Mitogenome of the Extinct Desert 'Rat-Kangaroo' Times the Adaptation to Aridity in Macropodoids

Michael Westerman

La Trobe University

Stella Loke

Deakin University

Mun Hua Tan

Deakin University

Benjamin Kear (✉ benjamin.kear@em.uu.se)

Uppsala University

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Abstract

The evolution of Australia's distinctive marsupial fauna has long been linked to the onset of continent-wide aridity. However, how this profound climate change event affected the diversification of extant lineages is still hotly debated. Here, we assemble a DNA sequence dataset of Macropodoidea – the clade comprising kangaroos and their relatives – that incorporates a complete mitogenome for the Desert 'rat-kangaroo', *Caloprymnus campestris*. This enigmatic species went extinct nearly 90 years ago and is known from a handful of museum specimens. *Caloprymnus* is significant because it was the only macropodoid restricted to extreme desert environments, and therefore calibrates the group's specialisation for increasingly xeric conditions. Our robustly supported phylogenies nest *Caloprymnus* amongst the bettongs *Aepyprymnus* and *Bettongia*. Dated ancestral area optimisations further reveal that the *Caloprymnus*-*Bettongia* lineage originated in nascent arid zone settings from the later-middle to early-late Miocene, ~12 million years ago (Ma), but subsequently dispersed into mesic habitats during the Pliocene and Pleistocene. This coincides with ancestral divergences amongst kangaroos in disparate woodland-forest and shrubland settings, but predates their adaptive radiation into proliferating grasslands during the late Miocene to Pliocene, after ~7 Ma. We thus demonstrate that protracted changes in both climate and vegetation likely staged the emergence of modern arid zone macropodoids.

Introduction

Arid zone marsupials are icons of Australia and have an inferred evolutionary history that extends back over some ~15 Ma¹. Nevertheless, the precise divergence timings of the major extant clades are ambiguous, as are the possible drivers behind their adaptive radiations²⁻¹³.

Macropodoids (Macropodiformes: Macropodoidea) ³/₄ the group encompassing living kangaroos, wallaroos, wallabies, pademelons and tree-kangaroos (Macropodidae), bettongs and potoroos (Potoroidae), the Musky rat-kangaroo (*Hypsiprymnodon moschatus*: Hypsiprymnodontidae), and their stem antecedents¹⁴ ³/₄ incorporate some of the most distinctive Australian arid zone marsupials, as epitomised by the famous Red kangaroo, *Osphranter rufus*¹⁵. The well-documented fossil record of this and other 'true kangaroos' (Macropodini) has been used to correlate arid zone macropodoid evolution with the expansion of intracontinental grasslands during the Pliocene and Pleistocene, from ~3–4 Ma^{3,9,12}. By contrast, the contemporary diversification of xeric-adapted bettongs is often overlooked, but has considerable significance because it includes the only example of an exclusively desert-inhabiting macropodoid, the Desert 'rat-kangaroo', which is alternatively referred to as the "Oolacunta"¹⁶ or Ngudlukanta¹⁷, *Caloprymnus campestris* (Figure 1A).

The first scientific specimens of *C. campestris* were collected from northeastern South Australia (Figure 1B) in 1842, with three preserved examples subsequently shipped to London for study¹⁸. These were dubbed '*Bettongia*' *campestris* by Gould¹⁹, although Thomas²⁰ later recognised '*B.*' *campestris* as morphologically distinct from *Bettongia*, and thus established a separate genus, *Caloprymnus*. No further

sightings of *C. campestris* were reported after this initial description, and the species was assumed to be extinct for some 90 years until Finlayson^{16,21} announced the “Rediscovery of *Caloprymnus campestris*” in 1931-1932, from the remote Kooncheera Dune¹⁷ region in the Sturt Stony Desert of far northeastern South Australia (Figure 1C). Since then, only a skin recovered sometime between 1902 and 1905 (Figure 1D) has been reidentified²², and various unsubstantiated live sightings made^{17,23,24}, with the most recent being in 2011²⁴ and 2013¹⁷, prompting unsuccessful surveys for the species in 2018 and 2019¹⁷. *Caloprymnus campestris* has otherwise been formally classified as Extinct by the IUCN (<https://www.iucnredlist.org/>) since 1994, with the probable cause being over-predation by feral dogs, cats and foxes²⁵.

