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On the composition of *Antechinomys* (Marsupialia: Dasyuridae): how many species?

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ABSTRACT

Morphological and molecular studies have consistently suggested that *Sminthopsis*, as currently defined, is rendered paraphyletic by the kultarr (*Antechinomys laniger*). They have also suggested a sister relationship between the kultarr and the long-tailed dunnart. Based on DNA sequence data from multiple mitochondrial and nuclear gene loci we reassign the long-tailed dunnart (formerly *Sminthopsis longicaudata*) to *Antechinomys*. Although there is good evidence of genetic structure within the kultarr (*A. laniger*), it does not correspond to the two currently recognised subspecies, *viz A. laniger laniger* and *A. l. spenceri*. We conclude that *Antechinomys* consists of two species, *A. laniger* and *A. longicaudatus*, consistent with morphology. We suggest that the observed genetic and morphological variation within *A. laniger* merits a more thorough investigation of more samples from across its range to resolve the taxonomy.

Keywords: *Antechinomys*, Australia, biogeography, Dasyuridae, kultarr, *Ningaui*, phylogeography, Sminthopsinae, Sminthopsini, *Sminthopsis*.

Introduction

The dasyurid tribe Sminthopsini, as currently recognised (AMTC 2021), comprises three closely related genera of small, largely arid-adapted dasyurids: *Antechinomys* (one species), *Ningaui* (three species) and *Sminthopsis* (19 species), whose taxonomic interrelationships have been difficult to ascertain. Molecular studies have consistently demonstrated that *Sminthopsis* is rendered paraphyletic by *Antechinomys* (Blacket *et al.* 1999; Krajewski *et al.* 2012; Westerman *et al.* 2016; Kealy and Beck 2017; García-Navas *et al.* 2020). First collected by Sir Thomas Mitchell in New South Wales on the plains between the Murray and Darling rivers, and described as *Phascogale lanigera* (Gould 1856), the kultarr was subsequently placed in the new genus *Antechinomys* (as *A. lanigera*) by Krefft (1867), where it was later joined by *A. spenceri*, based on specimens with larger ears and bullae collected by Baldwin Spencer during the Horn Expedition to central Australia (Thomas 1906).

Lidicker and Marlow (1970) confirmed the distinctness of these two species: *A. laniger* with a limited distribution in western New South Wales (NSW) and western Queensland (Qld) and *A. spenceri* with a much broader distribution across central and western Australia. These taxa, geographically separated from one another (see inset Fig. 1), were found to differ in morphology, relative size, habitat, and in the number of nipples in the pouch of females (eight in *laniger*, six in *spenceri*). Archer (1977), using additional specimens from localities not known to Lidicker and Marlow, challenged their findings and recognised only a single species within *Antechinomys*, arguing that the differences between *laniger* and *spenceri* forms were simply the result of geographic and habitat variation between allopatric populations of a single widely distributed taxon. He noted (Archer 1977, p. 27) that '... of the characters used by Lidicker and Marlow, nipple number alone seems not to overlap in the two forms'. Most recent authorities have followed Archer and recognised only a single species within *Antechinomys* but have, however, recognised the existence of two subspecies – *A. laniger laniger* from eastern Australia and *A. l. spenceri* from the Northern Territory, South Australia and Western Australia (Jackson and Groves 2015; AMTC 2021).

