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The impact of fossil calibrations, codon positions and relaxed clocks on the divergence time estimates of the native Australian rodents (Conilurini)

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ABSTRACT

The native rodents are the most species-rich placental mammal group on the Australian continent. Fossils of native Australian rodents belonging to the group Conilurini are known from Northern Australia at 4.5 Ma. These fossil assemblages already display a rich diversity of rodents, but the exact timing of their arrival on the Australian continent is not yet established. The complete mitochondrial genomes of two native Australian rodents, *Leggadina lakedownensis* (Lakeland Downs mouse) and *Pseudomys chapmani* (Western Pebble-mound mouse) were sequenced for investigating their evolutionary history. The molecular data were used for studying the phylogenetic position and divergence times of the Australian rodents, using 12 calibration points and various methods.

Phylogenetic analyses place the native Australian rodents as the sister-group to the genus *Mus*. The *Mus*–Conilurini calibration point (7.3–11.0 Ma) is highly critical for estimating rodent divergence times, while the influence of the different algorithms on estimating divergence times is negligible. The influence of the data type was investigated, indicating that amino acid data are more likely to reflect the correct divergence times than nucleotide sequences.

The study on the problems related to estimating divergence times in fast-evolving lineages such as rodents, emphasize the choice of data and calibration points as being critical. Furthermore, it is essential to include accurate calibration points for fast-evolving groups, because the divergence times can otherwise be estimated to be significantly older. The divergence times of the Australian rodents are highly congruent and are estimated to 6.5–7.2 Ma, a date that is compatible with their fossil record.

1. Introduction

A unique mammalian fauna evolved in Australia due to approximately 50 million years (Myr) of isolation. Australia is home to the marsupials and monotremes, which have for various reasons gone extinct on most other continents, except South America. In contrast, placental mammals apart from bats did not reach Australia until recently, allowing the successful radiation of marsupial mammals.

Two placental mammalian groups have been thriving in Australia, namely the bats and the murine rodents. Today Australia is home to over 70 species of native rodents (Musser and Carleton, 1993; Strahan, 1995; Tate, 1951; Watts and Aslin, 1981). The Australian rodents are traditionally divided into three groups: Conilurini, Uromyini and Hydromyini (Musser and Carleton, 1993; Watts and Aslin, 1981; Baverstock, 1984). The Conilurini (the pseudo-mouse group) is the most species-rich group and has a strictly Australian distribution. The genera Uromyini and Hydromyini have their main distribution in New Guinea, but some species of these genera – *Uromys*, *Melomys*, *Hydromys*, *Xeromys* – are also found on the Australian continent.

The fossil record of the native Australian rodents, the Conilurini, starts at 4.5 Ma (million years ago), but how and when the earliest rodents reached the Australian continent remains controversial. Australia has been an isolated continent since the last connection with Antarctica was severed in the Eocene. While Australia was drifting northwards and further away from Antarctica, it was getting closer to the Indonesian archipelago. At 5–10 Ma Australia and New Guinea came close enough to the Indonesian islands to facilitate the dispersal of animals (Hall, 2002). It is most likely that at this time the ancestral rodents migrated from South East Asia via Indonesia to the Australian continent. An older analysis of the fossil record suggests that the native rodents reached Australia in three waves (Baverstock, 1984; Hand, 1984). The first wave occurred 4.5 Ma and brought the Conilurini to Australia. Several 4.5 Myr old Pliocene localities such as

Abbreviations: Myr, million years; Ma, million years ago; mt, mitochondrial; kb, kilobase; nt, nucleotide; ca, amino acid; ML, maximum likelihood; GTR, general time reversible; MTD, Multidivtime; S. D., standard deviation; cdp, codon position.

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Rackham's Roost, Floraville and Chinchilla assemblages in Queensland have yielded a diversity of these first rodent fossils (Long et al., 2002). The second wave of rodents, introducing the water rats (Hydromyini) and the mosaic-tailed rats *Uromys* and *Melomys* to Australia occurred about 2 Ma and most likely originated from New Guinea (Long et al., 2002). The last addition to the Australian rodent fauna came with European settlement (Hand, 1984), which introduced the two common rat species *Rattus rattus* and *R. norvegicus* as well as the house mouse *Mus musculus*. Australia is also home to seven species of native rats of the genus *Rattus*, but it is not known how and when they reached the continent (Baverstock, 1984) or if they evolved on Australia.

A recent study (Rowe et al., 2008) examined the relationships of the Australian/New Guinean rodents using a mix of nuclear and mitochondrial data. Their results suggested that there had been at least nine rodent dispersals between New Guinea and Australia. Most interestingly, in this study a paraphyletic Conilurini was recovered with high support. The three Conilurini genera *Mesembriomys*, *Conilurus* and *Leporillus* clustered as a sister-group to Uromyini (Fig. 1A). This implies either two dispersals of 'Conilurini' to Australia, or alternatively that Uromyini may have evolved from a group of Australian conilurines.

The first Australian rodent bearing localities at 4.5 Ma already include over 13 species of rodents belonging to several genera (Long et al., 2002). The rich diversity of the early fossil rodents could indicate that rodents colonized Australia earlier than 4.5 Ma. The genera *Leggadina* and *Pseudomys* are among the earliest fossil rodents. Both these genera are represented in the Rackham's Roost assemblage as well as at a second locality in northern Queensland (Chinchilla Local Fauna), indicating that these genera were once widespread (Long et al., 2002). A study of albumin microcomplement fixation identified *Leggadina* as the sister-group to the remaining Conilurini (Watts et al., 1992). The most recent study, based on protein-coding data supported that *Leggadina* is the sister-group to a subgroup of Conilurini (Rowe et al., 2008) (Fig. 1B).

