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Intergeneric Relationships Among Macropodoidea (Metatheria: Diprotodontia) and The Chronicle of Kangaroo Evolution

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The superfamily of kangaroos (Macropodoidea) is comprised of the subfamilies Propleopinae, Hypsiprymnodontinae, Paleopotoroinae, Potoroinae, Bulungamayinae, Balbarinae, Macropodinae, and Sthenurinae. Of these, Hypsiprymnodontinae, Potoroinae, and Macropodinae are extant. Competing phylogenetic hypotheses unite potoroines with either hypsiprymnodontines or macropodines, with most recent workers following a classificatory scheme that recognizes Hypsiprymnodontidae (hypsiprymnodontines) and Macropodidae (macropodines + potoroines). To address phylogenetic relationships among living macropodoids, we analyzed sequences from three mitochondrial genes (12S rRNA, tRNA valine, 16S rRNA) and one nuclear gene (protamine P1). MtDNA and protamine P1 both support a basal split of *Hypsiprymnodon* from other macropodoids rather than an association of *Hypsiprymnodon* with potoroines. This suggests that bipedal hopping and a complex stomach evolved once among macropodids. Monophyly of the Macropodinae is supported. Among macropodines, there is support for a *Dorcopsis-Dorcopsulus* association. Potoroine monophyly is less clear, although among potoroines there is support for an association of *Bettongia* and *Aepyprymnus*. Divergence times were estimated using 12S rRNA, tRNA-valine, and 16S rRNA transversions and suggest that kangaroos separated from a possum-like ancestor approximately 38–44 million years ago. *Hypsiprymnodon* diverged from other macropodoids approximately 34 to 38 million years ago. In agreement with the fossil record, the diversification of potoroines predates the diversification of macropodines. The latter have radiated in association with the development of a more arid climate and emergent grasslands over the Australian continent.

KEY WORDS: Kangaroo phylogeny; Mitochondrial rRNA; protamine P1; Macropodoidea; Metatheria.

INTRODUCTION

Eight subfamilies of kangaroos (paleopotoroines, potoroines, propleopines, hypsiprymnodontines, bulungamayines, balbarines, macropodines, and sthenurines; Flannery, 1989) comprise the superfamily Macropodoidea in the Australasian marsupial order Diprotodontia. Of the eight kangaroo subfamilies, Hypsiprymnodontinae, Potoroinae, Macropodinae, and possibly Sthenurinae are extant. The status of Sthenurinae depends on the phylogenetic placement of the living genus *Lagostrophus* (see Flannery, 1989). The

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musky rat kangaroo (*Hypsiprymnodon moschatus*) is the only living representative of Hypsiprymnodontinae. Potoroinae is comprised of three extant genera containing the smaller bettongs and rat kangaroos; larger wallabies and 'true' kangaroos belong to the subfamily Macropodinae. Macropodines are represented by approximately 50 living species comprising 12 genera. With few exceptions, kangaroos are terrestrial herbivores that are adapted to a wide variety of habitats including tropical rainforests, alpine tussock grasslands, arid inland plains, and deserts. Macropodoids range in size from 0.5 to 90 kilograms and most modern forms exhibit a characteristic mode of locomotion, the bipedal hop. The radiation of macropodoids in Australia and New Guinea parallels radiations of herbivores in other parts of the world, such as artiodactyls and perissodactyls (Eisenberg, 1981).

Macropodoids ostensibly evolved from arboreal possum-like ancestors (Winge, 1941; Flannery, 1987, 1989; Springer and Woodburne, 1989; Szalay, 1994). Molecular data suggest separation of kangaroos from other diprotodontian lineages as much as 50 million years ago (Springer and Kirsch, 1991; Kirsch *et al.*, 1997; Burk *et al.*, 1998), but macropodoids do not appear in the fossil record until the late Oligocene (26-24 mya) (Woodburne *et al.*, 1993). In Australian formations of late Oligocene age, bulungamayines, paleopotoroines, potoroines and balbarines are present (Flannery and Rich, 1986; Flannery, 1989; Woodburne *et al.*, 1993). Among hypsiprymnodontids, giant rat kangaroos (i.e., propleopines) do not appear in the fossil record until the lastest Oligocene/early Miocene (Archer and Flannery, 1985) and hypsiprymnodontines first appear in the middle Miocene (Flannery and Archer, 1987). By the late Miocene, sthenurines and macropodines appear (Woodburne, 1967), coinciding with the disappearance of bulungamayines and balbarines from the fossil record (Prideaux, 1999).

Paleopotoroines, potoroines, propleopines, and hypsiprymnodontines have often been associated within the family Potoroidae, with bulungamayines variably included in this group as well (e.g., Flannery, 1989). Putative synapomorphies for Potoroidae include a masseteric canal that extends into the body of the dentary to below the posterior edge of P_3, an M_1 distinguished by a protostylid as the anterobuccal cusp, a proximoventral process on the fifth metatarsal, and female reproductive tract morphology (Pearson, 1946, 1950a, 1950b; Archer, 1984; Case, 1984; Flannery and Rich, 1986; Flannery, 1989). Within Potoroidae, Flannery and Rich (1986) tenuously united paleopotoroines and potoroines on the basis of a straight, anteroposteriorly oriented metaconid-protoconid crest. Ride (1993) and Wroe and Archer (1995) placed propleopines and hypsiprymnodontines (*Hypsiprymnodon*) as sister taxa within the family Hysiprymnodontidae. Hypsiprymnodontids share dental characteristics such as plagiaulacoid upper and lower premolars and a distinctive trigonid morphology of the deciduous premolar and the first molar (Ride, 1993). In addition, Ride (1993) and Wroe and Archer (1995) considered Hypsiprymnodontidae a sister lineage to all other kangaroos. Tarsal morphology (Szalay, 1994) and molecular data (Burk *et al.*, 1998) also support the separation of the hypsiprymnodontine lineage from a clade containing macropodines and potoroines. An implication of this hypothesis is that several craniodental and postcranial (i.e. the proximoventral process on the fifth metatarsal and female reproductive tract morphology) characters may be homoplastic or primitive in *H. moschatus* and other potoroines. This hypothesis also implies that bipedal hopping and other characters either evolved independently in potoroines and macropodines or were lost in hypsiprymnodontines after evolving in the common ancestor of all macropodids.

Flannery *et al.* (1983) and Flannery (1989) united Macropodinae, Balbarinae, and Sthenurinae in the family Macropodidae. A clade of these three lineages is supported by the loss of a steep molar gradient, loss of fine ridging on P_3, lophodont molars, and gain of a plantar fascia insertion on the anterior surface of the calcaneum (Flannery *et al.*, 1983; Flannery, 1989). Cooke (1997a) concluded that there was parallel evolution of lophodonty in balbarines versus bulungamayines, macropodines, and sthenurines and that balbarines may represent a basal macropodoid clade. Wroe *et al.* (1998) suggested that Propleopinae may represent the sister taxon to Balbarinae and that together these may be basal to all other macropodoids. Bulungamayines have been associated with potoroines and hypsiprymnodontids based on the presence of thickening of the dentine of I_1, a finely ridged P_3, a buccally expanded opening of the masseteric canal, and a dentary that is strongly convex below the cheek-tooth row (Flannery *et al.*, 1983). In contrast, bulungamayines share the presence of lophodont molars and an elongate P_3 with a straight occlusal edge with macropodines (Flannery *et al.*, 1983), suggesting an affinity of bulungamayines with macropodines. Cooke (1997b) examined new Miocene bulungamayine kangaroos and concluded that within this lineage there is a transition from a potoroine-like molar in basal species to a macropodine-like molar in derived species and asserted that bulungamayines are directly ancestral to macropodines.

Among extant potoroine genera, *Aepyprymnus* and *Bettongia* are united to the exclusion of *Potorous* in the tribe Bettongini. Bettongini is supported by possession of a postglenoid process, fusion of pedal digital pads into a single unit, a mastoid process of the periotic that projects above the promontory, and a discrete ectotympanic process (Flannery, 1989).

