Marsupial relationships and a timeline for marsupial radiation in South Gondwana

Maria A. Nilsson[a,*], Ulfur Arnason[a], Peter B.S. Spencer[b], Axel Janke[a]

[a]*Department of Cell and Organism Biology, Division of Evolutionary Molecular Systematics, University of Lund, S-223 62 Lund, Sweden*
[b]*Veterinary Biology, Murdoch University, Murdoch, WA, Australia 6150*

Abstract

Recent marsupials include about 280 species divided into 18 families and seven orders. Approximately 200 species live in Australia/New Guinea. The remaining species inhabit South America with some of these secondarily ranging into North America. In this study, we examine marsupial relationships and estimate their divergences times using complete mitochondrial (mt) genomes. The sampling, which includes nine new mtDNAs and a total number of 19 marsupial genomes, encompasses all extant orders and 14 families. The analysis identified a basal split between Didelphimorphia and remaining orders about 69 million years before present (MYBP), while other ordinal divergences were placed in Tertiary times. The monotypic South American order Microbiotheria (*Dromiciops gliroides*, Monito del Monte) was solidly nested among its Australian counterparts. The results suggest that marsupials colonized Australia twice from Antarctica/South America and that the divergence between Microbiotheria and its Australian relatives coincided with the geological separation of Antarctica and Australia. Within Australia itself, several of the deepest divergences were estimated to have taken place close to the Eocene/Oligocene transition.
© 2004 Elsevier B.V. All rights reserved.

Keywords: Marsupial evolution; Dromiciops; Notoryctes; South Gondwana

1. Introduction

Considering the low number of recent marsupial orders, one might expect that their relationships would be easily resolved. This has not been the case, however. One reason for this is that molecular studies with extensive species coverage have generally been based on limited amount of sequence data. Another reason is that the fossil record leading to recent marsupials is somewhat sparse, a circumstance that has made it difficult to corroborate morphological comparisons by fossil data. Information on the fossil fauna of Antarctica would be invaluable for the interpretation of marsupial evolution, but with Antarctica largely covered by ice such information is limited. Even Australia has, to date, produced few pre-Oligocene fossils (Archer et al., 1999).

Extant marsupials are commonly split into Ameridelphia and Australidelphia (Szalay, 1982). According to this scheme, Ameridelphia includes the two South American orders Didelphimorphia (opossums) and Paucituberculata (shrew opossums), while Australidelphia includes four Australian orders, Peramelemorphia (bandicoots), Diprotodontia (kangaroos, possums and koalas), Dasyuromorphia (carnivorous marsupials) and the monotypic Notoryctemorphia (*Notoryctes typhlops*, marsupial mole) plus the monotypic South American order Microbiotheria (Aplin and Archer, 1987).

The Ameridelphia/Australidelphia hypothesis, which was originally proposed on the basis of morphological comparisons (Szalay, 1982), has been generally supported in molecular analyses, while relationships within other parts

---

of the marsupial tree have been contentious. A particular problem in this context has been the root of the marsupial tree and the positions of the two monotypic orders Notoryctemorphia and Microbiotheria (Szalay, 1982; Kirsch et al., 1997; Palma and Spotorno, 1999; Phillips et al., 2001; Springer et al., 1998; Amrine-Madsen et al., 2003; Horovitz

Table 1
Species included in the analysis and their taxonomy

Infraclass—Marsupialia
Cohort—Ameridelphia
Order—Didelphimorphia
Didelphis virginiana (North American opossum, accession number Z29573)
Metachirus nudicaudatus (brown four-eyed opossum, AJ639866)
Thylamys elegans (llaca, AJ508401)
Monodelphis domestica (short-tailed grey opossum, AJ508398)
Order—Paucituberculata
Rhyncholestes raphanurus (Chilean shrew opossum, AJ508399)
Caenolestes fuliginosus (silky shrew opossum, AJ508400)
Cohort—Australidelphia
Order—Microbiotheria
Dromiciops gliroides (Monito del Monte, AJ508402)
Order—Notoryctemorphia
Notoryctes typhlops (marsupial mole, AJ639874)
Order—Dasyuromorphia
Sminthopsis douglasi (Julia Creek dunnart, AJ639867)
Phascogale tapoatafa (brush-tailed phascogale, AJ639869)
Order—Peramelemorphia
Macrotis lagotis (bilby, AJ639871)
Isoodon macrourus (northern brown bandicoot, AF358864)
Perameles gunnii (eastern barred bandicoot, AJ639872)
Order—Diprotodontia
Tarsipes rostratus (honey possum, AJ639868)
Pseudocheirus peregrinus (ringtail possum, AJ639870)
Potorous tridactylus (long-nosed potoroo, AJ639873)
Macropus robustus (wallaroo, Y10524)
Trichosurus vulpecula (brushtail possum, AF357238)
Vombatus ursinus (common wombat, AJ304828)
Infraclass: Eutheria
Mus musculus (mouse, J01420)
Dasypus novemcinctus (nine banded armadillo, Y11832)
Lemur catta (ring-tailed lemur, AJ421451)
Equus caballus (horse, X79547)
Ceratotherium simum (white rhinoceros, Y07726)
Balaenoptera musculus (blue whale, X72204)
Bos taurus (cow, J01394)
Infraclass: Monotremata
Zaglossus bruijnii (long-beaked echidna, AJ639865)
Ornithorhynchus anatinus (platypus, X83427)
Tachyglossus aculeatus (short-beaked echidna, AJ303116)
Non-mammals
Corvus frugilegus (rook, Y18522)
Rhea americana (greater rhea, Y16884)
Gallus gallus (chicken, NC_001323)
Iguana iguana (common iguana, AJ278511)
Eumeces egregius (mole skink, AB016606)
Alligator mississippiensis (American alligator, Y13113)
Crocodylus caiman (spectacled caiman, AJ404872)
Chelonia mydas (green seaturtle, AB012104)

