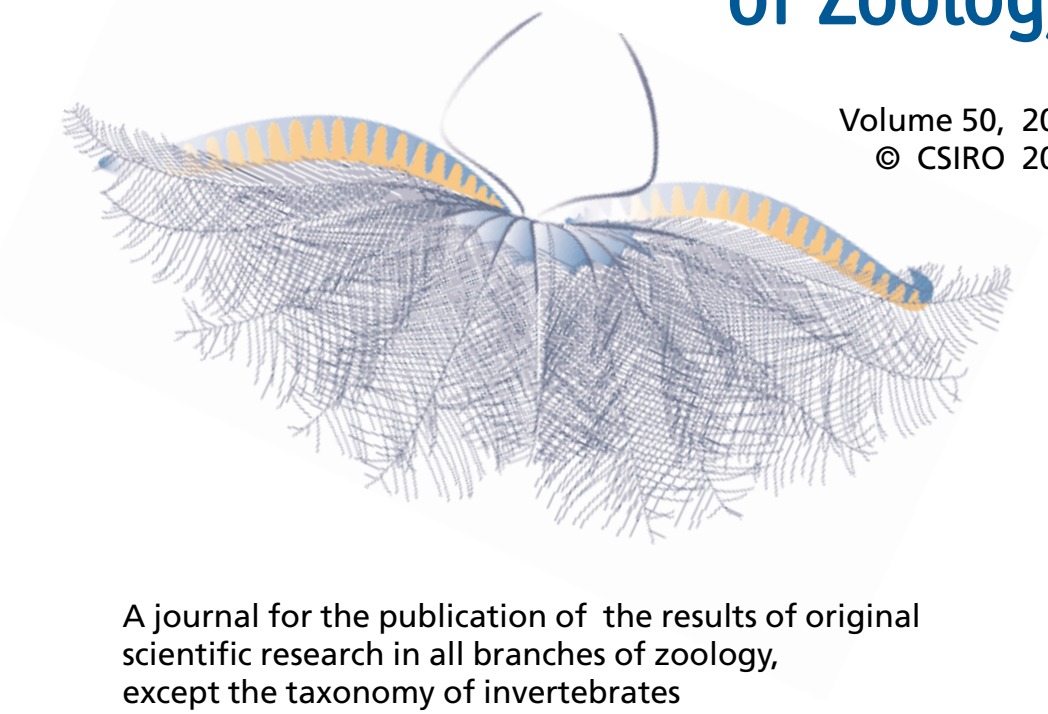
CSIRO Publishing

Australian Journal of Zoology



Volume 50, 2002

A journal for the publication of the results of original scientific research in all branches of zoology, except the taxonomy of invertebrates

All enquiries and manuscripts should be directed to:

Australian Journal of Zoology
CSIRO Publishing
PO Box 1139 (150 Oxford St)
Collingwood, Vic. 3066, Australia

Telephone: +61 3 9662 7622
Fax: +61 3 9662 7611
Email: publishing.ajz@csiro.au

Published by CSIRO Publishing
for CSIRO and the Australian Academy of Science

www.publish.csiro.au/journals/ajz

Australian Journal of Zoology, 2002, **50**, 25–37

Systematics and biogeography of pygmy possums (Burramyidae : *Cercartetus*)

M. J. Osborne[AB] *and L. Christidis*[A]

[A]Sciences Department, Museum Victoria, PO Box 666E, Melbourne, Vic. 3001, Australia.
[B]Department of Genetics, La Trobe University, Bundoora, Vic. 3083, Australia.

Abstract

Mitochondrial DNA sequences from the ND2 gene were used to investigate the systematic relationships within pygmy possums (*Cercartetus*) at the subspecies and species level. The phylogenetic relationships identified between *Cercartetus* species using partitioned ND2 sequences are in agreement with published morphological characters. *C. caudatus* was identified as the basal member of this assemblage, whilst *C. nanus* and *C. concinnus* are linked to the exclusion of *C. lepidus*. Molecular data identifies some inconsistencies in the assignment of subspecies within *Cercartetus*, suggesting that revision may be warranted.

Introduction

Five species of pygmy possums are currently recognised (Flannery 1994; Strahan 1995) and these are placed in two genera, *Burramys* (*B. parvus*) and *Cercartetus* (*C. concinnus*, *C. nanus*, *C. caudatus*, *C. lepidus*). The two genera comprise the family Burramyidae, with morphological characters (Archer 1984) and molecular evidence (Kirsch 1977; Baverstock *et al.* 1990) supporting the monophyletic association of these genera.

Within *Cercartetus*, Iredale and Troughton (1934) and Troughton (1957) initially included only two species (*C. concinnus* and *C. nanus*), with the other species (*caudatus*, *macrura* (now recognised as a subspecies of *caudatus*), and *lepidus*) placed within *Eudromicia*. Wakefield (1963) later combined *Eudromicia* with *Cercartetus* whereas Turnbull and Schram (1972) suggested that each of the *Cercartetus* species actually represented a monotypic genus. However, this was not formalised and subsequent workers have retained only a single genus (Archer 1984). The phylogenetic relationships between all species within the Burramyidae have been considered using morphological characters only (Archer 1984).

The members of *Cercartetus* occupy a diverse range of habitats throughout Australia and New Guinea (Fig. 1) and all species contain disjunct populations that are sometimes recognised as distinct subspecies (Wakefield 1963, 1970). A recent DNA study (Osborne *et al.* 2000) identified unexpected geographical differentiation within *B. parvus*. No such DNA studies have been conducted on *Cercartetus* to date, even though the generic- and species-level systematics of the group has been a source of considerable debate (e.g. Iredale and Troughton 1934; Wakefield 1963; Turnbull and Schram 1972; Archer 1984).

