enable assessment of whether divergences coincided with periods of environmental change, such as climatic variations (e.g., Delsuc et al. 2004; Douady and Douzery 2003) and fluctuations in sea level (Johnson et al. 2006; Mercer and Roth 2003). However, robust dates for divergences within Marsupialia have been unavailable because of incompleteness of the fossil record, notably a lack of sites from the Late Cretaceous and early Tertiary when most of the deep divergences are thought to have occurred.

Late Cretaceous marsupials from North America were relatively diverse and include taxa that have been tentatively assigned to extant lineages by some authors (Case et al. 2005; Kielan-Jaworowska et al. 2004), but they are known only from fragmentary material (largely isolated teeth) and their relationships to the marsupial crown-group remain controversial. In South America, there are no Late Cretaceous fossil sites containing marsupials; the oldest known South American marsupial is a single tooth from the earliest Paleocene (Goin et al. 2006). The Tiupampa fauna in Bolivia (Marshall and Muizon 1988; Muizon 1991) contains exceptionally well-preserved, articulated skeletons of several plesiomorphic marsupials, as well as more fragmentary remains of several other marsupial taxa. However, the Tiupampa fauna may be somewhat younger than usually assumed (Benton and Donoghue 2007; Gradstein et al. 2004), being middle (59.2–60.4 million years ago [mya]) rather than early (63–64.5 mya) Paleocene. In Australia, there is only a single mammalian fauna from the early Tertiary (the early Eocene [54.6 mya] Tingamarra fauna—Godthelp et al. 1992), and marsupial fossils from this site are highly fragmentary. The next oldest Australian sites contain diverse and often well-preserved marsupial fossils, but are some 30 million years younger (late Oligocene—Archer et al. 1999; Long et al. 2002). Attempts to determine divergence dates from fossil evidence also may be confounded by the existence of ''phylogenetic fuses'' (Cooper and Fortey 1998): morphological apomorphies that distinguish 2 lineages may not have evolved until a considerable time after those lineages diverged.

These factors suggest that molecular methods, which are not strictly tied to the known fossil record (although they do usually require fossils to act as calibration points), seem more likely to provide accurate divergence dates. However, previous molecular dates for marsupials have been based on dubious assumptions, such as rate homogeneity or the use of a single (often questionable) fossil calibration. Indeed, in a number of instances, molecular dates for particular marsupial clades have been considerably younger than their oldest fossil members (e.g., Nilsson et al. 2004; Sarich et al. 1982), assuming, of course, that these fossils have been correctly identified.

In an attempt to resolve these problems, I have combined molecular data from 3 recent phylogenetic analyses of marsupials (Amrine-Madsen et al. 2003; Asher et al. 2004; Nilsson et al. 2004) to produce a supermatrix of 7 nuclear and 15 mitochondrial genes. This is, to my knowledge, the largest molecular data set used so far to investigate higher-level marsupial relationships, and it is the 1st to use a Bayesian relaxed molecular clock approach to calculate divergence dates between the major groups of marsupials.

Materials and Methods

Initial data set.—Sequence data were taken from the following studies: Amrine-Madsen et al. (2003; matrix supplied by M. Springer), who sampled the nuclear genes apolipoprotein B (*APOB*), breast cancer 1 (*BRCA1*), interphotoreceptor retinoid binding-protein (*IRBP*), recombination activating gene 1 (*RAG1*), and Von Willebrand factor (*VWF*); Asher et al. (2004; matrix downloaded from doi:10.1016/j.ympev.2004.05.004), who sampled the nuclear genes *IRBP*, phosphoglycerate kinase 1 (*PGK1*), and protamine 1 (*P1*) and the mitochondrial genes cytochrome *b* (*CYTB*), 12S rRNA, 16S rRNA, tRNA valine, and reduced nicotinamide adenine dinucleotide dehydrogenase 2 (*NADH2*); and Nilsson et al. (2004; matrix supplied by M. Nilsson), who sampled the 12 mitochondrial H-strand protein-coding genes. The original alignments of all 3 matrices were maintained; for the data set of Asher et al. (2004), the original authors' static alignment produced by the program CLUSTAL was preferred over any of their direct-optimization alignments produced by the program POY (De Laet and Wheeler 2003). Portions of the matrix of Asher et al. (2004) that overlapped with those of the other 2 studies (i.e., *IRBP, CYTB*, and *NADH2*) were deleted, because taxon sampling was sparser in the former study. In total, sequences from 7 nuclear genes (*APOB, BRCA1, IRBP, PGK1, P1, RAG1*, and *VWF*) and 15 mitochondrial loci (12S rRNA, 16S rRNA, tRNA valine, and the 12 H-strand protein-coding genes) were included in this study (Appendix I). The ''Macropodinae'' terminal of Amrine-Madsen et al. (2003) was decomposed into separate *Macropus* and *Dendrolagus* sequences and combined with sequences for these 2 genera from the other 2 studies. Sequences of 3 genes, *P1, CYTB*, and 12S rRNA, for the thylacine *Thylacinus* were downloaded from GenBank and added manually to the alignment. In total, 22 marsupial ingroup taxa were included, with 2 monotreme (*Ornithorhynchus* and *Tachyglossus*) and 2 placental (Eulipotyphla and Xenarthra) outgroups. Five of the marsupial taxa comprised sequences from more than 1 genus: Didelphini (*Didelphis* and *Lutreolina*), Peramelinae (*Perameles* and *Isoodon*), Petauridae (*Petaurus* and *Dactylopsila*), Phascogalinae (*Phascogale* and *Antechinus*), and Pseudocheiridae (*Pseudocheirus* and *Pseudochirops*).

Choice of taxa for this study essentially replicates that of Asher et al. (2004). Some taxa suffer from relatively large amounts of missing data (notably *Dasyuroides* and *Thylacinus*, which are represented by only 2 and 3 genes, respectively), but all share at least 1 nuclear (*P1*) and 1 mitochondrial (12S rRNA) gene in common, thus avoiding the problem of non-overlapping sequences identified by Springer et al. (2004). The supermatrix also can be easily combined with the morphological data set of Horovitz and Sanchez-Villagra (2003) for total evidence analyses. Because recent work by Phillips et al.

(2006) suggests that base composition bias might not be accounted for by current likelihood models, 2 versions of the supermatrix were analyzed, 1 including 3rd codon positions of mitochondrial protein-coding genes (''full''; 20,121 base pairs [bp]) and 1 that excludes them (''no mt3''; 16,662 bp).

Data partitioning.—Three different partitioning strategies were applied to both the full and no mt3 data sets: a single partition for the entire supermatrix (''unpartitioned''); each gene assigned its own partition (''gene-partitioned''); and each codon position within each protein-coding gene and stems and loops within each ribosomal gene assigned their own partitions (''genes, codons, stems, loops [GCSL]-partitioned''). In the GCSL-partitioned analyses, the 250-bp noncoding region of *P1* identified by Queralt et al. (1995; see also Asher et al. 2004) also was assigned its own partition, and stem and loop regions of the ribosomal genes were defined using secondary structure masks from the Organellar Genome Retrieval (OGRe) database (Jameson et al. 2003). The 12 mitochondrial protein-coding genes were treated as a single supergene (which was further partitioned by codon position in the GCSL-partitioned analyses), rather than individual genes to avoid a very large number of partitions. Too many partitions risk overparameterization and greatly increase computation time and difficulty in reaching convergence in Bayesian analyses. Furthermore, base composition does not vary significantly among the 12 mitochondrial protein-coding genes (Nilsson 2006), so it seems reasonable to treat them as a single supergene.

Phylogenetic analysis.—Maximum-likelihood analyses were carried out using RAxML VI (Stamatakis 2006), which can implement partitioned analysis by applying 1 of 2 models to each partition: GTRGAMMA (general time reversible model with rate heterogeneity accommodated by a gamma distribution) or GTRCAT (as for GTRGAMMA, but rate heterogeneity is accommodated by a number of discrete rate categories). To maximize computational efficiency, the GTRMIX option of RAxML was used; this assumes the GTRCAT model (which is faster and less memory intensive than GTRGAMMA) when searching for tree topologies, but assumes the GTRGAMMA model when calculating the likelihood score of each topology. The RAxML analyses each comprised 100 tree search replicates (assuming default parameters) and were implemented using the perl script batchRAxML.

