Molecular Phylogenetics and Evolution 62 (2012) 97–108

Contents lists available at SciVerse ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Phylogenetic relationships of living and recently extinct bandicoots based on nuclear and mitochondrial DNA sequences

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ARTICLE INFO

Article history:
Received 4 November 2010
Revised 6 September 2011
Accepted 12 September 2011
Available online 11 November 2011

Keywords:
Marsupial
Bandicoot
Peramelemorphia
Phylogeny

ABSTRACT

Bandicoots (Peramelemorphia) are a major order of australidelphian marsupials, which despite a fossil record spanning at least the past 25 million years and a pandemic Australasian range, remain poorly understood in terms of their evolutionary relationships. Many living peramelemorphians are critically endangered, making this group an important focus for biological and conservation research. To establish a phylogenetic framework for the group, we compiled a concatenated alignment of nuclear and mitochondrial DNA sequences, comprising representatives of most living and recently extinct species. Our analysis confirmed the currently recognised deep split between *Macrotis* (Thylacomyidae), *Chaeropus* (Chaeropodidae) and all other living bandicoots (Peramelidae). The mainly New Guinean rainforest peramelids were returned as the sister clade of Australian dry-country species. The wholly New Guinean Peroryctinae was sister to Echymiperinae. The poorly known and perhaps recently extinct Seram Bandicoot (*Rhynchomeles*) is sister to Echymipera. Estimates of divergence times from relaxed-clock Bayesian methods suggest that living bandicoots originated in the late Oligocene or early Miocene, much earlier than currently thought based on fossils. Subsequent radiations within Peramelemorphia probably took place on the Australian mainland during the Miocene, with diversification of rainforest taxa on the newly emergent New Guinean landmasses through the middle-late Miocene and complete establishment of modern lineages by the early Pliocene.

1. Introduction

Although the evolutionary relationships of Peramelemorphia (commonly known as bandicoots) within Marsupialia have been clarified by recent molecular studies (Meredith et al., 2008), the taxonomy of its various genera and species is still confused. The 23 species of living or recently extinct bandicoots are currently classified into three families (Groves, 2005); Chaeropodidae, Thylacomyidae and Peramelidae. Peramelidae is the most diverse, comprising three subfamilies – the mainly New Guinean forest-dwelling Echymiperinae and Peroryctinae, and the largely Australian, dry-country Peramelinae. Echymiperinae contains *Echymipera*, *Rhynchomeles* and *Microperoryctes*; Peroryctinae includes only *Peroryctes*; Peramelinae accommodates *Isoodon* and *Perameles*.

In their concept of peramelemorphian relationships based on morphology, Groves and Flannery (1990) placed the endemic New Guinean *Peroryctes* together with *Microperoryctes*, *Echymipera* and *Rhynchomeles* in a distinct family (Peroryctidae) under the caveat that "…. three features… in which *Peroryctes* resembles the *Echymipera* clade… are most likely the result of convergence" (Groves and Flannery, 1990, p. 6). Since then, the broader genus-level evolution of these taxa has been studied using mitochondrial (mtDNA) and nuclear (nDNA) gene sequences (Westerman et al., 1999, 2001; Meredith et al., 2008). In contrast, comparatively little attention has been paid to peroryctin and echymiperin species, in part due to the inherent difficulty of obtaining useable tissue samples for DNA extraction. For example no tissues and few museum samples are presently available for the Mouse Bandicoot (*Microperoryctes murina*) or the recently described *Echymipera davidi* and *Echymipera echinista*. A similar problem exists for the enigmatic *Rhynchomeles prattorum*, which is known from a total of seven specimens collected in 1920 from the Mt. Mansuela area of central Seram. This island taxon has not been captured or seen since that time and may be extinct (Flannery, 1995). Tate (1948) and Groves and Flannery (1990) aligned *Rhynchomeles* with *Echymipera*, the latter citing several derived morphological characters uniting them.

Species identity, relationships and distributions within *Microperoryctes* are unclear. In addition to the rare *M. murina* (known only

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doi:10.1016/j.ympev.2011.09.009

from three specimens from the Weyland Range of West Papua), *Microperoryctes longicauda* and *Microperoryctes papuensis* are also recognised. Helgen and Flannery (2004) recently described a new form (*Microperoryctes aplini*) from the Vogelkop Peninsula of New Guinea and advocated the separation of *Microperoryctes ornata*, from *M. longicauda*.

The Giant Bandicoot *Peroryctes broadbenti* is the largest of all living bandicoots, weighing up to 5 kg, and is restricted to hill forests on the southeastern peninsula of Papua New Guinea. This bandicoot has been variously treated as a subspecies of the more common and widespread *Peroryctes raffrayana* (Tate, 1948; Laurie and Hill, 1954) or as a distinct species (Ziegler, 1977; George and Maynes, 1990; Flannery, 1995). Aplin et al. (2010) recently documented its distribution and morphological characters.

The primarily Australian genera *Perameles* and *Isoodon* are readily distinguishable using morphological criteria (e.g. Tate, 1948), but taxonomic boundaries within each are poorly defined. Currently three species of *Isoodon* are recognised – *I. auratus*, *I. macrourus* and *I. obesulus* – although up to ten have been established in the past (Westerman and Krajewski, 2001). Most of these geographically defined forms are regarded as subspecies (Strahan, 1995), but recent molecular work suggests that some (e.g. *I. o. peninsulae*) may warrant species status (Close et al., 1990; Pope et al., 2001). On the other hand, Pope et al. (2001) and Zenger et al. (2005) suggested that *Isoodon auratus* might be subordinate to *Isoodon obesulus* based on variation in a ~500 nucleotide mitochondrial D-Loop dataset.

Four species of *Perameles* are presently considered valid (*P. bougainville*, *P. eremiana*, *P. gunnii* and *P. nasuta*) one of which (*P. eremiana*) is recently extinct. *P. eremiana* has sometimes been considered as a sub-specific form of *Perameles bougainville* (Strahan, 1995) which itself, though formerly widespread with two or more distinct subspecies (Friend, 1990), is now restricted to a few localities in Shark Bay (Western Australia).

This paper provides the first concatenated nuclear and mitochondrial gene-sequence dataset designed to evaluate current evolutionary/biogeographical hypotheses relating to species within Peramelemorphia.

2. Materials and methods

2.1. Taxon sampling

We obtained tissue samples (Table 1) from most living bandicoot species together with the recently extinct taxa *R. prattorum*, *Perameles eremiana*, and *Chaeropus ecaudatus* (see Westerman et al., 1999; Meredith et al., 2008 for tissue source details on *C. ecaudatus*). An ear clip from *P. broadbenti* captured by hunters near Ajoa, New Guinea was stored in 95% ethanol after collection and prior to extraction. Dried muscle and connective tissue were obtained from the cranium of the *P. broadbenti* holotype (AM A3238), a scraping from a museum skin of *Rhynchomeles* (AM M29415), and a specimen of *P. eremiana* (C5864) from the National Museum of Victoria. For some geographically widespread species of *Peroryctes*, *Echymipera* and *Microperoryctes*, we utilised multiple exemplars from across the species' ranges in order to assess genetic variation. The four rare echymiperins *E. davidi* (Flannery, 1990), *E. echinista*, *Microperoryctes longicauda* (*sensu* Helgen and Flannery, 2004) and *M. murina* proved difficult to source and were therefore not included in the study (see Table 1). However, we did include a *Microperoryctes* specimen captured at Tembagapura (Snow Mts, West Papua) which may represent a novel species (K. Helgen, pers. commun.). We here note that animals identified as *M. longicauda* in previous studies (Westerman et al., 2001; Meredith et al., 2008) have since been referred to *M. ornata* as defined by Helgen and Flannery (2004); *M. longicauda* (*sensu* Helgen and Flannery, 2004) is thus restricted to specimens from the Vogelkop Peninsula.