At latest count, only 25 specimens of *C. campestris* are catalogued in museums worldwide²². This dearth of research material has led to uncertainty about potoroid interrelationships²⁶, as well as the concomitant chronicle of their arid zone evolution. Here, we therefore analyse the first complete mitochondrial (mt) genome of *C. campestris*, together with new mtDNA extracted for another historically extinct potoroid, the Broad-faced potoroo, *Potorous platyops*, which was last seen alive in 1875²⁷. Our novel data is used to construct a comprehensive phylogeny of crown potoroid species and subspecies within Macropodoidea. We also apply molecular clock calibrated ancestral area optimisations to infer both the timing and context of macropodoid habitat change over the last ~25 Ma.

Materials And Methods

Samples and sequencing. We obtained non-formalin-fixed liver samples from a male *Caloprymnus campestris* (Museums Victoria, Melbourne, Australia [NMV] C8981) that was collected in 1834 from Mulka cattle station in northeastern South Australia (Figure 1B). In addition, we processed dried skin samples of *Potorous platyops* (NMV C6770), which were recovered in 1874 from southwestern Western Australia²⁷. Our DNA extraction, PCR amplifications, sequencing and alignment procedures followed Westerman *et al.*^{27,28}. Whole genome libraries were prepared with the *Nextera* DNA flex library kit (Illumina, CA), incorporating 50 ng of input DNA. Sequencing was performed on the *Illumina* MiSeq platform using 2 x 300 bp V3 chemistry to generate 1.77 Gb of sequence data. Raw reads were trimmed for adapters and quality using *Trimmomatic* 0.36²⁹ (sliding window = 4:15; leading = 3; trailing = 3), and then assembled with *IDBA-UD* 1.1.1³⁰ (mink = 20; maxk = 300; min_contig = 500). The resulting *C. campestris* mitogenome was annotated using the *MITOS* webserver³¹ with start-stop positions for protein coding genes manually curated using *blastp* homologies extracted from the NCBI non-redundant (*nr*) database. We were unable to assemble a complete mitogenome for *P. platyops*, although partial NADH1, COX1/COX2 and 16S rRNA sequences (Supplementary Table S1) were added to the 12S rRNA and Cyt *b* gene components accessioned into *GenBank*²⁷.

Phylogenetic and molecular clock analyses. Phylogenetic relationships within Macropodoidea were examined using a mitogenome dataset including representatives of all potoroid species, together with *Hypsiprymnodon moschatus* and multiple species-level exemplars for selected macropodid genera (see

Supplementary Table S1). The Northern common cuscus, *Phalanger orientalis* (Phalangeridae), and Western pygmy possum, *Cercartetus concinnus* (Burramyidae), were added as non-macropodoid outgroups. To accommodate for recognised gene incongruence³², we compared these results with analyses of nuclear (n), and combined mitogenome/mtDNA/nDNA sequence datasets derived from *GenBank*, which integrated an expanded taxon sample of all potoroid species and subspecies (see Supplementary Tables S1 and S2). The mitogenomes were treated as a single partition, or alternatively sub-partitioned into 12S/16SrRNA stems and loops, pooled 1st, 2nd and 3rd protein codon positions, and 3rd codon positions with RY coding to allow for heterogeneity and saturation. A General Time Reversible gene partition model, *gamma* distribution and variable site proportions were determined using *jModelTest*³³ (Supplementary Table S3).