Bayesian analyses used MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003), which, unlike RAxML, allows specification of 1 of a wide range of models for each partition. Models for the Bayesian analyses were identified using the model selection program MrModeltest 2.2 (Nylander 2004), and models preferred by the Akaike information criterion were implemented (following Posada and Buckley [2004]; a list of models used for each partition is available from the author). For the MrBayes analyses, all parameters except topology were unlinked across partitions, and 2 independent runs (each comprising 1 ''cold'' and 3 ''heated'' chains) were run simultaneously, with trees sampled every 100th generation. The MrBayes analyses were run for either 1×10^6 (unpartitioned), 2.5×10^6 (gene-partitioned), or 12×10^6 (GCSL-partitioned) generations for both the full and no mt3 data sets. In all cases, stationarity had been reached by the end of the analysis (average standard deviation of split frequencies <0.01). Majority-rule consensus trees were constructed, with a burn-in period of either 1×10^5 (unpartitioned), 2.5×10^5 (gene-partitioned), or 10×10^6 (GCSL-partitioned) generations excluded. Bayes factors (Nylander et al. 2004), calculated as 2 times the difference in the harmonic means of the log likelihoods of the post–burn-in trees (obtained using the ''sump'' command in MrBayes), were examined to investigate whether particular partitioning schemes show a better fit to the data than do others.

Support values.—Support for different clades was calculated by 1,000 bootstrap replicates for the maximum-likelihood analyses (again using RAxML and batchRAxML together with the perl script bootStrip), and by posterior probabilities for the Bayesian analyses. Studies indicate that posterior probabilities may be misleadingly high, whereas bootstrap values are more conservative (Suzuki et al. 2002; Taylor and Piel 2004). However, Hall and Salipante (2007) have argued recently that neither measure reflects the probability of a particular clade. Statistical tests of alternative topologies may therefore be more informative than either of these support measures (Shimodaira and Hasegawa 1999).

Testing alternative topologies.—The different topologies found in the various analyses, as well as a number of others taken from other studies, were tested using the approximately unbiased (AU—Shimodaira 2002) and Kishino–Hasegawa (KH—Kishino and Hasegawa 1989) tests as implemented in CONSEL (Shimodaira and Hasegawa 2001). Both the full and no mt3 data sets were used for these tests. PAUP* version 4.0b10 (Swofford 2002) was used to calculate the site likelihoods for each of the test topologies, with the GCSL-partitioning scheme assumed and the appropriate model for each partition specified using the output from MrModeltest 2.2. The CONSEL analyses employed 10 batches of 1×10^5 bootstrap replicates and 1 of 1×10^6 bootstrap replicates.

Molecular dating analysis.—A Bayesian relaxed molecular clock method was used, as implemented by the program Multidivtime (Kishino et al. 2001; Thorne et al. 1998). General methodology follows Rutschmann (2005; see also Inoue et al. 2005), with a single F84 + gamma model applied to the entire supermatrix (incomplete overlap of sequences prevented a separate model being applied to each partition) and maximum-likelihood parameters estimated under PAML version 3.15 (Yang 1997). Because a partitioned approach could not be implemented in Multidivtime, only the no mt3 data set (which excludes a partition with a known base composition bias) was used for the calculation of dates. Following Benton and Donoghue (2007), a high number of constraints was specified (23 minima and 3 maxima) and a conservative approach was used when assigning dates: if based on absolute (radiometric) dating, dates were assumed to be the lower end of the 95% confidence interval (95% *CI*), where given; if based on relative (biostratigraphic) dating, dates were assumed to represent the lower bound, for example, late Oligocene was assumed to be the Oligocene–Miocene boundary. Ages of boundaries were taken from Gradstein et al. (2004). The root prior rttm (the mean of the prior distribution for the time from the ingroup root to the tips)

TABLE 1.—Minimum and maximum time constraints used in the Multidivtime dating analysis. Node numbers correspond to nodes in Figs. 3 and 4.

Node number	Age (mya)	Justification
1	162.5 (root prior)	*Amphilestes broderipii* and *Phascolotherium bucklandi*—oldest known theriiomorphs (Benton and Donoghue 2007)
2	124.6 (minimum)	*Sinodelphys szalayi* and *Eomaia scansoria*—oldest known metatherian (Luo et al. 2003) and eutherian (Ji et al. 2002), respectively
3	124.6 (maximum)	All splits within placental crown-group assumed to be younger than oldest known eutherian, *Eomaia*
3	58.5 (minimum)	*Riostegotherium yanei*—oldest known xenarthran (Rose et al. 2005)
4	124.6 (maximum)	All splits within marsupial crown-group assumed to be younger than oldest metatherian, *Sinodelphys*
4[a], 6[a], 7[a]	59 (minimum)	*Khasia cordillerensis*—oldest known microbiothere (Marshall and Muizon 1988)
4[b], 6[b]	58.5 (minimum)	Oldest known tarsals with the apomorphic didelphimorphian proximal calcaneocuboid facet (Szalay 1994) and the oldest known paucituberculate (Oliveira et al. 1996)
5	11.8 (minimum)	*Micoureus laventicus* (Goin 1997)—*Micoureus* is more closely related to *Monodelphis* than *Didelphis* (Jansa and Voss 2005; Steiner et al. 2005)
8[c]	71.2 (maximum)	Apparent absence of marsupials from South America, and no evidence of australidelphians in North America
7, 8, 9	23.03 (minimum)	Oldest known Australasian marsupials referable to modern orders (Archer et al. 1999; Long et al. 2002)
10	3.53 (minimum)	Cf. *Peroryctes* sp.—oldest known peroryctids (Turnbull et al. 2003)
11, 12	23.03 (minimum)	*Badjcinus turnbulli*—oldest known thylacinid (Muirhead and Wroe 1998)
13	4.45 (minimum)	*Antechinus* sp.—oldest known phascogaline (Archer 1982; Turnbull et al. 2003)
14	4.45 (minimum)	Cf. *Dasyurus* sp.—oldest known *Dasyurus* (Turnbull et al. 2003)
15	23.03 (minimum)	Oldest known vombatiforms and phalangeridans (Archer et al. 1999; Long et al. 2002)
16	23.03 (minimum)	Oldest known koalas and vombatoids (Long et al. 2002)
17	23.03 (minimum)	Oldest known petauroids, burramyids, phalangerids, and macropodoids (Archer et al. 1999; Long et al. 2002)
18	23.03 (minimum)	Oldest known petaurids and pseudocheirids (Archer et al. 1999; Long et al. 2002)
19	23.03 (minimum)	Oldest known burramyids, phalangerids, and macropodoids (Archer et al. 1999; Long et al. 2002)
20	4.45 (minimum)	*Dorcopsis wintercookorum*—oldest known *Dorcopsis* (Flannery et al. 1992)
21	4.45 (minimum)	*Thylogale ignis*—oldest known *Thylogale* (Flannery et al. 1992)
22	4.45 (minimum)	*Thylogale ignis*—oldest known *Thylogale* (Flannery et al. 1992)
23	23.03 (minimum)	Oldest known burramyids and phalangerids (Archer et al. 1999; Long et al. 2002)
24	23.03 (minimum)	Oldest known trichosurin (Crosby 2004)

[a] Used in the "all constraints," but not in the "no *Khasia* constraint" MultiDivTime analyses.
[b] Used in the "no *Khasia* constraint," but not in the "all constraints" MultiDivTime analyses.
[c] Not used in the "no 71.2 mya constraint" MultiDivTime analyses.

was set at 162.5 mya (the age of the monotreme–therian split) following the arguments of Benton and Donoghue (2007). A full list of calibrations is given in Table 1. Other Multidivtime parameters were calculated as follows (following Rutschmann 2005): rtrate (mean of prior distribution for the rate at the root node) = X/rttm, where X is the median amount of evolution from the root to tips; rtratesd (standard deviation of rtrtate) = 0.5 × rtrate; brownmean (mean of the prior distribution for the autocorrelation parameter, v) = 1/rttm; and brownsd (standard deviation of brownmean) = 1/rttm. Three independent Multidivtime analyses were run for 1×10^6 cycles with samples taken every 100 cycles after a burn-in period of 1×10^5 cycles. The estimated dates for each node varied <1% across the 3 runs in all analyses indicating that stationarity had been reached; the dates presented here are mean values for the 3 runs.

An additional analysis was carried out with the lower constraint based on *Khasia cordillerensis* (Table 1) excluded, as doubts have been raised (e.g., Szalay 1994) as to whether *Khasia* is indeed a microbiothere. When *Khasia* was excluded, the lower constraint on the Didelphimorphia–(*Caenolestes* + Australidelphia) and the *Caenolestes*–Australidelphia splits was 58.5 mya, based on the presence of didelphimorphian-type tarsals (Szalay 1994) and an undescribed paucituberculate (Oliveira et al. 1996) at Itaborai. Likewise, in this analysis the lower constraint on the Microbiotheria–Australasian radiation split was 23.03 mya, based on the oldest known members of the Australasian crown-group (Table 1). A further analysis was carried out with the 71.2 upper constraint on divergences within the Australasian radiation excluded, because the apparent absence of marsupials from South America before this date may be an artifact of the incomplete fossil record.