2.2. Molecular protocols and dataset assembly

Tissues were placed in 1.5 ml microfuge tubes with 0.4 ml 1 mM Tris-EDTA, to which 40 μl of 10% SDS was added. Following total digestion with proteinase K at 55 °C, the solution was shaken with chloroform: isoamyl alcohol (24:1) and centrifuged to separate aqueous and organic layers, DNA was precipitated from the aqueous phase with 2.5 volumes of ice-cold absolute ethanol. The precipitate was dried and resuspended in 1 mM Tris-EDTA and digested for 1 h with RNAse, then with Proteinase K for a further hour before re-extraction as above. Final DNA precipitates were resuspended in a small volume of 1 mM Tris, 1 mM EDTA. PCR amplification, direct sequencing, and sequence alignment procedures were as detailed in Krajewski et al. (1997), Burk et al. (1998), and Westerman et al. (1999). The nuclear protein coding genes ApoB, BRCA1, IRBP, RAG1, vWF have consistently been shown to resolve marsupial intergeneric relationships (Meredith et al., 2009) so these were sequenced along with the mitochondrial genes (12S rRNA, cytochrome *b* and the 3′ portion of 16S rRNA) from all taxa following the methodologies of Meredith et al. (2008) and Westerman et al. (2001), respectively.

Ingroup monophyly and the phylogenetic position of Peramelemorphia relative to various outgroup marsupial lineages has been demonstrated by Westerman et al. (2001) and Meredith et al. (2008). Consequently, except for molecular dating analyses, we employed only a limited number of outgroup taxa all from Dasyuromorphia (species of *Antechinus*, *Dasyurus*, *Phascogale*, *Phascolosorex*, *Planigale Myrmecobius* and *Thylacinus*). We constructed a dataset of 9979 nucleotides for representatives of each available bandicoot species incorporating five nuclear (6137 nucleotides) and three mitochondrial genes sequences (~3600 nucleotides representing almost 23% of the mitochondrial genome). 12S rRNA sequences (958 nucleotides) from a much larger sampling of *Microperoryctes* and *Peroryctes* as well as from a number of the available subspecies of *Isoodon* and *Perameles* were included in order to cover as much of their species' ranges as possible. Sequences were aligned as described in Meredith et al. (2008); protein-coding genes were conceptually translated to check for premature stop codons, frameshifts and indels. No indications of pseudogene amplifications were found. Alignments of incomplete sequences were filled out with the missing data code ("?") for phylogenetic analyses.

2.3. Phylogenetic analyses and statistical tests

Our rationale for combining nuclear genes in phylogenies of marsupials has been dealt with elsewhere (Meredith et al., 2010). Maximum parsimony (MP) was implemented with TNT (Goloboff et al., 2008) distributed by the Willi Hennig Society in order to generate bootstrap support values for 1000 pseudoreplicates of the large dataset. Maximum likelihood (ML) and Bayesian analyses were performed with RAxML 7.2.8 (Stamatakis, 2006), and MrBayes 3.1.2 (Huelsenbeck and Rondquist, 2001; Rondquist and Huelsenbeck, 2003), respectively. ML and Bayesian analyses were performed with unpartitioned and partitioned datasets. In the latter case we allowed each of seven partitions (ApoB, BRCA1, IRBP, RAG1, vWF, cytochrome b, mt rRNA) to have its own model of sequence evolution. Partitioned RAxML analyses employed a GTR + Γ model for each partition. Best-fit models of molecular evolution for Bayesian analyses were chosen using the Aikake Information Criterion as implemented by jModelTest (Posada, 2008) and were as follows: GTR + Γ (ApoB, BRCA1, RAG1, cytochrome b, mt rRNA); HKY + Γ (IRBP); and SYM + Γ (vWF). Support for nodes on

Table 1
Habitats and distributions of bandicoot species included in the study. Information on the five rare/extinct species not included in the study is provided for completeness.

Taxa	Included in study	Habitat (current/past)	Distribution (current/past)	Altitudinal range (in metres)
Chaeropus ecaudatus[a]	Yes	Arid/semi-arid	Australia	Lowland
Macrotis lagotis	Yes	Arid/semi-arid	Australia	Lowland
Peroryctes broadbenti	Yes	Closed forest	New Guinea	Lowland
P. raffrayana	Yes	Closed forest	New Guinea	0–4000
Microperoyctes aplini	Yes	Closed forest	New Guinea (Arfak Mts)	1900–2200
M. ornata	Yes	Closed forest	New Guinea	1000–3600
M. papuensis	Yes	Closed forest	New Guinea	1200–2650
Echymipera clara	Yes	Closed forest	New Guinea	0–1700
E. kalubu	Yes	Closed forest	New Guinea	0–1200
E. rufescens	Yes	Closed forest	New Guinea and Australia	0–2100
Rhynchomeles prattorum[a]	Yes	Closed forest	West Papua (Seram)	1800
Isoodon auratus	Yes	Arid/semi–arid	Australia	0–1200
I. macrourus	Yes	Open grassland/woodland/open forest	Australia and New Guinea	0–1200
I. obesulus	Yes	Heathland/woodland/open forest	Australia	0–1200
I. peninsulae	Yes	Woodland/open forest	Australia (Cape York Peninsula)	0–1200
Perameles bougainville	Yes	Semi-arid	Australia	Lowland
P. eremiana	Yes	Arid/semi-arid	Australia	Lowland
P. gunnii	Yes	Grassland	Australia	Lowland
P. nasuta	Yes	Heathland/open forest	Australia	Lowland
Rare/extinct taxa NOT included				
Macrotis leucura[a]	No	Arid/semi-arid	Australia	Lowland
Microperoryctes longicaudata	No	Closed forest	New Guinea (Arfak Mts)	1900–2200
M. murina	No	Closed forest	New Guinea (Weyland Range)	2500
Echymipera davidi	No	Closed forest	New Guinea (Kiriwina Is.)	Lowland
E. echinista	No	Closed forest	New Guinea (Trans Fly Plains)	Lowland

[a] Presumed extinct.

estimated trees was ascertained with 1000 bootstrap pseudoreplicates for MP and 500 pseudoreplicates for RAxML analyses. Bayesian analysis utilised random starting trees and two runs to assess chain convergence on the same posterior probability distribution. Chains were run for six million generations with a burnin of 1.5 million generations, and were sampled every 1000 generations.

The AU (approximately unbiased; Shimodaira, 2002), SH (Shimodaira–Hasegawa; Shimodaira and Hasegawa, 1999), and KH (Kishino–Hasegawa; Kishino and Hasegawa, 1989) tests implemented in Consel (Shimodaira, 2002; Shimodaira and Hasegawa, 2001) were used to evaluate alternative phylogenetic hypotheses. These included whether (1) *Rhynchomeles* is sister to *E. clara* (Tate, 1948), (2) *Isoodon peninsulae* is sister to *I. obesulus*, (3) *C. ecaudatus* is most closely related to *Perameles* (Muirhead, 1994), and (4) *Chaeropus* + *Macrotis* are sister to *Isoodon* + *Perameles* (Groves and Flannery, 1990).

2.4. Timetree analyses

Timetree analyses were performed with BEAST 1.6.1 (Drummond et al., 2006; Drummond and Rambaut, 2007) with the uncorrelated lognormal relaxed clock model. We selected multiple diprotodontian and dasyuromorphian outgroups for which fossils are known (Supplementary Table S1). Minimum and maximum constraints were assigned to 12 nodes based on phylogenetic bracketing and stratigraphic bounding as discussed by Meredith et al. (2010) and Springer et al. (2011) (Supplementary Table S2). Minimum and maximum constraints were soft-bounded and were modelled with a normal prior distribution that assigned 95% of the prior to the interval between the minimum and maximum and 2.5% of the prior to each tail. We assigned a Gamma prior (shape = 1, scale = 1) to the "ucld.mean" parameter for each partition. MCMC analyses were run for 100 million generations with a burnin of 20 million generations and sampling every 10,000 generations. ESS values were greater than 200 for all estimated parameters. We used TreeAnnotator 1.6.1 from the BEAST package to summarise the sample of trees generated by BEAST. Sampled trees were summarised with mean node heights. The ML tree from the partitioned RAxML analysis was used as a target tree.