An extensive radiation within the Macropodinae occurred within the last 5–10 million years and coincided with the drying out of Australia and the spread of grasslands (Flannery, 1989). Recent and rapid evolution has resulted in closely related macropodine genera whose mutual affinities have been difficult to resolve (Kirsch *et al.*, 1995). A putative basal split of New Guinean forest wallabies (*Dorcopsis* and *Dorcopsulus*) from other macropodines is supported by dental characteristics (Woodburne, 1967; Flannery, 1984), DNA hybridization (Springer and Kirsch, 1991; Kirsch *et al.*, 1995), and 12S rRNA and tRNA valine sequence analyses (Burk *et al.*, 1998). In addition, it is generally believed that species of *Thylogale* (pademelons) and *Petrogale* (rock wallabies) are closely related. Chromosomal similarities of *Petrogale* and *Thylogale* were shown by Sharman (1961) and Hayman and Martin (1974). Immunological studies (Kirsch, 1977) indicate that species of *Thylogale* are closely related to species of *Petrogale*. Microcomplement fixation data not only support an affiliation between *Thylogale* and *Petrogale*, but include the tree kangaroos (*Dendrolagus*) in this clade (Baverstock *et al.*, 1989). DNA hybridization provides some support for the association of *Dendrolagus*, *Petrogale*, and *Thylogale* (Kirsch *et al.*, 1995). Ziegler (1977) was the first to suggest that *Dendrolagus* may have arisen in New Guinea from an ancestor similar to modern *Petrogale* but provided no evidence in support of his hypothesis. The suggestion that tree kangaroos are closely related to *Thylogale* and/or *Petrogale* contrasts with earlier studies utilizing dental characteristics that proposed an affinity between tree kangaroos and the New Guinean forest wallabies *Dorcopsis* and *Dorcopsulus* (Bensley, 1903; Raven and Gregory, 1946; Tate, 1948).

Adaptive changes in stomach morphology and digestive physiology have played an important role in the evolutionary history of kangaroos (Hume, 1982). In *Hypsiprymnodon*

the stomach is simple. In contrast, the stomach of all other extant macropodoids is more complex and can be divided into three regions: the sacciform portion of the forestomach (the region adjacent to the esophagus entry point), the tubiform portion of the forestomach (the main tubular body, previously referred to as the midstomach), and the hindstomach (the gastric pouch and the adjacent region terminating at the pylorus) (Hume, 1982). The forestomach (sacciform and tubiform) functions as a fermentation chamber in which microorganisms assist in the digestion of plant material (Dawson, 1989). An enlarged sacciform region of the forestomach is believed to maximize plant fiber fermentation within a minimum volume, whereas an enlarged tubiform forestomach is less efficient at fiber digestion but decreases the minimum processing time of one gut volume of food (Freudenberger *et al.*, 1989). The stomach of potoroines is comprised of a large sacciform forestomach, a short tubiform forestomach and an acid-secreting hindstomach (Freudenberger *et al.*, 1989). The large sacciform forestomach of potoroines assists the digestion of any plant material in their diet of grass seeds, rhizomes, tubers, tap roots, hypogeous fungi, plant exudates, and invertebrate animal material (Hume, 1982; Seebeck, 1989). Consistent with their more nutritive and less fibrous diet, potoroines lack adaptations to abrasive diets, such as molar progression and the relative hypsodonty, which are found in macropodines (Sanson, 1989).

The foraging habits of macropodine genera range from a varied dicotyledonous browse (*sensu* Dawson, 1989; woody bushes and trees) to homogeneous monocotyledonous graze (grasses) (Sanson, 1989). Stomach and dental morphology among macropodines reflects the range of diets in this subfamily of kangaroos. Macropodines adapted to forest herbivory, such as pademelons (e.g. *Thylogale* spp.), possess a sacciform forestomach (similar to the potoroine sacciform forestomach) and a slightly enlarged tubiform forestomach thought to aid in the passage of less digestible plant material (Freudenberger *et al.*, 1989). In addition, pademelons have a lophodont, browsing grade dentition suitable for soft, non-abrasive forage (Sanson, 1989). Forest edge macropodines such as the swamp wallaby (*Wallabia bicolor*) and rock-wallabies (*Petrogale* spp.) are intermediate feeders of both browse material and grass (Freudenberger *et al.*, 1989). The tubiform forestomach of forest edge macropodines is enlarged, putatively to increase flow of the less digestible but more abundant grasses in their diet (Hume, 1982). Forest edge macropodine genera are more variable in dental morphology, possessing molars capable of both shearing and crushing (Sanson, 1989). This variability in dental morphology is thought to be correlated with the relative amounts of browse and grass ingested in the diet (Sanson, 1989). Grazing macropodines such as tammar wallabies (*Macropus eugenii*) and wallaroos (*M. robustus robustus*) have the most reduced sacciform forestomach and a greatly enlarged tubiform forestomach (Freudenberger *et al.*, 1989). Grazers possess molars with enhanced shearing capacity, curvature of the tooth row, and molar progression (Sanson, 1989).

To address macropodoid intergeneric relationships, the available mitochondrial sequences for kangaroos (Burk *et al.*, 1998) were extended to encompass 2.6-kilobases of the mitochondrial genome (12S rRNA, tRNA valine, and 16S rRNA) for 20 macropodoids representing 15 of the 16 extant genera. In addition, we report flanking, exonic, and intronic sequences for the nuclear protamine P1 gene from 18 macropodoids. Protamine sequences have previously been used to examine higher level marsupial relationships (Retief *et al.*, 1995b), as well as relationships within the marsupial family Dasyuri-

dae (Retief *et al.*, 1995a; Krajewski *et al.*, 1997a,b). Molecular sequences are used to examine previous hypotheses in kangaroo systematics and to reconstruct branching patterns and divergence times for living kangaroos. We also examine the ramifications of molecular phylogenetic analyses for understanding macropodoid evolution and integrate molecular, morphological, fossil, and paleoclimatic data into a chronicle of the evolutionary history of kangaroos.

MATERIALS AND METHODS

DNA samples from *Macropus giganteus*, *Macropus agilis* (agile wallaby), *Macropus parryi*, *Macropus rufus* (red kangaroo), *Wallabia bicolor* (swamp wallaby), *Thylogale stigmatica* (red-legged pademelon), *Petrogale xanthopus*, *Dendrolagus dorianus* (Doria's tree kangaroo), *Dendrolagus goodfellowi* (Goodfellow's tree kangaroo), *Peradorcas concinna* (Nabarlek), *Dorcopsulus vanheurni* (New Guinean forest wallaby), *Dorcopsis veterum* (brown dorcopsis), *Setonix brachyurus* (Quokka), *Onychogalea fraenata* (nail-tail wallaby), *Onychogalea unguifera* (Northern nail-tail wallaby), *Lagorchestes hirsutus* (rufous hare-wallaby), *Bettongia penicillata* (brush-tailed bettong), *Aepyprymnus rufescens* (rufous bettong), *Potorous longipes* (long-footed potoroo), *Hypsiprymnodon moschatus* (musky rat-kangaroo), *Trichosurus vulpecula* (brush-tailed possum), and *Spilocuscus rufoniger* were extracted from tissue as described in Kirsch *et al.* (1990). A 1.6 kb region containing the complete mitochondrial 16S ribosomal RNA gene was amplified from the taxa above (except *Onychogalea unguifera*) using the polymerase chain reaction (PCR) (Saiki *et al.*, 1988) with primers and cycling parameters as described in Springer *et al.* (1997). In addition, an 1.1 kb region containing the complete mitochondrial 12S ribosomal RNA and tRNA valine genes was amplified from *Macropus parryi* and *Petrogale xanthopus* with primers and cycling parameters as described in Springer *et al.* (1995). The protamine P1 gene, as well as 5′ and 3′ flanking regions, were amplified from macropodoid genomic DNA (taxa with protamine P1 accession numbers AF187533-AF187548, see Table I) using primer combinations of 052 and 033 as described in Retief *et al.* (1993) or 052 and PR-B (5′ GAACAATGCCGACCTGTCAA 3′) with an annealing temperature of 52°C. Amplified sequences were cloned into pCR II (Invitrogen) or pCR 2.1 (Invitrogen) using 2 μl of PCR product and 11 ng of vector in 10 μl reactions (12°C, overnight). Ligated products were transformed into INVαF *Escherichia coli* cells following the procedure of Hanahan (1983). Plasmid DNA was isolated from positive clones (Maniatis *et al.*, 1982), digested with *Eco*R1, and run on 1% agarose gels to determine which plasmids had inserts of the expected size.

DNA was sequenced in both directions using the dideoxy chain-termination method (Sanger *et al.*, 1977) with 35 S dATP and Sequenase 2.0 (Amersham Life Science). At least three clones were sequenced for a single individual from each species; in cases where there were differences (always <0.5%), the consensus is reported. Additional 12S rRNA, tRNA valine, and 16S rRNA sequences were obtained from GenBank. Table I gives accession numbers of all sequences included in analyses presented here.