Cercartetus caudatus (long-tailed pygmy possum) is found in the rainforests of north-eastern Queensland between Townsville and the Daintree River and on the coastal plains in *Eucalyptus–Melaleuca* forest between the Daintree River and Cooktown. The distribution of *Cercartetus caudatus* also extends to the Central Cordillera of New Guinea. Flannery (1994) suggested that these distinct populations might actually represent several unrecognised species; however, only two subspecies are formally recognised: *C. c. macrurus* (Australia) and *C. c. caudatus* (New Guinea).

The distribution of *Cercartetus concinnus* (western pygmy possum) extends from the south-west of Western Australia and coastal South Australia (including Kangaroo Island)

 10.1071/ZO01071 0004-959X/02/010025

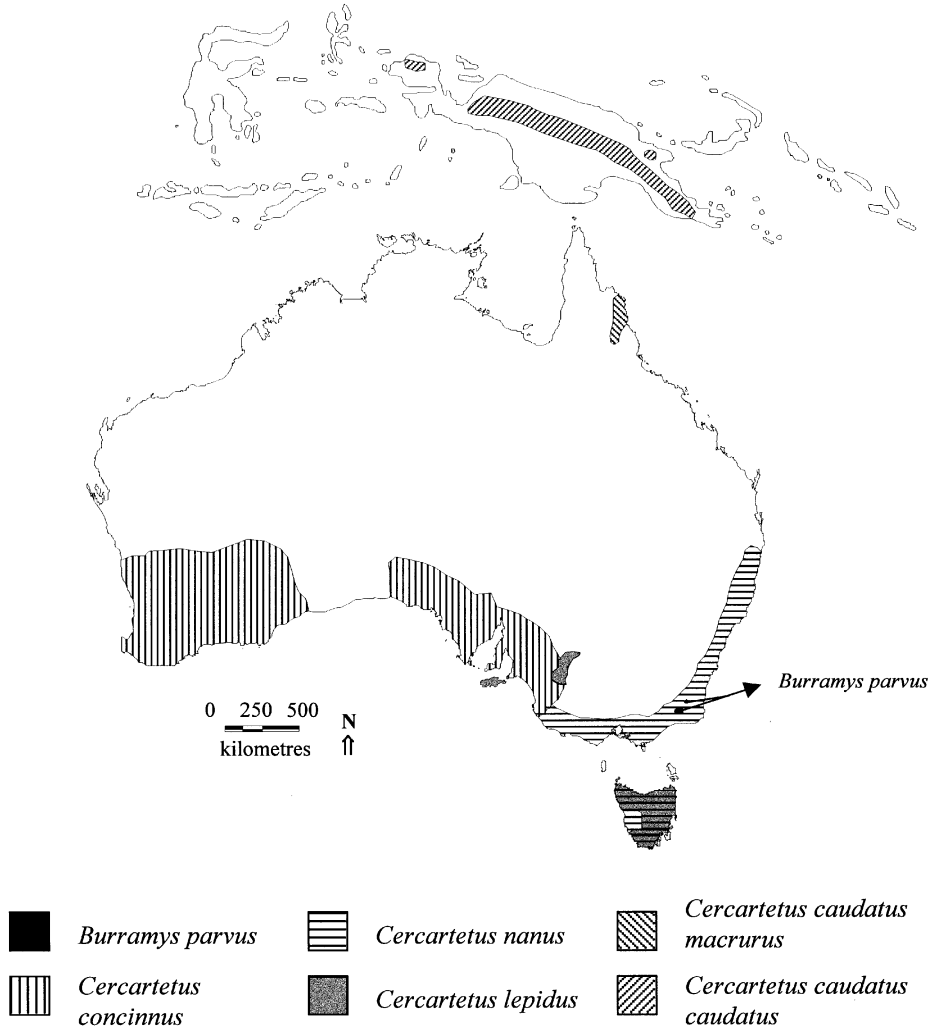


Fig. 1. Distribution map for *Burramys* and *Cercartetus* species in Australia and New Guinea.

to north-western Victoria and adjoining areas in New South Wales. This species is found predominantly in heath, mallee and sclerophyll forest but has also been found in mulga–saltbush vegetation (Smith 1983). These populations have been described as distinct subspecies – *C. c. concinnus* (South Australia, Victoria and New South Wales) and *C. c. minor* (Western Australia) – because of an apparently disjunct distribution (Wakefield 1963). However, a specimen of *C. concinnus* has been reported from approximately the middle of the distributional gap between the known ranges of this species in Western Australia and South Australia (Bolam 1923), as were remains of recent geological age (Lundelius 1957).

The smallest species, *Cercartetus lepidus* (little pygmy possum), was thought to be endemic to Tasmania until 1964, when a specimen was recorded from Kangaroo Island (Aitken 1967). *C. lepidus* is now known to occur in a wide range of habitats from the dry

sclerophyll forests of Kangaroo Island and eastern Tasmania, the wet sclerophyll forests in western Tasmania, and the mallee scrub of north-western Victoria and South Australia.

Cercartetus nanus (eastern pygmy possum) also has a mainland (south-eastern Queensland through to south-eastern South Australia) and Tasmanian distribution with a subspecific distinction made between these two disjunct populations: *C. n. unicolor* and *C. n. nanus* respectively (Wakefield 1963). Wakefield (1970) also suggested that there is an east–west divide on the basis of craniometric data, with *C. nanus* from western and central Victoria tending to be smaller than those from eastern Victoria and New South Wales.

In this study, sequence from the mitochondrial nicotinamide dehydrogenase subunit-two gene (ND2) was used to elucidate systematic relationships within *Cercartetus* at the species and subspecies level.