Tree building employed Maximum likelihood and Bayesian methods implemented in *RaxML* 7.2.8³⁴, *MrBayes* 3.2.7³⁵ and *BEAST* 1.8.2³⁶ with node support calculations based on 1000 bootstrap pseudoreplicates (%) and Bayesian Posterior Probabilities (BPP), respectively. Time-trees were constructed in *BEAST* 1.8.2³⁶ with relaxed clocks and the minimum–maximum node age constraints listed in the Supplementary Information. Up to 95% of the normal prior distributions were assigned to the interval between minimum and maximum, with 2.5% to each tail. *Gamma* priors (shape = 1; scale = 1) were assigned to the “ucl.d.mean” parameter for each partition. MCMC analyses were run for 50 x 10⁶ generations with a burn-in of 10 x 10⁶ generations and sampling every 10 x 10³ generations. ESS values were >200 for all estimated parameters. *TreeAnnotator* 1.8.2 (<https://www.beast2.org/treeannotator/>) was used to summarise the tree sample with mean node heights.

Ancestral area analyses. Ancestral area optimisations were scaffolded onto the time-calibrated *BEAST* tree with S-DIVA and Bayesian Binary MCMC (BBM) approaches³⁷ implemented in *RASP* 4³⁸. Four Markov Chains (default heating values) were run over 5 x 10⁶ generations with sampling frequency and burn-in fixed at 500. Among-site rate variation was set to ‘gamma’ and state frequencies utilized a ‘fixed (JC)’ model. Habitat codes followed standardised vegetation units⁶: A = humid forest (rainforest and/or ‘wet’ sclerophyll dominant); B = woodland (‘dry’ sclerophyll dominant); C = shrubland (primarily chenopodiaceous shrublands); and D = grassland-desert (semi-arid/arid grasslands dominant and/or desert).

Results And Discussion

The *Caloprymnus campestris* mitogenome (16,866 bp) is ordered with 13 protein-coding genes, two ribosomal (r)RNA genes, 21 transfer (t)RNAs, and a non-coding AT-rich control region, which follows the typical configuration for marsupials^{39,40}. The tRNAs are arranged around the origin of the L strand (A-C-W-O_L-N-Y) and intersected between the NADH2 and COX1 genes. Substitution of the anticodon GCC for tRNA^{ASP} (*trnD*) is also consistent with RNA-editing⁴¹.

Maximum likelihood and Bayesian analyses of our mitogenome dataset produce unanimous resolution of Macropodoidea with Potoroidae as the sister to Macropodidae (Supplementary Figures S1–S6). This pivotal higher-level grouping is consistent with other crown macropodoid phylogenies^{12,42–45}, and warrants a new taxonomic definition⁴⁶, which we coin as Macropodia, new clade, herein (Table 1; Supplementary Information). Bootstrap and BPP support is >90% for almost all constituent nodes except those uniting: (1) the extinct short-faced kangaroo, *Simosthenurus occidentalis*, with the Banded hare-wallaby, *Lagostrophus fasciatus*, as basally branching macropodids (partitioned/non-partitioned bootstrap = 58/63%; *MrBayes*partitioned/non-partitioned BPP = 0.54/0.56; *BEAST*partitioned/non-partitioned BPP = 1/1); (2) the Quokka, *Setonix brachyurus*, with other macropodines (bootstrap = 48/60%; *MrBayes* BPP = 1/1; *BEAST* BPP = 0.63/0.72); (3) grey kangaroos in the genus *Macropus* with *Osphranter rufus* and brush wallabies representing the genus *Notamacropus* (bootstrap = 80/64%; *MrBayes* BPP = 0.99/1; *BEAST* BPP = 0.99/0.96); and (4) *O. rufus* with *Notamacropus* (bootstrap = 58/55%; *MrBayes* BPP = 0.81/1; *BEAST* BPP = 0.72/0.76). As found by previous studies^{5,12,27,42–47}, Potoroinae comprises potoroos within the genus *Potorous* and is distinguished from its sister clade, which we designate Bettonginae⁴⁸ to include the Rufous bettong, *Aepyprymnus rufescens*, as the basally branching sister to *C. campestris* and the species of *Bettongia* (Table 1). Alternative monophyly of *C. campestris* with either *A. rufescens*^{49,50}, or the species of *Potorous*^{27,46} were tested using topological constraints in *PAUP** 4.0b10⁵¹ (Supplementary Table S4), but decisively rejected ($P < 0.0001^{***}$). Taxonomically, therefore, we conclude that the original classification of Gould's Desert 'bettong'¹⁹ as generically consistent with *Bettongia* is feasible, but defer any formal nomenclatural amendment pending a detailed morphological re-evaluation.

