Mitogenome of the extinct Desert ‘rat-kangaroo’ times the adaptation to aridity in macropodoids

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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 arid conditions. Our robustly supported phylogenies nest Caloprymnus amongst the bettongs Aepyprymnus and Bettongia. Dated ancestral range estimations further reveal that the Caloprymnus-Bettongia lineage originated in nascent xeric settings during the middle to late Miocene, ~12 million years ago (Ma), but subsequently radiated into fragmenting mesic habitats after the Pliocene to mid-Pleistocene. This timeframe parallels the ancestral divergences of kangaroos in woodlands and forests, but predates their adaptive dispersal into proliferating dry shrublands and grasslands from the late Miocene to mid-Pleistocene, after ~7 Ma. We thus demonstrate that protracted changes in both climate and vegetation likely staged the emergence of modern arid zone macropodoids.

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)—the group encompassing living kangaroos, wallaroos, wallabies, pademelons and tree-kangaroos (Macropodidae), bettongs and potoroos (Potoroidae), the Musky rat-kangaroo (Hypsiprymnodon moschatus: Hypsyprymnodontidae), and their stem antecedents—incorporate some of the most distinctive Australian arid zone marsupials, as epitomised by the famous Red kangaroo, Osphranta rufus15. 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 Ma9,12. 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” or Ngudlukanta, Caloprymnus campestris (Fig. 1A).

The first scientific specimens of C. campestris were collected from northeastern South Australia (Fig. 1B) in 1842, with three preserved examples subsequently shipped to London for study18. These were dubbed ‘Betongia’ campestris by Gould19, although Thomas later20 recognised ‘B. campestris as morphologically distinct from Betongia, 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 Finlayson16,21 announced the “Rediscovery of Caloprymnus campestris” in 1931–1932, from the remote Kooncheera Dune region in the Sturt Stony Desert of far northeastern South Australia (Fig. 1C). Since then, only a skin recovered

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sometime between 1902 and 1905 (Fig. 1D) has been reidentified, and various unsubstantiated live sightings made, with the most recent in 2011 and 2013 prompting unsuccessful surveys for the species in 2018 and 2019. *Caloprymnus campestris* has otherwise been classified as Extinct by the IUCN (https://www.iucnredlist.org/) since 1994, with the probable cause being over-predation by feral dogs, cats, and foxes.

At latest count, only 25 specimens of *C. campestris* are catalogued in museums worldwide. This dearth of research material has led to uncertainty about potoroid interrelationships, as well as the concomitant chronicle of their arid zone evolution. Here, we therefore analyse the first complete mitochondrial (mt) genome of *C. campestris* showing a 'jump-up' escarpment and ephemeral drainage channel lined by riparian vegetation in the distance (photograph reproduced with permission from Michael Letnic, University of New South Wales). (D) Preserved skin of *Caloprymnus campestris* (Australian Museum, Sydney [AM] M21674) from Killalpaninna in northeastern South Australia (photograph reproduced with permission from Mark Eldridge, AM).

**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 (Fig. 1B). Our DNA extraction, PCR amplifications, sequencing and alignment procedures followed Westerman et al. Whole genome libraries were prepared with the Nextera DNA flex library kit (Illumina, CA), incorporating 50 ng of input DNA per sample. Sequencing was performed on the Illumina MiSeq platform using 2 × 300 bp V3 chemistry to generate 4,445,476 read pairs and
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-macropodid outgroups. To accommodate for recognised gene incongruence, we then compared these results with analyses of nuclear (n), and combined mitogenome/noDNA/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/16S rRNA 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 jModelTest49 (Supplementary Table S3).

Ancestral area analyses. Distributional areas were optimised onto the time-calibrated BEAST consensus tree and analysed using the R package BioGeoBEARS37 to compare alternative biogeographical range models, and a Bayesian Binary MCMC (BBM) approach38,39 to reconstruct ancestral ranges in RASP40. Area codes (Supplementary Table S4) followed standard units6 but were refined to represent a generalised vegetation map41: A = humid forest (rainforest and/or ‘wet’ sclerophyll dominant) prevalent throughout eastern coastal Australia, western Tasmania and New Guinea; B = woodland (‘dry’ sclerophyll dominant) prevalent throughout northern, eastern and southwestern inland Australia and northeastern Tasmania; C = shrubland (Acacia and chenopodiaceous shrubland dominant) prevalent throughout central and central-western Australia; and D = grassland-desert (arid grasslands and/or desert dominant) prevalent in central and central-northern Australia. The maximum number of ancestral areas was restricted to three because this equalled the maximum number of areas occupied by our terminal taxa at any given node.