Methods

Samples

Specimens examined, source of samples, collection localities and GenBank accession numbers are listed in Table 1. Representatives of all species, subspecies and most geographically disjunct populations of *Cercartetus* were examined, except for *C. nanus nanus* from Tasmania. Published sequences of *Burramys parvus* were included (Osborne *et al.* 2000). Published sequences (Osborne and Christidis 2001) from the common ringtail (*Pseudocheirus peregrinus*) and brushtail (*Trichosurus vulpecula*) possums were included as outgroup taxa.

Sequence and phylogenetic analyses

Genomic DNA was extracted from tissue or hair samples following the methods of Gemmell and Akiyama (1996). ND2 was sequenced using the methods and primers described in Osborne and Christidis (2001). Double-stranded sequence was obtained from a representative of each taxon.

ND2 sequences were aligned manually. Transition : transversion ratios were obtained using MEGA (Molecular Evolutionary Genetic Analyses) Version 2.1 (Kumar *et al.* 2001). Saturation plots were constructed by plotting the number of transitions and transversions against Kimura (1980) two-parameter

Table 1. Species and common name, voucher or specimen location, collection locality information and GenBank Accession numbers of specimens used in this study

Institutions: AM, Australian Museum; MV, Museum Victoria; SAM, South Australia Museum; TM, Tasmanian Museum; QMV, Queen Victoria Museum, Tasmania; WAM, Western Australian Museum; MU, Melbourne University; LU, La Trobe University. Collection localities: SA, South Australia; NSW, New South Wales; NG, New Guinea; NP, National Park. Double-stranded sequence was obtained from individuals marked with an asterisk

Species	Common Name	Voucher	Locality	GenBank Acc.
Cercartetus nanus	Eastern pygmy possum	C31224 (MV)	Marlo, Eastern Victoria	–
		CN5 (MU)*	Bear Gully, south Gippsland	AF425977
Cercartetus lepidus	Little pygmy possum	29473 (SAM)*	NNE Lucindale, SA	AF425976
		Clep1 (TM)	Tasmania	–
		7047 (QVM)	Nunamurra, Tasmania	–
Cercartetus concinnus	Western pygmy possum	M32456 (AM)*	Mallee Cliffs NP, western NSW	AF25975
		M55432 (WAM)	Toodyay, Western Australia	–
		M55433 (WAM)	Toodyay, Western Australia	–
Cercartetus caudatus	Long-tailed pygmy possum	DNA (LU)*	Australia (locality unknown)	–
		EBU24767 (AM)	Milne Bay Province, NG	AF25978

genetic distances. Comparisons between ingroup and outgroup taxa were also included as this assists in the identification of saturated data (Griffiths 1997). Phylogenetic analyses were conducted using PAUP 4.0b4a (Swofford 2000).

Unweighted parsimony analyses were performed for total and partitioned data using the heuristic search option (random addition, 10 replicates). Data partitions included: weighting third-codon position transitions twice that of transversions, exclusion of third-codon position transitions, exclusion of all third-codon position substitutions and exclusion of all transitions (transversion parsimony). Branch support was assessed by the decay index value (d) (Bremer 1988), as obtained using the programme Auto Decay (Eriksson 1998) and with the bootstrap approach (Felsenstein 1985) with 1000 replicates.

Maximum-likelihood trees (using empirical base frequencies) were constructed using the heuristic search option (with 10 random additions) under HKY85 model (Hasegawa *et al.* 1985). Branch support was assessed by 100 bootstrap replicates.

Neighbour-joining analyses (Saitou and Nei 1987) were conducted using Kimura two-parameter (Kimura 1980) and HKY85 (Hasegawa *et al.* 1985) distances. Branch support was assessed by 1000 bootstrap replicates.

Molecular clock calibrations

An average rate of 2% mtDNA sequence divergence per million years (Brown *et al.* 1979) has often been used to estimate divergence times between marsupial lineages from cytochrome-*b* (e.g. Krajewski *et al.* 1997). However, evidence of saturation at the third-codon positions of ND2 in possums (Osborne and Christidis 2001) suggests that this rate will under-estimate divergences (as multiple substitutions at the one position will not be recorded in the divergence estimate) for the deeper nodes within the phylogeny. In such cases, Springer (1997) has advocated a linear regression approach using transversion distances calculated by the Tamura–Nei method (Tamura and Nei 1993).

In previous studies the co-occurrence of fossils assigned to *Trichosurus* and *Strigocuscus* (Flannery and Archer 1987) have been used as a calibration point for molecular clock estimates (e.g. Kirsch *et al.* 1997; Springer 1997). However, there is uncertainty regarding the real assignment of the fossils placed in *Strigocuscus* and in *Trichosurus* (K. Crosby, personal communication). Furthermore, the ages of the Riversleigh fossil assemblages have not been accurately determined. Consequently, the co-occurrence of lineages at Riversleigh were not used here as calibration points.

Instead, three different calibration points were used. The first was the co-occurrence of fossil macropodines and potoroines at Etadunna dated at 24–26 million years (Woodburne *et al.* 1993). The second was the co-occurrence of *Petaurus* species from the Pliocene Hamilton Local Fauna (Turnbull *et al.* 1987) dated by radiometric methods at 4.46 ± 0.1 million years (Rich 1991). These times reflect the lower limit of divergence times as the first occurrence of two extant lineages in the fossil record yields an upper bound of divergence but can severely under-estimate real divergence times (Marshall 1990). The third point was obtained from the molecular study of Janke *et al.* (1997), who constructed a mammalian phylogeny using 12 mitochondrial coding-genes. This tree was calibrated using the separation between cetaceans and artiodactyls as evidenced from the fossil record. The morphological changes accompanying the transition from terrestrial to aquatic life and the large body size of these taxa (making fossils easier to locate) results in the allocation of a timing for this split within a narrower time frame than is possible for many other lineages (Arnason *et al.* 1996). From their study of mammalian divergences, Janke *et al.* (1997) estimated a time of divergence of 75 (±7.1) million years for the split between the two marsupials examined, *Didelphis virginiana* and *Macropus robustus*. This was used as the third point for the regression analysis of the present study. ND2 transversion distances were calculated from the following sources: *Macropus* and *Didelphis* (Janke *et al.* 1997), *Petaurus* (Osborne and Christidis 2001), and *Potorous* (Osborne, unpublished).