All 3 analyses (i.e., all constraints, no *Khasia* constraint, and no 71.2 mya constraint) were repeated using a reduced data set excluding Burramyidae, *Dasyuroides*, *Dorcopsis*, *Thylacinus*, and *Thylogale*; these taxa were represented by fewer than 6 of the 22 genes (Appendix I), which may lead to inappropriate branch lengths if these genes are evolving particularly slowly or rapidly relative to the average rate across all 22 genes.

Analysis of unrepresented basal branch lengths (UBBLs).—Following Teeling et al. (2005), I calculated unrepresented basal branch lengths (UBBLs) for several lineages to quantify the incompleteness of the fossil record implied by the molecular divergence dates (Appendix II). UBBL was calculated as 1 − (age of oldest fossil assignable to a particular branch divided by age of divergence of that branch) and converted into a percentage by multiplying by 100. Unlike the dating analysis, in which it is preferable to specify conservative minimum divergence dates (Benton and Donoghue 2007), the point estimate age (rather than the lower bound of the 95% *CI*) of fossils from radiometrically dated sites was used in the UBBL analysis, and the ages of sites estimated by biocorrelation were assumed to be the midpoint of the assigned age range (following

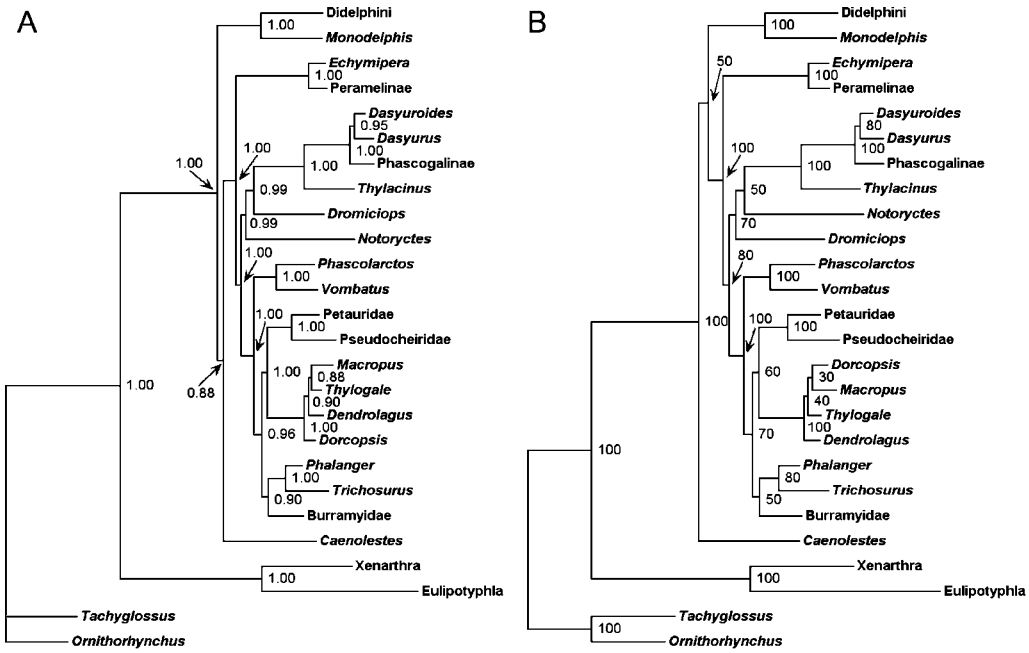

Fig. 1.—Topologies that result from A) Bayesian analysis of the full data set without partitioning, and B) maximum-likelihood analysis of the full data set using the GCSL-partitioning scheme. The Bayesian tree is a 50% majority rule consensus tree. Numbers in A are Bayesian posterior probabilities, and in B are maximum-likelihood bootstrap values.

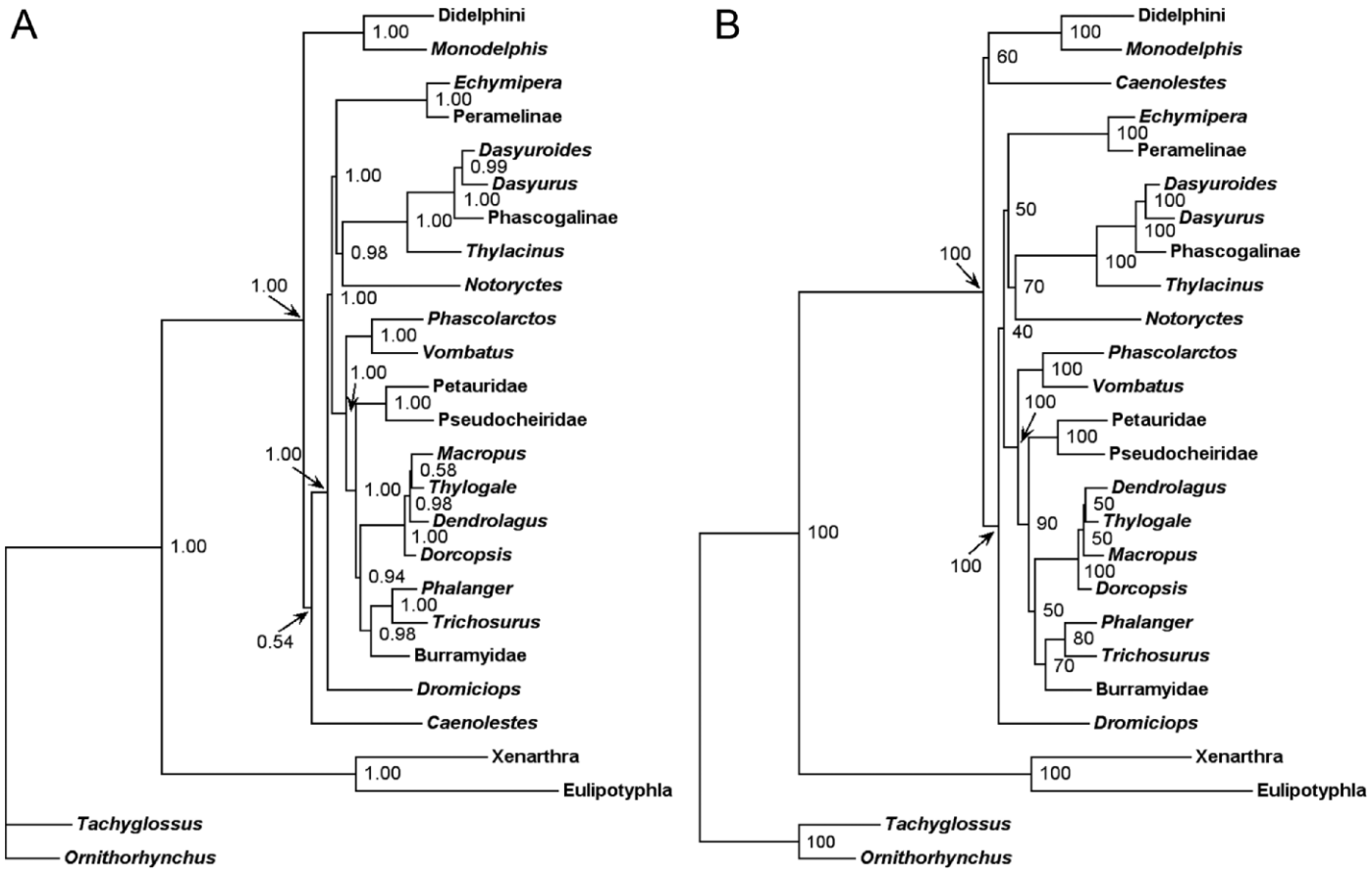

Fig. 2.—Topologies that result from A) Bayesian analysis of the full data set using the GCSL-partitioning scheme, and B) maximum-likelihood analysis of the no mt3 data set using the GCSL-partitioning scheme. Other details are as in Fig. 1.