2.5. Evolution of geographic ranges in bandicoots

Geographic ranges and habitat preferences of current and recently extinct bandicoot species are shown in Table 1. Areas of origin of species and clades were determined using both minimum area change (MAC) parsimony (Springer et al., 2011) and dispersal–extinction–cladogenesis (DEC) (Ree and Smith (2008). The former was performed with Mesquite (Maddison and Maddison, 2010) in conjunction with a step matrix that allowed for a maximum occupancy of four areas (Australia, New Guinea, Seram Island, Tasmania). DEC is a likelihood-based method and was performed on the same four areas. By contrast with MAC parsimony, DEC takes advantage of branch lengths in reconstructing ancestral areas.

3. Results

3.1. Sequences from museum tissues

Preserved museum tissues (other than those of *C. ecaudatus*) tended to yield only partial mitochondrial DNA sequences (12S rRNA from most specimens, some/all of cytochrome *b*, and ~580 nucleotides from the 3′ portion of 16S rRNA). From *R. prattorum* and *M. aplini*, as well as from three New Guinean and Australian species with large geographic ranges, we obtained sequences from the 12S rRNA gene (*R. prattorum*, *M. aplini*) or 12S gene plus some/all sequences for cytochrome *b* and ~580 nucleotides from the 3′ portion of 16S rRNA gene (see Supplementary Table S1). Unfortunately, no sequence data were obtainable for the Lesser Bilby (*M. leucura*), which is probably extinct; only complete 12S rRNA, partial 16S rRNA and partial RAG1 nuclear gene sequences were obtained for *C. ecaudatus* and only 12S rRNA, partial 16SrRNA and partial cytb for *E. rufescens* from Yuro (Supplementary Table S1).

3.2. Molecular phylogenetic structure of peramelemorphia

Meredith et al. (2008) noted the occurrence of a number of synapomorphic insertions or deletions (indels) in peramelemorphian nuclear genes that support monophyly of this order. However, this earlier work was limited in that it included only a single exemplar

from each genus; the current analysis incorporates representatives of most available species. We confirm the characterisation of Peramelemorphia by three deletions in the BRCA1 gene relative to other marsupials (positions 481–495, 1720–1728 and 2029–2031). Other unique BRCA1 indels include: 9 bp and 3 bp inserts in Thylacomyidae (positions 832–840 and 2278–2280); a 3 bp deletion in Peramelidae (2227–2229); an expanded 9 bp deletion across the same region in *Microperoryctes* (2224–2232); a 6 bp deletion in *Isoodon* (1072–1077); a 6 bp insert in Peramelinae (2188–2193); a 15 bp deletion in *Peroryctes* (2323–2337); and a 15 bp insertion in *P. bougainville* (2053–2064). We also discovered two deletions (6 bp and 9 bp respectively) in the vWF gene of *Peroryctes* (positions 570–575 and 589–597).

Previous studies using limited taxon coverage and either mitochondrial or nuclear gene sequences alone (Westerman et al., 1999, 2001; Meredith et al., 2008), showed monophyly of Peramelemorphia and suggested a basal split between Thylacomyidae and Peramelidae. The relationships of *Chaeropus* to other bandicoots was not clear; mitochondrial 12S rRNA sequences suggest it was sister to all other bandicoots (Westerman et al., 1999, 2001), nuclear RAG1 sequences suggest that it is sister to Peramelidae (Meredith et al., 2008), though neither had very strong bootstrap support. Our expanded Bayesian analyses of the concatenated nuclear plus mitochondrial data matrix yielded a well-resolved tree (Fig. 1) in which *C. ecaudatus* (Chaeropodidae) is sister to *Macrotis lagotis* (Thylacomyidae) plus Peramelidae. Table 2 shows the bootstrap support values for various nodes in the tree for each of the analyses. AU tests implemented in Consel (Shimodaira, 2002) showed no support for the earlier suggestions that *Chaeropus* is closely related to *Perameles* (Muirhead, 1997) or that *Chaeropus* + *Macrotis* is sister to *Isoodon* + *Perameles* (Groves and Flannery, 1990) (Table 3).

Within Peramelidae three subfamilies (Peroryctinae, Echymiperinae and Peramelinae) were resolved, with Peroryctinae sister to Echymiperinae (Fig. 1). Peroryctinae comprised *P. broadbenti* and *P. raffrayana.* Although only 12SrRNA sequences were used for the additional exemplars of both species, the data nevertheless shows a clear genetic distinctness between *P. raffrayana* and *P. broadbenti* as well as considerable sub-structuring within *P. raffrayana.*

Monophyly of Echymiperinae (*Echymipera*, *Rhynchomeles* and *Microperoryctes*) was strongly supported by our molecular data,

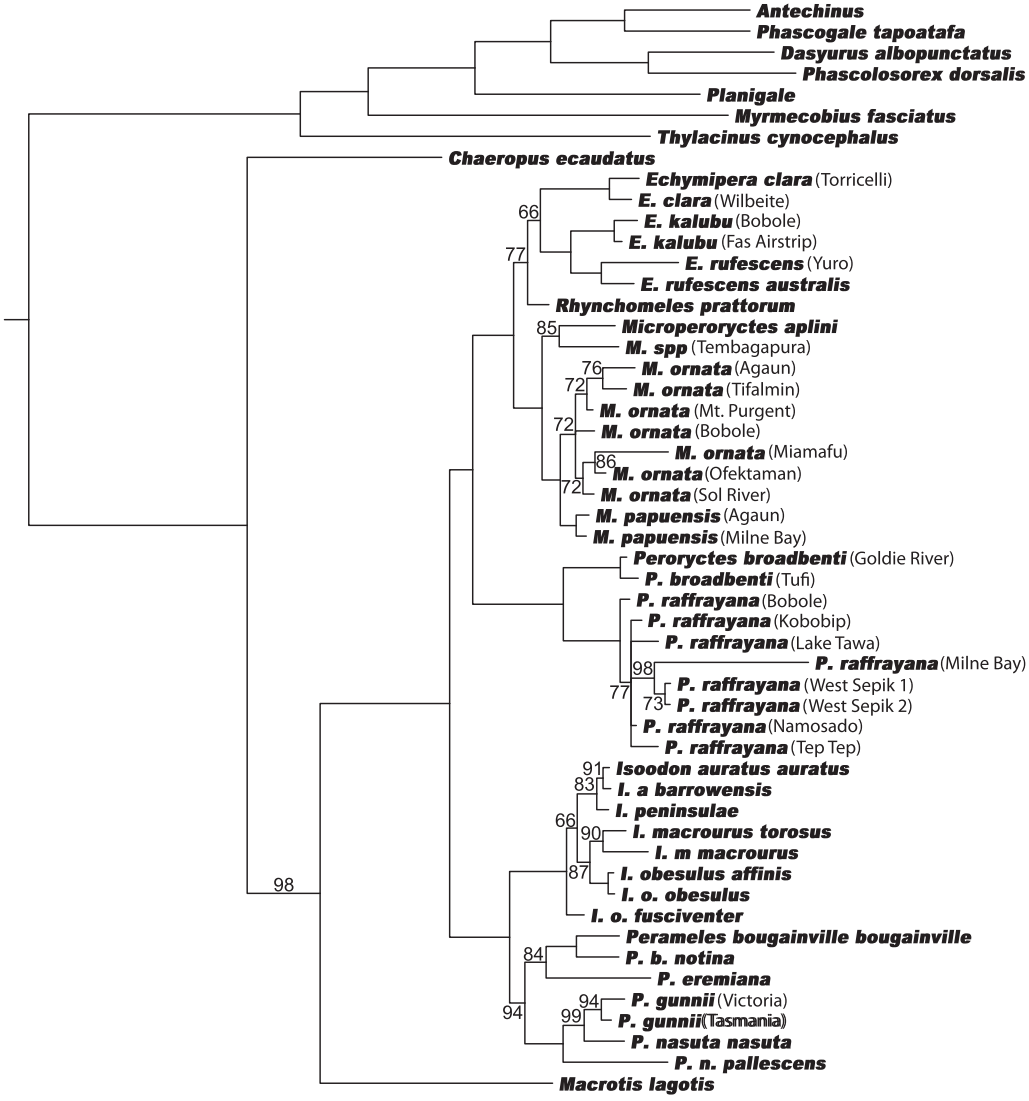

Fig. 1. Bayesian tree obtained from the combined nuclear and mitochondrial DNA sequences in which each gene was given its own model of sequence evolution. Only Bayesian Posterior Probabilities (BPP) less than 1.00 are shown. See Table 2 for MP and RAxML bootstrap support percentages and BPPs. Per = Peroryctinae.