and Sánchez-Villagra, 2003; Nilsson et al., 2003; Asher et al., 2004).

The current study is based on phylogenetic analysis of complete mitochondrial (mt) genomes from species shown in Table 1. In addition to allowing analysis of marsupial inter and intra ordinal relationships based on complete mitochondrial genomes, the data set made it possible to estimate the times of various marsupial divergences and to examine these in the context of marsupial zoogeography and Gondwanan geology.

2. Materials and methods

The new sequences were established using polymerase chain reaction (PCR) methodology and primer walking (see Nilsson et al., 2003 for details). The study includes 19 marsupials, three monotremes, seven placental mammals and eight non-mammalian amniotes that were used to establish the root of the mammalian tree. Table 1 lists the species that were included in the analysis. The placental sampling was particularly aimed at including slowly evolving mt genomes and genomes connected to well-established palaeontological calibration points.

The phylogenetic analyses were carried out at both the amino acid (aa) and nucleotide (nt) levels. Nt sequences were analyzed using first and second codon positions (ignoring leucine transitions at first position) and coding third codon positions as R/Y (purines/pyrimidines). The analyses were based on 12 out of 13 mt protein-coding genes. The NADH6 gene which is located on the opposite strand relative to the other protein-coding genes, is not included as its nt composition deviates from that of the other 12 genes. Two data sets were used: one including mammals plus non-mammalian amniote taxa, the other including mammals only. After removing gaps and potentially non-homologous sites the size of the mammalian data set was 3459 aa (10,377 nt). The corresponding size of the amniote (mammalian+non-mammalian) data set was 3259 aa (9777 nt). Differences in aa and nt composition were examined using a χ^2-test, as implemented in the TREE-PUZZLE program package (Strimmer and von Haeseler, 1996). The compositional bias of the mammalian aa and nt (excluding 3rd codon positions) was not significant.

The aa sequences were analyzed using the mt-REV24 model (Adachi and Hasegawa, 1996), while the TN-93 model (Tamura and Nei, 1993) was applied to the nt sequences. Maximum likelihood (ML) analysis was performed using TREE-PUZZLE (Strimmer and von Haeseler, 1996), the MOLPHY package (Adachi and Hasegawa, 1996) and MrBayes 3.0 (Huelsenbeck and Ronquist, 2001). Maximum parsimony (MP) and neighbor joining (NJ) analyses were carried out as implemented in PHYLIP (Felsenstein, 1993). An exhaustive ML search constraining the 19 marsupial taxa into eight groups was performed in order to identify the best ML tree among the 135,135 possible rooted topologies. Confidence values were calculated by bootstrapping NJ and

MP analysis. The probability of the likelihood trees were evaluated applying the Shimodaira–Hasegawa test (pSH) as implemented in the PAL package (Drummond and Strimmer, 2001). Quartet-PUZZLE support values (QP) and Bayesian probability (Bp) values were also calculated. If not otherwise stated, likelihood analyses were based on aa sequences, assuming rate homogeneity. Rate heterogeneity models assuming eight classes for variable sites were applied to both aa and nt sequences, using the above mentioned models of sequence evolution.

Divergence times were estimated from branch lengths of the aa ML tree, taking rate differences into consideration and by a penalized log-likelihood method as implemented in the "r8s" program (Sanderson, 2002) version 1.60. Different smoothing factors and algorithms as well as the non-parametric rate smoothing method were tested on the data set.