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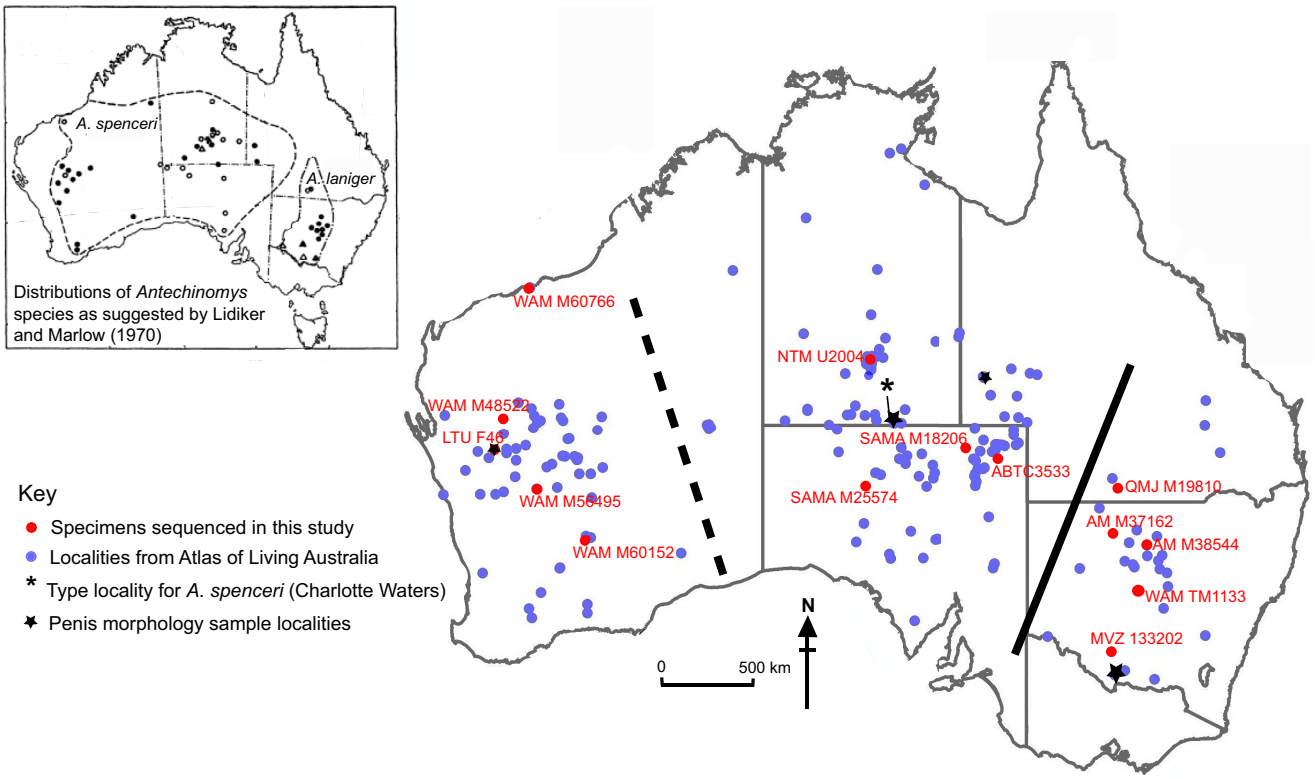

Fig. 1. Distribution of *Antechinomys laniger* in Australia. Inset shows the distributions of *A. laniger* and *A. spenceri* as indicated by Lidicker and Marlow (1970). Specimens sequenced in the present study are indicated in red. Localities from the Atlas of Living Australia (2020) are shown by blue dots. Positions of major biogeographic discontinuities are indicated by solid and broken lines. The solid line corresponds to the boundary between *A. laniger* and *A. spenceri* in the inset; the broken line indicates approximate location of the boundary between Western Australian forms and eastern congeners seen in this study. The type locality for *A. laniger spenceri* is show by an asterisk, but no precise locality is known for *A. l. laniger* (see text).

Based on a cladistic analysis of >30 external and craniodental characters, Archer (1981) further suggested that *Antechinomys* should have subgeneric status within *Sminthopsis*. Allozymes and albumin immunology (Baverstock *et al.* 1982, 1989), mitochondrial and nuclear gene sequences (Krajewski *et al.* 1997, 2012) and penis morphology (Woolley 1984; Krajewski *et al.* 2012) have all failed to produce evidence to support such a relationship. Most studies have consistently identified three distinct genera within Sminthopsini (*Antechinomys*, *Ningaui* and *Sminthopsis*) although their inter-relationships are unclear. Even the monophyly of *Sminthopsis* itself is in doubt since both *Antechinomys* and *Ningaui* render *Sminthopsis* paraphyletic in recent molecular analyses (Baverstock *et al.* 1982, 1989; Blacket *et al.* 1999; Krajewski *et al.* 2012). DNA sequence data have consistently suggested a sister relationship between *Antechinomys* and the long-tailed dunnart (*S. longicaudata*) (Krajewski *et al.* 2012), a finding in accord with an earlier study of 79 craniodental characters by Van Dyck *et al.* (1994).

Little is known about the variability of *Antechinomys* across its range apart from the early morphological studies referred to above. We therefore sought to clarify the status of the two currently recognised subspecies (*A. laniger laniger* and *A. l. spenceri*) by sequencing multiple mitochondrial and nuclear gene loci from specimens collected at various localities within the known range. We also sought to clarify the relationships of *Antechinomys* within Sminthopsinae by including multiple exemplars of the long-tailed dunnart (*S. longicaudata*) in our analyses and by expanding the DNA dataset of Krajewski *et al.* (2012) with information from two new nuclear gene loci (ω-globin and vWF).