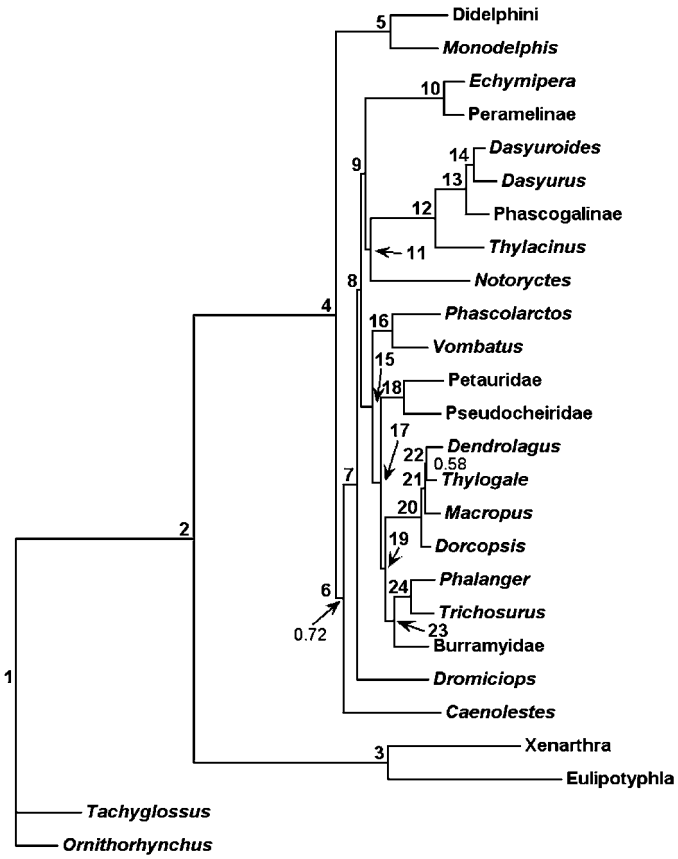

FIG. 3.—Topology that results from Bayesian analysis of the no mt3 data set using the GCSL-partitioning scheme. Numbers to the left of the nodes correspond to constraints used in the Multidivtime analyses (Table 1). All Bayesian posterior probability values are ≥0.98, unless otherwise indicated. Other details are as in Fig. 1.

the dates given by Gradstein et al. [2004]), for example, late Oligocene = 25.715 mya ($\bar{X}$ of 23.03–28.4 mya).

RESULTS

The unpartitioned Bayesian analysis (Fig. 1A) and all maximum-likelihood analyses (Fig. 1B) of the full data set recover a broadly similar topology in which Peramelemorphia is sister to all other australidelphians (= Eometatheria) and *Dromiciops* forms a clade with either Dasyuromorphia (unpartitioned Bayesian and unpartitioned maximum-likelihood analyses; Fig. 1A) or Dasyuromorphia + *Notoryctes* (gene- and GCSL-partitioned maximum-likelihood analyses; Fig. 1B). By contrast, gene-partitioned and GCSL-partitioned Bayesian analyses of the full data set (Fig. 2A) and all Bayesian and maximum-likelihood analyses of the no mt3 data set (Figs. 2B and 3) support Peramelemorphia as the sister to Dasyuromorphia + *Notoryctes* and place *Dromiciops* as the sister to all other australidelphians. The only differences between the topologies presented in Fig. 2A (no mt3 data set, GCSL-partitioning, Bayesian), Fig. 2B (full data set, GCSL-partitioning, maximum-likelihood), and Fig. 3 (no mt3 data set, GCSL-partitioning, Bayesian) concern the position of *Caenolestes* (sister to Didelphimorphia in Fig. 2B, but sister to Australidelphia in Figs. 2A and 3) and relationships within macropodines (*Thylogale* is sister to *Dendrolagus* in Figs. 2B and 3, but sister to *Macropus* in Fig. 2A). These alternatives are statistically indistinguishable (Table 2), and I focus my discussion on the tree in Fig. 3 (no mt3 data set, GCSL-partitioning, Bayesian), because this represents the topology and data set used in the molecular dating analysis.

When the probable microbiothere *Khasia* is not used as a fossil constraint, molecular dates become 1–6% younger, whereas removal of the 71.2 mya upper constraint on the diversification of Australasian marsupials results in an 8–12% increase in age (Table 3). Exclusion of the 5 taxa with the most missing data (Burramyidae, *Dasyuroides*, *Dorcopsis*, *Thylacinus*, and *Thylogale*) generally results in somewhat (~2–8%) younger dates, although some nodes instead show a slight increase in age (Table 3). In the absence of compelling evidence that *Khasia* is not a microbiothere or that marsupials were present in South America before 71.2 mya, I will assume for the purposes of discussion the divergence dates that result when all constraints (Table 1; Fig. 4) are enforced.

DISCUSSION

Although relatively strongly supported by both bootstrapping and posterior probabilities, the positions of Peramelemorphia and *Dromiciops* in Figs. 1A and 1B are most likely anomalous and result from misleading signals in the 3rd codon positions of the mitochondrial protein-coding genes. Increased partitioning of the full data set, which is strongly supported by Bayes factors ≫ 10 (Nylander et al. 2004), results in a different topology (Fig. 2A) that is largely identical to that from the no mt3 data set, albeit only when Bayesian analysis is used. The topologies in Fig. 1 also are rejected by both the AU and KH tests when the no mt3 data set is assumed (Table 2). Finally, Phillips et al. (2006) found clear support for a close relationship between Peramelemorphia and Dasyuromorphia (and for *Dromiciops* as the sister to the Australasian radiation) when the analysis was corrected for base composition bias. The inability of maximum-likelihood analysis (as implemented by RAxML) to recover the inferred correct topology, even under the GCSL-partitioning scheme (Fig. 1B), was unexpected and may relate to the fact that maximum-likelihood model parameters must be specified before the analysis, whereas they are estimated during the analysis in the Bayesian approach. Also, RAxML only allows use of the GTR + gamma model for each partition, rather than other models that may be more appropriate.

Figure 3 is fully resolved, and all but 1 of the interordinal divergences are strongly supported by Bayesian posterior probabilities (BPP ≥ 0.98), although equivalent bootstrap values from the maximum-likelihood analysis using the same data set and partitioning scheme (Fig. 2A) are low for some nodes. The deepest split within marsupials (BPP = 1.00) is between Didelphimorphia and a clade comprising *Caenolestes* and Australidelphia. This arrangement is congruent with recent molecular phylogenies (Amrine-Madsen et al. 2003; Nilsson et al. 2004), the morphological analysis of Horovitz and Sanchez-Villagra (2003), and the combined analysis of Asher et al. (2004). However, the *Caenolestes* + Australidelphia clade is

Table 2.—Results of approximately unbiased (AU—Shimodaira 2002) and Kishino–Hasegawa (KH—Kishino and Hasegawa 1989) tests of alternative topologies as calculated by CONSEL (Shimodaira and Hasegawa 2001) assuming both the full and no mt3 (excluding 3rd codon positions of mitochondrial protein-coding genes) data sets. *P*-values less than 0.05 (indicating that the topology is statistically rejected by the data set) are indicated with asterisks. The first 5 topologies are from Figs. 1–3, with the remainder from the studies cited.

Topology	Reference	Full data set		No mt3 data set	
		AU	KH	AU	KH
Full, unpartitioned, Bayesian	Fig. 1A	0.084	0.076	0.031*	0.026*
Full, GCSL-partitioned, ML	Fig. 1B	0.118	0.071	0.047*	0.036*
No mt3, GCSL-partitioned, ML	Fig. 2A	0.48	0.299	0.529	0.371
Full, GCSL-partitioned, Bayesian	Fig. 2B	0.577	0.38	0.608	0.405
No mt3, Bayesian, GCSL-partitioned	Fig. 3	0.708	0.414	0.774	1
Didelphimorphia + *Caenolestes* (= Ameridelphia)	—	0.473	0.298	0.526	0.372
Caenolestes sister to rest of Marsupialia	Baker et al. 2004; Kirsch et al. 1997; Szalay and Sargis 2001, 2006	0.732	0.586	0.654	0.481
Perameleomorphia sister to rest of Australidelphia (= Eometatheria)	Asher et al. 2004: figure 1; Kirsch et al. 1997	0.408	0.267	0.261	0.154
Diprotodontia + Perameleomorphia (= Syndactyla excluding *Notoryctes*)	Szalay 1994; Szalay and Sargis 2001, 2006	0.012*	0.024*	0.016*	0.011*
Diprotodontia + (Perameleomorphia + *Notoryctes*) (= Syndactyla including *Notoryctes*)	Szalay 1994; Szalay and Sargis 2001, 2006	0.038*	0.036*	0.037*	0.023*
Perameleomorphia + *Notoryctes*	Horovitz and Sanchez-Villagra 2003; Warburton 2003	0.3	0.186	0.3	0.143
Dromiciops + Diprotodontia	Horovitz and Sanchez-Villagra 2003, Cardillo et al. 2004	0.339	0.236	0.27	0.159
Dromiciops + Dasyuromorphia (= Gondwanadelphia)	Szalay 1994; Szalay and Sargis 2001	0.022*	0.021*	0.009*	0.008*
Dromiciops + (Dasyuromorphia + *Notoryctes*)	—	0.004*	0.014*	0.029*	0.004*
Dromiciops + Perameleomorphia	Asher et al. 2004: figure 2—right	0.075	0.063	0.074	0.048*
Dromiciops + (Perameleomorphia + (Dasyuromorphia + *Notoryctes*))	Nilsson et al. 2004	0.025*	0.04*	0.023*	0.017*
Macropodoidea + Petauroidea	Amrine-Madsen et al. 2003	0.208	0.175	0.227	0.159
Petauroidea + (Burramyidae + Phalangeridae) (= possum monophyly)	—	0.498	0.36	0.515	0.376
Burramyidae sister to rest of Diprotodontia	Asher et al. 2004: figure 1	0.036*	0.063	0.041*	0.02*