3. Results and discussion

3.1. Some characteristics of the new sequences

The new mt genomes (see Table 1) essentially conform to those of other marsupials (Nilsson et al., 2003) and
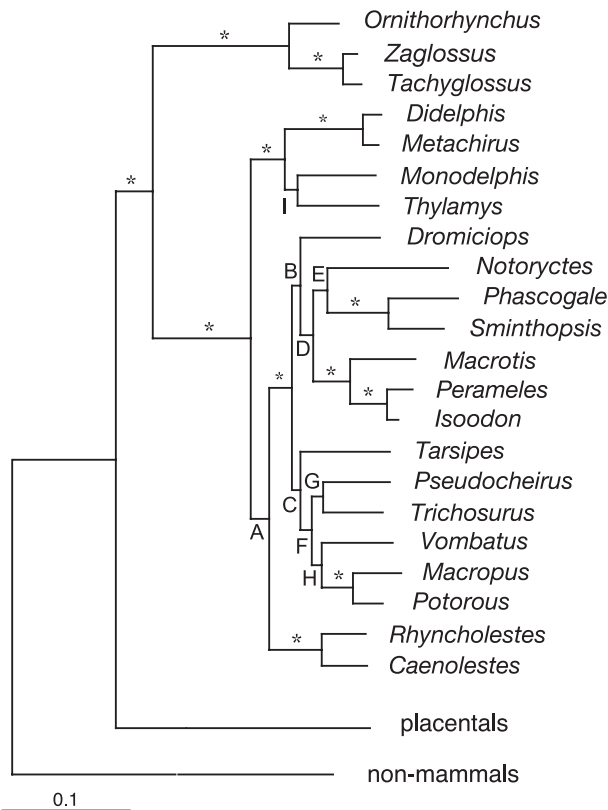

Fig. 1. Marsupial ML relationships with branch lengths based on the mammalian data set (3459 aa). The figure is a composite in that basal relationships among Marsupialia, Monotremata and Placentalia were established using the amniote data set (3259 aa). Clades that received 100% support by all methods are marked with a star (*). Support values of remaining branches (A-I) are shown in Table 1.

Table 2
Support values for branches A-I in Fig. 1

	LBP	Bprhom	Bprhet
A	73	100	61
B	90	100	100
C	93	100	100
D	95	100	57
E	73	100	<50
F	98	100	100
G	94	63	<50
H	59	<50	<50
I	64	100	56

Local bootstrap probability (LBP), Bayesian probability under the assumption of rate homogeneity (Bprhom), Bayesian probability under the assumption of rate heterogeneity using eight classes of variable sites calculated on 300,000 generations (aa) (Bprhet).

monotremes (Janke et al., 2002). However, the two species of the family Dasyuridae (order Dasyuromorphia) have an intergenic spacer between the genes for ATPase6 and COIII. This spacer, which is 65 nt long in *Sminthopsis douglasi* and 35 nt in *Phascogale tapoatafa*, has no counterpart in other mt sequences. The sequences do not resemble tRNA genes and do not fold into a stable secondary structure. The presumed pseudogenic tRNA-Lys of the South American brown four-eyed opossum (*Metachirus nudicaudatus*) can be folded into a structure similar to that of a functional tRNA (not shown), but such a structure does not occur in any Australian marsupial or the South American *Dromiciops*. It is noteworthy that the tRNA-Lys sequences in the two families Thylacomyidae (*Macrotis*) and Peramelidae (*Isoodon* and *Perameles*) of the Australian order Peramelemorphia are identical in spite of the depth of their divergence.

3.2. Phylogeny

The first step of the phylogenetic analysis was to root the mammalian tree using eight non-mammalian taxa. After this step, marsupial relationships were examined by rooting their tree with a collection of three monotremes and seven placental mammals. Both data sets resulted in the same best marsupial maximum-likelihood (ML) tree. The latter, mammalian, data set was 200 amino acids (aa) longer than the amniote data set, yielding increased support for most branches.

Fig. 1 shows marsupial relationships as obtained in ML analysis of the mammalian aa data set. Bayesian analysis of aa and nt sequences using rate homogeneity identified the same tree with high probabilities for ordinal branches. Resolution among some Diprotodontia lineages was limited in both rate homogeneity and rate heterogeneity analyses. Bayesian analysis with rate heterogeneity reduced the support for a sister group relationship between Didelphimorphia and remaining marsupials, and favoured a grouping of Notoryctemorphia and Peramelemorphia. Other deeper-level relationships were the same in rate homogeneity and rate heterogeneity analyses. This included the maximum support

Table 3
Statistical evaluation of 10 different topologies of marsupial interordinal relationships

Tree	Rate homogeniety				Rate heterogeniety		
	ΔlnL	S.E.	pBoot	pSH	ΔlnL	S.E.	pSH
1	0.00	0.00	0.27	1.00	0.67	±6.9	0.97
2	10.2	±14.4	0.10	0.82	0.00	±0.00	1.00
3	213.1	±37.1	0.00	0.00	108.71	±22.6	0.00
4	27.8	±12.3	0.00	0.60	14.96	±10.3	0.63
5	17.7	±13.9	0.06	0.79	12.02	±11.0	0.74
6	7.5	±24.1	0.23	0.84	5.13	±16.0	0.81
7	85.2	±38.0	0.01	0.06	59.09	±26.9	0.04
8	52.7	±36.4	0.07	0.29	41.36	±21.5	0.12
9	127.2	±40.7	0.00	0.005	69.01	±24.2	0.14
10	68.4	±20.0	0.00	0.09	38.18	±14.7	0.12