Table 2
Summary of bootstrap support percentages and Bayesian posterior probabilities for the nuclear plus mitochondrial multigene dataset for maximum parsimony (MP), maximum likelihood (RAxML) and Bayesian analyses. Unpartitioned analyses treated the entire concatenation as a single gene; partitioned analyses allowed each of seven partitions to have its own model of molecular evolution.

Tree Node	MP	RaxML partitioned	RaxML unpartitioned	MrBayes partitioned	MrBayes unpartitioned
1. Peramelemorphia	100	100	100	100	100
2. Macrotis + Peramelidae	40	81	90	98	98
3. Peramelidae	98	99	100	100	100
4. Peroryctinae + Echymiperinae	49	98	98	100	100
5. Echymiperinae	100	98	99	100	100
6. *Echymipera* plus *Rhynchomeles*	95	64	64	77	67
7. *Echymipera*	73	47	47	66	74
8. *E. kalubu* + *E rufescens*	87	100	100	100	100
9. *Microperoryctes*	100	96	95	100	100
10. *M. papuensis* + *M. ornata*	80	97	98	100	100
11. *M. ornata*	–	–	–	72	71
12. *M. aplini* + *M.sp.* 'Tembagapura'	44	73	72	85	77
13. Peroryctinae	100	100	100	100	100
14. *Peroryctes broadbenti*	100	100	100	100	100
15. *P. raffrayana*	100	100	100	100	100
16. Peramelinae	99	99	99	100	100
17 *Isoodon*	100	100	100	100	100
18. *Isoodon auratus* + *I. peninsulae*	39	36	32	83	88
19. *Isoodon auratus*	92	52	47	91	95
20. *Perameles*	88	79	63	94	95
21. *P. eremiana* + *P. bougainville*	86	56	52	84	83
22. *P. gunnii* + *P. nasuta*	56	81	81	99	100

Table 3
Results of AU, weighted Kishino–Hasegawa (KH), and weighted Shimodaira–Hasegawa (SH) tests of alternative hypotheses.

Tree	Probability		
	AU test	Weighted KH test	Weighted SH test
1 Best tree			
2. *Rhynchomeles* sister to *E. clara* (Tate, 1948)	0.429	0.402	0.402
3. *Chaeropus* sister to *Perameles*	2e−05***	8e−05***	8e−05***
4. (*Macrotis* + *Chaeropus*) sister to (*Isoodon* + *Perameles*) (Groves and Flannery, 1990)	8e−43***	0***	0***
5. *I. obesulus* sister to *I. peninsulae*	8e−35***	0***	0***

*** $P < 0.001$.

as was monophyly of *Microperoryctes*. Our analyses resolved *Rhynchomeles* as sister to *Echymipera*, although we were unable to exclude the possibility that *R. prattorum* is sister to *E. clara* as suggested by Tate (1948). Within *Echymipera*, *E. clara* is the most divergent species in our Bayesian analyses in accord with the morphological studies of Tate (1948) and George and Maynes (1990). *E. rufescens* is sister to *E. kalubu*.

Based on the concatenated nuclear and mitochondrial DNA sequences, *Microperoryctes* was resolved in all analyses. The tree shows two major clades within the genus; one comprising *M. aplini* from the Arfak Mountains of the Vogelkop Peninsula and the Tembagapura animal from the Snow Mountains of West Papua, the other comprising *M. ornata* and *M. papuensis*. The inclusion of multiple 12S rRNA haplotypes from a much larger sampling of *M. ornata* to cover the species' range suggests a complex pattern of genetic variability within this putative species.

The mainly Australian peramelin genera *Isoodon* and *Perameles* are clearly divergent from the New Guinean forest bandicoots in all analyses and are always reciprocally monophyletic. Within *Isoodon*, both subspecies of the Golden Bandicoot (*I. auratus auratus* and *I. a. barrowensis*) along with *I. peninsulae* form a clade relative to *Isoodon macrourus* and *I. obesulus* though with only strong bootstrap support in the Bayesian analyses. Our combined nuclear and mitochondrial DNA sequences provide no support for the contention that *I. auratus* should be subsumed within *I. obesulus*, as suggested by Pope et al. (2001) and Zenger et al. (2005). In addition, although *I. peninsulae* is represented only by mitochondrial sequences in our study, it is genetically divergent from *I. obesulus* as suggested by Close et al. (1990) and Pope et al. (2001). Although *I. peninsulae* has often been posited as a subspecies of *I. obesulus*, our analyses consistently placed it together with *I. auratus*. Trees positing a sister relationship between *I peninsulae* and *I. obesulus* were significantly worse than the best one (Table 3). Within *Perameles*, *P. eremiana*, robustly nested with *P. bougainville* despite the former being represented only by 12S rRNA sequences and these desert-adapted bandicoots are clearly divergent from the eastern-barred and long-nosed species. The Cape York subspecies of *Perameles nasuta* (*P. n. pallescens*) fell as the immediate sister taxon to this clade rather than to *P. n. nasuta*.

3.3. Divergence dates

The results of the BEAST analysis are shown in Fig. 2 and Table 4. What is particularly clear from our analyses (Fig. 2) is that all bandicoot divergences are old, with the initial splits of Thylacomyidae and Chaeropodidae from Peramelidae occurring sometime in the later Oligocene (~26 million years ago). All major extant bandicoot families and genera were established by the middle Miocene.

3.4. Areas of origin

Fig. 3 shows the results of the MAC Parsimony analysis using geographic distributions of extant and recently extinct bandicoots. The MAC Parsimony and dispersal–extinction–cladogenesis (DEC) results both suggest that Australia is the ancestral area of origin for all modern bandicoots and that PNG is the area of origin of