Our maximum likelihood, Bayesian and time-tree analyses of the nDNA (Supplementary Figures S7–S9) and combined mitogenome/mtDNA/nDNA datasets (Figure 2; Supplementary Figures S10–S12) yield broadly compatible topologies, with the basal divergence of potorooids and macropodids, and subsequent split between potoroines and bettongines both occurring during the early Miocene (Table 2; Supplementary Table S4). Notably, this concurs with divergence estimates derived using different dating methods and constraints^{12,42–46,52}. Furthermore, while our S-DIVA and BBM ancestral area optimisations correlate the early Oligocene (or late Eocene based on nDNA⁴¹; Supplementary Table S4) emergence of crown macropodoids with predominantly humid wooded habitats⁵³, the earliest radiation of potorooids, together with members of the macropodid subclades Sthenurinae and Lagostrophinae + Macropodinae, all coordinate with dispersals into shrubland and woodland-forest mosaics (Figure 2; Supplementary Figures S13 and S14). These potentially included 'mallee-like'⁵⁴ sclerophyll communities, which propagated throughout central Australia from the early to middle Miocene⁵³.

The globally recognised⁵⁵ middle to late Miocene climatic transition from equable to increasingly cool, dry conditions^{53,56} coincides with potoroine speciations in mesic environments throughout southern Australia^{27,52}. These are tracked by our S-DIVA and BBM optimisations, which infer occupation of primarily wooded habitats after the early-late Miocene (Supplementary Figures S13 and S14). This is also

concurrent with the incipient desertification of inland Australia⁵⁷, which may have promoted genetic segregation of *Potorous platyops* and Gilbert's potoroo, *Potorous gilbertii*, in central-southern⁵⁸ and southwestern Australia, versus the Long-nosed potoroo, *Potorous tridactylus*, and basally branching Long-footed potoroo, *Potorous longipes*, in southeastern Australia⁵². Additionally, we show that regional subspecies distinctions within *P. tridactylus* were completed by the early to middle Pleistocene (Table 2; Supplementary Table S5). Curiously, though, Cyt *b* K2P variation (Supplementary Table S6) implies substantially less genetic difference between the Tasmanian *P. tridactylus apicalis* and northeastern mainland *P. tridactylus tridactylus* (1.93%), in comparison to the southeastern mainland *P. tridactylus trisulcatus* (4.21%). Indeed, these values approximate those contrasting *P. tridactylus tridactylus*/*P. tridactylus trisulcatus* with *P. gilbertii* (2.69/5%), *P. platyops* (4.1/5%), and *P. longipes* (5.84/5.69%), supporting inferences of cryptic taxa⁵², but in our opinion, only up to species-level.