Differences in log-likelihood (ΔlnL), standard error (S.E.), bootstrap probability (pBoot), Shimodaira–Hasegawa probability (pSH).

for the branch joining the South American order Microbiotheria and the four Australian orders Notoryctemorphia, Dasyuromorphia, Peramelemorphia and Diprotodontia.

Table 2 shows ML and MrBayes support values for branches (A-I in Fig. 1), other than those receiving maximum support. Distance and maximum parsimony methods showed a tendency to place Notoryctemorphia and Dasyuromorphia in more basal positions in the marsupial tree than other methods. These trees are therefore not directly comparable with the tree in Fig. 1 and their values have not been included in Table 2. As evident in Fig. 1, the notoryctemorph and dasyuromorph branches are longer than those of other marsupials and it is possible that the faster evolutionary rates of these two lineages have affected their positions in the MP and distance analysis.

The mammalian data set was subjected to an exhaustive search after constraining the marsupial representation to eight operational taxonomic units (OTUs): Didelphimorphia, Paucituberculata, Microbiotheria, Notoryctemorphia, Dasyuromorphia, Peramelemorphia, *Tarsipes*, and Diproto-

dontia less *Tarsipes*. The exhaustive search yielded the same tree as shown in Fig. 1.

Table 3 shows the results of a comparison between the best ML trees and alternative relationships using different evolutionary models. The tested topologies are shown in Fig. 2. The positions of Didelphimorphia and Paucituberculata remained unresolved under the rate heterogeneity model with trees 1 and 2 (Fig. 2, Table 3) receiving virtually identical log-likelihood values. Monophyly of both South American and Australian marsupials was statistically rejected (tree 3) as was also the position of Microbiotheria as the sister group of all Australian marsupials (tree 4). A sister group relationship between Microbiotheria and Diprotodontia (tree 5) has been favoured in some previous analyses (Kirsch et al., 1997; Horovitz and Sánchez-Villagra, 2003), but that relationship was not supported by the current data set. As evident in Fig. 1, the branch (B) that joins Microbiotheria, Peramelemorphia, Notoryctemorphia and Dasyuromorphia is short as is also the Diprotodontia branch (C). As a result of this, a transfer of the basal diprotodont taxon *Tarsipes* into a position as the sister group of Microbiotheria is not significantly rejected (not shown). Similarly, a transfer of Dasyuromorphia onto the Microbiotheria branch (tree 6) results in a tree that is not distinctly worse than the best tree, suggesting a diversification within a narrow temporal window into Microbiotheria, Peramelemorphia, Notoryctemorphia and Dasyuromorphia. Distance and maximum parsimony methods show a tendency to place Notoryctemorphia and Dasyuromorphia near the Didelphimorphia. These topologies (trees 7 and 8) remained unsupported in ML analyses, however.

The position of Peramelemorphia has been contentious for a long time. Using a single plesiomorphic character, polyprotodont dentition, Peramelemorphia has been grouped with Dasyuromorphia (Owen, 1866), while studies heavily resting upon the shared character syndactyly (the
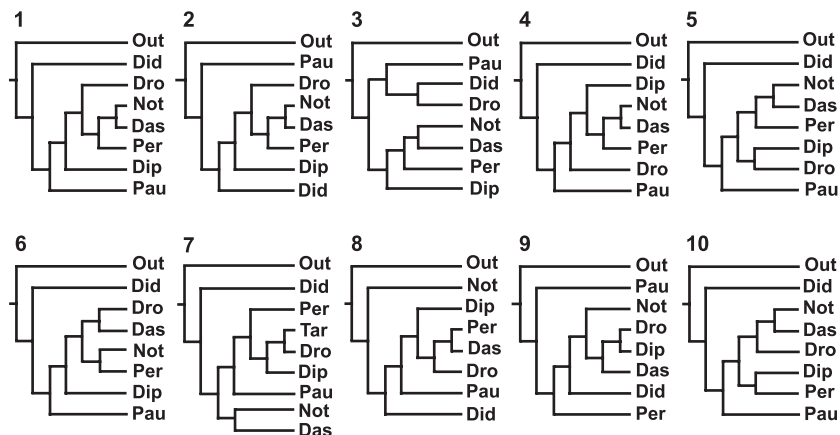