A plot of this regression is shown in Fig. 2. From this plot, the equation

$$y = d(3.42 \pm 0.13) - (2.72 \pm 0.30)$$

is obtained, where d is transversion distance calculated by the Tamura–Nei (1993) method and y is divergence time in millions of years.

Results

Sequences

Sequence was obtained for the ND2 gene (1040 base pairs) for ten individuals representing all four species of *Cercartetus*. In comparisons involving *Burramys* and *Cercartetus* there

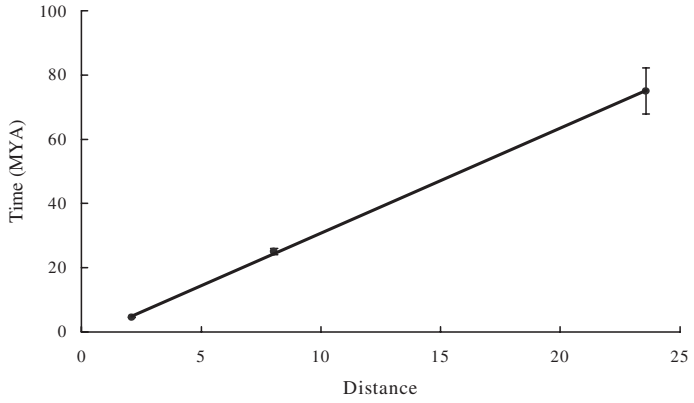


Fig. 2. Regression of transversion distances (Tamura and Nei 1993) plotted against divergence times (in millions of years).

were 408 variable sites, of which 356 were parsimony informative. Of the parsimony-informative characters, 241 were at the third codon, 86 at the first codon and 29 at the second codon.

Transition : transversion ratios were skewed towards transitions for all ingroup comparisons (Table 2). Ratios ranged from 1.35 to 1.46 for comparisons between *Burramys* and *Cercartetus*. Within *Cercartetus*, ratios ranged between 1.44 and 2.11. In outgroup comparisons involving *Pseudocheirus peregrinus*, ratios were biased in favour of transversions (0.90–1.02), whilst comparisons involving *Trichosurus vulpecula* were skewed toward transitions (1.05–1.24).

Sequence divergences (Table 2) between *Burramys* and *Cercartetus* ranged from 24.18% and 27.72%. Within subspecies of *Cercartetus* divergences ranged from 0.50% to 6.93%. Between recognised subspecies and species of *Cercartetus* divergences ranged from 0.80% to 8.28% and from 18.83% to 23.50%, respectively.

Transversions at all codon positions and transitions at the second codon position accumulated linearly with genetic distance for ingroup comparisons (Fig. 3). Transitions at the first and third codon positions approached an asymptote with increasing genetic distance, especially those involving ingroup to outgroup comparisons.

Phylogenetic analyses

Maximum-parsimony, maximum-likelihood and neighbour-joining analyses produced trees with an identical topology (Fig. 4, Table 3), in which *Burramys* was basal to the clade containing *Cercartetus*. *Cercartetus lepidus* and *C. nanus* clustered together (bootstrap support of 60–69%) and *C. concinnus* was the sister taxon of this group (bootstrap support of 67–70%). *C. caudatus* was basal within *Cercartetus* (bootstrap support of 96–99%). This topology was also produced when third-codon position transversions were weighted twice that of transitions at this position. Parsimony analysis of partitioned data sets where all third-codon position changes, third-codon position transitions or all transitions were excluded, changed the topology slightly (Fig. 5, Table 3): *C. nanus* now grouped with *C. concinnus* to the exclusion of *C. lepidus* (bootstrap support of less than 50–55%).

Table 2. Percentage sequence divergence for ND2

Pairwise comparisons corrected using Kimura two-parameter method are given above the diagonal. The average transition to transversion ratio between species is given below the diagonal. Population localities are as follows: Aust., Australia; Vic.,Victoria; NSW, New South Wales; Tas., Tasmania; SA, South Australia; WA, Western Australia; NG, New Guinea

Taxon		Taxon													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	*B. parvus* (Vic.)		0.80	25.66	24.18	25.66	24.74	26.94	27.72	25.70	26.31	26.58	26.60	28.31	28.82
2	*B. parvus* (NSW)	–		25.96	24.47	25.81	24.88	26.94	27.72	25.70	26.43	26.70	26.72	28.29	28.67
3	*C. caudatus* (Aust.)	1.36	–		8.28	21.54	20.98	23.15	23.42	22.78	20.23	20.92	20.93	31.69	30.27
4	*C. caudatus* (NG)	–	–	–		22.98	22.26	22.80	23.50	23.01	20.23	21.06	20.93	30.83	28.81
5	*C. nanus* 31224 (Vic.)	1.35	–	1.62	–		2.22	20.35	20.63	19.24	20.28	19.86	19.74	33.09	30.11
6	*C. nanus* 5 (Vic.)	–	–	–	–	–		20.07	20.63	18.83	19.78	19.36	19.51	31.95	29.60
7	*C. lepidus* 1 (Tas.)	1.64	–	1.76	–	2.11	–		1.81	6.93	20.16	20.16	20.17	31.23	28.64
8	*C. lepidus* 7047 (Tas.)	–	–	–	–	–	–	–		6.81	20.44	20.44	20.45	31.71	28.64
9	*C. lepidus* (SA)	–	–	–	–	–	–	–	–		20.07	20.07	20.08	30.53	27.92
10	*C. concinnus* (NSW)	1.46	–	1.44	–	2.02	–	1.55	–	–		0.90	0.80	28.84	26.35
11	*C. concinnus* 55432 (WA)	–	–	–	–	–	–	–	–	–	–		0.50	29.74	27.22
12	*C. concinnus* 55433 (WA)	–	–	–	–	–	–	–	–	–	–	–		29.60	26.78
13	*P. peregrinus*	0.96	–	0.93	–	1.02	–	0.90	–	–	0.91	–	–		27.91
14	*T. vulpecula*	1.22	–	1.24	–	1.12	–	1.05	–	–	1.11	–	–	0.79	