Preliminary multiple alignments were generated by using CLUSTAL W (Thompson *et al.*, 1994). MtDNA sequence alignments were modified following secondary structure models for 12S rRNA (Springer and Douzery, 1996), tRNA valine (Anderson *et al.*, 1982) and 16S rRNA (Burk, 1999) genes. In both the mitochondrial RNA and protamine P1 gene

Table I. Taxa and GenBank Accession Numbers[a]

Taxa	MtDNA	Protamine P1
Macropodoidea		
Macropodidae		
Macropodinae		
*Macropus giganteus**	AF187885	L35333
Macropus agilis	AF027986	na
*Macropus parryi**	AF187887	AF187533
*Macropus rufus**	AF027985	L35447
Macropus rufogriseus	na	L35329
Macropus eugenii	na	L35450
Macropus robustus	Y10524	na
*Wallabia bicolor**	AF027987	L35328
*Thylogale stigmatica**	AF027991	AF187534
*Petrogale xanthopus**	AF187886	AF187535
*Peradorcas concinna**	AF027993	AF187538
*Dorcopsulus vanheurni**	AF027994	AF187539
*Dorcopsis veterum**	AF027995	AF187540
*Dendrolagus dorianus**	AF027989	AF187536
*Dendrolagus goodfellowi**	AF027990	AF187537
*Setonix brachyurus**	AF027988	AF187541
*Onychogalea unguifera**	AF027992 (12S-val)	AF187543
*Onychogalea fraenata**	AF187889 (16S)	AF187542
*Lagorchestes hirsutus**	AF027996	AF187544
Potoroinae		
*Bettongia penicillata**	AF027998	AF187546
*Aepyprymnus rufescens**	AF027999	AF187547
*Potorous longipes**	AF028000	AF187548
Hypsiprymnodontidae		
*Hypsiprymnodon moschatus**	AF027997	AF187545
Outgroup taxa		
Phalanger orientalis	U33496	na
Pseudochirops cupreus	na	L35334
Spilocuscus maculatus	AF108220 (12S-val)	na
Spilocuscus rufoniger	AF187890 (16S)	na
*Trichosurus vulpecula**	AF031823, AF187888	L32744
*Phascolarctos cinereus**	U61076, AF166344	U87789
Vombatus ursinus	U61078, AF102811	na

[a] Asterisks (*) indicate taxa used in combined mtDNA+Protamine P1 dataset. na, not available.

sequences, there are regions with complex indels that are difficult to align. Because positional homology in these regions is uncertain, we excluded these regions prior to phylogenetic analyses following the recommendation of Swofford *et al.*, (1996). These regions are indicated in the alignments that are available from M.S.S. (*mark.springer@ucr.edu*).

Phylogenetic trees were estimated by using parsimony, minimum evolution, maximum likelihood, and quartet puzzling. All of these analyses, as well as bootstrapping (Felsenstein, 1985), Kishino-Hasegawa tests (Kishino and Hasegawa, 1989), and partition homogeneity tests (Huelsenbeck *et al.*, 1996), were performed with PAUP*4.0b2 (Swofford, 1998). Tree bisection-reconnection (TBR) was selected as the branch swapping algorithm. A single randomized input order was used in maximum likelihood searches; 10 randomized input orders were used in parsimony searches. In parsimony analysis gaps were coded as missing data. All bootstrap tests included 500 replications except for maximum likelihood

test (200 replications), unless otherwise indicated. Minimum evolution trees were obtained using logdet (Lockhart *et al.*, 1994) and maximum likelihood distances under the HKY85 model (Hasegawa *et al.*, 1985) of sequence evolution. Maximum likelihood analyses also employed the HKY85 model. Quartet puzzling analyses were performed using 10,000 puzzling steps. Maximum likelihood and quartet puzzling utilized estimated transition to transversion ratios of 2.8 : 1 for mtDNA analyses, 1.3 : 1 for protamine P1 analyses, and 2.5 : 1 for combined mtDNA + protamine P1 analyses. Maximum likelihood estimates of transition to transversion ratios represent averages of ratios that were estimated from parsimony, minimum evolution, and maximum likelihood trees.

Two methods were used to estimate divergence times from mtDNA using mtDNA transversions. Previous work has shown that transversions are less susceptible to saturation effects over longer lookback times (Springer and Douzery, 1996). First, HKY85 pairwise distance matrices were obtained for transversions without any adjustments for relative rate differences. The mean of all pairwise comparisons between macropodine and potoroine taxa was calculated for each matrix. The clock was calibrated using the date of the macropodine—potoroine split specified by Woodburne *et al.* (1993) at 23–24 million years before present. Next, all pairwise comparisons were divided by percent divergence per million years yielding a pairwise matrix of divergence times.

Hillis et al. (1996) have enumerated potential sources of error in molecular clock calculations. The second method for estimating divergence times that we employed is aimed at evaluating the effects of lineage-specific rate variation. In the second method, the same calibration date was used in conjunction with relative rate-corrected distances. Pairwise distances were obtained as above and each taxon was assigned a correction factor to account for its relative rate in comparison to *Hypsiprymnodon* following the approach of Arnason *et al.* (1996). Each pairwise distance was then adjusted by multiplying the original value by the average of each taxon's correction factor. Rate adjustments for all pairwise comparisons were used to generate a pairwise matrix of divergence times using the macropodine-potoroine calibration. In both methods, divergence times were obtained by calculating means of all appropriate pairwise comparisons.

RESULTS

Alignments, Secondary Structure, and Base Composition

The mtDNA data set contains 2,654 total aligned nucleotide positions. We recognize 40 stems in 12S rRNA, 4 stems in tRNA-valine, and 53 stems in 16S rRNA. The mtDNA alignment was partitioned into 1152 stem positions and 1502 loop positions. Ambiguous regions of the alignment were excluded prior to phylogenetic analyses. Only one ambiguous position occurs within the stem partition; all other ambiguous positions were loop positions. Analyses of mtDNA sequences, containing 20 macropodoids and 5 outgroup taxa, included 2,412 aligned nucleotide positions (stem positions = 1151; loop positions = 1261). The 1151 stem positions among all taxa contain 219 variable positions (110 parsimony informative). In contrast, loops (1261 bp) contain 436 variable sites (329 parsimony informative) among all taxa. Mean base composition of stems (G = 24.9%; A = 25.1%; T = 27.0%; C = 23.0%) was more uniform than for loops (G = 11.3%; A = 47.1%; T = 21.2%; C = 20.3%). Loops are higher in adenine and lower in guanine.

The aligned protamine P1 data set included sequences from 21 macropodoids and 3 outgroup taxa and was 669 nucleotide positions in length. Ambiguous regions of the protamine P1 alignment were excluded leaving 591 aligned nucleotide positions; of these, there were 167 variables sites and 63 parsimony informative sites. Following junctions delimited by Retief et al. (1995a,b) for different regions of the protamine P1 gene, the 591 aligned positions were distributed as follows: 5′ untranslated region = 94 bp (37 variable; 9 informative); exon 1 = 132 bp (28 variable; 13 informative); intron 1 = 133 bp (48 variable; 23 informative); exon 2 = 75 bp (8 variable; 2 informative); and 3′ untranslated region = 157 bp (45 variable; 16 informative).

None of the strongly supported nodes (bootstrap value >90%) from phylogenetic analyses of the protamine P1 data set (Macropodoidea, Macropodidae, *Bettongia* + *Aepyprymnus*, *Notamacropus*, and *Onychogalea*; see Table II) conflicted with nodes that received strong support from the mtDNA data set. In addition, a partition homogeneity test indicates that the mtDNA data set and the protamine P1 data set are not significantly heterogeneous ($p = 0.76$). As a result, analyses were conducted with a combined data set (mtDNA + protamine P1) that included representatives of 18 macropodoids and 2 outgroup taxa. Of 3323 aligned nucleotide positions, 320 positions were excluded due to alignment ambiguity. Of the remaining 3303 positions, 764 were variable and 400 were parsimony informative.