Fig. 2. Ten topologies that were statistically evaluated using the Shimodaira–Hasegawa test. The log-likelihood values and bootstrap probabilities are found in Table 3. Outgroup (Out; Eutheria and Monotremata), Didelphimorphia (Did), Paucituberculata (Pau), *Dromiciops* (Dro), Notoryctemorphia (Not), Dasyuromorphia (Das), Peramelemorphia (Per), Diprotodontia (Dip; excluding *Tarsipes* in tree 7), *Tarsipes rostratus* (Tar).

joining of the second and third digit of the hind feet) have joined Peramelemorphia and Diprotodontia (Bensley, 1903), or grouped them with Diprotodontia and Notoryctemorphia into the Syndactyla (Szalay, 1982, 1994). In a more recent morphological study, Peramelemorphia and Notoryctemorphia were identified as sister groups (Horovitz and Sánchez-Villagra, 2003). DNA/DNA hybridization analyses (Kirsch et al., 1997) have placed Peramelemorphia as the sister group of Didelphimorphia and all Australian marsupials plus Microbiotheria, while other molecular studies have favoured a sister group relationship between Paucituberculata and Peramelemorphia (Palma and Spotorno, 1999). The current analyses identified a sister group relationship between Dasyuromorphia and Notoryctemorphia (branch E in Fig. 1 and Table 1) and between Dasyuromorphia/ Notoryctemorphia and Peramelemorphia (branch D). The DNA/DNA hybridization findings (Kirsch et al., 1997) (tree 9) are statistically rejected by the mitogenomic data set. Similarly, the results are inconsistent with the syndactyly tree, i.e., Peramelemorphia and Diprotodontia as sister groups (tree 10).

In addition to providing information on relationships among marsupial orders, the current study has allowed examination of some intraordinal divergences. The Diprotodontia is the most species rich marsupial order with some 120 living species. The order is commonly divided into two suborders, Vombatiformes and Phalangerida. The current diprotodont representation includes one vombatiform, the wombat, *Vombatus ursinus*, and five phalangerids. The analyses identified a basal diprotodont split between the phalangerid honey possum, *Tarsipes rostratus*, and the remaining species (wombat and phalangerids other than *Tarsipes*). The branch (F) separating the honey possum and remaining diprotodonts received strong support (Table 1). The resolution among the common ringtail possum, the brush-tail possum, the wombat and the Macropodoidea (*Macropus* and *Potorous*) is limited, suggesting that these taxa diverged within a narrow temporal window. However, the strong support for branch F, which separates the honey possum from the other phalangerids and the wombat, challenges the traditional Diprotodontia division into Vombatiformes and Phalangerida.

3.3. Divergence times

The current taxon sampling has allowed a more detailed examination of the times of various marsupial divergences than was previously possible. The times of marsupial divergences were calculated using three calibration points, (i) A/C-60: the split between ruminant artiodactyls and cetaceans (actually Cetacea/Hippopotamidae) set at 60 million years before present (MYBP) (Arnason and Gullberg, 1996; Arnason et al., 1996); (ii) E/R-50: the intraordinal perissodactyl split between Equidae and Rhinocerotidae set at 50 MYBP (Arnason et al., 1998), and (iii) the time of the split between Metatheria and Eutheria set at

135 MYBP. The solid palaeontological record behind both A/C-60 and E/R-50 has led to their common acceptance for estimating the times of various mammalian divergences, while M/E-135, which yields datings consistent with both A/C-60 and E/R-50, is palaeontologically supported by the age, ≈ 125 MY, of the oldest eutherian fossil described to date (Ji et al., 2002) and with the age, ≈ 125 MY, of the recently described oldest marsupial fossil, *Sinodelphys szalayi* (Luo et al., 2003). As many of the early fossil marsupials are of uncertain taxonomic status (for detailed discussion, see Wroe et al., 2000), these have not been included as calibration points. Changing the rate smoothing method (penalized likelihood or non-parametric) in the r8s-program had only limited effect on the estimates of divergence times. The divergence times differed by less than 5–10%, which is within the range of the accompanying confidence intervals. The divergence times as estimated by the r8s-program were also similar to those based on distances, taking into account rate variation among lineages (see Arnason et al., 1998).

The estimated times of various marsupial divergences and their confidence intervals are shown in Fig. 3. The calculations placed the basal marsupial divergence, i.e., the split between Didelphimorphia and remaining orders, at 69 MYBP, slightly earlier than estimated in a recent mitogenomic study (Nilsson et al., 2003). All other divergences were dated to Tertiary times. The origin of Australidelphia and the intra-Australidelphia divergence between Diprotodontia and remaining Australidelphia orders were close to the Paleocene/Eocene boundary, while many of the deepest intraordinal divergences among Australian marsupials fell in the period from Middle Eocene to Early Oligocene. The split between the South American *Dromiciops gliroides* (Microbiotheria) and its closest Australian relatives was dated to 46 MYBP. The molecular estimates placed the split between *Macropus* and *Potorous* ≈ 20 MYBP (17–24), in agreement with the palaeontological date (23 MYBP) of this divergence (Woodburne et al., 1993). The consistency between the molecular estimate and the palaeontological age of this divergence, strengthens the confidence in the molecular estimates of the remaining marsupial divergences.