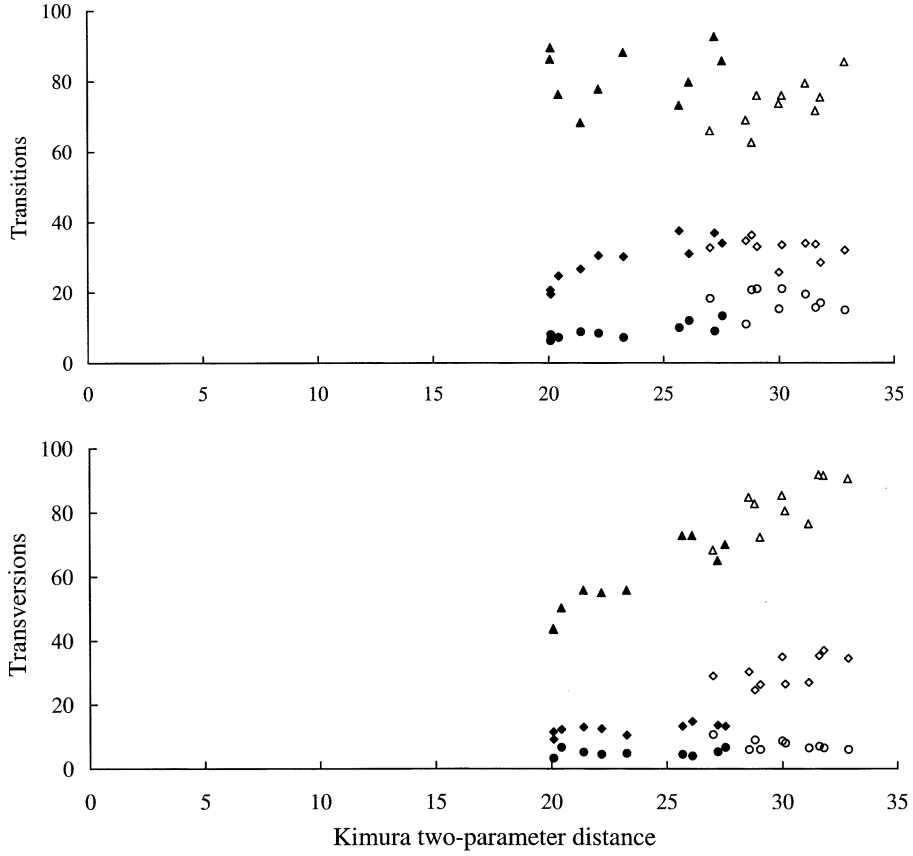


Fig. 3. Saturation plots for ND2. The number of transitions and transverions at each codon position is plotted against the genetic distance (Kimura two-parameter method). Ingroup comparisons: ◆, 1st position; ●, 2nd position; ▲, 3rd position. Ingroup to outgroup comparisons: ◇, 1st position; ○, 2nd position; △, 3rd position. The outgroups are *Pseudocheirus peregrinus* and *Trichosurus vulpecula*.

Discussion

Phylogenetic relationships

Monophyly of *Cercartetus* relative to *Burramys* was strongly supported by the ND2 sequence data and this is consistent with the presence of a shared cranial feature in all members of *Cercartetus*: expansion of the alisphenoid hypotympanic cavity (Archer 1984). ND2 sequences identified *C. caudatus* as basal within *Cercartetus*, which is also concordant with phylogenetic arrangements based on morphological characters (Archer 1984). Moreover, *C. caudatus* shares several features with unnamed fossil *Cercartetus* from the Miocene assemblage at Riversleigh (Archer *et al.* 1991; Brammall and Archer 1999).

Phylogenetic analyses of total ND2 sequence data identified a sister relationship between *C. lepidus* and *C. nanus*, although this relationship received only moderate bootstrap support. Several dental (reduction of P(1–2)/(1–2), loss of M5/5, specialised trignoid and reduced paraconid–cristid cusp–crest complexes) and cranial (expansion by

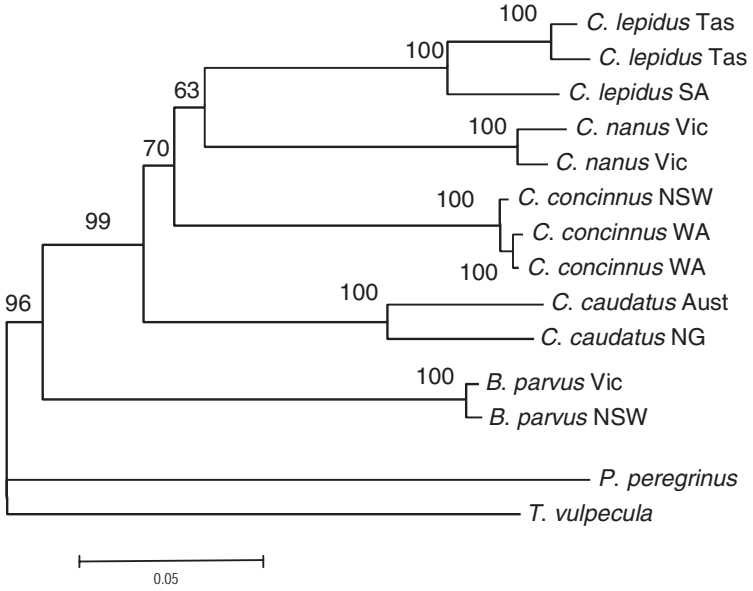


Fig. 4. Neighbour-joining tree for ND2. Bootstrap values are given above branches (identical topologies were obtained for maximum-parsimony and maximum-likelihood analyses). Localities are given after the species name (abbreviations follow those used in Table 2).