Phylogenetic Relationships Among Extant Kangaroos

Figures 1–3 depict phylograms based on maximum likelihood for the mtDNA, protamine P1, and mtDNA + protamine P1 data sets, respectively. Bootstrap values are reported in Table II for a variety of phylogenetic methods. Support values for quartet puzzling are also reported in Table II. Macropodoidea monophyly was supported with values ranging from 62–100%. Support for the monophyly of Macropodidae to the exclusion of *Hypsiprymnodon* ranged from 51–100% in analyses of the individual data sets and was 100% in four of five analyses of the combined mtDNA + protamine P1 data set. Among macropodids, Macropodinae monophyly was well supported in mtDNA analyses (99–100%) and analyses of the combined mtDNA + protamine P1 data set (78–100%). Support for potoroine monophyly ranged from 6–55%. Within Macropodinae, an association of tree kangaroos (*Dendrolagus*) with rock wallabies (*Petrogale* and *Peradorcas*) was supported with values ranging from 65–83% (mtDNA), 5–41% (protamine P1), and 66–86% (mtDNA + protamine P1). In contrast, support for an association of tree kangaroos (*Dendrolagus*) with New Guinean forest wallabies (*Dorcopsis* and *Dorcopsulus*) never exceeded 1%. Support for a clade containing all macropodines except the New Guinea forest wallabies ranged from 37–55% in mtDNA analyses, but received no support from the protamine P1 data set. Similarly, support values for monophyly of the genus *Macropus* ranged from 36–93% in mtDNA analyses, but was 0% in all protamine P1 analyses. Support for the association of *Wallabia* and *Macropus* ranged from 41–93% (mtDNA), 22–75% (protamine P1), and 65–83% (mtDNA + protamine P1). Within *Macropus*, support for the subgenus *Notamacropus* ranged from 70–99% with mtDNA and 93–99% with protamine P1; support for the subgenus *Osphranter* was lower (range = 22 to 67% with mtDNA). *Dendrolagus* monophyly received support from mtDNA (94–99%), but not protamine P1 (0–8%). However in the combined analyses,

Table II. Support Values for Select Clades Based on Different Phylogenetic Methods[a]

Clade	Mitochondrial DNA						Protamine P1[a]					MtDNA + Protamine P1					
	MP	ME (ML)	ME (LD)	ML	QP	Mean	ME (ML)	ME (LD)	ML	QP	Mean	MP	ME (ML)	ME (LD)	ML	QP	Mean
Macropodoidea	71	73	70	62	99	75	99	100	100	98	99	88	93	90	85	95	90
Macropodidae	95	99	99	99	100	98	96	95	51	98	85	100	100	100	100	81	96
Potoroinae	42	41	31	48	20	36	55	52	6	24	34	24	24	15	33	21	23
Bettongia + *Aepyprymnus*	79	98	97	96	100	94	98	98	93	98	97	95	100	99	96	90	96
Macropodinae	100	99	100	99	99	99	42	31	44	82	50	100	100	100	100	78	96
Tree Kangaroos (TKs)	99	97	94	99	96	97	2	1	0	8	3	98	98	97	99	94	97
Rock Wallabies (RWs)	34	66	63	37	69	54	1	1	0	8	3	39	56	51	28	68	48
New Guinean Forest Wallabies (NGFWs)	98	100	100	97	98	99	14	10	0	33	14	100	100	100	99	99	100
TKs + RWs	70	80	82	65	83	76	35	41	5	26	27	69	86	85	66	83	78
TKs + NGFWs	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
All macropodines except NGFWs	52	46	47	55	37	47	0	0	0	0	0	46	52	47	54	40	48
Macropus + *Wallabia*	41	64	63	47	93	62	64	65	22	75	57	65	83	81	72	82	77
Macropus	51	45	36	66	93	58	0	0	0	0	0	56	44	36	58	81	55
Notamacropus	70	92	90	73	99	85	99	99	93	98	97	na	na	na	na	na	na
Osphranter	36	32	22	39	61	38	na	na	na	na	na	na	na	na	na	na	na
Onychogalea	na	na	na	na	na	na	100	100	100	100	100	na	na	na	na	na	na

[a] Bootstrap support values are given for parsimony, minimum evolution, and maximum likelihood. Puzzle support values are given for quartet puzzling. Maximum parsimony bootstrap analysis for the protamine P1 dataset was computationally impractical. MP, maximum parsimony; ME (ML), minimum evolution with maximum likelihood distances (estimated transition : transversion ratio); ME (LD), minimum evolution with logdet distances; ML, maximum likelihood; QP, quartet puzzling; na, not applicable given limitations of taxonomic sampling.

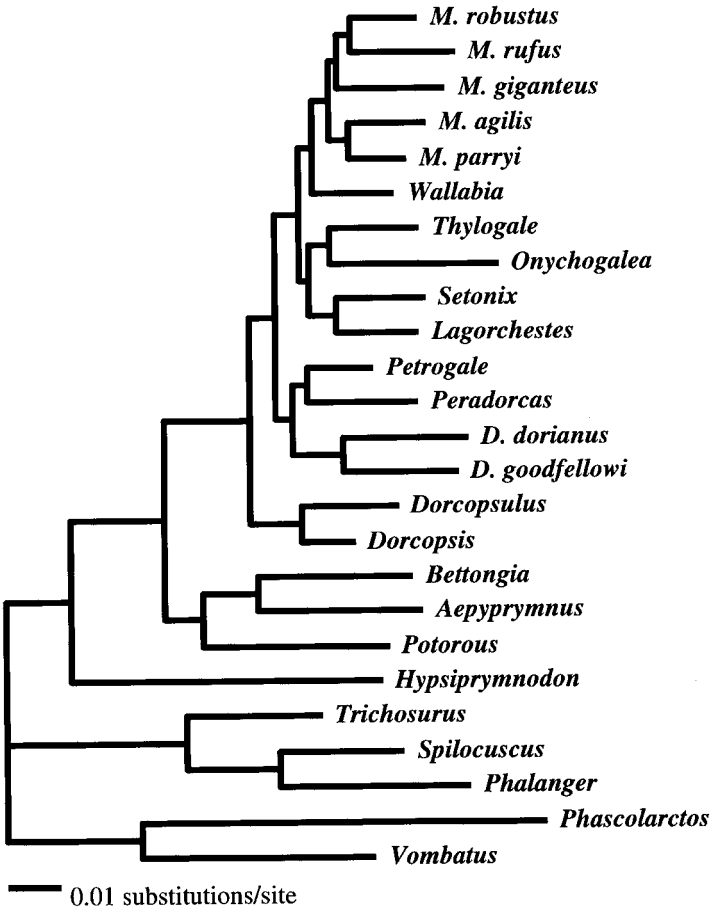

Fig. 1. Maximum likelihood phylogram (HKY85 model with TS : TV = 2.8 : 1) based on the mtDNA data set.

support values for *Dendrolagus* remained high (94–99%). Within Potoroinae, an association of the genera *Bettongia* and *Aepyprymnus* to the exclusion of *Potorous* was supported by all data sets with values ranging from 79–100%.

Statistical tests that evaluated *a priori* hypotheses are reported in Tables III (parsimony) and IV (maximum likelihood). Mitochondrial DNA sequences provide significant support for Macropodidae monophyly under maximum likelihood; with the combined data set, both parsimony and maximum likelihood tests rejected the best trees without Macropodidae. The best trees without Macropodinae monophyly were rejected in maximum likelihood tests with both the mtDNA data set and the combined data set. Tree kangaroo monophyly was supported under maximum likelihood with the combined data set. Protamine P1 data provide significant support for monophyly of the superfamily Macropodoidea in tests based on parsimony and maximum likelihood. Other *a priori* hypotheses could be neither accepted nor rejected (Table III and IV).

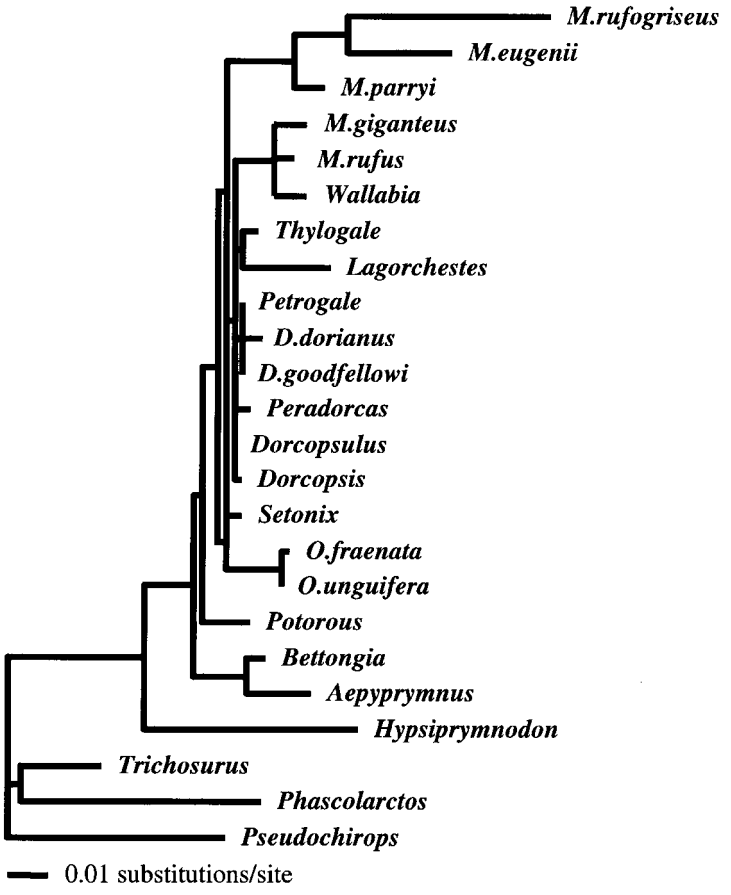

Fig. 2. Maximum likelihood phylogram (HKY85 model with TS : TV = 1.3 : 1) based on the protamine P1 data set.