Despite currently limited DNA sequence coverage for the extinct Finlayson's⁵⁹ Desert bettong, *Bettongia anhydra*⁶⁰, we derive unequivocal support (Figure 2; Supplementary Figures S1–S12) for the monophyly of *Bettongia* spp. (bootstrap = >90%; BPP = 1), together with close relationships between the woodland-forest dwelling Eastern bettong, *Bettongia gaimardi*, Northern bettong, *Bettongia tropica*, and Brush-tailed bettong, *Bettongia penicillata penicillata* (bootstrap = >99%; BPP = 1). Only a few hundred Cyt *b* (or control region) nucleotides are available for the Woylie, *Bettongia penicillata ogilbyi*⁶¹. Nevertheless, our S-DIVA and BBM optimisations suggest seminal late Miocene divergences of *Bettongia* spp. in open xeromorphic habitats (Supplementary Figures S13 and S14), followed by a Pliocene to as recent as middle Pleistocene radiation of *B. gaimardi* + *B. tropica* + *B. penicillata* subsp. (Table 2; Supplementary Table S5) in conjunction with mesic habitat variegation (Supplementary Figures S13 and S14). We attribute this to vicariant 'reversions'⁵ in eucalypt woodlands and forests^{62–64}, which contracted and fragmented with intensifying aridification over the Pliocene–Pleistocene interval⁶⁵.

Bettongia is karyotypically conservative, retaining the 2n=22 chromosomal number of most macropodoids^{66,67}. Conversely, chromosomal fission in *P. longipes* has produced 2n=24, while fusions (and inversions) in *P. tridactylus* and *P. gilbertii* manifest unusual reductions to 2n=12, 13⁶⁸. *Aepyprymnus rufescens*, on the other hand, exhibits a unique karyotypic increase to 2n=32, which is the highest for any marsupial⁶⁸, and presumably reflects its independent evolution since the middle or early-late Miocene (Table 2; Supplementary Table S5). Although the chromosomal arrangement of *C. campestris* is unknown, our robustly supported (bootstrap = >97%; BPP = 1) later-middle to early-late Miocene split from *Bettongia* (Table 2; Supplementary Table S5) suggests a similarly protracted ancestry, yet with genetic differentiation that approaches intrageneric levels within *Bettongia* spp. (Cyt *b* K2P variation being as little as 6.91% compared to *B. penicillata*: Supplementary Table S6). Significantly, our S-DIVA and BBM optimisations correlate the *C. campestris*-*Bettongia* divergence with an invasion of open-xeric environments (Supplementary Figures S13 and S14), perhaps incorporating arid chenopod shrublands and gallery woodlands that spread across central Australia from the late Miocene^{53,54,57}. The coeval radiation of macropodines is otherwise linked to disparate woodland-forest and shrubland

settings (Supplementary Figures S13 and S14), with speciation among dorcopsins and dendrolagins in humid forests coincident with uplift of the New Guinean landmass^{3,69,70}, and macropodins in sclerophyllous woodlands, chenopodiaceous shrublands, and xeric grasslands (Supplementary Figures S13 and S14), which proliferated after the late Miocene–Pliocene⁵³ coincident with the prevalence of grazing kangaroos⁹.

Conclusions

Our characterisation of the complete mitogenome for *Caloprymnus campestris* provides an ecological diversification timescale for bettongs and potoroos within the context of crown macropodoid evolution. Most importantly, we show that the unambiguously monophyletic *C. campestris*-*Bettongia* lineage originated with the onset of increasingly arid climates during later-middle to early-late Miocene^{53–57,71}, and corresponds with the deepest divergences of Australia's arid zone biota around ~15 Ma¹. This contrasts with the largely late Miocene to Pleistocene radiation of kangaroos, whose abundance in modern arid zone habitats has been attributed to grazing adaptations and the spread of grasslands during the Pliocene and Pleistocene^{3,9,12}. Clearly, therefore, the appearances of Australia's distinctive arid zone macropodoids were staged over some ~5–7 Ma (based on comparative maximum–minimum confidence intervals for the divergences of *C. campestris* and *Osphranter rufus*: Table 2), and likely occurred in response to a complex interplay of abiotic and biotic drivers involving both climate and vegetation change.