BioGeoBEARS comparisons proceeded with likelihood ratio testing of ‘Jumping dispersal events (+ J)’, which have been considered inappropriate for dispersal-extinction-cladogenesis (DEC) models42. However, the three parameter Bayesian inference of historical biogeography for discrete areas (BAYAREALIKE) + J model (P = 0.0006) received overwhelmingly highest support (AICc = 199.6; AICc_wt = 0.98) for conferring best statistical likelihood on our data (Supplementary Table S5). Finally, we accommodated for connectivity by designating a dispersal multiplier of ‘1’ for adjacent areas (A-B-C)43 versus non-adjacent areas (A-D)41, which were assigned a value of ‘0.5’.

Our BBM analyses utilised 10 MCMC chains with default temperature 0.1, and run over 5 × 10^6 generations with sampling frequency and burn-in fixed at 1000. Model settings included ‘Gamma (+ G)’ for among-site rate variation, and ‘Fixed (JC)’ for state frequencies.

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 marsupials43,44. The tRNAs are arranged around the origin of the L strand (A-C-W-O2-N-Y) and intersected between the NADH2 and COX1 genes. Substitution of the anticodon GCC for tRNAASP (trnD) is also consistent with RNA-editing45.

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 accords with other crown macropodid phylogenies41,46,49, 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
Table 1. Phylogenetic definitions for Macropodiformes, including Macropodia, new clade, and other selected constituent subclades. Conceptual explanations and phylogenetic definition registration details are provided in the Supplementary Information. *Extinct.

| Clade                  | Definition                                                                 | Type       |
|------------------------|-----------------------------------------------------------------------------|------------|
| Macropodiformes        | Most inclusive clade including Balbaroo nalima*, Hypsiprymnodon moschatus, Potorous tridactylus and Macropus giganteus, but excluding Cercartetus concinnus and Phalanger orientalis | Stem       |
| Balbaridae*            | Most inclusive clade including Balbaroo nalima*, but excluding Hypsiprymnodon moschatus, Potorous tridactylus and Macropus giganteus | Stem       |
| Macropodidae           | Least inclusive clade including Hypsiprymnodon moschatus, Potorous tridactylus and Macropus giganteus | Crown      |
| Hypsiprymnonodontidae  | Most inclusive clade including Hypsiprymnodon moschatus and Propleopus oscilantes*, but excluding Balbaroo nalima*, Potorous tridactylus and Macropus giganteus | Stem       |
| Hypsiprymnonodontinae  | Most inclusive clade including Hypsiprymnodon moschatus, but excluding Propleopus oscilantes* | Stem       |
| Propleopinae*          | Most inclusive clade including Propleopus oscilantes*, but excluding Hypsiprymnodon moschatus | Stem       |
| Macropodia, new clade  | Least inclusive clade including Potorous tridactylus and Macropus giganteus, but excluding Hypsiprymnodon moschatus | Crown      |
| Potoroidea             | Least inclusive clade including Potorous tridactylus and Aepyprymnus rufescens, but excluding Hypsiprymnodon moschatus and Macropus giganteus | Crown      |
| Potorinae              | Least inclusive clade including Potorous tridactylus, but excluding Aepyprymnus rufescens | Crown      |
| Bettonginae            | Least inclusive clade including Aepyprymnus rufescens, but excluding Potorous tridactylus | Crown      |
| Macropodidae           | Most inclusive clade including Simothemurus occidentalis*, Lagostrophus fasciatus and Macropus giganteus, but excluding Potorous tridactylus and Hypsiprymnodon moschatus | Crown      |
| Sthenurinae*           | Most inclusive clade including Simothemurus occidentalis*, but excluding Lagostrophus fasciatus and Macropus giganteus | Crown      |
| Lagostrophinae         | Most inclusive clade including Lagostrophus fasciatus, but excluding Simothemurus occidentalis* and Macropus giganteus | Stem       |
| Macropodinae           | Most inclusive clade including Macropus giganteus, but excluding Simothemurus occidentalis* and Lagostrophus fasciatus | Stem       |
| Dorcopini              | Least inclusive clade including Dorcopsis hageni, but excluding Dendrolagus lumholtzi and Macropus giganteus | Crown      |
| Dendrolagini           | Least inclusive clade including Dendrolagus lumholtzi, but excluding Dorcopsis hageni and Macropus giganteus | Crown      |
| Macropodini            | Least inclusive clade including Macropus giganteus, but excluding Dorcopsis hageni and Dendrolagus lumholtzi | Crown      |