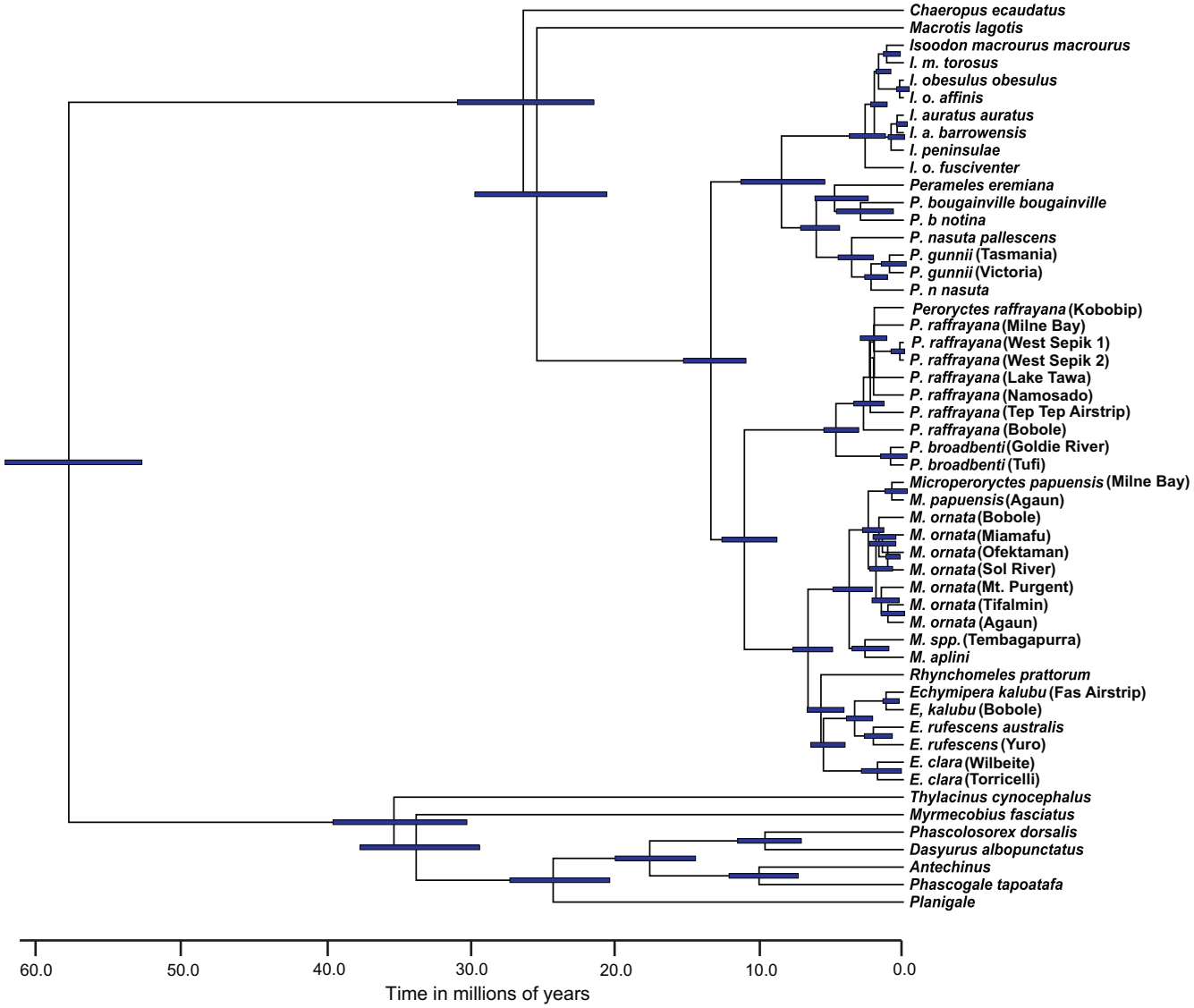

Fig. 2. Molecular divergence times (in millions of years) with associated confidence limits for peramelemorphians based on the analysis of partitioned concatenated sequences of five nuclear and three mitochondrial genes using BEAST v1.6.1. Minimum and maximum values for 12 nodes that were constrained in molecular dating analyses are given in Supplementary Table S2.

Table 4
Molecular divergence date estimates in millions of years before present based on partitioned data using BEAST v1.6.1.

Node	BEAST divergence estimates (in millions of years)
Dasyuromorphia v Peramelemorphia	58.3 (53.2–62.7)
Chaeropus v *Macrotis* + Peramelidae	26.7 (21.9–31.3)
Macrotis v Peramelidae	25.8 (20.9–30.1)
Peramelinae v Peroryctinae + Echymiperinae	13.8 (11.3–15.7)
Peroryctinae v Echymiperinae	11.4 (9.2–12.9)
Peroryctes broadbenti v *P. raffrayana*	5.1 (3.5–5.9)
Rhynchomeles + *Echymipera* v *Microperoryctes*	7.0 (5.3–8.1)
Rhynchomeles v *Echymipera*	6.1 (4.5–7.1)
E. clara v *E. kalubu* + *E. rufescens*	6.0 (4.5–6.8)
E. kalubu v *E. rufescens*	3.8 (2.5–4.4)
E. kalubu (Bobole) v *E kalubu* (Fas)	1.6 (0.7–1.8)
M. aplini + *M. spp* v *M. ornata* + *M. papuensis*	4.2 (2.6–5.3)
M. aplini v *M. spp* (Tembagapura)	3.1 (1.4–4.0)
M. ornata v *M. papuensis*	2.8 (1.8–3.2)
Isoodon v *Perameles*	8.9 (5.9–11.7)
I. auratus + *I. peninsulae* v *I. macrourus* + *I. obesulus*	2.4 (1.5–2.7)
I. macrourus v *I. obesulus*	2.1 (0.3–2.3)
Peroryctes bougainville + *P. eremiana* v *P. gunnii* + *P. nasuta*	6.5 (4.8–7.5)
P. bougainville v *P. eremiana*	5.2 (2.9–6.5)
P. gunnii v *P. nasuta*	4.0 (2.5–4.9)

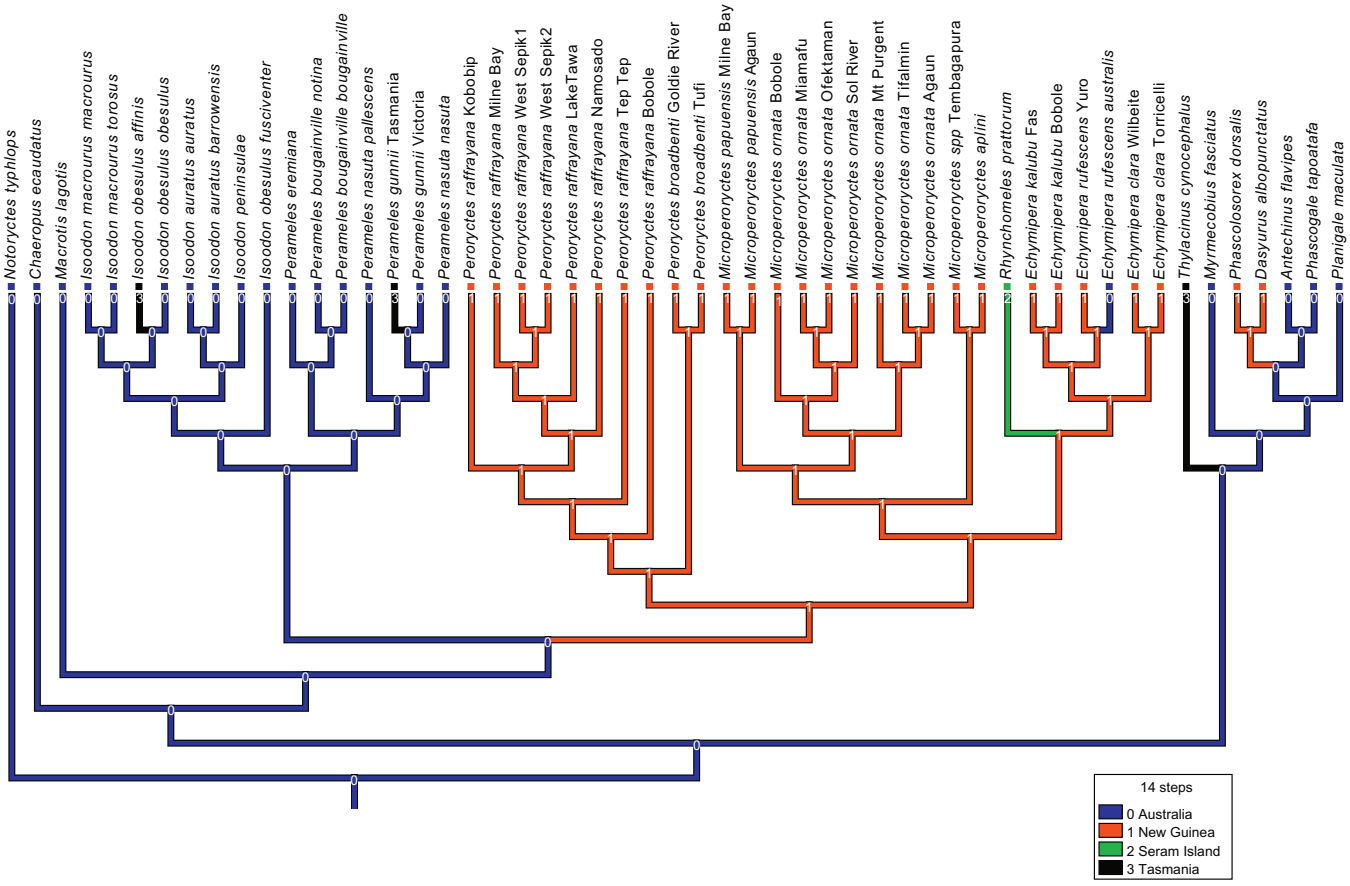

Fig. 3. Ancestral area reconstruction for extant and recently extinct peramelemorphians using MAC Parsimony (Springer et al., 2011) with the step-matrix in Supplementary Table S3. Areas are colour-coded as follows: Australia, blue; Papua New Guinea, red; Seram Island, green; Tasmania, black. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

forest bandicoots (Peroryctinae and Echymiperinae). In contrast, the DEC probability that Australia is the ancestral area of origin of forest bandicoots is very low (0.3258) although our analyses did not include data on known fossil peroryctids, the oldest of which are from the early Pliocene Hamilton Local Fauna of Victoria (see below).