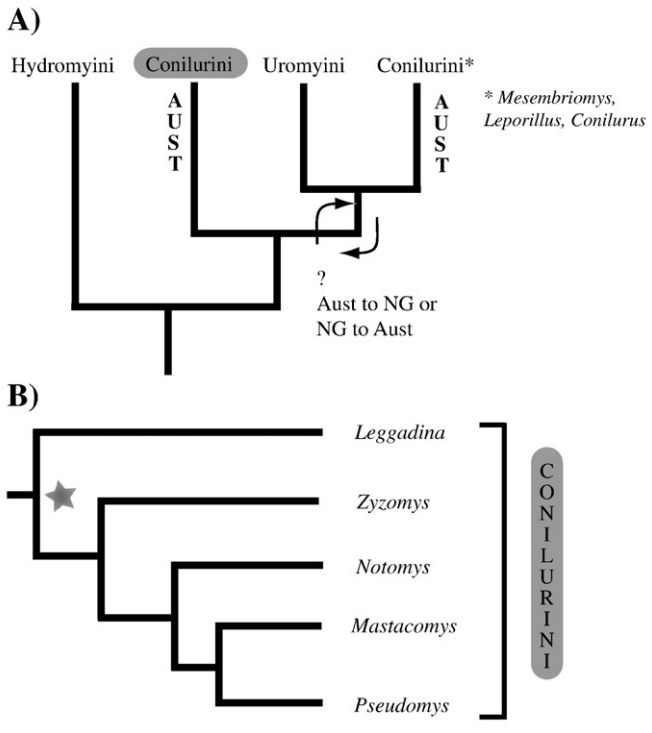

Fig. 1. A) The phylogenetic tree showing the relationships between the Australian and New Guinean rodents based on 12 kb of protein-coding data (Rowe et al., 2008). A detailed description of the relationship inside Conilurini is depicted in Fig 1B. Aust: Australia; NG: New Guinea. B) The relationship between the Conilurini genera based on a study of 12 kb of protein-coding data (Rowe et al., 2008). The star indicates the split investigated by mitochondrial data.

Until now divergence times of the Australian native rodents have been dated by using only a few local calibration points and a single method (Rowe et al., 2008). A general complication with divergence time estimations is that individual studies sometimes produce different dates for the same split (Pulquério and Nichols, 2007). The reason behind this is not exactly known. It is probable that dissimilar divergence time estimates are the result of the choice of dating algorithms, calibration points, different taxon sampling, and treatment and choice of data. The problem is prominent for the estimate of muroid rodent divergence times, and especially for the *Mus* and *Rattus* divergence. While the fossil record supports a divergence time between 11–12 Ma between the two species (Benton and Donoghue, 2007), molecular studies have suggested much older dates, ranging between 16–35 Ma (e.g. Janke et al., 1994; Arnason et al., 2002; Springer et al., 2003; Jansa et al., 2006). Only a few studies have provided divergence time estimates between *Mus* and *Rattus* that are reasonably congruent with the fossil record (Douzery et al., 2003; Steppan et al., 2004; Rowe et al., 2008). The muroid tribe Conilurini is phylogenetically closely related to *Mus* and *Rattus* and therefore constraining the *Mus–Rattus* age will greatly influence the divergence estimates of the Conilurini. We have investigated the impact of the calibration points, data type and methods on the estimates of divergence times, for assisting to select among these features for dating.

The complete mitochondrial (mt) genomes from two native Australian rodents of the Conilurini tribe, namely *Leggadina lakedownensis* and *Pseudomys chapmani* have been sequenced and analysed to establish their phylogenetic relationship and the temporal origin of these native Australian rodents. The mitogenomic study will provide an estimate of the evolutionary age of the deepest split in Conilurini and allows investigating the congruence of the molecular age with the fossil record. In addition, we have extended the taxon sampling of Murinae, by sequencing the mt genome of *R. rattus* and including *Mus musculus domesticus* and *M. m. molossinus* mt genome sequences in the analyses. The molecular clock has been calibrated with twelve well-established mammalian calibration points (Benton and Donoghue, 2007) (Table 1) thus providing a robust framework for the divergence time estimates. When possible we used calibration points with soft bounds because these appear to be superior for estimating divergence times (Yang and Rannala, 2006).

2. Material and methods

2.1. DNA sequencing

The mt genomes of *Leggadina lakedownensis* and *Pseudomys chapmani* were PCR amplified using conserved, rodent specific primers that were constructed from published mt genomes. The amplicons were 5–7 kb (kilo base) long fragments which overlapped by at least 100 nt (nucleotides). The PCR fragments were purified

Table 1
A list of the 12 calibration points that is included in the molecular divergence time estimation.

Calibration point	Age in Ma	Node
Mus–Conilurini	7.3–11.0	B
Rattus	<6.4	D
Murinae	>11.0	E
Rodentia	>55.8	L
Glires	61.5–100.5	O
Perissodactyla	50.0–58.0	P
Carnivora	43.0–63.8	Q
Carnivora–Perissodactyla	62.3–71.2	R
Cetartiodactyla	>55.0	T
Placentalia	95.3–113	V
Marsupialia	61.5–71.2	W
Theria	124.6–138.4	X