relatively weakly supported (BPP = 0.72), and neither the AU nor KH test rejects a monophyletic Ameridelphia (recovered in the equivalent maximum-likelihood analysis with 60% bootstrap support; Fig. 2A) or Didelphimorphia + Australidelphia. The latter clade was favored by Szalay and Sargis (2001, 2006) on morphological grounds and has been found in some molecular studies, for example, Baker et al. (2004) and Kirsch et al. (1997). Thus, the relationships of caenolestids remain uncertain, and inclusion of sequence data from 1 or both of the other extant caenolestid genera (*Lestoros* and *Rhyncholestes*) may help increase resolution by breaking up the long branch leading to *Caenolestes*. At least part of this uncertainty also may be due to rooting difficulties, because studies suggest that the position of the root is often the most difficult region of a molecular phylogeny to resolve robustly (Sanderson and Shaffer 2002). Additional sources of molecular data, such as retrotransposon insertions, insertions–deletions, or other rare genomic changes (Rokas and Holland 2000), may be required to root the marsupial tree with confidence.

Within Australidelphia, the South American *Dromiciops* is sister to a monophyletic Australasian clade (BPP = 1.00; bootstrap = 100). The alternative positions for *Dromiciops* recovered by Asher et al. (2004—sister to all Australasian marsupials except Perameleomorphia) and Nilsson et al. (2004—sister to *Notoryctes* + Dasyuromorphia + Perameleomorphia) are most likely erroneous, because both studies used mitochondrial genes without correcting for the biases identified by Phillips et al. (2006). The topology of Nilsson et al. (2004) is rejected by both the AU and KH tests, whereas the topology of Asher et al. (2004) is not (Table 2).

Within the Australasian radiation, the 1st divergence is between the diverse order Diprotodontia and a clade comprising the 3 Australasian ''polyprotodont'' orders, Perameleomorphia, Notoryctemorphia, and Dasyuromorphia (BPP = 1.00; bootstrap = 40). This topology agrees with that of Amrine-Madsen et al. (2003) and Phillips et al. (2006), and it indicates that syndactyly (fusion of digits II and III and their integration into a single functional digit—Weisbecker and Nilsson 2006) evolved independently in these 2 orders (contra Szalay 1994). Although syndactyly is a derived feature present in all diprotodontians and perameleomorphians, a monophyletic Syndactyla (Szalay 1982, 1994), whether including or excluding

TABLE 3.—Divergence dates estimated using Multidivtime (node numbers are given in Figs. 3 and 4). Two different data sets were used, 1 including all sequences and the other excluding the 5 taxa with most incomplete data (Burramyidae, *Dasyuroides*, *Dorcopsis*, *Thylacinus*, and *Thylogale*). Multidivtime analyses were carried out using all constraints listed in Table 1 (''All constraints''), excluding *Khasia* as the oldest known microbiothere (''No *Khasia* constraint''), or excluding the 71.2 mya upper constraint on divergences within the Australasian radiation (''No 71.2 mya constraint''). Divergence dates from the analysis using all sequences and all constraints are illustrated in Fig. 4.

	No mt3 data set, all sequences			No mt3 data set, 5 most incomplete taxa deleted		
Node	All constraints	No *Khasia* constraint	No 71.2 mya constraint	All constraints	No *Khasia* constraint	No 71.2 mya constraint
2	197.2 ± 23.5 (154.9–246.9)	193.8 ± 24.5 (150.1–245.5)	215.4 ± 33.1 (159.2–285.5)	198.9 ± 22.6 (159.6–247.2)	190.6 ± 23.6 (151.5–242.6)	216.2 ± 32.8 (163.4–287.8)
3	78.2 ± 11.5 (60.3–104.0)	77.0 ± 11.5 (59.8–103.4)	85.1 ± 14.7 (61.3–116.7)	77.9 ± 11.3 (60.3–103.3)	75.0 ± 11.1 (59.5–101.1)	84.2 ± 14.7 (61.1–116.5)
4	80.6 ± 5.2 (70.7–90.0)	79.0 ± 6.3 (65.8–89.7)	90.3 ± 12.7 (71.5–118.8)	79.3 ± 5.5 (70.2–90.1)	75.0 ± 7.5 (62.6–89.1)	88.4 ± 13.0 (70.9–118.0)
5	44.4 ± 4.5 (35.9–53.4)	43.4 ± 4.9 (34.1–53.1)	50.4 ± 8.7 (37.1–70.3)	42.4 ± 4.4 (34.4–51.5)	39.8 ± 5.3 (30.6–50.5)	47.9 ± 8.5 (35.4–67.5)
6	76.5 ± 4.7 (67.5–84.7)	74.9 ± 5.8 (62.7–84.4)	85.9 ± 12.1 (68.1–113.0)	75.1 ± 5.0 (67.0–84.5)	70.9 ± 7.0 (59.4–83.8)	83.9 ± 12.4 (67.6–112.3)
7	67.4 ± 3.9 (59.8–73.6)	66.0 ± 4.9 (55.5–73.5)	75.9 ± 10.8 (60.3–100.2)	65.8 ± 4.2 (59.3–73.3)	62.0 ± 6.2 (51.7–73.0)	73.6 ± 11.0 (59.5–99.1)
8	65.2 ± 3.7 (57.9–70.9)	63.8 ± 4.7 (53.7–70.8)	73.3 ± 10.4 (58.3–96.8)	63.5 ± 4.0 (57.1–70.7)	59.9 ± 6.0 (49.9–70.5)	71.2 ± 10.7 (57.5–95.8)
9	63.4 ± 3.7 (56.3–69.2)	62.1 ± 4.6 (52.3–69.1)	71.4 ± 10.2 (56.8–94.3)	61.6 ± 4.0 (55.2–68.8)	58.1 ± 5.8 (48.3–68.5)	69.1 ± 10.4 (55.7–93.1)
10	11.7 ± 1.5 (9.1–15.1)	11.5 ± 1.6 (8.7–14.9)	13.2 ± 2.5 (9.4–19.0)	11.3 ± 1.5 (8.8–14.5)	10.6 ± 1.6 (7.9–14.0)	12.8 ± 2.5 (9.1–18.7)
11	60.1 ± 3.6 (53.2–66.1)	58.9 ± 4.4 (49.6–65.9)	67.6 ± 9.7 (53.8–89.8)	58.3 ± 3.9 (51.8–65.5)	54.9 ± 5.6 (45.4–65.1)	65.4 ± 10.0 (52.3–88.3)
12	26.0 ± 2.2 (23.2–31.4)	25.7 ± 2.2 (23.1–31.1)	28.6 ± 4.5 (23.2–39.7)	—	—	—
13	11.6 ± 1.6 (8.8–15.2)	11.4 ± 1.6 (8.7–15.1)	12.8 ± 2.5 (9.1–18.9)	10.6 ± 1.3 (8.3–13.5)	10.0 ± 1.5 (7.5–13.2)	12.0 ± 2.3 (8.6–17.5)
14	7.8 ± 1.5 (5.3–11.1)	7.7 ± 1.5 (5.2–11.0)	8.6 ± 2.1 (5.5–13.5)	—	—	—
15	57.1 ± 3.4 (50.5–62.9)	55.9 ± 4.3 (47.1–62.8)	64.3 ± 9.3 (50.9–85.2)	55.4 ± 3.7 (49.2–62.3)	52.2 ± 5.3 (43.3–61.8)	62.1 ± 9.5 (49.7–84.0)
16	40.7 ± 3.3 (34.4–47.0)	39.8 ± 3.7 (32.5–46.9)	45.8 ± 7.1 (35.0–62.1)	39.2 ± 3.4 (33.0–45.8)	36.9 ± 4.2 (29.5–45.2)	44.0 ± 7.2 (33.7–60.6)
17	51.1 ± 3.2 (44.9–57.0)	50.0 ± 4.0 (41.8–56.8)	57.4 ± 8.4 (45.2–76.4)	49.0 ± 3.5 (43.0–55.8)	46.2 ± 4.8 (38.2–55.2)	55.0 ± 8.5 (43.4–74.7)
18	32.4 ± 3.1 (26.6–38.7)	31.7 ± 3.4 (25.3–38.5)	36.4 ± 6.0 (27.0–50.1)	30.8 ± 3.1 (25.3–37.2)	29.1 ± 3.5 (23.6–36.4)	34.6 ± 6.0 (25.7–48.5)
19	47.1 ± 3.2 (41.1–53.0)	46.0 ± 3.8 (38.3–52.8)	52.9 ± 7.9 (41.3–70.7)	45.0 ± 3.4 (39.1–51.6)	42.4 ± 4.5 (34.8–51.0)	50.5 ± 8.0 (39.5–68.9)
20	16.7 ± 2.3 (12.6–21.7)	16.3 ± 2.4 (12.0–21.4)	18.7 ± 3.7 (12.9–27.2)	—	—	—
21	14.7 ± 2.1 (10.9–19.2)	14.3 ± 2.2 (10.3–19.0)	16.4 ± 3.3 (11.1–24.0)	17.1 ± 2.5 (12.6–22.4)	16.1 ± 2.6 (11.5–21.8)	19.2 ± 3.9 (13.1–28.2)
22	13.1 ± 2.0 (9.4–17.4)	12.7 ± 2.1 (9.0–17.2)	14.6 ± 3.1 (9.7–21.6)	—	—	—
23	40.0 ± 3.7 (33.0–47.4)	38.8 ± 4.2 (30.6–46.9)	44.5 ± 7.4 (33.0–61.3)	—	—	—
24	28.1 ± 3.2 (23.3–35.3)	26.5 ± 4.1 (18.7–34.9)	30.4 ± 6.2 (20.2–44.4)	27.1 ± 4.1 (19.5–35.4)	25.5 ± 4.3 (17.8–34.5)	30.4 ± 6.2 (20.4–44.7)