4. Discussion

4.1. Phylogenetic relationships of bandicoots

Our phylogenetic analyses include the largest current sampling of living and recently extinct bandicoot species and thus serve to clarify relationships at all taxonomic levels within Peramelemorphia. Groves and Flannery's (1990) prediction that Thylacomyidae and Chaeropodidae (*sensu* Tate, 1948; Meredith et al., 2008) are sister-lineages is not confirmed, nor can we find support for a sister relationship between *Chaeropus* plus *Macrotis* and *Isoodon* plus *Perameles* as suggested by Groves and Flannery (1990). Rather, *Macrotis* (Thylacomyidae) and *Chaeropus* (Chaeropodidae) are divergent from all other bandicoots, having separated from a bandicoot common ancestor in the mid-late Oligocene. This is considerably earlier than suggested by the current fossil record, in which the earliest known *Chaeropus* fossil occurs in upper Pleistocene deposits (Muirhead and Godthelp, 1996) and the earliest putative thylacomyid, *Ischnodon australis*, is from the middle Pliocene (Stirton, 1957). Significantly, our molecular datings imply that these most arid-adapted of all bandicoot lineages may have arisen well before desert habitats proliferated across Australia in the Pliocene (Martin, 2006). The hypothesis that *Macrotis* had an exceptionally long period of independent evolution also accords with recently published karyotypic evidence (Westerman et al., 2010). The karyotype of *M. lagotis* is unique amongst peramelemorphians in possessing sixteen autosomes and a multiple sex-chromosome system (XX♀:XY_1Y_2♂) (Westerman et al., 2010) rather than the $2n = 14$ (XX♀:XY♂) karyotype of all other bandicoots.

At first sight, the difference between suggested times for origins of modern bandicoots based on fossils and on molecular dates seems difficult to reconcile. However, given that the Oligocene–Miocene yaralids are only distinguished from other contemporaneous, or even some modern peramelids by a small number of plesiomorphic characters (Kear et al., submitted for publication); it is possible that they are not so distantly related to extant bandicoot families. If this is true then the large number and variability of Oligo-Miocene bandicoot fossils (see Muirhead, 1994; Campbell, 1976) would be consistent with an origin and diversification of modern peramelemorphs in the mid-late Oligocene of Australia. Surprisingly, most of the currently recognised bandicoot genera and species also seem considerably older than traditionally thought, with most modern species already in existence by the late Pliocene.

Despite its low taxonomic diversity, Peroryctinae contains considerable genetic variability within both of its constituent species. Nevertheless *P. broadbenti* and *P. raffrayana* consistently resolve into reciprocally monophyletic lineages, thereby providing molecular support for the contention that *P. broadbenti* is indeed specifically distinct (Ziegler, 1977; Flannery, 1995; Aplin et al., 2010).

Echymiperinae contains the highest taxic and eco-morphological diversity of the peramelid subfamilies. Our molecular analyses universally resolve *Rhynchomeles* as sister to *Echymipera*, though

we cannot exclude the possibility that it is sister to *E. clara* as suggested by Tate (1948). This conclusion is in accord with previous morphological studies (e.g. Thomas, 1920; Tate, 1948; Groves and Flannery, 1990; Table 2a), which distinguish *Rhynchomeles* from *Echymipera* on the basis of a few cranial features (long and slender muzzle, very small last molars, reduction of upper incisors from five to four) that might be related to diet. *E. clara*, characterised by male-biased sexual dimorphism in body size, canine length and hypertrophy of the 3rd premolar (George and Maynes, 1990; Aplin et al., 2010), is the most divergent species both morphologically and genetically. Until DNA sequences are available for the recently described *E. davidi* (found only on Kiriwina Island in the Trobriand Group) and *E. echinista* (known from only two animals from the Western Province of Papua New Guinea Menzies, 1990), little can be said about their phylogenetic relationships.

Microperoryctes has recently been reviewed by Helgen and Flannery (2004), who not only recognised a new species (*M. aplini*) from the Arfak Mountains of the Vogelkop Peninsula but also suggested that *M. ornata* should be distinguished from *M. longicauda*. In addition, the morphologically distinct specimen of *Microperoryctes* (AM M30723), taken from Tembagapura in the Snow Mountains of West Papua which is possibly the largest *Microperoryctes* and occurs in sympatry with *M. ornata* (K. Helgen, pers. commun.) might represent an as yet undescribed new species; a conclusion supported by our results that place it as sister to *M. aplini* (Fig. 1). The molecular phylogenetic relationships of *M. murina* and *M. longicaudata* (*sensu* Helgen and Flannery, 2004) await availability of DNA sequences. The inclusion in our study of multiple exemplars of *M. ornata* covering its wide species' range reveals a great deal of genetic variability within this species.

Our phylogenetic structure of Peramelinae obtained from the nuclear plus mitochondrial sequences clearly differentiates *Isoodon auratus* from *I. macrourus* and *I. obesulus*. This finding is in stark contrast with the hypothesis of Pope et al. (2001) and Zenger et al. (2005) who, on the basis of a short sequence of mitochondrial control region sequences, proposed that *I. auratus* should no longer be considered a species. Interestingly, Pope et al. (2001) and Zenger et al. (2005) also showed that the close relationship between *I. auratus* and *I. obesulus* applied to Western Australian exemplars of the latter (*I. o. fusciventer*) rather than to eastern Australian (*I. o. obesulus* and *I. o. affinis*) animals, a relationship also noted in Fig. 1, where our Western Australian animal did not nest with either of the two eastern *I. obesulus* subspecies. Introgression of mtDNA following rare hybridization between *I. auratus* and *I. obesulus* in the western part of Australia might explain this pattern of relationships.

Although as yet based only on mitochondrial sequences, our specimen of *Isoodon peninsulae* is apparently more closely related to *I. auratus* than to *I. obesulus*. This accords with results from some prior morphological (Thomas, 1922; Lyne and Mort, 1981), allozyme (Close et al., 1990) and control-region studies (Pope et al., 2001), all of which favour specific distinction for *I. peninsulae*. Further taxonomic examination of this pan-continental genus is therefore critical, especially given the catastrophic declines observed in regional populations of many of its constituent species.

Within *Perameles*, *P. eremiana*, and *P. bougainville* comprise a genetically distinct, arid-adapted radiation relative to *P. gunnii* and *P. nasuta*, which typically occupy higher rainfall areas (Strahan, 1995). Consistent with morphology, our molecular results demonstrate that *P. eremiana* and *P. bougainville* are closely related, though mtDNA diversity within this group exceeds that observed in *Isoodon*. Accordingly, we urge caution in treating all of the small-bodied, arid adapted *Perameles* as a single species.