Materials and methods

Taxon sampling

Multiple kultarr specimens were chosen to cover the species' continent-wide range and to include representatives of both currently recognised forms (Supplementary Table S1). Genomic DNA were extracted from 14 tissue samples from specimens held by the Western Australian Museum, the Australian Biological Tissue Collection (South Australian Museum), the Queensland Museum, the Australian Museum, the Museum and Art Gallery of the Northern Territory and the

Museum of Vertebrate Zoology, Berkeley. Tissues included liver samples stored either fresh at −80°C or in 70–100% EtOH, a fresh ear-clip taken from an animal that was released in the field, and a skin sample taken from a museum specimen (MVZ133202). This last specimen was included in Lidicker and Marlow's (1970) study as representative of *A. laniger*.

DNA extractions and amplifications were performed as described in Umbrello *et al.* (2017). New DNA sequences were obtained for two nuclear genes – ß fibrinogen intron 7 (*bfib7*) and intron 2 of ω-globin – and for three mitochondrial genes – cytochrome *b* (*cytb*), *12S* rRNA (*12S*), and control region (CR). PCR cycling conditions follow Krajewski *et al.* (1997) for *12S* and *cytb*, with CR and *bfib7* following the same conditions as *cytb*. For the skin sample taken from MVZ133202, 0.3 μL of a 10% BSA solution was added to the PCR reagent mix with 2 μL template DNA and *12S* amplified in three short fragments. The Australian Genome Research Facility (Perth) carried out DNA purification and bidirectional sequencing. Assembly, quality control and alignment of sequences was performed in Geneious Prime 2020.2.4 (https://www.geneious.com) as described in Umbrello *et al.* (2017).

All novel sequences were added to a database including representatives of all recognised sminthopsin species as well as five specimens of *S. longicaudata*. Four species of *Planigale* (*P. maculata*, *P. tenuirostris*, *P. gilesi* and *P.* sp1) were chosen as outgroups for the sminthopsins. All new sequences were deposited in GenBank (see Table S1 for details).

Data analysis

Our data matrix comprised sequence data from four mitochondrial (CR, *cytb*, *12S* and *16S* rRNA) and six nuclear gene loci (IRBP, Protamine P1, ω-globin, *bfib7*, e-globin and vWF exon 28). Prior to analysis, sequences were aligned and checked for premature stops in protein coding genes: none were found. The data were then analysed using either maximum likelihood (ML as implemented in RAxML 7.2.8: Stamatakis 2006) or Bayesian methods (as implemented in MrBayes v3.2.7: Rondquist *et al.* 2012). Sequences were initially treated as either a single unpartitioned block or separated into 10 gene partitions (*IRBP*, *ProtP1*, *bfib7*, ε-globin, ω-globin, *vWF*, CR, *cytb*, *12S* and *16S* rRNA) each with its own model of sequence evolution (Table S2) as determined by AIC in jModeltest (Posada 2008). To improve phylogenetic signal retention, we then partitioned the two rRNA genes into stems and loops according to the model of Burk *et al.* (2002) and protein coding genes by codon position, introns and 3′ and 5′ URL regions where appropriate, with 3rd codon positions coded as RY to allow for transitional saturation at this site. Bayesian analyses utilised random starting trees and two simultaneous runs of four Markov chains (one cold and three heated using default heating values) applied for 5 million generations with sampling every 1000th generation. The first 1.0×10^6 generations were discarded as burn-in, and remaining trees were used to construct a majority-rule consensus tree. Nodes with posterior probabilities (PP) >0.95 were deemed to be strongly supported and PP = 0.90–0.95 moderately supported. Support for nodes in the phylogenetic trees were also estimated by 1000 non-parametric bootstrap pseudoreplications (in RAxML) with nodes >90% deemed strongly supported and values >70% deemed moderately supported.

Network analysis

To investigate broad scale phylogeographic patterns in the mitochondrial sequence data statistical parsimony TCS haplotype networks (Clement *et al.* 2002) were built using the software program PopART (Population Analysis with Reticulate Trees; http://popart.otago.ac.nz). PopART excludes gaps and unknown bases (N, ? and –) from the analysis so these regions were removed from the alignments before building networks. A TCS network was built for the concatenated mitochondrial genes (since they are linked loci) of all *Antechinomys* samples, with samples grouped based on the Australian State of collection. TCS networks for the individual *12S*, *cytb* and CR sequence alignments are shown in Fig. S5.

Results

Shared indels in *bfib7*

Antechinomys and *S. longicaudata* samples share a 5-base deletion near the start of the intron (bases 115–119), which is not seen in other sminthopsins. In addition, *Antechinomys* has three unique deletions including a 3-base deletion (nucleotides 218–220), a 6-base deletion (nucleotides 681–686) and a 194-base deletion (nucleotides 738–932) not found in any other sminthopsin species, although *S. longicaudata* has a 7-base deletion in this same region (nucleotides 784–800).