Notoryctes (which may or may not be syndactylous), is rejected by both the KH and AU tests (Table 2). *Notoryctes* is the sister-group of Dasyuromorphia (BPP = 0.98; bootstrap = 70). Recent morphological studies (Horovitz and Sanchez-Villagra 2003; Warburton 2003) have linked *Notoryctes* with Peramelemorphia, and a *Notoryctes* + Peramelemorphia clade is not rejected by KH and AU tests (Table 2). Tarsal material of a fossil notoryctid from early Miocene Faunal Zone B (previously ''System B''—Arena 2004; Travouillon et al. 2006) sites at Riversleigh also shares distinctive apomorphies (not present in the more derived *Notoryctes*) with peramelemorphians (R. M. D. Beck, in litt.). *Notoryctes* is the sole extant representative of the order Notoryctemorphia and is extremely autapomorphic; it thus represents both a molecular and morphological long branch that may be difficult to place robustly with either morphological or sequence data. Other kinds of molecular data may be required to resolve the affinities of *Notoryctes* conclusively. Within Dasyuromorphia, Dasyuridae is monophyletic relative to the thylacine *Thylacinus* (BPP = 1.00; bootstrap = 100).

In agreement with morphological data (e.g., Aplin and Archer 1987), Diprotodontia comprises 2 major clades: Vombatiformes (the koala and wombats; BPP = 1.00; bootstrap = 100) and Phalangerida (the various families of extant ''possums'' and kangaroos; BPP = 1.00; bootstrap = 90). An alternative topology, with Burramyidae sister to other diprotodontians (Asher et al. 2004), is rejected by both the AU and KH tests with the no mt3 data set, but only by the AU test for the full data set (Table 2). Petauroidea, a clade comprising pseudocheirids and petaurids, is monophyletic (BPP = 1.00; bootstrap = 100) and also is well supported by morphology (Aplin and Archer 1987). A 2nd possum clade consisting of Burramyidae and Phalangeridae (BPP = 0.98; bootstrap = 70) also was seen in the DNA-hybridization trees of Springer and Kirsch (1991) and Kirsch et al. (1997), a maximum-likelihood analysis of *RAG1* sequences by Baker et al. (2004), and morphological analyses of phalangeridans by Crosby (2004). ''Possums,'' as a whole, appear to be a paraphyletic assemblage (although possum monophyly cannot be rejected; Table 2) because kangaroos are the sister-group of the Burramyidae + Phalangeridae clade (BPP = 0.98; bootstrap = 50). Plesiomorphic macropodoids, burramyids, and phalangerids all possess a hypertrophied 3rd premolar that may represent a morphological synapomorphy of this clade.

Divergence dates.—On initial examination, the divergence dates in Fig. 4 and Table 3 seem congruent with the known fossil record; the dates are not implausibly ancient (i.e., older than the Late Cretaceous), nor are any lineages younger than the oldest known fossils referred to them. In contrast, at least some of the molecular dates of Nilsson et al (2004), who used a penalized log-likelihood method and a relatively young estimate of 135 mya for the divergence between marsupials and placentals (compared to 186–193 mya calculated by van Rheede et al. [2006]), appear incongruent with the fossil record. For example, their estimated age for the divergence of Microbiotheria is 46 mya, but the oldest known fossil microbiothere, *Khasia cordillerensis* from Tiupampa (Marshall and Muizon 1988), is approximately 60 million years old (however, there are some doubts as to whether *Khasia* is indeed a microbiothere—Szalay 1994). Below, I undertake a more detailed examination of the divergence dates to identify the degree of congruence with the fossil record and to determine whether particular divergences coincided with major environmental changes.

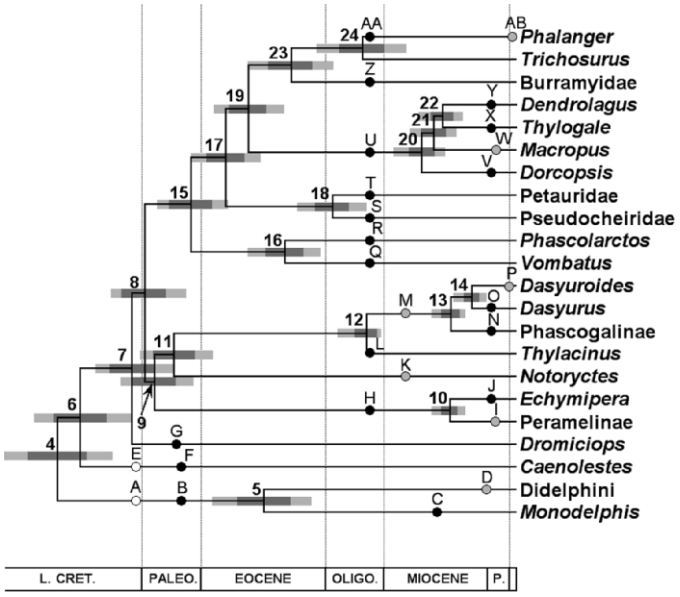

FIG. 4.—Modified version of Fig. 3 with dates calculated by Multidivtime assuming full constraints and including all taxa (estimated dates from all the Multidivtime analyses are given in Table 3). Monotreme and placental outgroups have been pruned. Dark gray bars represent 1 *SD* either side of the point estimate, and light gray bars represent the 95% *CI*. Circles on branches and their associated letters represent the earliest known fossils assignable to that lineage and used to calculate unrepresented basal branch lengths (UBBLs; Appendix II). Black circle = fossil confidently assigned to that lineage and used to calibrate the Multidivtime analysis (Table 1); gray circle = fossil confidently assigned to that lineage but not used to calibrate the Multidivtime analysis; white circle = fossil only tentatively assigned to that lineage.

The 2 deepest splits within marsupials are here estimated as occurring 80.6 mya (Didelphimorphia–other marsupials) and 76.5 mya (*Caenolestes*–Australidelphia), that is, in the Campanian of the Late Cretaceous. If the apparent absence of marsupials from the Alamitian (~71.2 mya) faunas of South America is accurate, then these 2 divergences must have occurred in Laurasia (probably North America, given that marsupials appear to have been rare in Eurasia during the Late Cretaceous), and members of 3 separate marsupial lineages (didelphimorphians, paucituberculates, and australidelphians) dispersed from North America to South America during the latest Cretaceous–earliest Paleocene. This total does not include fossil groups, such as borhyaenoids and polydolopimorphians, that may lie outside the marsupial crown-group. Fossil evidence indicates that as many as 4 separate placental lineages also dispersed from North to South America during the latest Cretaceous–earliest Paleocene (Muizon 1991; Muizon and Cifelli 2000), as did other terrestrial vertebrates

such as hadrosaurs and snakes (Case et al. 2000, 2005), suggesting the existence of a relatively robust connection between the 2 continents during this period.