Our mtDNA data failed to associate the two currently recognised subspecies of *Perameles nasuta*. Instead, the Cape York subspecies *P. n. pallescens* consistently resolved as sister to the *P. bougainville* + *P. eremiana* lineage, rather than with the typical southern *P. n. nasuta*. A qualitative examination of craniodental morphology in *P. nasuta* by K. Aplin (unpublished data) indicated multiple diagnostic differences between the subspecies of *P. nasuta* and no clear synapomorphic traits that would unite them. Consequently unity of *P. nasuta* should no longer be assumed and the subspecies complex requires more rigorous testing.

4.2. Divergence times and the evolution of Peramelemorphia

The minimum date for the origin of bandicoots is widely assumed to be at least 54 million years ago (MYA), based on the presence of possible peramelemorphian fossils in the Eocene (Godthelp et al., 1992; Archer and Hand, 2006; Woodburne and Case, 1996). Meredith et al. (2008) suggested a timeframe of ~20 MYA for the diversification of modern bandicoot taxa. Our current estimates suggest a slightly older estimate for the basal bandicoot divergence event of ~27 MYA. Though these Eocene fossils may be bandicoots, the earliest and most primitive formally described peramelemorphian fossil species are from the late Oligocene–early Miocene and include *Yarala kida* (Schwarz, 2006) and *Y. burchfieldi* (Muirhead, 2000). These species are thought to be only distantly related to extant bandicoots and have been referred to a separate superfamily Yaraloidea (Archer and Hand, 2006; Schwarz, 2006; Muirhead, 1994; Muirhead and Filan, 1995). Yaraloids were small-bodied and lacked many of the cranio-dental specializations present in modern species (Muirhead, 1994; Muirhead and Filan, 1995). Muirhead (1994) posited that the potentially rainforest-specialised yaraloids went into decline and were eventually replaced by modern dry-zone adapted peramelemorphians during the middle Miocene-early Pliocene. This replacement coincided with the collision of Australia and the Pacific and Eurasian plates in the middle Miocene, a process that not only uplifted the New Guinean Cordillera and Timor but radically altered rainfall patterns across northern Australia and prompted the contraction of many rainforest habitats (Hill, 2004; White, 1994, 2006). In accordance with this idea, the oldest fossil representatives of extant bandicoot lineages appear in the early Pliocene with possible examples of Peroryctinae (*cf. Peroryctes tedfordi*; Turnbull et al., 2003), Peramelinae (*Perameles bowensis*; Muirhead et al., 1997), and Thylacomyidae (*Ischnodon australis*: Stirton, 1957). These records imply that the modern perameloid radiation occurred only shortly before the end of the Miocene. However, our molecular divergence estimates suggest a substantially more ancient, possibly mid-late Oligocene timing for this early phase of evolution. Indeed identification of advanced bandicoot fossils that resemble modern taxa in the late Oligocene–early Miocene deposits of central and northern Australia (Campbell, 1976; Case, 2001) suggests that we should expect to see representatives of extant families in the stratigraphically oldest peramelemorphian assemblages. The popular idea of 'faunal turnover' amongst bandicoots in the Miocene–Pliocene and the concomitant genesis of 'modern' lineages therefore may be overly simplistic.

During the Palaeogene, peramelemorphians probably inhabited the aseasonal rainforest habitats dominating much of mainland Australia (White, 2006), although some xeric floras are known from the Eocene in both the central and northwest parts of the continent. It is plausible that the early diverging, arid-adapted thylacomyids and chaeropodids could have originated in or been associated with these more xeric regions. The appearance of Peramelidae and rapid diversification of Peramelinae, Peroryctinae and Echymiperinae in the middle Miocene broadly coincides with a period of increasing climatic seasonality and the advent of more open, sclerophyll habitats in mainland Australia (Woodburne and Case, 1996; Travouillon et al., 2009). Conversely, Flannery (1988) argued that the radiation of Peroryctinae and Echymiperinae

(Peroryctidae *sensu* Groves and Flannery, 1990) occurred only after the vicariant isolation of an ancestral form on the newly created New Guinean landmass. Based on the geological reconstructions of Dow (1976), Flannery (1988) regarded the southern portion of what is now New Guinea as emergent and continuous with northern Australia from the late Palaeocene to the early Oligocene. Under this scenario, creation of a discrete island, the nubbin of a future New Guinea, was understood to have occurred via oblique collision of the Australian and Pacific Plates, causing a buckling of the northern margin of the Australian craton and inundation across the newly created Papuan Basin. In Flannery's view, this vicariance signalled the origin of separately evolving insular biotas, including the ancestors of such groups as peroryctin and echymiperin bandicoots. Ancestral area analyses using MAC parsimony (Springer et al. (2011) and DEC (Ree and Smith, 2008) based as they are on current bandicoot distributions, appear to support this scenario (but see below). These clades probably diversified following subsequent expansion of the New Guinean landmass during uplift of the Papuan Fold and Thrust Belt, together with accretion of terranes (e.g. the Papuan Peninsula, Vogelkop, Torricelli and Adalbert-Finisterre Terranes; Pigram et al., 1989; Davies et al., 1987) and oceanic volcanic landmasses from island arcs along the northern coast and the Banda Arc. Under Flannery's model, dry land connections back to northern Australia were not possible until the onset of the Pleistocene glacial cycles, which caused sea levels to fall sufficiently for the Torres Strait to become emergent lowlands. However it is precisely the timings of these back-migrations from New Guinea to Australia and the appearance of sibling lineages that have caused several authors to question Flannery's (1988) hypothesis (see Aplin et al., 1993; Kirsch and Springer, 1993). Aplin et al. (1993, 2010) reported stratigraphical evidence for the exposure of the Torresian Plain prior to the Pleistocene. More significantly, refinement of stratigraphy and sequence dating in the Papuan Basin has revealed fundamental flaws in the palaeogeographic scenario of (1976) that formed the basis of Flannery's model.

New evidence summarised by Quarles van Ufford and Cloos (2005) suggests that prior to the middle Miocene (c. 12 MYA), only small areas of emergent land were present off the northern margin of the Australian continent, and that these were remote from the northern coastline and separated from it by deepwater barriers. Two areas of emergent land were identified – the 'Kemum High' in the west (Dow et al., 1988) and the 'Arafura High' in the east (Davies et al., 1987). Today these landmasses are incorporated into the Bird's Head and Papuan Peninsula as a result of the 'Central Range orogeny' of Quarles van Ufford and Cloos (2005) that commenced ~12 MYA and caused the mountainous spine of New Guinea to emerge *de novo* north of the Australian continental margin. Subsequent uplift of the Central Cordillera, together with accretion of various terranes, caused further enlargement of the emergent landmass (Quarles van Ufford and Cloos, 2005) and shallowing of the intervening marine sedimentary basins.

Since the late Miocene, episodic expansion and contraction of the Antarctic ice cap (Ciesielski et al., 1982) caused global sea-level fluctuations of sufficient magnitude to postulate the establishment of transient dry land connections between northern Australia and New Guinea (Hodell et al., 1986). Exactly how many such connections existed and when they occurred is not known for certain. However, prolonged connectivity is likely to have first occurred during the latest Miocene, coincident with the Messinian Crisis in the Mediterranean (Hodell et al., 1986), when global sea levels are thought to have fallen by 40–60 m. Under this new paleogeographic scenario, the notion of an early vicariant component in the New Guinean biota holds no currency. Prior to the late Miocene, dispersal into New Guinean 'proto-islands' was possible only for groups that could cross wide water barriers, probably including several groups of frogs, reptiles (Keogh et al., 1998), birds, bats and possibly murid rodents (Rowe et al., 2008). Bandicoots and other medium to large terrestrial mammals are unlikely to have been part of the New Guinean fauna until such time as land connections became established. We also note that whilst the ancestral area analyses described above appear to support a New Guinean origin for peroryctin and echymiperin bandicoots, our analyses were based only on the geographic distributions of living and recently extinct species. Clearly the results would be affected by knowledge of the geographic distributions of the oldest fossils for each lineage. Fossil peroryctins and echymiperins are currently poorly known, but if the Pliocene fossils from the Hamilton Local Fauna of Victoria identified as cf. *Peroryctes tedfordi* and cf. *P.* spp. (Turnbull et al., 2003) are correctly designated, then the oldest peroryctin fossils would have an Australian mainland distribution.