Unfortunately, little is known about the biology of *C. campestris* or other extinct 'Desert bettongs', such as *Bettongia anhydra*⁶⁰, and the Nullarbor dwarf bettong⁷², *Bettongia pusilla*⁷³. Nonetheless, early eye-witness reports state that *C. campestris* inhabited sparsely vegetated gibber plains¹⁶. The diet of *C. campestris* is also uncertain²³, but might have been varied^{16,23} similar to the extant arid zone Boodie, *Bettongia lesueur*⁷⁴, and *Bettongia penicillata*⁶¹, which consume a range of plant matter, fungi and insects^{75,76}. *Caloprymnus campestris* was thus probably an important 'ecosystem engineer'⁶⁰, whose tragic loss is compounded by dramatic range reductions and the Near Threatened (*Bettongia gaimardi*, *B. lesueur*, *Potorous tridactylus*), Vulnerable (*Potorous longipes*), Endangered (*Bettongia tropica*), Critically Endangered (*B. penicillata*, *Potorous gilbertii*), or Extinct (*B. anhydra*, *C. campestris*, *Potorous platyops*) IUCN Red listings (<https://www.iucnredlist.org/>) for 10 out of the 11 named non-fossil crown potoroids. The extinction susceptibility of *C. campestris* was presumably exacerbated by its limited distribution (only four recognised collection²², and 13 potential sighting localities¹⁷ within a ~350 km radius) and desert specialisation, which when coupled with habitat modification and the introduction of exotic species via European pastoralism⁷⁷, underscores the extreme conservation sensitivity of Australia's unique arid zone marsupials and the urgent need to document their now dwindling multi-million-year evolutionary histories.

Declarations

Data availability

All data generated or analysed during this study were included in the article.

Ethics declarations

Approval for animal experiments. No live animal subjects were used for experiments in this study. All extinct animal tissues were obtained and analysed following approved institutional procedures. All experiments were performed in accordance with institutional guidelines and regulations.

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Author contributions

M.W. conceived the study, collected and analysed the data, and wrote the paper. S.L. and M.H.T. analysed the data. B.P.K. analysed the data and wrote the paper.

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Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at <https://doi.org/>

Correspondence and requests for materials should be addressed to M.W. or B.P.K.

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Tables

Table 1. Phylogenetic definitions for Macropodiformes, including Macropodia, new clade, and other selected constituent subclades. *Extinct. Conceptual explanations are provided in the Supplementary Information.

Clade	Definition	Type
Macropodiformes	most inclusive clade including <i>Balbaroo nalima</i> * and <i>Hypsiprymnodon moschatus</i> , but excluding <i>Cercartetus concinnus</i> and <i>Phalanger orientalis</i>	stem
Balbaridae*	most inclusive clade including <i>Balbaroo nalima</i> *, but excluding <i>Hypsiprymnodon moschatus</i> , <i>Potorous tridactylus</i> and <i>Macropus giganteus</i>	stem
Macropodoidea	least inclusive clade including <i>Hypsiprymnodon moschatus</i> , <i>Potorous tridactylus</i> and <i>Macropus giganteus</i>	crown
Hypsiprymnodontidae	least inclusive clade including <i>Hypsiprymnodon moschatus</i> , but excluding <i>Potorous tridactylus</i> and <i>Macropus giganteus</i>	crown
Hypsiprymnodontinae	most inclusive clade including <i>Hypsiprymnodon moschatus</i> , but excluding <i>Propleopus oscillans</i> *	stem
Propleopinae*	most inclusive clade including <i>Propleopus oscillans</i> *, but excluding <i>Hypsiprymnodon moschatus</i>	stem
Macropodia, new clade	least inclusive clade including <i>Potorous tridactylus</i> and <i>Macropus giganteus</i> , but excluding <i>Hypsiprymnodon moschatus</i>	crown
Potoroidae	least inclusive clade including <i>Potorous tridactylus</i> and <i>Aepyprymnus rufescens</i> , but excluding <i>Hypsiprymnodon moschatus</i> and <i>Macropus giganteus</i>	crown
Potorinae	least inclusive clade including <i>Potorous tridactylus</i> , but excluding <i>Aepyprymnus rufescens</i>	crown
Bettonginae	least inclusive clade including <i>Aepyprymnus rufescens</i> , but excluding <i>Potorous tridactylus</i>	crown
Macropodidae	most inclusive clade including <i>Simosthenurus occidentalis</i> *, <i>Lagostrophus fasciatus</i> and <i>Macropus giganteus</i> , but excluding <i>Potorous tridactylus</i> and <i>Hypsiprymnodon moschatus</i>	stem
Sthenurinae*	most inclusive clade including <i>Simosthenurus occidentalis</i> *, but excluding <i>Lagostrophus fasciatus</i> and <i>Macropus giganteus</i>	stem
Lagostrophinae	most inclusive clade including <i>Lagostrophus fasciatus</i> , but excluding <i>Simosthenurus occidentalis</i> * and <i>Macropus giganteus</i>	stem
Macropodinae	most inclusive clade including <i>Macropus giganteus</i> , but excluding <i>Simosthenurus occidentalis</i> * and <i>Lagostrophus fasciatus</i>	stem
Dorcopsini	least inclusive clade including <i>Dorcopsis hageni</i> , but excluding <i>Dendrolagus lumholtzi</i> and <i>Macropus giganteus</i>	crown
Dendrolagini	least inclusive clade including <i>Dendrolagus lumholtzi</i> , but excluding <i>Dorcopsis hageni</i> and <i>Macropus giganteus</i>	crown
Macropodini	least inclusive clade including <i>Macropus giganteus</i> , but excluding <i>Dorcopsis hageni</i> and <i>Dendrolagus lumholtzi</i>	crown