spongiosa of lateral zygomatic region of the squamosal bone and greatly reduced tympanic sinuses) characters, instead, unite *C. nanus* with *C. concinnus* (Archer 1984). When rapidly evolving DNA characters such as transitions and third-codon position changes were excluded from analysis then a sister relationship between *C. nanus* and *C. concinnus* was evident. Given the concordance between the partitioned molecular data and the morphological characters, a sister relationship between *C. nanus* and *C. concinnus* is likely. However, additional sequence data are required to resolve the discrepancy between the analyses based on the complete and partitioned ND2 data sets.

The large DNA distances observed between species of *Cercartetus* (18–23%) are in sharp contrast to micro-complement fixation of albumin data, which found there to be only

Table 3. Tree statistics for parsimony analyses

Characters, no. of informative characters; length, no. of trees; CI, consistency index; RI, retention index; RC, rescaled consistency index for the ND2 data. Analyses: MP, Maximum parsimony; 1,0, excluding third position transitions; Ex 3rd, excluding all third position substitutions; 2,1, weighting third-position transversions twice that of transitions; and Ex Ti, excluding all transitions

Analyses	Characters	Length	CI	RI	RC
MP	385	922 (1)	0.620	0.720	0.447
1,0	391	527 (1)	0.624	0.734	0.458
Ex 3rd	187	242 (1)	0.702	0.788	0.554
2,1	391	1223 (1)	0.608	0.711	0.433
Ex Ti	184	333 (1)	0.553	0.711	0.393

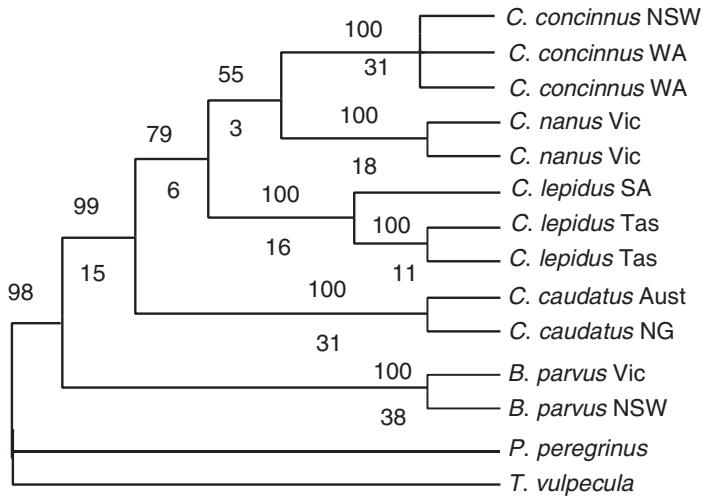


Fig. 5. Parsimony tree produced when third-position transitions were excluded. Bootstrap (above branches) and Bremer (below) support values are given. Localities are given after the species name (abbreviations follow those used in Table 2).

one or two amino acid substitutions between *C. lepidus*, *C. nanus* and *C. concinnus* (Baverstock *et al.* 1990). The DNA sequence data are more consistent with the suggestion of Turnbull and Schram (1972), that, on the basis of dental characters, each of the four species of *Cercartetus* should be placed in separate genera. Archer's view (1984) was that if all species of *Cercartetus* represent a monophyletic group (and they do) there is no compelling reason to separate each species into a monotypic genus. Nevertheless, the disparity in the amount of genetic divergence that separates different taxonomic ranks is evidently not consistent within the diprotodontids. For example, within the Petauroidea, the level of divergence between the genera *Dactylopsila* and *Dactylonax* is *c.* 20% and that between *Petauroides* and *Pseudocheirus* is *c.* 24% (Osborne and Christidis 2001). A case could be made that *Cercartetus* should be spilt into two or more genera to better reflect evolutionary divergences.

Subspecies

The ND2 sequence data identified each of the currently recognised species of *Cercartetus* as monophyletic, but significant genetic differentiation was observed within some species. Most diverged (8%) were *C. caudatus macrurus* and *C. caudatus caudatus* from Australia and New Guinea respectively. A similarly high level of DNA sequence divergence (*c.* 7%) was recorded between the mainland and Tasmanian populations of *C. lepidus*. Although the two disjunct populations are not currently recognised as separate subspecies, they clearly could be on the basis of DNA divergence.

The level of divergence observed between populations of both *C. caudatus* and *C. lepidus* is similar to, or greater than, that recorded between congeneric species of *Petaurus* (Osborne and Christidis 2001), *Phalanger*, *Spilocuscus* and *Trichosurus* (Osborne and Christidis 2002). Thus, a case could be made that the two currently recognised subspecies of *C. caudatus* and the mainland and Tasmania populations of *C. lepidus* may

in fact be best treated as separate species. Further sampling and molecular analysis and the consideration of morphological characters needs to be undertaken to clarify the taxonomy of this group.

Cercartetus nanus also has a disjunct distribution, occurring on the mainland and Tasmania. These disjunct populations are sometimes recognised as separate subspecies (Flannery 1994; Strahan 1995): *C. n. unicolor* on the mainland and *C. n. nanus* in Tasmania. Unfortunately, only the mainland form was available for examination in the present study and so the status of the two forms cannot yet be resolved.