Evidence of crown marsupials in the Late Cretaceous of North America is equivocal given the limited fossil material currently known, but the dates presented here are congruent with the assignment of *Glasbius* to Paucituberculata (Kielan-Jaworowska et al. 2004) and *Nortedelphys* to Didelphimorphia (Case et al. 2005), in which case UBBL is 17.4% and 13% for Didelphimorphia and Paucituberculata, respectively (Appendix II). Unequivocal didelphimorphians (identified by the presence of the distinctive proximal calcaneocuboid facet of the tarsus seen in all extant didelphids—Szalay 1994) and paucituberculatans (Oliveira et al. 1996) are 1st known from the late Paleocene (58.7–59.2 mya) Itaborai fauna in Brazil, but have not been identified in the slightly older (59.2–60.4 mya) Tiupampa fauna. If the Itaboraian fossils are preferred as the earliest known members of these orders, then UBBL increases to 26.9% and 22.9% for Didelphimorphia and Paucituberculata, respectively (Appendix II). Efforts to discover earlier records of these orders are hampered by a lack of South American marsupials older than those from Tiupampa, with the exception of the highly fragmentary Punta Peligro fauna (possibly early Paleocene) and a single tooth from the early Paleocene Lefipan Formation (Goin et al. 2006). UBBLs for the crown didelphimorphians *Monodelphis* (68.4%) and Didelphini (87.8%) are high, probably because didelphids are poorly defined on dental grounds (the family is characterized by a highly plesiomorphic dentition), making referral of isolated teeth to the family or to specific genera difficult.

The split between microbiotheres and the Australasian marsupials is estimated as 67.4 mya. There is no evidence of microbiotheres or any member of the Australasian radiation in the Late Cretaceous North American deposits, which may be indirect evidence that this split occurred in Gondwana. However, stem australidelphians most likely had a relatively plesiomorphic tribosphenic dentition (perhaps similar to that of the early Eocene Australian marsupial *Djarthia murgonensis*—R. M. D. Beck, in litt.), and unequivocal australidelphian synapomorphies are restricted to the tarsus, suggesting that it may be impossible to identify them from isolated teeth alone. The oldest known australidelphian is the probable microbiothere *Khasia cordillerensis* from Tiupampa; hence, UBBL for Microbiotheria is only 11.3% (Appendix II).

Although the estimated age of the 1st split within the Australasian marsupials (65.2 mya) is almost immediately after the Cretaceous–Tertiary boundary, this is not necessarily evidence of a causal link between these 2 events. Whether this split occurred in South America, Australia, or Antarctica is uncertain. However, given that the modern Australasian radiation appears to be monophyletic, and that there is no fossil evidence of members of any Australasian order in South America or the middle Eocene of Seymour Island in western Antarctica, an Australasian or eastern Gondwanan center of origin seems likely. If so, the date of this split represents the minimum age for the arrival of marsupials in Australasia–eastern Gondwana, which therefore must have occurred between 65.2 ($\pm$ 3.7; 95% CI = 57.9–70.9) and 71.2 mya. All 4 extant Australasian orders—Peramelemorphia, Dasyuromorphia, Notoryctemorphia, and Diprotodontia—are estimated to have diverged by the age of the early Eocene Tingamarra fauna (54.6 mya). However, no undoubted members of any of these orders have been identified among the marsupial specimens recovered from this site; hence UBBL for these 4 orders is 59.4%, 57.2%, 67.5%, and 60.6%, respectively (Appendix II). A single upper molar from Tingamarra was tentatively identified as a possible bandicoot (Archer et al. 1999), but it lacks unequivocal peramelemorphian dental synapomorphies. A 2nd upper molar from Tingamarra reported as an undoubted peramelemorphian (Beck et al. 2006) is in fact a misattributed specimen from a younger site. Woodburne and Case (1996) suggested that the bunodont Tingamarran marsupial *Thylacotinga* is a peramelemorphian, but it also lacks unequivocal apomorphies that would distinguish it from other bunodont forms such as polydolopimorphians. Given that the most distinctive feature of peramelemorphian molars—a highly invasive, incomplete centrocrista that breaches the ectoloph—is not present in the late Oligocene *Yarala burchfieldi* (Muirhead and Filan 1995), identifiable dental synapomorphies of Peramelemorphia may not have evolved until long after the early Eocene. If so, fossil peramelemorphians may be present at Tingamarra but cannot be recognized as such. This explanation also may apply to Notoryctemorphia; although notoryctemorphians are estimated as originating at 60.0 mya, the only known fossil notoryctid (from the middle Miocene of Riversleigh—Long et al. 2002) is considerably more plesiomorphic than *Notoryctes*, suggesting that many characteristic notoryctid specializations may have evolved much later than the early Eocene. The absence of notoryctids from the late Oligocene Faunal Zone A of Riversleigh is more surprising, given that a relatively derived notoryctid is present in the middle Miocene Faunal Zone B. However, Faunal Zone A sites appear biased (whether taphonomically or ecologically) toward large-bodied taxa such as thylacinids and larger diprotodontians (H. Godthelp, University of New South Wales, pers. comm.).

A detailed analysis of marsupial dental characters by Godthelp et al. (1999) failed to identify dental apomorphies that distinguish dasyuromorphians from other marsupials with relatively unspecialized tribosphenic dentitions. Thus, dasyuromorphians may be represented by some of the hundreds of isolated teeth now recovered from Tingamarra but cannot be distinguished unequivocally from other dentally plesiomorphic groups. Within Dasyuromorphia, the dasyurid–thylacinid divergence date of 26.0 mya is congruent with presence of the plesiomorphic thylacinids *Nimbacinus* and *Badjcinus* in late Oligocene Riversleigh Faunal Zone A deposits and the dasyurid *Barinya* (which shows relatively derived dasyurid apomorphies of the petrosal—Wroe 1999) in middle Miocene Faunal Zone B deposits. UBBL for Thylacinidae is only 1.1%, yet Muirhead and Wroe (1998) identified 6 thylacinid apomorphies in *Badjcinus*. This suggests either that morphological evolution in the earliest thylacinids was quite rapid, that Riversleigh Faunal Zone A sites may be somewhat younger than the 25.7 mya assumed here, or that the molecular divergence date

calculated here is an underestimate resulting from the high proportion of missing data for *Thylacinus*.

The estimated divergence dates for the split between phascogaline and dasyurine dasyurids (11.6 mya) and between peramelid and peroryctid bandicoots (11.7 mya) are almost identical and coincide with the middle–late Miocene boundary, which saw a major drop in sea level (Haq et al. 1987). These dates explain the absence of these groups from Faunal Zones A–C (late Oligocene–middle Miocene) sites at Riversleigh; they are 1st seen in the fossil record only in the Pliocene, probably because of a lack of Australian sites from the late Miocene.

Within Diprotodontia, the point estimate for the Vombatiformes–Phalangerida split is 57.1 mya, implying that these lineages had diverged by the estimated age of the Tingamarra fauna. Both vombatiforms and phalangeridans possess apomorphically enlarged ''diprotodont'' 1st lower incisors that were presumably present in their last common ancestor, but no such teeth (or any other specimens that can be referred unequivocally to Diprotodontia) have been found at Tingamarra. However, the standard deviation for the Vombatiformes-Phalangerida split is 3.4 million years and the 95% *CI* is 50.5–62.9 mya, and so it may in fact postdate Tingamarra. Drummond et al. (2006) used a different relaxed molecular clock method that (unlike Multidivtime) does not employ rate autocorrelation and estimated a slightly younger age for this split, at about 48 mya.

Both koalas and vombatoids (but not vombatids), estimated here as having diverged 40.5 mya, are known from the late Oligocene deposits of Riversleigh, as are petaurids and pseudocheirids (divergence date = 32.2 mya), and burramyids and phalangerids (divergence date = 39.4 mya). Crosby (2004) identified trichosurins among the late Oligocene Riversleigh phalangerids, congruent with the 26.9 mya divergence date estimated for the trichosurin–phalangerin split presented here, hence UBBL for trichosurins is only 8.5%. However, undoubted phalangerins have not been found at Riversleigh or indeed any fossil site older than the Pleistocene, resulting in a very large UBBL of 96.6%. Phalangerins may have evolved in New Guinea or Sulawesi and invaded Australia during the Pleistocene (Raterman et al. 2006). The earliest undoubted macropodoids are from the late Oligocene (Archer et al. 1999; Long et al. 2002), and an undescribed macropodine is known from a middle Miocene Faunal Zone C site at Riversleigh (B. N. Cooke, Queensland University of Technology, pers. comm.). The radiation of the macropodines, which appears to have started in the middle Miocene (16.7 mya), coincided with the mid-Miocene climatic optimum (Gradstein et al. 2004; Zachos et al. 2001) and the 1st appearance of grasslands in Australia (Martin 2006). Late Miocene macropodines have yet to be found, but, as already mentioned, few Australian sites of this age are known.