Beyond the bootstrap and statistical analyses presented above, there were unique nucleotide synapomorphies that provide support for select macropodoid clades. At position 464 in the protamine alignment, all macropodoids, including *Hypsiprymnodon*, share character state A, whereas the three outgroups share character state C. At position 510 in the protamine alignment, *Hypsiprymnodon* shares character state A with the three outgroup taxa; all other macropodoids have a G at this position. Position 397 in the mt alignment also shows a unique synapomorphy for all macropodoids (A) excepting *Hypsiprymnodon*, which shares character state G with all of the outgroup taxa at this site. Macropodines are united together by a unique change (A) at position 1133 in the mt alignment; potoroines, *Hypsiprymnodon*, potoroines, and all of the outgroup taxa share a C at this site. At position 397 in the mt alignment, all macropodines excepting *Dorcopsis* and *Dorcopsulus* share a G whereas *Dorcopsis* and *Dorcopsulus* share character state A with potoroines, *Hypsiprymnodon*, and all of the outgroup taxa. *Aepyprymnus* and *Bettongia* exhibit unique synapomorphies at positions 601, 602, 641, and 648 in the protamine alignment.

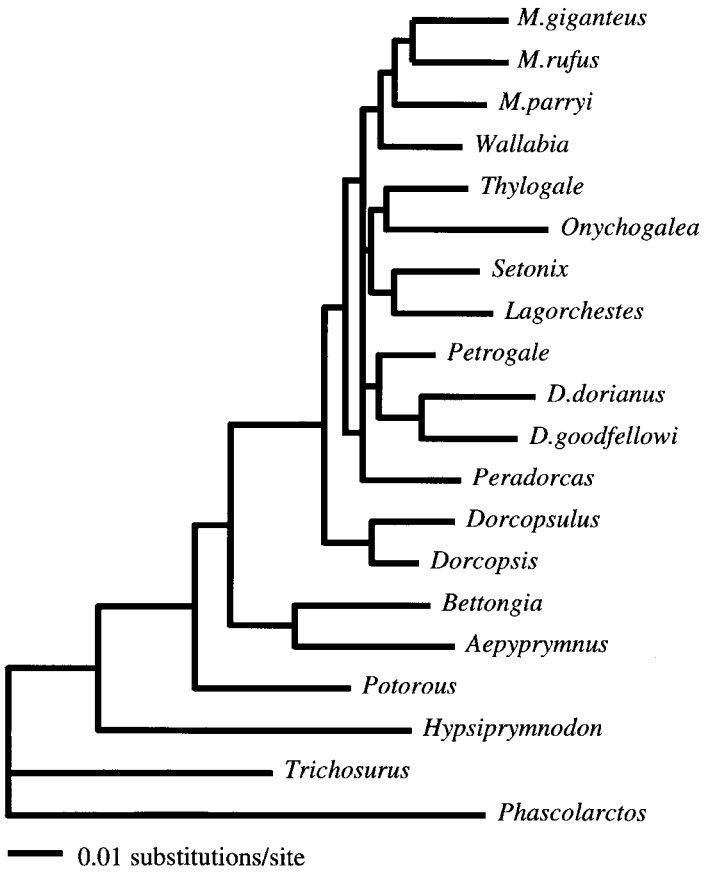

Fig. 3. Maximum likelihood phylogram (HKY85 model with TS : TV = 2.5 : 1) based on the combined mtDNA + protamine P1 data sets.

Divergence Times

HKY85 transversion distances for the mt data were as follows: 0.00335 to 0.01481 within Macropodinae; 0.01613 to 0.02262 within Potoroinae; 0.03417 to 0.04318 between Macropodidae and *Hypsiprymnodon*; and 0.03503 to 0.05331 between macropodoids and the outgroups. Divergence times based on HKY85 transversions, with and without rate corrections, are given in Table V. Divergence times ranged from 3.4 to 43.8 mya without relative-rate corrections and 3.7 to 38.4 mya with relative-rate corrections. Kangaroos separated from non-macropodoid diprotodontians in the range of 38 to 44 mya. *Hypsiprymnodon* separated from Macropodidae in the range of 34 to 38 mya. Among potoroines, *Potorous* diverged from *Bettongia* and *Aepyprymnus* at approximately 22 mya in the early Miocene, nearly concurrent with the calibrated macropodine—potoroine divergence (23.5 mya); *Bettongia* and *Aepyprymnus* diverged approximately 15 to 17 mya. Among macropodines, intrageneric divergence times (i.e. within *Dendrolagus* and *Macropus*) were estimated at 3–4 mya and divergence times among closely affiliated

Table III. Results of Statistical Tests[a]

	Mitochondrial RNA					Protamine P1					MtRNA + Protamine P1				
Hypothesis	w/ clade	w/o clade	WS	T	KH	w/ clade	w/o clade	WS	T	KH	w/ clade	w/o clade	WS	T	KH
Macropodoidea monophyly	1961	1965	0.716	0.641	0.595	241	249	0.058	0.034*	0.034*	1741	1752	0.336	0.253	0.253
Macropodidae monophyly	1961	1971	0.217	0.233	0.134	241	243	0.727	0.480	0.480	1741	1760	0.026*	0.050*	0.049*
Potoroinae monophyly	1962	1961	1.000	0.870	0.851	242	241	1.000	0.529	0.529	1744	1741	0.699	0.738	0.729
Bettongia + *Aepyprymnus*	1961	1965	0.530	0.463	0.402	241	246	0.156	0.135	0.135	1741	1751	0.253	0.204	0.204
Macropodinae monophyly	1961	1972	0.173	0.192	0.134	241	241	1.000	1.000	1.000	1741	1758	0.146	0.100	0.100
Tree Kangaroo (TK) monophyly	1961	1973	0.228	0.177	0.402	241	241	1.000	1.000	1.000	1741	1752	0.091	0.063	0.063
Rock Wallaby (RW) monophyly	1962	1961	1.000	0.846	0.825	241	241	1.000	1.000	1.000	1741	1742	0.979	0.899	0.898
New Guinean Forest Wallaby (NGFW) monophyly	1961	1970	0.362	0.329	0.266	241	241	1.000	1.000	1.000	1741	1751	0.326	0.231	0.231
TKs + RWs	1961	1964	0.702	0.666	0.622	241	241	1.000	1.000	1.000	1741	1742	0.894	0.922	0.909
TKs + NGFWs	1968	1961	0.349	0.490	0.431	242	241	1.000	0.528	0.528	1747	1741	0.244	0.149	0.139
All macropodines except NGFWs	1961	1963	0.716	0.840	0.816	243	241	0.845	0.358	0.358	1742	1741	0.987	0.890	0.891
Macropus + *Wallabia*	1962	1961	1.000	0.869	0.851	241	242	ns[b]	ns[b]	ns[b]	1741	1743	0.899	0.809	0.808
Macropus monophyly	1961	1962	1.000	0.892	0.876	243	241	ns[c]	ns[c]	ns[c]	1741	1743	1.000	0.793	0.795
Notamacropus	1961	1965	0.595	0.488	0.432	241	246	0.213	0.128	0.128	na	na	na	na	na
Osphranter	1961	1962	1.000	0.892	0.876	na	na	na	na	na	na	na	na	na	na

[a] Asterisks (*) indicate significance at $p = 0.05$. KH, Kishino–Hasegawa; T, Templeton; WS, winning-sites; na, not applicable.
[b] The *Macropus* + *Wallabia* constraint yielded 48,039 most parsimonious trees that were not significantly different (ns) from the best tree.
[c] The *Macropus* monophyly constraint yielded 17,667 most parsimonious trees that were not significantly different (ns) from the best tree.