macropodines (bootstrap = 48/60%; MrBayes BPP = 1/1; BEAST BPP = 0.63/0.72); (3) grey kangaroos in the genus Macropus with Ophshrnuter 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, as Potorinae comprises potoroos within the genus Potorous and is distinguished from its sister clade, which we designate Bettonginae, to include the Rufous bettong, Aepyprymnus rufescens, as the basally branching sister to C. campesi and the species of Betongia (Table 1). Alternative monophyly of C. campesi with either A. rufescens or the species of Potorus, were tested using topological constraints in PAUP* 4.0b10 (Supplementary Table S6), but decisively rejected (P < 0.0001***). Taxonomically, therefore, we conclude that the original classification of Gould’s Desert ‘bettong’ as generically consistent with Betongia 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 (Fig. 2; Supplementary Figures S10–S12) yield broadly compatible topologies, with the basal divergence of potoroids and macropodids, and subsequent split between potorines and bettongines both occurring from the latest Oligocene to earliest-middle Miocene (Table 2; Supplementary Table S7). Notably, this concurs with divergence times derived using different dating methods and constraints. 12,46–50,56. Furthermore, while our BioGeoBEARS and BBM ancestral range estimations correlate the latest Eocene (or mid-Eocene using nDNA: Supplementary Table S7) to late Oligocene emergence of crown macropodoids with predominantly humid forest habitats (> 50% probability values from BAYAREALIKE + J [A] = 65.76%; BBM [A] = 61.31%; Supplementary Tables S8 S8 and S9), the initial radiation of potoroids (BAYAREALIKE + J [B/A] = 45.42/25.55%; BBM [B/AB] = 42.62/28.9%), together with the macropodid subclades Sthenurinae (BAYAREALIKE + J [B] = 82.31%; BBM [B] = 66.1%) and Lagostrophinae + Macropodinae (BAYAREALIKE + J [B] = 70.45%; BBM [B/BC] = 41.79/27.29%) are coordinated with earlier Miocene dispersals into woodland dominated mosaics (Fig. 2; Supplementary Tables S8–S11; Supplementary Figures S13 and S14). These potentially included ‘mallee-like’ sclerophyll communities, which propagated throughout central Australia from the early to middle Miocene.

The globally recognised middle to late Miocene climatic transition from equable to increasingly cool, dry conditions coincides with potorine speciations into mesic environments throughout southern Australia. These are tracked by our BioGeoBEARS and BBM estimates, which infer occupation of primarily woodland and forest habitats after the earliest-late Miocene (Supplementary Tables S7–S9; Supplementary Figures S13 and S14). This is concurrent with the incipient desertification of inland Australia, which may have promoted genetic
Figure 2. Time calibrated phylogeny of crown Macropodoidea (filled black diamond) showing divergence of *Caloprymnus campestris* (bold type) within Bettonginae (black open circle), and correlated against a schematic of changing palaeohabitats across the late Oligocene–Holocene interval (modified from Kear et al. and Den Boer et al.). 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 2.2.1/MrBayes 3.2.7/RAXML 7.2.8 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.
versus the Long-nosed potoroo, *Potorous tridactylus* that regional subspecies distinctions within *P. tridactylus* substantially less genetic difference between the Tasmanian *P. tridactylus apicalis* (Table 2; Supplementary Table S7). Curiously, though, *Cynocephalus* segregation of the extinct Broad-faced potoroo, *Potorous platyops* *Extinct.* on the partitioned mitogenome/mtDNA/nDNA dataset. See Fig. 2 for node number references and the Supplementary Information for other dating analyses. *Extinct.* in our opinion, only up to species-level. Indeed, these values approximate those contrasting (1.93%), in comparison to the southeastern mainland *tridactylus tridactylus* and basally branching Long-footed potoroo, *Potorous gilbertii* with *P. tridactylus tridactylus/P. tridactylus trisulcatus* (4.21%). Indeed, these values approximate those contrasting *P. tridactylus tridactylus/P. tridactylus trisulcatus* with *P. gilbertii* (2.69/5%), and *P. longipes* (5.84/5.69%), supporting inferences of cryptic taxa56, but in our opinion, only up to species-level.