Antechinomys specimens from Queensland and NSW (QMJ M19810; AM M38544, M37162 and WAM TM1133) all share a unique 3 base deletion not seen in any of the South Australian or Western Australian animals.

S. longicaudata specimens have two unique deletions (at nucleotides 415–420, and 1337–1344) as well as a unique 12 base insert (nucleotides 945–957) relative to other sminthopsins. We noted some indel variability within long-tailed dunnarts where sequences of the bfib7 locus were obtained from five other individuals. One of these (WAM M56543) had an 11-base deletion not seen in any other individual, and one (WAM M61176) lacked the 8-base deletion (1337–1344) seen in all other *S. longicaudata* specimens.

Sequence analysis

Bayesian trees derived for each individual gene locus and for concatenated mitochondrial and concatenated nuclear genes are shown in Figs S1–S4. There were no major differences in topology for nodes with moderate to good support and all trees supported a sister relationship between *A. laniger* and *S. longicaudata*. For subsequent analyses the sequence data were therefore concatenated over mitochondrial and nuclear gene loci as noted above. Partitioning of the sequence data by codon position, stems and loops, URL and introns the phylogenetic tree (Fig. 2) showed four well-resolved clades: *Ningaui; Antechinomys* plus *S. longicaudata;* a clade comprising the 'Macroura' group species (*Sminthopsis crassicaudata*, *S. bindi*, *S. douglasi*, *S. macroura*, *S. virginiae*), and a clade comprising all other currently recognised *Sminthopsis* species (*S. murina*, *S aitkeni*, *S. archeri*, *S. butleri*, *S. dolichura*, *S. gilberti*, *S. griseoventer*, *S. leucopus*, and *S. ooldea*, *S. granulipes*, *S. hirtipes*, *S. psammophila* and *S. youngsoni*).

All kultarr and long-tailed dunnart specimens included in our study resolve as sister taxa with strong support (1.0 BPP, 100% bootstrap) and are themselves likely sister to *Ningaui*, though this is less well supported (Fig. 2). As noted above, both kultarrs and the long-tailed dunnarts share features of intron 7 of the beta fibrinogen gene locus not seen in other sminthopsins. Little genetic variability was evident between the *S. longicaudata* specimens in our study, a finding in contrast with that seen in *A. laniger*. Currently no DNA sequence information is available for long-tailed dunnart specimens from either South Australia or the Northern Territory for comparison with the WA specimens in our study.

The final length of the trimmed concatenated mtDNA sequence alignments used in the mitochondrial haplotype network analysis was 2170 bp, and the trimmed length