It is generally assumed that Gondwanan marsupials are the descendants of North American marsupials, despite the fundamental morphological differences between the two groups (Muizon et al., 1997). The 75 MY South American Los Alamitos fauna contains neither marsupials nor eutherians (Flynn and Wyss, 1998). The earliest South American marsupial fossil localities are Tiupampa (64.5–63 MYBP) and Punta Peligro (62.5–61 MYBP) (Muizon et al., 1997; Pascual and Ortiz Jaureguizar, 1990; Bonaparte et al., 1993). The contents of the Los Alamitos fauna suggest that the South American marsupials are younger than 75 MY. It is thus conceivable that the marsupial colonization of South America took place between 75 and 65 MYBP.

Mitogenomic analyses place most ordinal eutherian divergences in pre-Cretaceous–Tertiary (K/T) times (Arna-
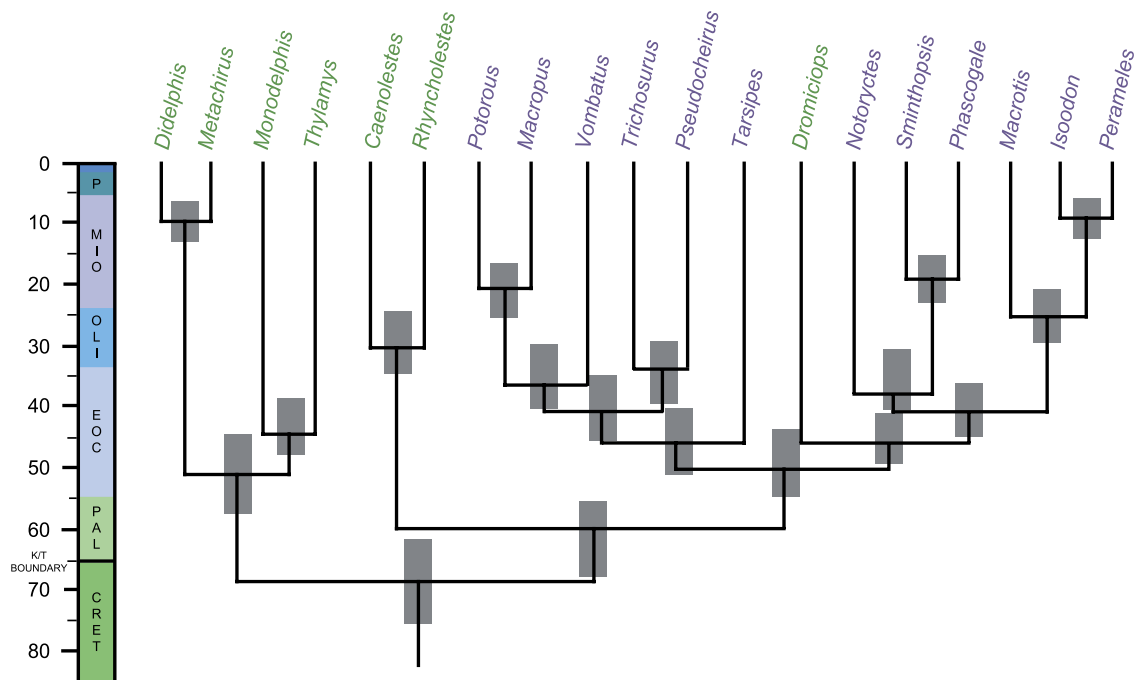
Fig. 3. Marsupial divergence times. The branch lengths have been scaled in proportion to divergence times. Shaded bars indicate a confidence interval of 1 S.D. Green names refer to South American species and blue to Australian species. Cretaceous (Cret), Palaeocene (Pal), Eocene (Eoc), Oligocene (Oli), Miocene (Mio), Pliocene (P).

son et al., 2002; Arnason and Janke, 2002). In comparison, the current estimates place the deepest divergence among extant marsupials, that between Didelphimorphia and remaining orders, at 69 MYBP, with all other ordinal divergences falling in the Tertiary. The split between Paucituberculata and remaining marsupials (the four Australian orders plus Microbiotheria) was placed at ≈ 60 MYBP, a dating that is consistent with the appearance of Paucituberculata 58 MYBP and the age, 55 MY, of the oldest Australian marsupial fossils (Godthelp et al., 1992). However, at this date morphological characters diagnostic for extant Australidelphia orders had not yet developed (Godthelp et al., 1999).