Despite the currently limited DNA sequence coverage for the extinct Finlayson’s62 Desert bettong, *Bettongia anhydra*63, we derive unequivocal support (Fig. 2; Supplementary Figures S1–S12) for the monophyly of *B. anhydra*63, (BAYAREALIKE + J [CD] = 98.12%; BBM [BCD] = 81.72%) together with close relationships between the woodland-forest dwelling *B. anhydra*63, (BAYAREALIKE + J [CD] = 98.54%; BBM [AB] = 94.82%), and basally branching Long-footed potoroo, *Potorous longipes*, in southeastern Australia66. Additionally, we show that regional subspecies distinctions within *P. tridactylus* were completed by the latest Pliocene to mid-Pleistocene (Table 2; Supplementary Table S7). Curiously, though, Cyt b K2P variation (Supplementary Table S12) 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%).

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

| Node | Divergence | Time estimate |
|------|------------|---------------|
| 1    | 30.49      | (24.75–36.58) |
| 2    | 21.91      | (18.15–25.66) |
| 3    | 18.68      | (15.36–22.01) |
| 4    | 12.71      | (10.36–15.23) |
| 5    | 9.62       | (7.57–11.92)  |
| 6    | 8.98       | (7.11–11.11)  |
| 7    | 7.12       | (5.46–8.95)   |
| 8    | 2.2        | (1.57–2.97)   |
| 9    | 14.62      | (11.79–17.4)  |
| 10   | 12.23      | (9.77–14.72)  |
| 11   | 8.8        | (6.29–11.73)  |
| 12   | 7.67       | (5.86–9.51)   |
| 13   | 2.46       | (1.81–3.17)   |
| 14   | 1.76       | (1.25–2.33)   |
| 15   | 19.21      | (15.75–22.62) |
| 16   | 18.6       | (15.32–21.9)  |
| 17   | 13.54      | (11.19–15.96) |
| 18   | 7.14       | (5.41–9.05)   |
| 19   | 12.6       | (10.35–14.78) |
| 20   | 11.51      | (9.55–13.68)  |
| 21   | 8.99       | (8.01–11.69)  |
| 22   | 7.55       | (6.04–9.14)   |
| 23   | 7.79       | (6.17–9.42)   |
| 24   | 11.95      | (9.81–14.02)  |
| 25   | 11.46      | (9.47–13.52)  |
| 26   | 10.38      | (8.59–12.29)  |
| 27   | 7.53       | (5.92–9.12)   |
| 28   | 9.52       | (7.86–11.25)  |
| 29   | 8.84       | (7.3–10.44)   |
| 30   | 8.11       | (6.68–9.58)   |
| 31   | 3.84       | (2.81–4.94)   |
| 32   | 7.81       | (6.43–9.25)   |
| 33   | 6.57       | (5.29–7.9)    |
| 34   | 7.33       | (6.01–8.7)    |
| 35   | 5.22       | (4.11–6.37)   |

Despite the currently limited DNA sequence coverage for the extinct Finlayson’s62 Desert bettong, *Bettongia anhydra*63, we derive unequivocal support (Fig. 2; Supplementary Figures S1–S12) for the monophyly of *Bettingia* 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*64. Nevertheless, our BioGeoBEARS and BBM estimates suggest a latest middle to probably late Miocene divergence of *B. anhydra* (BAYAREALIKE + J [CD] = 98.54%; BBM [CD] = 55.06%) and the Boodie, *Bettongia lesueur*, (BAYAREALIKE + J [CD] = 98.12%; BBM [BCD] = 81.72%)
in xeromorphic habitats (Table 2; Supplementary Tables S7–S9; Supplementary Figures S13 and S14), followed by Pliocene to as recent as mid-Pleistocene radiations of *B. gaimardi* (BAYAREALIKE + J [CD] = 90.65%; BBM [BCD/BC] = 27.79/23.75%) and *B. tropica* + *B. penicillata* subsp. (BAYAREALIKE + J [CD] = 79.96.12%; BBM [BCD/BC] = 28.32/21.98%) coupled with increasing habitat variegation14. We correlate this with vicariant 'reversions'3 into eucalypt woodlands and forest65–67 (Supplementary Tables S10 and S11), which contracted and fragmented with intensifying aridification over the Pliocene–Pleistocene interval14.