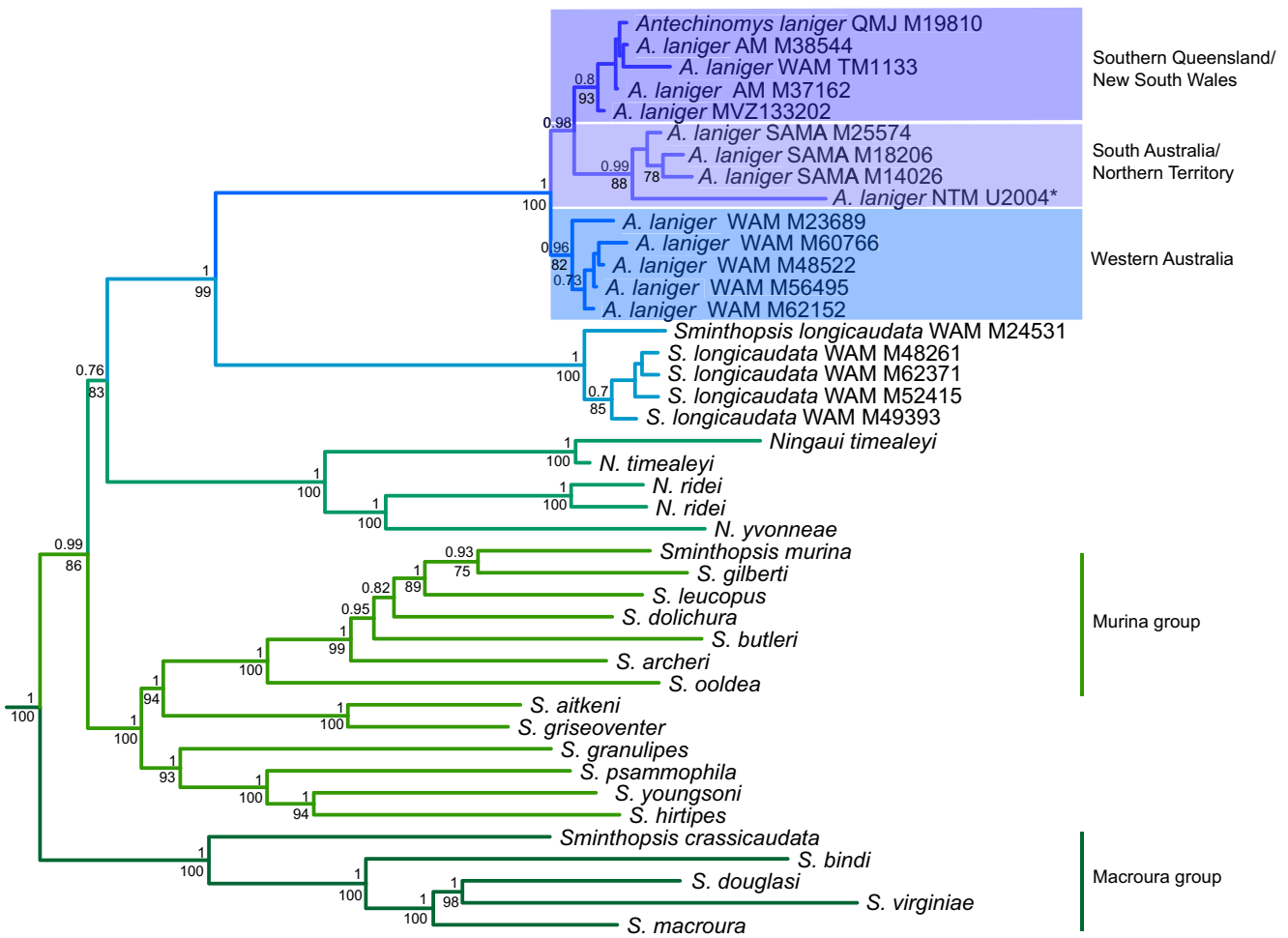

Fig. 2. Phylogenetic tree derived from Bayesian analysis of DNA sequences for codons of proteins-coding genes and stems and loops of ribosomal RNA genes (see text). Abbreviations for individual specimens: AM, Australian Museum, Sydney; QM, Queensland Museum, Brisbane; SAMA, South Australian Museum, Adelaide; WAM, Western Australian Museum, Perth. Values at nodes correspond to Bayesian Posterior Probabilities (>0.7 BPP, above line) or Bootstrap values (>70% support, below line). *Indicates partial *12S* sequence only available for NTM U2004.

of the individual mtDNA alignments presented in Fig. S5 were CR L = 384 bp, *cytb* L = 738 bp, and *12S* L = 803 bp. The mtDNA networks resulted in our samples falling into one or other of three clearly differentiated haplogroups (Fig. 3, Fig. S5). These groups corresponded to the geographic region that samples were collected from: one that included WA only, another of NSW and Qld only, and the third included samples from SA. There was no haplotype sharing between these three haplogroups. For all three mitochondrial genes, the SA haplogroup was the most differentiated of the three, with multiple nucleotide changes with the WA, NSW and Qld samples. A similar result was found with the mitochondrial gene trees placing the SA samples as a sister lineage to WA + NSW and Qld, but this placement was not reflected in the nuclear sequence data.

Estimates of the differences between the three kultarr groupings observed in our analyses were obtained using genetic distances calculated from the *cytb* sequences using the Kimura 2-parameter (K2P) model of nucleotide substitution (Kimura 1980). K2P values for *cytb* distance between the South Australian and Qld/NSW specimens (5.81%) is slightly smaller than that between SA and WA animals (7.16%), whereas K2P values between individuals *within* these three geographic areas are much smaller (0.26% within SA, 1.04% within NSW/Qld and 2.59% within WA). K2P distances (5.81–7.16%) seen between the three *Antechinomys* lineages, though much less than the >18% differences seen between kultarrs and other *Sminthopsis* and *Ningaui* species, nevertheless suggest considerable genetic divergence between them. The distinctness of the NSW and QM M19810 specimens from all others was further evidenced by the shared 3 bp deletion in the *bfib7* intron, which is not seen in either South Australian or Western Australian specimens. To date, only limited *12S* rRNA sequences have been obtained for NTM U2004 and MVZ 133202 but these individuals are closely related to the South Australian and NSW specimens respectively.