The split between the Australian order Diprotodontia and the branch including the South American order Microbiotheria (Dromiciops) and the Australian orders Peramelemorphia, Dasyuromorphia and Notoryctemorphia was dated to 50 MYBP, while the basal diprotodont divergence, that between Tarsipes and the remaining members of the order, was dated to 46 MYBP. The same estimate was obtained for the split between Dromiciops and its Australian relatives (Peramelemorphia, Dasyuromorphia and Notoryctemorphia). Antarctica and Australia were geologically connected until ≈ 45 MYBP (Li and Powell, 2001). Thus, the molecular dating of the divergence coincides with the separation of Australia from Antarctica, suggesting that the divergence between Dromiciops and its Australian relatives was vicariance related and the probable result of the geological separation of Australia and Antarctica. In the context of the current estimates, it is of interest that the

dating of the divergence between Microbiotheria and Peramelemorphia/Notoryctemorphia/Dasyuromorphia coincides with a previous molecular dating (45 MYBP) of the divergence between the South American rhea and the Australian cassowary (Härlid et al., 1998).

The cooling of Earth's climate during the Cenozoic has been related to the effects of continental drift and changes in oceanic circulation. The climatic change following the Eocene/Oligocene transition has been well documented in the terrestrial mammalian faunas of North America, Europe and Asia and is known as "La Grande Coupure" (Stehlin, 1909). It may also have affected the rapid diversification of modern whales 35–30 MYBP as seen by both molecular (Arnason et al., 2004) and palaeontological findings (Fordyce and de Muizon, 2001). The gaps in the faunal record of South America (Flynn and Wyss, 1998; Flynn et al., 2003) and Australia (Archer et al., 1999) have not allowed a comparison between climate and fauna in these regions, similar to that in the Northern Hemisphere. There are, nevertheless, indications that the Australian flora changed markedly following the Eocene/Oligocene transition and a recently described fossil assemblage from South America suggests a swift climatic change in the Southern Hemisphere (Flynn et al., 2003). It is not possible, however, to decide whether climatic change, geographical isolation or a combination of these factors had the primary effect on marsupial evolution. "La Grande Coupure" coincides with several of the divergences within Diprotodontia and the divergence between the two extant genera of the South American Paucituberculata. It would therefore be of interest to examine if a more extensive

taxon sampling would show additional divergences that would coincide with this climatic change. It is possible that the long branches leading to some Australian marsupial groups are the result of elimination of lineages, which, in a more stable environment, might have left a mark in marsupial palaeontology and the marsupial molecular tree.

Acknowledgements

References

Adachi, J., Hasegawa, M., 1996. MOLPHY Version 2.3: Programs for molecular phylogenetics based on maximum likelihood. Comp. Sci. Monogr. vol. 28. Inst. Stat. Mathematics, Tokyo, pp. 1–150.

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.

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

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.

Arnason, U., Gullberg, A., 1996. Cytochrome *b* nucleotide sequences and the identification of five primary lineages of extant cetaceans. Mol. Biol. Evol. 13, 407–417.

Arnason, U., Janke, A., 2002. Mitogenomic analyses of eutherian relationships. Cytogenet. Genome Res. 96, 20–32.

Arnason, U., Gullberg, A., Janke, A., Xu, X., 1996. Pattern and timing of evolutionary divergences among hominoids based on analyses of complete mtDNAs. J. Mol. Evol. 43, 650–661.

Arnason, U., Gullberg, A., Janke, A., 1998. Molecular timing of primate divergences as estimated by two non-primate calibration points. J. Mol. Evol. 47, 718–727.

Arnason, U., Adegoke, J.A., Bodin, K., Born, E.W., Esa, Y.B., Gullberg, A., Nilsson, M., Short, R.V., Xu, X., Janke, A., 2002. Mammalian mitogenomic relationships and the root of the eutherian tree. Proc. Natl. Acad. Sci. U. S. A. 99, 8151–8156.

Arnason, U., Gullberg, A., Janke, A., 2004. Mitogenomic analyses provide new insights into cetacean origin and evolution. Gene 333, 27–34.

Asher, R.J., Horovitz, I., Sánchez-Villagra, M.R., 2004. First combined cladistic analysis of marsupial mammal interrelationships. Mol. Phylogenet. Evol. 33, 240–250.

Bensley, B.A., 1903. On the evolution of the Australian Marsupialia; with remarks on the relationships of the marsupials in general. Trans. Linn. Soc. London 9, 83–214.

Bonaparte, J.F., Van Valen, L.M., Kramartz, A., 1993. La fauna local de Punta Peligro, Paleoceno Inferior, de la Provincia del Chubut, Patagonia, Argentina. Evol. Monogr. 14, 1–61.

Drummond, A., Strimmer, K., 2001. PAL: an object-oriented programming library for molecular evolution and phylogenetics. Bioinformatics 17, 662–663.

Felsenstein, J., 1993. Phylogenetic Inference Programs (PHYLIP), University of Washington and University Herbarium, University of California, Seattle WA/Berkeley, CA.