Table 2. Estimated divergence times (Ma) with confidence intervals for crown macropodoid clades based on the partitioned mitogenome/mtDNA/nDNA dataset. See Figure 2 for node number references and the Supplementary Information for other dating analyses.

Node	Divergence	Time estimate
1	Hypsiprymnodontidae v. Macropodia	30.92 (23.94-39.53)
2	Macropodidae v. Potoroidae	21.9 (17.87-26.12)
3	Potorinae v. Bettonginae	18.8 (15.37-22.49)
4	<i>Potorous longipes</i> v. other <i>Potorous</i>	13.39 (10.22-16.46)
5	<i>Potorous platyops</i> v. other <i>Potorous</i>	11.66 (7.75-14.86)
6	<i>Potorous gilbertii</i> v. other <i>Potorous</i>	9.9 (2.38-12.56)
7	<i>Potorous tridactylus tridactylus</i> v. other <i>Potorous tridactylus</i> subsp.	7.57 (0.95-9.63)
8	<i>Potorous tridactylus apicalis</i> v. <i>Potorous tridactylus trisulcatus</i>	2.12 (0.42-2.98)
9	<i>Aepyprymnus rufescens</i> v. other Bettonginae	14.51 (11.53-17.68)
10	<i>Caloprymnus campestris</i> v. <i>Bettongia</i> spp.	12.05 (9.37-14.84)
11	<i>Bettongia anhydra</i> v. other <i>Bettongia</i>	8.61 (6.09-11.65)
12	<i>Bettongia leseuer</i> v. other <i>Bettongia</i>	7.54 (5.55-9.6)
13	<i>Bettongia gaimardi</i> v. other <i>Bettongia</i>	2.45 (1.67-3.42)
14	<i>Bettongia tropica</i> v. <i>Bettongia penicillata</i>	1.7 (1.02-2.55)
15	<i>Simosthenurus occidentalis</i> v. other Macropodidae	18.94 (15.19-22.94)
16	<i>Lagostrophus fasciatus</i> v. Macropodinae	18.25 (14.22-22.53)
17	Dorcopsini v. other Macropodinae	13.08 (9.8-16.13)
18	<i>Dorcopsulus vanheurni</i> v. <i>Dorcopsis hageni</i>	6.82 (2.14-9.68)
19	Dendrolagini v. Macropodini	12.14 (9.6-14.76)
20	<i>Thylogale billardieri</i> v. other Dendrolagini	11.02 (5.97-13.83)
21	<i>Petrogale</i> spp. v. <i>Dendrolagus</i> spp.	9.38 (5.41-11.85)
22	<i>Petrogale brachyotis</i> v. <i>Petrogale xanthopus</i>	7.09 (0.34-8.68)
23	<i>Dendrolagus lumholtzi</i> v. <i>Dendrolagus dorianus</i>	7.33 (0.35-8.89)
24	<i>Onychogalea unguifera</i> v. other Macropodini	11.51 (8.5-14.34)
25	<i>Setonix brachyurus</i> v. other Macropodini	11 (5.1-13.39)
26	<i>Lagorchestes</i> spp. v. other Macropodini	10.04 (8.27-11.78)
27	<i>Lagorchestes hirsutus</i> v. <i>Lagorchestes conspicillatus</i>	7.18 (0.79-8.96)
28	<i>Wallabia bicolor</i> v. other Macropodini	9.21 (7.63-10.89)