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APPENDIX I

GenBank accession numbers for all gene sequences used in this analysis. All sequences and alignments except those for *Thylacinus* (which were added manually) are taken from Amrine-Madsen et al. (2003), Asher et al. (2004), and Nilsson et al. (2004). Abbreviations for gene names are given in text.

	APOB	*BRCA1*	*IRBP*	*P1*	*PGK1*	*RAG1*	*VWF*	12S rRNA	16S rRNA	tRNA valine	12 mt protein-coding genes
Ornithorhynchus				Z26849				X83427	X83427	X83427	X83427
Tachyglossus				Z26848				AJ303116	AJ303116		AJ303116
Eulipotyphla											
Erinaceus								X88898	X88898	X88898	
Talpa	AY243373	AY121756				AF447515	AY121762				
Uropsilus			AY057831								
Xenarthra											
Bradypus	AF548427	AF284002	U48708			AY243401	U31603				
Dasypus								Y11832	Y11832	Y11832	Y11832
Burramyidae											
Cercatetus					AF011242						
Burramys								AF108223	AF108223	AF108223	
Caenolestes	AY243418	AF355794	AF025381	L35332	AF011240	AY243384	AY243403	U61072	AF102808	U61072	AJ508400
Dasyuroides				AJ508400				AF009888			
Dasyurus	AY243430	AY243452	AY243439	L35341	AF011239	AY243398	AY243414	AF009890	AF166349	AF166349	
Dendrolagus	AY243422		AY243435	AF187537	AF011237	AY243388		AF027990	AF027990	AF027990	
Didelphini											
Didelphis	AF548432	AF497261	Z11814	L17007	AF011232		AF226848	Z29573	Z29573	Z29573	Z29573
Lutreolina						AY243390					
Dorcopsis				AF187540				AF027995	AF027995	AF027995	
Dromiciops	AY243423	AY243446	AF025384	L35449	AF011238	AY243389	AY243407	U61073	U97341	U61073	AJ508402
Echymipera	AY243420	AF355796	AF025383		AF011230	AY243386	AY243405	U97342	U97342	U97342	
Macropus		AF284033		L35447	AF011261		AJ224670	Y10524	Y10524	Y10524	Y10524
Monodelphis	AY243431	AY243453	AF257694	L35448	AF011260	U51897	AY243415	AF166346	AF166346	AF166346	AJ508398
Notoryctes	AY243424	AY243447	AF025385	L35446	AF011254	AY243391	AY243408	U61075	AF102810	U61075	AJ639874
Peramelinae											
Isoodon					AF011227						
Perameles	AY243426	AY243450	AY243437	L35305		AY243394	AY243411	AF166347	AF166347	AF166347	AJ639872
Petauridae											
Dactylopsila					AF011235						
Petaurus	AY243433	AY243455	AY243441			AY243400	AY243417	U21181			
Phalanger	AF548431	AY243449	AY243436		AF011250	AY243393	AY243410	AF108222	AF108222	AF108222	
Phascogalinae											
Antechinus					AF011245						
Phascogale	AY243427	AF355795	AF025382	L35327		AY243395	AY243412	U33497	AF102809	AF102809	AJ639869
Phascolarctos	AY243421	AY243445	AY243434	U87789		AY243387	AY243406	U61076	AF166344	U61076	
Pseudocheiridae											
Pseudocheirus		AY243448									AJ639870
Pseudochirops	AY243425		AF025387	L35334	AF011252	AY243392	AY243409	AF102812	AF102812	AF102812	
Thylacinus				U87140				U87405			M99452 (*CYTB* only)
Thylogale				AF187534	AF011266			AF027991	AF027991	AF027991	
Trichosurus				L32744				AF357238	AF357238	AF357238	AF357238
Vombatus	AY243429	AF284031	AF284031			AY243397	AF497260	NC_003322	NC_003322	NC_003322	AJ304828

Appendix II

Unrepresented basal branch lengths (UBBLs—Teeling et al. 2005) of selected lineages, with fossils used to calculate UBBLs indicated. The UBBL value for a particular lineage is the proportion of the age of that lineage for which fossils are unknown. Age ranges are taken from Gradstein et al. (2004). Fossils are indicated on Fig. 4 by the letters in parentheses after the fossil names.

Lineage	Earliest fossil	Age (mya)	Reference	UBBL (%)
Didelphimorphia	*Nortedelphys* spp. (A)	66.55 (late Maastrichtian = 65.5–67.6)	Case et al. 2005	17.4
	IMG V and IMG XII (B)	58.95 (Itaboraian = 58.7–59.2)	Szalay 1994	26.9
Monodelphis	*Micoureus laventicus* (C)	14.05 (Laventan–Friasian = 11.8–16.3)	Marshall 1976; Goin 1997	68.4
Didelphis	*Didelphis pattersoni* (D)	5.4 (Montehermosan = 4–6.8)	Simpson 1974	87.8
Paucituberculata	*Glasbius* spp. (E)	66.55 (Late Maastrichtian = 65.5–67.6)	Kielan-Jaworowska et al. 2004	13.0
	Unnamed paucituberculate (F)	58.95 (Itaboraian = 58.7–59.2)	Oliveira et al. 1996	22.9
Microbiotheria	*Khasia cordillerensis* (G)	59.8 (Tiupampan = 59.2–60.4)	Marshall and Muizon 1988	11.3
Peramelemorphia	*Yarala burchfieldi* (H)	25.715 (late Oligocene = 23.03–28.4)	Muirhead and Filan 1995	59.4
Peramelidae	*Perameles allinghamensis* (I)	3.615 (Allingham Formation = 3.6–3.63)	Archer and Wade 1976	69.1
Peroryctidae	Cf. *Peroryctes* spp. (J)	4.46 (Hamilton Fauna = 4.46)	Turnbull et al. 2003	61.9
Notoryctemorphia	Unnamed notoryctid (K)	19.5 (early Miocene = 15.97–23.03)	Archer et al. 1999; Long et al. 2002	67.6
Thylacinidae	*Badjcinus turnbulli* (L)	25.715 (late Oligocene = 23.03–28.4)	Muirhead and Wroe 1998	1.1
Dasyuridae	*Barinya wangala* (M)	19.5 (early Miocene = 15.97–23.03)	Wroe 1999	25
Phascogalinae	*Antechinus* sp. (N)	4.46 (Hamilton Fauna = 4.46)	Archer 1982; Turnbull et al. 2003	61.6
Dasyurus	Cf. *Dasyurus* sp. (O)	4.46 (Hamilton Fauna = 4.46)	Turnbull et al. 2003	42.8
Dasyuroides	*Dasyuroides achilpatna* (P)	1.81 (late Pliocene–early Pleistocene = 1.81)	Archer 1982	76.9
Vombatidae	Vombatoid spp. (Q)	25.715 (late Oligocene = 23.03–28.4)	Archer et al. 1999; Long et al. 2002	36.8
Phascolarctidae	Phascolarctid spp. (R)	25.715 (late Oligocene = 23.03–28.4)	Archer et al. 1999; Long et al. 2002	36.8
Pseudocheiridae	Pseudocheirid spp. (S)	25.715 (late Oligocene = 23.03–28.4)	Archer et al. 1999; Long et al. 2002	20.6
Petauridae	Petaurid spp. (T)	25.715 (late Oligocene = 23.03–28.4)	Archer et al. 1999; Long et al. 2002	20.6
Macropodidae	Macropodoid spp. (U)	25.715 (late Oligocene = 23.03–28.4)	Archer et al. 1999; Long et al. 2002	45.4
Dorcopsis	*Dorcopsis wintercookorum* (V)	4.46 (Hamilton Fauna = 4.46)	Flannery et al. 1992	73.3
Macropus	*Macropus* (*Osphranter*) *pavana* (W)	3.615 (Allingham Formation = 3.6–3.63)	Bartholomai 1978	75.4
Thylogale	*Thylogale ignis* (X)	4.46 (Hamilton Fauna = 4.46)	Flannery et al. 1992	66.0
Dendrolagus	Cf. *Dendrolagus* sp. (Y)	4.46 (Hamilton Fauna = 4.46)	Flannery et al. 1992	66.0
Burramyidae	*Burramys brutyi* (Z)	25.715 (late Oligocene = 23.03–28.4)	Brammall and Archer 1997	35.7
Trichosurini	Unnamed trichosurins (AA)	25.715 (late Oligocene = 23.03–28.4)	Crosby 2004	8.5
Phalangerini	Pleistocene phalangerins (AB)	0.955 (Pleistocene = 0.01–1.81)	Crosby et al. 2004	96.6