Our ~11.5 MYA divergence estimate for the peroryctin/echymiperin split (range = 9.2–13 MYA) is consistent with the revised paleogeographic scenario introduced above and indicates that the primary diversification of these lineages must have occurred in Australia. As noted above, this is consistent with the recovery of 'peroryctid-like' fossils (*sensu* Groves and Flannery, 1990) in the early Pliocene (~4.46 MYA) Hamilton Local Fauna of southern Victoria (Turnbull and Lundelius, 1970; Turnbull et al., 2003), where an associated pollen assemblage suggests a mosaic of Aurucariaceae mesotherm rainforest and more open sclerophyllous communities (Macphail, 1996). Presence of 'peroryctids' in this context is consistent with the new scenario presented above, as is the recent discovery of a number of 'peroryctid' bandicoots in the middle Pleistocene fossil record of central-eastern Queensland (Hocknull et al., 2007). These admittedly meagre finds are nonetheless sufficient to demonstrate that peroryctin and/or echymiperin bandicoots may have survived until quite late in parts of Australia, presumably harboured by diminishing coastal rainforest habitats as the continental interior underwent aridification (White, 2006). We predict that other, much older, fossils currently regarded as being 'archaic' bandicoots will prove to be referrable to modern crown-group lineages, consistent with our molecular dates.

In a similar situation to New Guinea, the island of Seram is thought to have emerged above sea level after the rise of Timor some 5–6 MYA (Fortuin and de Smets, 1991; Polhemus and Polhemus, 1998; Hall, 2002). Seram's highlands were created in the late Pliocene (M. Novick, pers. commun.) and today there are deep-water barriers between Seram, Misool, and the Vogelkop Peninsula that represent tectonic trenches formed during the last 1–2 MYA (Pairault et al., 2003). *R. prattorum* (or its ancestor) diverged from *Echymipera* about 6 MYA and must therefore have had a trans-water arrival on Seram and remained/remains the only bandicoot present on the island. No fossils attributable to *Rhynchomeles* are known from elsewhere in New Guinea or associated islands, although Flannery et al. (1995) reported remains from the Holocene of Halmahera (Moluccas Islands) that shared some similarities with *Rhynchomeles*. If correct, these fossils might document an earlier, wider distribution of *Rhynchomeles'* ancestral stock that then spread to Seram following the radiation of *Echymipera* in the later Miocene.

Today, the earliest diverging species of *Echymipera*, *E. clara*, is limited to northern regions of the New Guinean mainland, including various terranes (e.g. the Torricelli Range) that relatively recently accreted to the New Guinean landmass. Given the considerable antiquity of the *E. clara* lineage (diverged ~6 MYA), its present distribution must surely be a relictual one. Certainly there seems little support for Zeigler's (1977, p. 128) contention that *E. clara* "evolved from a primitive *E. kalubu* stock stranded on one of the northern coast ranges during a sea incursion in, perhaps, the late Pliocene at the same time that *E. rufescens* arose".

Microperoryctes longicauda (*sensu* Helgen and Flannery, 2004) and *M. aplini* both seem to be restricted to the Vogelkop Mountains

that represent another quite recent accretion to New Guinea. However, unlike the north coastal terranes that presumably only appeared in the late Pliocene, the Vogelkop has a much longer terrestrial history prior to docking (Dow et al., 1988; Aplin et al., 1999). Consequently it is possible that species of *Microperoryctes* are additional relictual endemics from a formerly discrete landmass.

Within Australia, *Perameles* and *Isoodon*, had diverged from one another by the later Miocene (~9 MYA), probably as a response to the same climatic and tectonic stimuli that led to the radiation of primary lineages within Echymiperinae. Once again, our estimates for this split considerably predate the current fossil record, which does not extend past the Pliocene (Muirhead et al., 1997; Mackness et al., 2000). As with the Australo-New Guinean rainforest taxa, there appears to have been a diversification within both genera during the late Miocene to middle Pliocene. These events are consistent with the spread of dry sclerophyll woodlands and grasslands throughout the continental interior (Martin, 2006). The dispersal of peramelins to the savannah habitats of the New Guinean Trans Fly plains and southern Papua during the Pleistocene presumably occurred when falling sea levels a-periodically exposed the Torresian Plain up until about 18,000 ya.

The molecular divergence date recovered for *Isoodon auratus* and *I. peninsulae* from *I. obesulus/I. macrourus* (~2.4 MYA) is at odds with previous work (Pope et al., 2001; Zenger et al., 2005) that considers that the "levels of genetic divergence among populations of *I. obesulus*, and *I. auratus*. . ..support the idea that there was once a single, geographically continuous species across much of Australia that has *in recent times* (our italics) suffered range reduction and subsequent population isolation" (Pope et al., 2001, p. 425). Indeed, our results suggest that almost no pair of currently recognised living bandicoot species has a common ancestor that occurs more recently than the latest Pliocene (a phenomenon also reported in dasyurid marsupials: Krajewski et al., 2000). If major episodes of climatic change are indeed coincident with this distinct period of cladogenesis, as is often claimed for major peramelemorphian radiations in the middle Miocene (e.g. Muirhead, 1994, 1999), then why is there no comparable signal from the dramatic Pleistocene record? Might the 'missing' Pleistocene divergences in bandicoots simply be masked by cryptic speciation? That this could be the case is suggested by our preliminary genetic and morphological studies within *Microperoryctes*, *Peroryctes*, *Echymipera*, *Perameles* and I*soodon*. The signature of relatively recent climate-driven cladogenesis in bandicoots thus may be discovered as phylogeographic patterning or cryptic species diversity.

4.3. Concluding remarks

Surprisingly, most of the currently recognised bandicoot species seem considerably older than traditionally thought, with few being more recent than the middle Pliocene. Indeed, genetic divergence within the New Guinean *E. kalubu* (Bobole v Fas populations) is comparable in age to the divergence of *Isoodon macrourus* and *I. obesulus* of the Australian mainland, suggesting that speciation in New Guinean peramelemorphians may be more ancient than previously suspected.

The comprehensive phylogenetic and molecular clock dating analyses undertaken by this study clearly demonstrates that (1) the New Guinean forest bandicoots are not – as frequently alluded – primitive bandicoots relative to more specialised Australian taxa; (2) that they are not relics of mid-Tertiary Australian bandicoot diversity, and (3) they are not the product of *in situ* isolated evolution on the island of New Guinea. Rather, they are *derivatives* of an originally Australian radiation of rainforest bandicoots that diversified through the mid-Miocene to Quaternary, and which, having reached the newly emergent landmasses of New Guinea during the latest Miocene to Pleistocene, and persisted into the Holocene in New Guinea because of its largely rainforest vegetation cover. In addition, the New Guinean peramelemorphian radiation provides a spectacular example of ongoing diversification on an island where rapid uplift has created geographical and topographical complexity, sustaining a network of microhabitats suitable for exploitation by a diverse range of taxa.

Acknowledgments

We would like to thank Matt Faure of CCRI Madang, PNG for kindly supplying an ear clip of the Giant Bandicoot and the Ajoa village hunters who, in **not** eating everything from this animal, made this work possible! We also thank staff of the Australian Museum, Sydney, who made available tissue from their specimens of *P. broadbenti* and *R. prattorum* as well as other material. Joan Dixon kindly made material available from the Museum of Victoria. The South Australian Museum, Museum of Vertebrate Zoology, Berkeley, and the University of Kansas also kindly supplied tissue samples from their collections. Steve Hamilton provided tissue from *E kalubu* and *E. rufescens* animals collected from the Trans Fly region and James Menzies supplied a skin sample of *E. echinista* as well as the *M. aplini* tissue. For these kindnesses we thank them. Mark Adams and Lisa Pope made samples of various *Isoodon* and *Perameles* subspecies available for the 12S rRNA work. M.S.S. acknowledges support from NSF (EF0629860).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2011.09.009.

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