*Bettongia* is karyotypically conservative, retaining the 2n = 22 chromosomal number of most macropodoids60,61. 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 = 12,13,71. *Aepyprymnus rufescens*, on the other hand, exhibits a unique karyotypic increase to 2n = 32, which is the highest for any marsupial71, 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 S12). Significantly, our BioGeoBEARS (BAYAREALIKE + J [CD] = 87.73%) and BBM ([CD] = 53.41%) estimates correlate the *C. campestris-Bettongia* divergence with a seminal invasion of xeric environments (Supplementary Tables S8–S12; Supplementary Figures S13 and S14), perhaps incorporating arid Chenopod shrublands that spread across central Australia from the middle to late Miocene41,57–60,66. The coeval radiation of macropodines is otherwise linked to predominantly woodland and forest settings (Table 2; Supplementary Tables S8–S12; Supplementary Figures S13 and S14). This includes dorcopsins (BAYAREALIKE + J [BA] = 54.21%, BBM [AB/BA] = 38.93/25.2%) and dengrolins (BAYAREALIKE + J [BA] = 49.35/34.51%, BBM [AB/ABC] = 47.93/26.27%) diverging coincident with uplift of the New Guinean landmass2,3,73, and macropodines which initially diversified in woodland habitats (BAYAREALIKE + J [B] = 95.79%; BBM [B] = 77.19%), but subsequently expanded into open shrublands and eventually grasslands (e.g., *Osphranter rufus*: BAYAREALIKE + J [B] = 51.33%, BBM [BC/BCD] = 31.62/24.84%) after the late Miocene to as recent as Pliocene to mid-Pleistocene (Table 2; Supplementary Table S7), thereby presaging the modern prevalence of grazing kangaroos8.

Conclusions

Our characterisation of the complete mitogenome for *Caloprymnus campestris* provides an ecological diversification timescale for bettongs and potoros within the context of crown macropodid evolution. Most importantly, we show that the unambiguously monophyletic *C. campestris-Bettongia* lineage probably originated with the onset of increasingly arid intracontinental climates during the middle to late Miocene41,57–60,64, corresponding with the deepest diversifications 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 Pleistocene9,12. Clearly, therefore, the appearances of Australia’s distinctive arid zone macropodoids were staged over some ~ 3–6 Ma (based on minimum–maximum confidence interval differences for *C. campestris* versus *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*, and the Nullarbor dwarf bettong*[^2]*. Nonetheless, early eye-witness reports state that *C. campestris* inhabited sparsely vegetated gibber plains8. The diet of *C. campestris* is also uncertain2, but might have been varied25 similar to the extant arid zone *Bettongia lesueurii* and *Bettongia penicillata*44, which consume a range of plant matter, fungi and insects8,9,27. *Caloprymnus campestris* was thus probably an important ‘ecosystem engineer’63 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 platypus*) IUCN Red listings ([https://www.iucnredlist.org/](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 pastoralism68, 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.

Ethical approval and informed consent. No live animal subjects were used for experiments in this study. All extinct animal tissues were obtained and their use approved by the La Trobe University Animal Ethics Committee (AEC). All experiments were performed in accordance with institutional guidelines and regulations.

Data availability

Raw FASTQ files for the *Caloprymnus campestris* mitogenome assembly have been uploaded onto the Mendeley Data repository ([https://data.mendeley.com/](https://data.mendeley.com/)) under [https://doi.org/10.17632/88t7v7gfz.1](https://doi.org/10.17632/88t7v7gfz.1). The consensus *C. campestris* mitogenome (MT66337) and other macropodid DNA sequences are available from GenBank (Supplementary Tables S1 and S2).

Received: 9 November 2021; Accepted: 25 March 2022
Published online: 06 April 2022
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Acknowledgements
We dedicate this paper to the late Joan Dixon (1937–2019) of NMV, who was a distinguished researcher on Australian mammals and provided access to the liver sample (NMV C8981) of Caloprymnus campestris. Constructive comments from the Editor, Robin Beck (University of Salford) and two anonymous reviewers improved our manuscript.

Author contributions
M.W. conceived the study, collected and analysed the data, and wrote the paper. S.L. and M.I.T. analysed the data and wrote the paper.
Funding
Open access funding provided by Uppsala University. Aspects of this work were supported by a Swedish Research Council Young Researcher grant (2011–3637), and a Swedish Research Council Project grant (2020-3423) to B.P.K.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-022-09568-0.

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