Discussion

Archer (1981), in his revision of *Sminthopsis*, conferred subgeneric status on *Antechinomys* and considered it to have a close relationship with *S. crassicaudata*. A consistent feature of molecular studies of sminthopsins over the last 40 years has been the observation that *Antechinomys* and *Ningaui* render *Sminthopsis* paraphyletic (Blacket *et al.* 1999; Krajewski *et al.* 2012; Westerman *et al.* 2016; Kealy and Beck 2017; García-Navas *et al.* 2020). Nuclear and mitochondrial DNA sequences, whether taken individually or concatenated, demonstrate a well-supported sister relationship between the kultarr (*A. laniger*) and the long-tailed dunnart (*S. longicaudata*). This relationship was also observed in the allozyme study of Baverstock *et al.* (1982) and the morphological study of Van Dyck *et al.* (1994). This latter study demonstrated a clear relationship between *A. laniger* and *S. longicaudata*, contrasting with the *S. longicaudata*–*S. ooldea*, *S. murina*, *S. leucopus*, and *S. crassicaudata*–*A. laniger* clades suggested by Archer (1981). Morphologically,

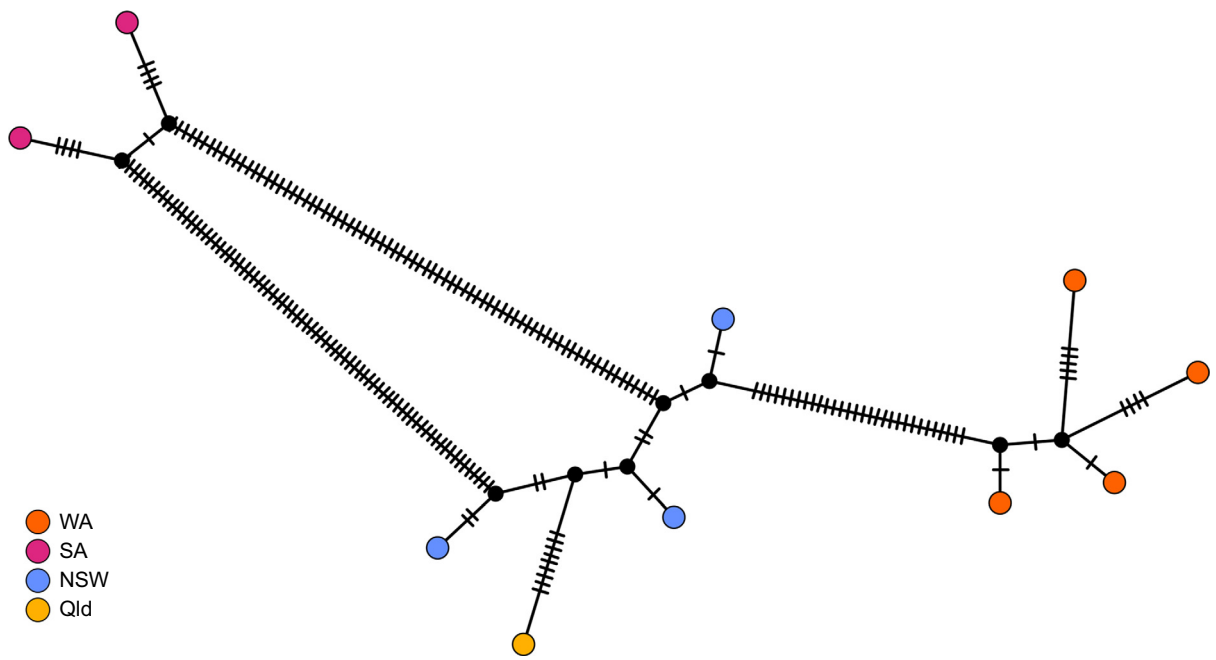

Fig. 3. Neighbour-joining TCS haplotype network for concatenated mtDNA sequences (L = 2170 bp) of *Antechinomys laniger* samples (n = 10) from Western Australia (orange), South Australia (magenta), New South Wales (blue) and Queensland (yellow). Nodes are shown as black circles and node differences are indicated by hatch marks.

A. laniger and *S. longicaudata* share a number of synapomorphies, including features of their upper canines, inflated alisphenoid tympanic wings, expanded periotic wings of the alisphenoid mastoid and tail longer than the snout–vent length.