Table IV. Results of Statistical Tests[a]

	Mitochondrial RNA			Protamine P1			MtRNA + Protamine P1		
	-Ln likelihood			-Ln likelihood			-Ln likelihood		
Hypothesis	w/ clade	w/o clade	KH	w/ clade	w/o clade	KH	w/ clade	w/o clade	KH
Macropodoidea monophyly	13893.736	13895.508	0.903	2178.838	2201.392	0.030*	13586.321	13605.641	0.201
Macropodidae monophyly	13893.736	13930.678	0.021*	2178.838	2184.507	0.600	13586.321	13639.091	0.001*
Potoroinae monophyly	13893.736	13894.465	0.955	2179.270	2178.838	0.869	13590.651	13586.321	0.804
Bettongia + *Aepyprymnus*	13893.736	13916.089	0.192	2178.838	2191.811	0.165	13586.321	13618.547	0.168
Macropodinae monophyly	13893.736	13944.038	0.006*	2178.838	2185.143	0.318	13586.321	13656.454	0.013*
Tree Kangaroo (TK) monophyly	13893.736	13932.457	0.071	2178.838	2178.838	1.000	13586.321	13624.674	0.014*
Rock Wallaby (RW) monophyly	13893.736	13894.013	0.972	2178.840	2178.838	0.480	13587.662	13586.321	0.882
New Guinean Forest Wallaby (NGFW) monophyly	13893.736	13921.975	0.091	2178.838	2178.838	1.000	13586.321	13619.639	0.113
TKs + RWs	13893.736	13899.620	0.739	2178.838	2178.838	1.000	13586.321	13591.546	0.737
TKs + NGFWs	13916.071	13893.736	0.199	2186.326	2178.838	0.249	13614.738	13586.321	0.168
All macropodines except NGFWs	13893.736	13901.135	0.630	2183.737	2178.838	0.739	13586.321	13593.390	0.465
Macropus + *Wallabia*	13893.736	13896.089	0.850	2179.111	2178.838	0.974	13586.321	13595.235	0.371
Macropus monophyly	13893.736	13907.617	0.482	2187.576	2178.838	0.441	13586.321	13587.299	0.948
Notamacropus	13893.736	13905.707	0.308	2178.838	2200.427	0.056	na	na	na
Osphranter	13893.736	13895.168	0.861	na	na	na	na	na	na

[a] Asterisks (*) indicate significance at $p = 0.05$. Abbreviations as in Table III.

Table V. Divergence Times[a] of Macropodoid Clades Based on Transversion Distances

Comparison	Relative Rate Adjustment	
	Without Rate Adjustments	With Rate Adjustments
1. Outgroup to macropodoids	43.8	38.4
2. *Hypsiprymnodon* to macropodids	37.9	33.5
3. *Potorous* to *Bettongia/Aepyprymnus*	22.3	22.1
4. *Bettongia* to *Aepyprymnus*	16.6	15.3
5. RWs to TKs[b]	6.0	6.4
6. *D. dorianus* to *D. goodfellowi*	4.3	4.3
7. *Dorcopsis* to *Dorcopsulus*	5.6	5.9
8. *M. agilis* to *M. parryi*	3.4	3.7
9. *Petrogale* to *Peradorcas*	4.3	4.8

[a] Divergence times are in millions of years. Transversion distances were calculated under the HKY85 model.
[b] RW = Rock Wallaby; TK – Tree Kangaroo.

genera (*Petrogale* and *Peradorcas*; *Dorcopsulus* and *Dorcopsis*) were slightly older (4–6 mya).

DISCUSSION

Mitochondrial DNA Versus Protamine P1

The mtDNA sequences provide more resolution than the protamine P1 sequences. Nevertheless, protamine P1 does provide independent support for some of the same clades that are supported by mtDNA including Macropodoidea, Macropodidae, *Bettongia* + *Aepyprymnus*, and subgenus *Notamacropus*. Less resolution from the protamine P1 data set is not surprising given its smaller size (591 nucleotide positions) relative to the mtDNA data set (2412 nucleotide positions). Indeed, for the 18 macropodoids and two outgroups shared in common between the mtDNA and protamine data sets, we performed variable length bootstrap analyses (minimum evolution with maximum likelihood distances) and resampled both mtDNA and protamine at the size of the smaller protamine data set. Protamine provided higher bootstrap support for Macropodoidea monophyly (100% versus 45%), Macropodidae monophyly (95% versus 83%), *Aepyprymnus* + *Bettongia* (99% versus 72%), and *Macropus* + *Wallabia* (55% versus 20%). MtDNA provided higher support for Macropodinae monophyly (95% versus 46%), *Dorcopsis* + *Dorcupsulus* (93% versus 14%), and *Dendrolagus* monophyly (74% versus 2%). Average bootstrap values across the tree were higher for protamine (52%) than mtDNA (42%). Thus, the protamine and mtDNA data sets provide comparable levels of phylogenetic information when corrections for data set size are employed.

Phylogenetic Relationships Among Extant Kangaroos

Results presented here add to evidence regarding the phylogenetic position of *Hypsiprymnodon*. Traditionally, *Hypsiprymnodon* was placed in the subfamily Potoroinae based on a variety of morphological characters (Archer, 1984; Flannery, 1989). Our placement of *Hypsiprymnodon* as a sister taxon to all other extant kangaroos is consistent with Szalay's (1994) analysis of tarsal morphology and subsequent reclassification of *Hypsiprymnodon* into a separate family within Macropodoidea. Baverstock *et al.* (1989) and Burk *et al.* (1998) also found that *Hypsiprymnodon* was distinct from other macropodids on the basis of microcomplement fixation data and mtDNA sequences, respectively. Burk *et al.* (1998) concluded that morphological and life history evidence also provided greater support for a separation of *Hypsiprymnodon* from other kangaroos than for an association of *Hypsiprymnodon* with Potoroinae. The additional taxa and nucleotide sequences included in the present study demonstrate increased support for Macropodidae (macropodines + potoroines) to the exclusion of *Hypsiprymnodon*. Further, statistical tests with parsimony and maximum likelihood provide significant support for macropodid monophyly.

Neither mitochondrial DNA nor protamine P1 sequences resolved whether or not potoroines are monophyletic (see Table II). Flannery's (1989) morphological characters that support potoroine monophyly, all affiliated with the periotic, are questionable because available periotic material was limited and constancy within taxa could not be determined. Szalay (1994; p. 259) stated that while he did not contest the "phyletic distinctness" of potoroines, there are no significant properties of the foot "that would support such a monophyly independent of a macropodid ancestry." Within Potoroinae, Bettongini (*Bettongia* + *Aepyprymnus*) is supported by both mtDNA data (mean support value = 93%), protamine P1 data (mean support value = 97%), and morphological characters (e.g. postglenoid process and discrete ectotympanic process; Flannery, 1989).

DNA hybridization studies support the separation of *Dorcopsulus* from the other macropodines that were examined (Springer and Kirsch, 1991; Kirsch *et al.*, 1995). In addition, dental characteristics (Woodburne, 1967; Flannery, 1984) and 12S rRNA and tRNA-valine sequences (Burk *et al.*, 1998) support a basal split of *Dorcopsulus* and *Dorcopsis* from other macropodines. A clade consisting of all macropodines excepting the New Guinea forest wallabies was recovered in several of our analyses (see Figs. 1 and 3), but never with strong bootstrap support. We do note that there is a putative synapomorphy for all macropodines excepting *Dorcopsis* and *Dorcopsulus* at position 397 in the mtDNA data set.

An association of the two rock wallabies, *Petrogale* and *Peradorcas*, does not receive strong bootstrap support from either mtDNA (34–69%) or protamine P1 (0–8%). Although our study does not begin to include enough species to address the monophyly of rock wallabies, it is interesting to note that Thomas (1904) suggested removing *Peradorcas* from the genus *Petrogale* based on the presence of continually erupting molars in *Peradorcas*. Our results, in agreement with other molecular studies (Baverstock *et al.*, 1989; Kirsch *et al.*, 1995), favor an association of tree kangaroos with rock wallabies. We find no support for an association of tree kangaroos and New Guinea forest wallabies (i.e., support values <1%) as was suggested based on dental characteristics in the first half of the 20th century (Bensley, 1903; Raven and Gregory, 1946; Tate, 1948). More recent

studies of dental characters, such as Flannery (1984), suggest that shared dental features of tree kangaroos and New Guinea forest wallabies (e.g., large canines, low crowned molars with weak linking) are primitive rather than derived features within Macropodinae.