Flannery (1994) recognised two subspecies of *C. concinnus*, *C. c. concinnus* in south-west Western Australia, and *C. c. minor* in South Australia, western Victoria and western New South Wales. Conversely, Wakefield (1970) and Strahan (1995) did not recognise any subspecies. The low level of divergence that was recorded between individuals of *C. concinnus* from western Australia and south-western New South Wales supports the latter view. There was less than 1% divergence between the two disjunct populations, which was only slightly higher than that which was identified between two individuals from the same locality (Toodyay, Western Australia).

Fossil evidence, divergence dates and biogeography

Dates calculated from the ND2 sequence data (see Methods) suggest that *Burramys* and *Cercartetus* diverged 29–32 million years ago, corresponding to the Oligocene period (23–35 million years ago). Fossils of *Burramys* have been described from the Oligocene–Miocene Riversleigh assemblage (*Burramys brutyi*) (Brammall and Archer 1997), from the late Oligocene Ngama Local Fauna (*Burramys wakefieldi*) (Pledge 1987; Woodburne *et al.* 1993) and from the Miocene Geilston Bay Fauna (Tedford *et al.* 1975). A fossil *Cercartetus* has also been identified from a Miocene Riversleigh assemblage (Archer *et al.* 1991; Brammall and Archer 1999). Thus, the divergence dates for *Cercartetus* and *Burramys* are consistent with fossil evidence. The extant species of *Cercartetus* are estimated to have diverged from each other *c.* 16–27 million years ago.

The mainland and Tasmanian populations of *C. lepidus* and the Australian and New Guinean populations of *C. caudatus* are estimated to have diverged *c.* 3–4 million years ago. These times are similar to those estimated by Blackett *et al.* (2000) for the divergence between Australian and New Guinean lineages of *Planigale*. Although it could be argued that the Pleistocene sea level fluctuations account for the divergence between the Australian and New Guinean forms of *C. caudatus*, and the mainland and Tasmanian forms of *C. lepidus*, the estimated divergence times indicate that fragmentation of the ancestral populations occurred prior to the Pleistocene. Although mainland *C. lepidus* is now restricted to western Victoria and South Australia, fossil remains found in eastern Victoria and in New South Wales (Wakefield 1963; Wakefield 1967; Turnbull and Schram 1972) also indicate that *C. lepidus* had a more extensive distribution as recently as the Pleistocene.

Rainforests dominated Australia in the Oligocene and early Miocene periods, which is reflected in the high level of marsupial diversity found in the Riversleigh fossil deposit (Archer *et al.* 1989; Archer *et al.* 1999). During the middle to late Miocene, rainfall declined and conditions became cooler and this resulted in fragmentation of closed forest habitats. Sclerophyllous vegetation was present in the west and heath was also present in some regions (Hill *et al.* 1999). The increase in habitat types and fragmentation may have facilitated the diversification of *Cercartetus*. The presumed most primitive *Cercartetus* species are adapted to rainforest habitats (Riversleigh fossil *Cercartetus* and extant *C. caudatus*) and the more recently diverged lineages are adapted to either sclerophyllous

forests (*C. nanus*) or drier habitats such as mallee and heathlands (*C. lepidus* and *C. concinnus*). This trend from mesic to xeric forms has been identified in various bird species (Schodde and Calaby 1972) and is thought to reflect the vegetation changes that occurred in response to climatic shifts.

Acknowledgments

Our sincere thanks are extended to the following people for lending or donating the samples used in this study: D. O'Meally (Australian Museum), S. Donnellan (South Australian Museum), A. Burbidge (CALM, Western Australia), D. Pemberton (Tasmanian Museum), T. Gordon (Queen Victoria Museum), T. Mitchell (DNRE, Victoria), S. Ward (University of Melbourne), M. Westerman (La Trobe University), I. Mansergh (DNRE, Victoria), L. Broome (NPWS, NSW), and D. Heinze (La Trobe University). N. Murray (La Trobe University) and J. Norman provided helpful advice during the course of this study. Thanks are also extended to S. Wyithe. Museum Victoria and La Trobe University provided funding for the project. M. Osborne was supported by a La Trobe University Postgraduate Scholarship.

References

Aitken, P. F. (1967). *Cercartetus lepidus* (Thomas) an addition to the fauna of Kangaroo Island. *Records of the South Australian Museum* **15**, 575–576.

Aplin, K. P., and Archer, M. (1987). Recent advances in marsupial systematics with a new syncretic classification. In 'Possums and Opossums: Studies in Evolution'. (Ed. M. Archer.) pp. xv–lxxii. (Surrey-Beatty & the Zoological Society of New South Wales: Sydney.)

Archer, M. A. (1984). The Australian marsupial radiation. In 'Vertebrate Zoogeography and Evolution in Australasia'. (Eds M. Archer and G. Clayton.) pp. 633–808. (Hesperian Press: Perth.)

Archer, M. A., Godthelp, H., Hand, S., and Megirian, D. (1989). Fossil mammals of Riversleigh, northwestern Queensland: preliminary overview of biostratigraphy, correlation and environmental change. *Australian Journal of Zoology* **25**, 29–65.

Archer, M., Hand, S. J., and Godthelp, H. (1991). 'Riversleigh, the Story of Animals in Ancient Rainforests of Inland Australia.' (Reed Books: Australia.)

Archer, M. A., Arena, R., Bassarova, M., Black, K., Brammall, J., Cooke, B., Creaser, P., Crosby, K., Gillespie, A., Godthelp, H., Gott, M., Hand, S. J., Kear, B., Krikmann, A., Mackness, B., Muirhead, J., Musser, A., Myers, T., Pledge, N., Wang, Y., and Wroe, S. (1999). The evolutionary history and diversity of Australian mammals. *Australian Mammalogy* **21**, 1–45.