This sister relationship between the kultarr and the long-tailed dunnart in a clade only distantly related to other species of *Sminthopsis* (Van Dyck *et al.* 1994; Krajewski *et al.* 2012; Westerman *et al.* 2016; Kealy and Beck 2017; García-Navas *et al.* 2020) mirrors Troughton's (1964) suggestion that *S. longicaudata* may not be a member of *Sminthopsis*, but rather 'may represent some annectant generic form'. We note here that Kealy and Beck (2017, p. 248) are in error in stating that '*Sminthopsis* is paraphyletic, with *Antechinomys* sister to *S. crassicaudata*' a statement that plainly contradicts their own phylogenetic trees. In view of the consistent results from DNA, allozyme and morphological studies referred to above, Eldridge *et al.* (2019) suggested that the taxonomy of *Sminthopsis* needs revision. As a first step, we recommend that the long-tailed dunnart be removed from *Sminthopsis* and be placed in *Antechinomys*, as indicated below. *Antechinomys* would now comprise two species: *A. laniger* and *A. longicaudatus*. While clearly sister taxa, they differ in both DNA sequences and morphology but share six unique, unreversed synapomorphies – four associated with the upper canines and two with the alisphenoid bone (Van Dyck *et al.* 1994). They are thus readily distinguishable from one another. Their last common ancestor probably existed in the later Miocene about 6–8 million years ago (Westerman *et al.* 2016; Kealy and Beck 2017; García-Navas *et al.* 2020; Beck *et al.* 2022), enough time for each to have developed distinct phenotypes in adapting to their arid habitats. Woolley *et al.* (2007) showed that penis structure of the long-tailed dunnart (Form 9) differs from that of all other species of *Sminthopsis*. It is also very different from that of the kultarr (Woolley, unpubl. obs.). Preliminary observations on the three-lobed tip of the penis of the kultarr suggest differences that may be characteristic for each of the western, central and eastern forms shown in Fig. 1. Fresh adult male specimens from the south-eastern part of the range of the kultarr are required to resolve this matter. The two species of the newly defined *Antechinomys* form a distinct monophyletic lineage within Sminthopsini, supporting their generic distinction from both *Ningaui* and *Sminthopsis*, as suggested by Celik *et al.* (2019) for the distinction of *Wallabia* from *Notamacropus*.

Family DASYURIDAE

Genus *Antechinomys* Krefft, 1866

Generic diagnosis

Small dasyurids like *Sminthopsis* but limbs and thin tail relatively longer. Cranium narrow, rostrum narrow and elongate; palatal vacuities; C^1 premolariform; alisphenoid and periotic tympanic wings variously enlarged. Tail longer than snout–vent length and with terminal brush. We note that the unique absence of a fifth toe (hallux) on the hind foot of *Antechinomys laniger* must now be considered an autapomorphy (*sensu* Hennig 1966) for this species and is therefore no longer diagnostic of genus *Antechinomys*.

Type species: *Phascogale lanigera* Gould, 1856 (= *Antechinomys laniger* (Gould, 1856))

Included species: *Antechinomys longicaudatus* (Spencer, 1909), comb. nov.

Genetic variability within *A. laniger*

Virtually all earlier molecular studies of the kultarr and the long-tailed dunnart were characterised by the inclusion of only a single exemplar of each species, hence our desire to include multiple exemplars of both *A. laniger* and *S. longicaudata* in the study to investigate possible genetic variability within each species. As shown in Fig. 2, there was no obvious variation among *S. longicaudata* samples, consistent with the study of Umbrello *et al.* (2020), in which >30 long-tailed dunnarts showed no obvious phylogeographic structure within Western Australia. We note, however, that, to date, no DNA sequence data are available for long-tailed dunnart specimens from either South Australia or the Northern Territory.

What then of the kultarr? Although previous morphological studies on *Antechinomys* have shown some morphological variation across the species' range (Lidicker and Marlow 1970; Archer 1977), there has been no consensus on whether this represents multiple taxonomic units. Lidicker and Marlow (1970) proposed that *Antechinomys* comprised two species – *A. laniger* (found in only a small area of eastern Australia east of the main Barrier Range of New South Wales and the Grey Range of Queensland), and *A. spenceri* (more widely distributed from central and western Australia). Archer (1977) suggested that these differences were better regarded as being subspecific in nature.

Our analyses of samples from across the range of *Antechinomys* clearly demonstrate the presence of three distinct genetic lineages within it; one comprising the five Western Australian animals, one including our samples from South Australia and the Northern Territory, and a third comprising the New South Wales and Queensland specimens. These lineages differ in their mitochondrial and nuclear gene sequences and although K2P distances for *cytb* (based on ~2% sequence divergence per million years) probably underestimate divergence times, the observed differences shown above suggest divergences during the Plio-Pleistocene ~2.5–3.5 million years ago. The Western Australian specimens are distinct from all others and form a major sublineage within *A. laniger*. The geographic boundary between the two major genetic lineages of kultarr (broken line in Fig. 1) is, however, not congruent with that suggested for the two currently recognised subspecies

(see below and inset Fig. S1). Rather, it lies well to the west – somewhere in the deserts between Western Australia and South Australia/Northern Territory and thus firmly *within* the area normally recognised as containing only *A. l. spenceri*.