Macropus monophyly is supported by chromosome number ($2n = 16$, except *M. barnardus* and *M. rufus*; Rofe, 1978), morphological characters (Dawson and Flannery, 1985; Flannery, 1989), and molecular studies (Richardson and McDermid, 1978; Kirsch, 1977). Although the chromosome morphology of *Wallabia* is distinct from *Macropus* ($2n = 10, 11$; Hayman and Martin, 1974), *Wallabia* has been known to hybridize with *M. agilis* (Smith *et al.*, 1979) and has been placed within *Macropus* based on DNA hybridization (Kirsch *et al.*, 1995) and MC'F (Baverstock *et al.*, 1989). Despite our limited sampling of *Macropus* species, it is interesting to note results pertaining to *Macropus* monophyly and the position of *Wallabia* relative to this group. Although bootstrap support values for *Macropus* monophyly and an affinity between *Macropus* and *Wallabia* are low (Table II), *Macropus* species and *Wallabia* were consistently associated in our analyses. Typically, *Wallabia* represents a sister taxon to a monophyletic *Macropus*; however, in some analyses, *Wallabia* is placed within *Macropus*. Hypothesis testing was unable to demonstrate statistical significance for either arrangement (Table III and Table IV). Among *Macropus* species, there was some support for *M. (Notamacropus) agilis* + *M. (Notamacropus) parryi* based on mtDNA and *M. (Notamacropus) rufogriseus* + *M. (Notamacropus) eugenii* + *M. (Notamacropus) parryi* based on protamine P1.

Molecular Divergence Times and the Fossil Record

Case (1989) proposed a family level radiation among arboreal possums in the mid- to late Eocene in response to an increase in arboreal habitat diversity (a change from podocarp- to *Nothofagus*-dominated forest). Molecular data place the divergence of kangaroos from other phalangeriforms in the Eocene (mtDNA: 38–44 mya; DNA hybridization: 54–55 mya; Springer and Kirsch, 1991, Kirsch *et al.*, 1997). MtDNA data further suggest that hypsiprymnodontids separated from other extant lineages in the range of 34 to 38 million years. In contrast, the fossil record for kangaroos is younger. The first macropodoid, a plesiomorphic potoroine (*Kyeema mahoneyi*), is known from the stratigraphically lowest zone within the Etadunna Formation (24–26 mya; Prideaux, 1999; Case, in press). Hypsiprymnodontids do not appear until the latest Oligocene/early Miocene in Riversleigh System B (Prideaux, 1999). This discrepancy between molecular estimates and the fossil record may be explained by the depauperate Cenozoic fossil record in Australia prior to the latest Oligocene (Kirsch *et al.*, 1997; Archer *et al.*, 1989). Also, fossil macropodoids from late Oligocene demonstrate that potoroines and macropodines were already distinct and hint at an earlier evolutionary history yet to be uncovered in the fossil record.

Sudden cooling at the end of the Eocene marks the onset of the drying of the Australian continent (White, 1994). In the Oligocene, ice build up in Antarctica resulted in a decrease of rainfall and the introduction of climates with seasonal aridity in Australia (White, 1994). During the Oligocene, there was a decline of moist, *Nothofagus*—dominated rainforests (Martin, 1994) and an expansion of drier sclerophyll (leathery leaf) woodlands, sedgeland, and reed swamp communities (Macphail *et al.*, 1994). These conditions may have promoted an increase in the terrestrial marsupial fauna (Case, 1989). Molecular estimates for the separation of *Hypsiprymnodon*

from other macropodoids at 34–48 million years ago are in line with predictions concerning the expected increase in the terrestrial fauna during the Oligocene. Molecular divergence times among potoroine genera (15 to 22 mya) coincide with an increasingly drier and more seasonal climate in Australia during the late Oligocene to medial Miocene (Prideaux, 1999).

The first undisputed appearance of a macropodine (*Dorcopsoides fossilis*) occurs in the late Miocene Alcoota local fauna (Woodburne, 1967). Evidence for a macropodine radiation [Hamilton and Bow local faunas (Flannery and Archer, 1984; Flannery *et al.*, 1992)] appears in the fossil record of the early Pliocene (Flannery and Hann, 1984). In agreement with the fossil record, mtDNA evidence supports a recent radiation of macropodine genera with diversification concentrated in the last five to ten million years. This radiation coincides with further drying of the Australian climate and expansion of grasslands in the late Miocene and Pliocene (Case, 1989; Megirian, 1992; Prideaux, 1999).

Character Evolution in Macropodoidea

The ancestor of all kangaroos is believed to have been a small, arboreal, possum-like marsupial (Winge, 1941; Flannery, 1987, 1989; Springer and Woodburne, 1989; Szalay, 1994). Like modern possums, this ancestor probably had a prehensile tail and opposable hallux, facilitating movement through the trees. *Hypsiprymnodon moschatus* is thought to possess numerous primitive features of an early macropodoid (Szalay, 1994). The sister group relationship of *Hypsiprymnodon* to other kangaroos provides insight into the evolutionary origins of morphological features associated with the early transition into a more terrestrial habitat. *Hypsiprymnodon* shares morphological characters associated with the transition to terrestrial locomotion with all other kangaroos (e.g. stepped calaneocuboid joint, elongated foot, and aspects of the transverse tarsal joint; see Table VI). These features confer transverse stability and enhance flexion-extension in the tarsus (Szalay, 1994). Unlike other kangaroos, however, *Hypsiprymnodon* maintains a quadrupedal gait (Johnson and Strahan, 1982) and lacks specializations associated with bipedal hopping (e.g. loss of hallux, loss of prehensile tail, enlarged hindlimbs; see Table VI). In addition, macropodids developed features that restrict mobility of the lower limb joints to a fore-aft direction [e.g. plantar and medial extension of the auxiliary calcaneocuboid facet, a calcaneofibular facet, and a well-developed, concave, medial astragalotibial facet for the distal articular surface of the medial malleolus (Flannery, 1984; Szalay, 1994)].

In light of molecular data, along with the conclusions of Szalay (1994) and Ride et al. (1997) based on morphology that there is a basal split between hypsiprymnodontids and macropodids, the most parsimonious explanation is that bipedal hopping evolved once following the divergence of hypsiprymnodontids (hypsiprymnodontines and propleopines) from a macropodid clade containing potoroines, bulungamayines, balbarines, macropodines, and sthenurines. Ride *et al.* (1997) found that the form and disposition of the pectoral and deltoid ridges and the degree of humeral torsion distinguish humeri of *Propleopus oscillons* from bipedal macropodiforms. Similarities among *P. oscillons* and *H. moschatus* humeri led Ride *et al.* (1997) to conclude that *P. oscillans* was a quadrupedal bounder. Postcranial elements have yet to be described for balbarines and bulungamayines; however, Bishop (1997) concluded that sthenurines were bipedal hoppers based on a comparative analysis of macropodoid pes.

Table VI. Character States Related to Locomotory Habit

	Taxa			
	Hypsiprymnodon	Macropodidae	*Dendrolagus*	Reference
1. Loss of Hallux	No	Yes	Yes	Szalay, 1994
2. Loss of digital pads	No	Yes	Yes	Flannery, 1989
3. Reduction of 2nd and 3rd digits on foot	No	Yes	Yes	Szalay, 1994
4. Loss of prehensile tail	No	Yes	Yes	Szalay, 1994; Flannery et al., 1995
5. Forelimbs much shorter than hindlimbs	No	Yes	No	Johnson and Strahan, 1982; Flannery, 1989; Szalay, 1994
6. Lengthened tibial-fibular contact	No	Yes	No	Flannery and Szalay, 1982
7. Stepped calcaneocuboid joint	Yes	Yes	De-emphasized	Flannery and Szalay, 1982; Szalay, 1994
8. Foot elongate	Yes	Yes	No	Szalay, 1994
9. Transverse tarsal joint: flexion/extension articulation	Yes	Yes	No (medio/lateral articulation	Szalay, 1994

Tree kangaroos represent a reinvasion of an arboreal habitat, having arisen from terrestrial macropodine ancestors. Not surprisingly, tree kangaroos possess adaptations to arboreality (e.g. longer, stronger forelimbs; see Table VI). However, rather than a reversion to arboreal specializations presumably present in the arboreal ancestor of all kangaroos (e.g. prehensile tail or hallux), tree kangaroos have modified ancestral macropodine features into arboreal adaptations (Szalay, 1994; see Table VI). For example, the stepped calcaneocuboid joint of macropodoids is de-emphasized to increase rotational ability of the foot allowing a better grip on branches (Flannery and Szalay, 1982). In addition, the emphasis of the transverse tarsal joint has shifted from flexion-extension of terrestrial kangaroos to mediolateral mobility in tree kangaroos (Szalay, 1994).