29	<i>Protemnodon anak</i> v. other Macropodini	8.47 (4.35-10.44)
30	<i>Macropus</i> spp. v. other Macropodini	7.77 (3.72-9.57)
31	<i>Macropus giganteus</i> v. <i>Macropus fuliginosus</i>	3.63 (1.42-5.24)
32	<i>Notamacropus</i> spp. v. <i>Osphranter</i> spp.	7.47 (2.12-9.31)
33	<i>Notamacropus eugenii</i> v. <i>Notamacropus parma</i>	6.29 (1.01-7.76)
34	<i>Osphranter rufus</i> v. other <i>Osphranter</i> spp.	7 (2.27-9.01)
35	<i>Osphranter robustus</i> v. <i>Osphranter bernardus</i>	4.97 (0.82-6.58)

Figures

Figure 1

(A) Painting of *Caloprymnus campestris* as illustrated by Gould⁷⁸ (image in public domain). (B) Estimated historical distribution of *C. campestris* (grey shaded area) and localities from which specimens were collected: (1) Koonchera; (2) Ooroowillanie; (3) Mulka; (4) Killalpaninna (based on data from Google Maps and OZCAM Online Zoological Collections of Australian Museums: <https://ozcam.org.au/>). (C) Sturt Stony Desert gibber plain habitat of *C. campestris* showing a 'jump-up' escarpment and ephemeral drainage channel lined by riparian vegetation in the distance (photograph courtesy Michael Letnic, University of New South Wales). (D) Preserved skin of *Caloprymnus campestris* (Australian Museum, Sydney [AM] M21674) from Killalpaninna in northeastern South Australia²² (photograph courtesy Mark Eldridge, AM).

Figure 2

Time calibrated phylogeny of crown Macropodoidea (◆) showing divergence of *Caloprymnus campestris* (bold type) within Bettonginae (●), and correlated against a schematic of changing palaeohabitats across the late Oligocene–Holocene interval (modified from Kear et al.6 and Den Boer et al.79). Topology is based on the partitioned mitogenome/mtDNA/nDNA dataset. Bayesian posterior probability (<1.0) and bootstrap (<100%) support values (regular type) derived using Beast 1.8.236/MrBayes 3.2.735/RAXML

7.2.834 are indicated at relevant nodes. Branch colours denote major clades: Hypsiprymnodontidae (purple); Macropodia, new clade (burgundy); Potoroidae (pink); Potoroinae (orange); Bettonginae (ochre); Macropodidae (red); Sthenurinae (green); Lagostrophinae (yellow); Macropodinae (light blue); Dorcopsini (grey) Dendrolagini (brown); Macropodini (dark blue). *Extinct taxa. See Table 2 for node number references (bold type) and the Supplementary Information for other analyses. Graphics produced with Adobe CC2021 by B.P.K.

Supplementary Files

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