Although the kultarr is widespread across the arid inland of Australia, no specimens have been collected from the Great Sandy, Gibson and Great Victoria Deserts, the area corresponding with the major genetic discontinuity between the 'western' and 'eastern' groups of kultarrs. This same region coincides with a gap in the distribution of the 'western' lineage of *S. macroura* from its 'eastern' conspecifics (Blacket *et al.* 2001; Umbrello *et al.* 2020) that may mark a major biogeographic barrier (Byrne *et al.* 2008; Rix *et al.* 2015) The eastern Australian boundary separating our South Australian/NT samples and those from NSW/Qld (solid line in Fig. 1) coincides with the boundary between *A. laniger* and *A. spenceri* shown by Lidicker and Marlow (1970), with *A. laniger* being restricted to a small region east of the Darling River in NSW and east of the Grey Range in Queensland (see inset Fig. 1).

The key morphological feature generally used to distinguish the two currently recognised subspecies of kultarr is nipple number – 8 in '*laniger*', 6 in '*spenceri*'. The four animals we sequenced as representing *A. laniger* (AM M37162, M38544, QMJ M19810 and WAM TM1133) were all males and were not included in Lidicker and Marlow's original study. They could not, therefore, be confirmed as '*laniger*' and so we included DNA sequences from an individual (MVZ M133202 from El Trune, NSW) clearly identified by these authors as an exemplar of *A. laniger*. Although two of our South Australian animals (SAMA M18206, SAMA M14026, Sturt Stony Desert, SA) came from areas inhabited by the '*spenceri*' form, they too were male. A third individual (SAMA M25574, Cadney Park, SA), an adult female with five of six nipples elongated and stained pouch fur, was representative of *A. spenceri*. Our NT specimen (NTM U2004 from Lilla Creek Station, NT) came from close to the type locality for *A. spenceri* (25°55′S, 134°55′E, Charlotte Waters, NT). Six adult female specimens held in the Western Australian Museum (Perth) were examined and all had six nipples. It is clear therefore that the genetic differentiation in our samples of *A. laniger* cannot be explained simply in terms of the two currently recognised subspecies/forms and clearly demonstrates the existence of a third major genetic lineage which is restricted to Western Australia.

The NSW and Qld specimens in our study are, genetically, closely related to MVZ 133202 (from El Trune Station, NSW) but differ markedly from the South Australian/NT specimens. Thus, although the geographic boundary identified by Lidicker and Marlow may well separate *some* specimens of *Antechinomys* that differ in nipple number, it does not correspond with the major genetic boundary identified in our study, a boundary that lies much further west. Thus, division of *A. laniger* specimens into only two subspecies differing in nipple number, as currently portrayed, is too simple. Indeed, one must question the validity of this character as a diagnostic trait since females from both South Australian/NT and Western Australian lineages all have six nipples yet differ dramatically in their DNA sequences.

Many studies have shown that nipple number in dasyurids can vary within species – sometimes dramatically (Cockburn *et al.* 1983; Rhind *et al.* 2001; How *et al.* 2002; Beckman and Lill 2007; Beckman *et al.* 2007; Lada *et al.* 2008; Draper 2017). Population studies on species of *Antechinus* have shown that although nipple number in females may be under strong genetic control, there is no evidence that populations of *A. agilis*, *A. flavipes*, *A swainsonii* or *A. mimetes* (as *A. swainsonii*) whose females differ in this character or other aspects of external morphology, are genetically distinct. Thus, adjacent populations of *A. agilis* in the Otway Ranges of Victoria may differ markedly in nipple number (6 or 10), in external traits and in microsatellite frequencies suggestive of species differences, but they still interbreed and show gene flow between them (Beckman *et al.* 2007). This is also true for populations of *A. mimetes* in the same region, where there is no significant difference between mitochondrial gene sequences despite obvious morphological differences (including nipple number) (Beckman and Lill 2007). It is therefore unlikely that differences in nipple number in *A. laniger* are of diagnostic value, especially when the boundary separating nipple numbers does not coincide with that separating broader genetic sequence differences. Although *A. laniger laniger* may be applied to the eastern form, given the large divergence seen in our study between the central and western exemplars, *A. l. spenceri* as currently applied and based on the type specimen from Charlotte Waters (NT), needs reassessment.

Supplementary material

Supplementary material is available online.

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