Interestingly, Flannery *et al.* (1995) conclude that one lineage of tree kangaroo, *D. mbaiso*, has re-adapted to a terrestrial lifestyle. In comparison to other tree kangaroos, *D. mbaiso* possesses a gracile skeleton, a relatively long hindfoot, relatively extensive tibia-fibula contact, and an elongate proximal tibia epiphysis (Flannery *et al.*, 1995). If *D. mbaiso* indeed is a derived member of the genus, it appears that evolutionary reversion between arboreal and terrestrial lifestyles and associated specializations, although imperfect, has occurred multiple times in the history of macropodoids.

The forage of macropodoids has three basic grades: mixed invertebrate and very low fiber plant diet, low fiber leaf diet (browsers), and high fiber grass diet (grazers) (Sanson, 1989). Hypsiprymnodontines and propleopines display a more primitive dental morphology; in *Hypsiprymnodon*, this morphology is correlated with an omnivorous diet

(Wroe *et al.*, 1998). Hypsiprymnodontines and propleopines did not develop lophodont molars; members of these lineages were likely characterized by a simple stomach similar to that of extant *H. moschatus*. Potoroines possess premolars that function as longitudinal shearing blades and tubercular molars. Within this lineage, Sanson (1989) noted increased development of shearing edges in molars of *Bettongia* and *Aepyprymnus*, which correlate with an increase of plant material in the diet. In fact, *Aepyprymnus* shows incipient lophodonty (Sanson, 1989). Potoroine stomach morphology is more complex than that of *Hypsiprymnodon*, with a complex forestomach designed for storage and fermentation of plant material (Freudenberger *et al.*, 1989).

The diet of browsing kangaroos is heterogeneous in nature, requiring the teeth to perform several different actions in order to effectively masticate a wide variety of plant material (Sanson, 1989). Strict browsers maintain permanent premolars that function as shearing blades in addition to lophodont molars capable of shearing and grinding action (Sanson, 1989). In contrast, macropodoids that graze on a more uniform substrate of grasses possess smaller premolars and shift the masticatory emphasis to molars of enhanced shearing capacity (Sanson, 1989). Dental morphology provides insight into the diet preferences of fossil kangaroos lineages. Balbarines, although fully bilophodont, retain plesiomorphic dental characteristics (e.g. P_3 ridgelets similar to P_3 morphology of *H. moschatus*; Flannery and Rich, 1986) and were presumably browsers. Bulungamayines possess finely ridged bulbous premolars that are similar to those of the present-day macropodine browsers, *Dorcopsis* and *Dorcopsulus* (Flannery *et al.*, 1983). Sthenurines developed craniodental specializations (i.e., anterior-oriented pull of the masseter musculature, rigidly ankylosed dentaries, wide premolars, and a laterally curved molar row; Prideaux, 1999), which led to increased occlusal force capabilities utilized for mastication of tough browse vegetation. Macropodinae is comprised of browsers, intermediate browser/grazer forms, and grazers. In all browsers and grazers, we infer the presence of a complex stomach adapted to digesting plant material. However, stomach morphology reflecting adaptations to a homogeneous grass diet (i.e. reduced sacciform forestomach and a greatly enlarged tubiform forestomach; Freudenberger *et al.*, 1989) may have developed only in the macropodine lineage.

From late Eocene onward, Australia became increasingly arid with grasslands appearing by the middle Miocene. The increase of grasslands provided an abundant supply of poorly nutritive food. Energy requirements relative to body mass decrease with increasing body size (Freudenberger *et al.*, 1989). Because gut capacity tends to be a constant proportion of body mass, large animals are more likely than small animals to be able to use plant material of low nutritive value (Freudenberger *et al.*, 1989). Consequently, smaller species tend to feed on high quality foods, medium size species are more generalist and take in a wide variety of low fiber content vegetation, and larger species are specialized grass eaters (Dawson, 1989). The appearance of larger kangaroos (better able to exploit grasses as a food source) in the medial to late Miocene fossil record is correlated with the appearance of grasslands in the middle Miocene (Prideaux, 1999; White, 1994). The increase in global aridity during the late Tertiary influenced the evolution of grasslands worldwide. Grazing adaptations among kangaroos (e.g., increase in body size, development of dental shearing systems, and development of plant fermentation chambers in the stomach) parallel evolutionary changes among placental grazers on other continents [e.g., North American horses (MacFadden, 1992)].

The Chronicle of Macropodoid Evolution

Molecular phylogenies and divergence datings, paleobotanical information and paleoclimatic reconstructions, the fossil record of kangaroos, and trends in the evolution of morphological characters within Macropodoidea provide insights into kangaroo evolution. Given these lines of evidence, we suggest the following chronicle for the history of kangaroos.

In the early to medial Eocene, a possum-like protomacropodoid began its descent from the trees to the rainforest floors. In the medial to late Eocene, global cooling led to a shift from a warm, moist Australian climate, in which non-seasonal rainforests prevailed, to a climate that became progressively cooler and drier (Woodburne and Case, 1996). Macropodoid evolution appears to have been driven by changes in vegetation resulting from the progressive drying out of Australia that commenced in the Eocene.

All kangaroos share tarsal modifications associated with the transition from arboreal to terrestrial locomotion (Szalay, 1994). These modifications most likely developed in the ancestral lineage of all kangaroos prior to the divergence of propleopines and hypsiprymodontines from the remaining kangaroo lineages. MtDNA derived divergence times estimate the divergence of hypsiprymnodontines from macropodines and potoroines at 34–38 mya. By the end of the Eocene, early kangaroos probably had tarsal modifications appropriate for terrestrial locomotion similar to modern day *H. moschatus*. Hypsiprymnodontines remained small, with simple stomachs capable of digesting an omnivorous diet. Today hypsiprymnodontines persist in refuge rainforest habitat of north Queensland, Australia (Flannery, 1984). In contrast, propleopines developed into giant rat kangaroos that survived until their extinction at the end of the Pleistocene (Prideaux, 1999). Their phylogenetic association with hypsiprymnodontines (Ride, 1993; Wroe and Archer, 1995) suggests that propleopines may have retained a simple stomach as well. In addition, the dentition of propleopines does not appear adapted to a diet high in plant material, and there has even been speculation about whether giant rat kangaroos were carnivorous (Archer and Flannery, 1985; Ride *et al.*, 1997; Wroe *et al.*, 1998).

Throughout the Oligocene a decrease in rainfall resulted in drier, more open forests (*Nothofagus* declined and sclerophyllous plant taxa increased; Martin, 1994; White, 1994). Subsequent to the hypsiprymnodontid-macropodid split, but prior to the macropodine-potoroine split, specializations such as bipedal hopping and a more complex stomach capable of fermenting structural plant material evolved. Given the competing hypotheses for the phylogenetic placement of Bulungamayinae, Balbarinae, and Sthenurinae, these specializations likely characterized these macropodid taxa as well. Molecular and fossil data suggest that potorines diversified soon after the macropodine-potoroine split, and potoroines persist in the modern Australian fauna as small bettongs and rat-kangaroos occupying habitats that range from rainforest to grasslands.

The late Oligocene to medial Miocene was a period of climatic oscillation. Although there were wetter intervals, overall the climate of Australia became increasingly drier and more seasonal (Prideaux, 1999). Bulungamayines, balbarines, macropodines, and sthenurines all possess lophodont molars. Whether lophodonty evolved once or more than once remains unclear. However, lophodonty probably developed in response to an increase in the availability of low fiber plant material (browse) in the late Oligocene to medial Miocene. Of the four lophodont lineages of kangaroos, bulungamayines and bal-

barines represent earlier forms that disappeared by the late Miocene, followed by the appearance of macropodines and sthenurines in the medial to late Miocene.

Sthenurines and the macropodines also included forms adapted to non-rainforest habitats that began to cover much of Australia by the Pliocene. Sthenurines were the first larger sized wallabies to appear in the fossil record. Sthenurines were rare until they radiated in the Plio-Pleistocene, following the development of craniodental specializations for obtaining and masticating browse material (Prideaux, 1999). Like the propleopines, sthenurines disappeared by the end of the Pleistocene. Within Macropodinae, almost all the modern genera appear in the Pliocene fossil record (Flannery and Archer, 1984; Flannery *et al.*, 1992; Flannery and Hann, 1984), suggesting that macropodines underwent a spectacular radiation at this time. This diversification of macropodines coincided with the appearance of grasslands in Australia (Case, 1989; Megirian, 1992; Prideaux, 1999). Many macropodines became specialized grazers, developing dental and stomach specializations adapted to a high fiber diet of grasses (e.g. *Macropus giganteus*; Sanson, 1989). Like modern potoroines, present day macropodines occupy habitats as diverse as rainforest (both terrestrial and arboreal forms) to grassy dunes and deserts.

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