Flynn, J.J., Wyss, A.E., 1998. Recent advances in South American mammalian paleontology. Trends Ecol. Evol. 13, 449–454.

Flynn, J.J., Wyss, A.R., Croft, D.A., Charrier, R., 2003. The Tinguiririca fauna, Chile: biochronology, paleoecology, biogeograhy, and a new earliest Oligocene South American land mammal age. Palaeogeogr. Palaeoclimatol. Palaeoecol. 195, 229–259.

Fordyce, E., de Muizon, C., 2001. Evolutionary history of cetaceans: a review. In: Mazin, J.-M., de Buffrénil, V. (Eds.), Secondary adaptation of tetrapods to life in water. Verlag Dr. Friedrich Pfeil, Munich, pp. 169–233.

Godthelp, H., Archer, M., Cifelli, R., Hand, S.J., Gilkeson, C.F., 1992. Earliest known Australian Tertiary mammal fauna. Nature 356, 514–516.

Godthelp, H., Wroe, S., Archer, M.A., 1999. New marsupial from the early Eocene Tingamarra local fauna of Murgon, Southeastern Queensland: a prototypical Australian marsupial? J. Mamm. Evol. 6, 289–313.

Härlid, A., Janke, A., Arnason, U., 1998. The complete mitochondrial genome of *Rhea americana* and early avian divergences. J. Mol. Evol. 46, 669–679.

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

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

Janke, A., Magnell, O., Wiezcorek, G., Westerman, M., Arnason, U., 2002. Phylogenetic analysis of 18S rDNA and mitochondrial genomes of the wombat, *Vombatus ursinus*, and the spiny anteater, *Tachyglossus aculeatus*: increased support for the Marsupionta hypothesis. J. Mol. Evol. 54, 71–80.

Ji, Q., Luo, Z., Yuan, C., Wible, J.R., Zhang, J., Georgi, J.A., 2002. The earliest known eutherian mammal. Nature 416, 816–822.

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.

Li, Z.X., Powell, C.McA., 2001. An outline of the palaeogeographic evolution of the Australasian region since the beginning of the Neoproterozoic. Earth-Sci. Rev. 53, 237–277.

Luo, Z., Ji, Q., Wible, J.R., Yuan, C., 2003. An early cretaceous tribosphenic mammal and metatherian evolution. Science 302, 1934–1939.

Muizon, C., Cifelli, R.L., Paz, R.C., 1997. The origin of the dog-like borhyaenoid marsupials of South America. Nature 389, 486–489.

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

Owen, R., 1866. On the anatomy of vertebrates vol. II. Longmans, Green, London.

Palma, R.E., Spotorno, A.E., 1999. Molecular systematics of marsupials based on the rRNA 12S mitochondrial gene: the phylogeny of didelphimorphia and of the living fossil microbiotheriid *Dromiciops gliroides* Thomas. Mol. Phylogenet. Evol. 13, 525–535.

Pascual, R., Ortiz Jaureguizar, E., 1990. Evolving climates and mammal faunas in Cenozoic South America. J. Hum. Evol. 19, 23–60.

Phillips, M.J., Lin, Y.H., Harrison, G.L., Penny, D., 2001. Mitochondrial genomes of a bandicoot and a brushtail possum confirm the monophyly of australidelphian marsupials. Proc. R. Soc. Lond., B 268, 1533–1538.

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

Springer, M.S., Westerman, M., Kavanagh, J.R., Burke, A., Woodburne, M.O., Kao, D.J., Krajewski, C., 1998. The origin of the Australasian marsupial fauna and the phylogenetics affinities of the enigmatic Monito del Monte and marsupial mole. Proc. R. Soc. Lond., B 265, 2381–2386.

Stehlin, H.G., 1909. Remarque sur les faunules de mammiféres des couches éocènes et oligocènes du bassin de Paris. Bull. Soc. Géol. Fr. 9, 488–520.

Strimmer, K., von Haeseler, A., 1996. Quartet puzzling: a quartet maximum likelihood method for reconstructing tree topologies. Mol. Biol. Evol. 13, 964–969.

Szalay, F.S., 1982. A new appraisal of marsupial phylogeny and classification. In: Archer, M. (Ed.), Carnivorous marsupials. Roy. Zool. Soc., Sydney, pp. 621–640.

Szalay, F.S., 1994. Evolutionary history of the marsupials and an analysis of osteological characters. Cambridge University Press, New York.

Tamura, K., Nei, M., 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol. 10, 512–526.

Woodburne, M.O., McFadden, 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.

Wroe, S., Ebach, M., Ahyong, S., de Muizon, C., Muirhead, J., 2000. Cladistic analysis of dasyuromorphian (Marsupialia) phylogeny using cranial and dental data. J. Mammal 81 (4), 1008–1024.