Arnason, U., Gulberg, A., and Xu, X. (1996). Pattern and timing of evolutionary divergences among hominoids based on analyses of complete mtDNAs. *Journal of Molecular Evolution* **43**, 650–661.

Baverstock, P. R., Krieg, M., and Birrell, J. (1990). Evolutionary relationships of Australian marsupials as assessed by albumin immunology. *Australian Journal of Zoology* **37**, 273–287.

Blackett, M. J., Adams, M., Krajewski, C., and Westerman, M. (2000). Genetic variation within the marsupial genus *Planigale*. *Australian Journal of Zoology* **48**, 443–459.

Bolam, A. G. (1923). 'The Trans-Australian Wonderland.' (Barker and Co.: Melbourne.)

Brammall, J., and Archer, M. (1997). A new Oligocene–Miocene species of *Burramys* (Marsupialia, Burramyidae) from Riversleigh, north-western Queensland. *Memoirs of the Queensland Museum* **41**, 247–268.

Brammall, J., and Archer, M. (1999). Living and extinct petaurids, acrobatids, tarsipedids and burramyids (Marsupialia): relationships and diversity through time. *Australian Mammalogy* **21**, 24–25.

Bremer, K. (1988). The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. *Evolution* **42**, 795–803.

Brown, W. M., George, M., and Wilson, A. C. (1979) Rapid evolution of animal mitochondrial DNA. *Proceedings of the National Academy of Sciences, USA* **76**, 1967–1971.

Eriksson, T. (1998). 'Auto-decay. Version 4.02.' (Department of Botany, Stockholm University: Stockholm.) (Programme distributed by the author.)

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Flannery, T. (1994). 'Possums of the World. A Monograph of the Phalangeroidea.' (GEO Productions: Sydney.)

Flannery, T., and Archer, M. (1987). *Strigocuscus reidi* and *Trichosurus dicksoni*, two new fossil phalangerids (Marsupialia: Phalangeridae) from the Miocene of northwestern Queensland. In 'Possums and Opossums: Studies in Evolution'. (Ed. M. Archer.) pp. 527–536. (Surrey Beatty & the Royal Zoological Society of New South Wales: Sydney.)

Gemmell, N. J., and Akiyama, S. (1996). An efficient method for the extraction of DNA from vertebrate tissue. *Trends in Genetics* **12**, 338–339.

Griffiths, C. S. (1997). Correlation of functional domains and rates of nucleotide substitution in cytochrome-*b*. *Molecular Phylogenetics and Evolution* **7**, 352–365.

Hasegawa, M., Kishino, H., and Yano, T. (1985). Dating of the human–ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution* **8**, 888–891.

Hill, R. S., Truswell, E. M., McLoughlin, S. M., and Dettman, M. E. (1999). Evolution of Australian flora: fossil evidence. In 'The Flora of Australia. Vol. 1. Introduction'. (Eds A. E. Orchard and H. S. Thompson.) pp. 251–320. (ABRS & CSIRO Publishing: Melbourne.)

Iredale, T., and Troughton, E. Le G. (1934). A checklist of mammals recorded from Australia. *Memoirs of the Australian Museum* **6**, 1–122.

Janke, A., Xu, X., and Arnason, U. (1997). The complete mitochondrial genome of the wallaroo (*Macropus robustus*) and the phylogenetic relationship among Monotremata, Marsupialia and Eutheria. *Proceedings of the National Academy of Sciences*, *USA* **94**, 1276–1281.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* **16**, 111–120.

Kirsch, J. A. W. (1977). The comparative serology of Marsupialia, and a classification of marsupials. *Australian Journal of Zoology*, *Supplementary Series* No. 52, 152 pp.

Kirsch, J. A. W., Lapointe, F., and Springer, M. S. (1997). DNA hybridisation studies of marsupials and their implications for metatherian classification. *Australian Journal of Zoology* **45**, 211–280.

Krajewski, C., Blackett, M., Buckley, L., and Westerman, M. (1997). A multigene assessment of phylogenetic relationships within the dasyurid marsupial subfamily Sminthopsinae. *Molecular Phylogenetics and Evolution* **8**, 236–248.

Kumar, S., Tamura, K., Jakobsen, I., and Nei, M. (2001). 'MEGA: Molecular Evolutionary Genetic Analysis. Version 2.1.' (University Park: Pennsylvania.)

Lundelius, E. (1957). Additions to knowledge of the ranges of Western Australian mammals. *Western Australian Naturalist* **5**, 173–182.

Marshall, C. R. (1990). The fossil record and estimating divergence times between lineages: maximum divergence times and the importance of reliable phylogenies. *Journal of Molecular Evolution* **30**, 400–408.

Osborne, M. J., and Christidis, L. (2001). Molecular phylogenetics of Australo-Papuan possums and gliders (Family Petauridae). *Molecular Phylogenetics and Evolution* **20**, 211–224.

Osborne, M. J., and Christidis, L. (2002). Molecular relationships of the cuscuses, brushtail and scaly-tailed possums. *Australian Journal of Zoology* **50**, in press.

Osborne, M. J., Norman, J. A., Christidis, L., and Murray, N. (2000). Genetic distinctness of isolated populations of an endangered marsupial, the mountain pygmy possum, *Burramys parvus*. *Molecular Ecology* **9**, 609–613.

Pledge, N. (1987). A new species of *Burramys* Broom (Marsupialia: Burramyidae) from the middle Miocene of South Australia. In 'Possums and Opossums: Studies in Evolution'. (Ed. M. Archer.) pp. 725–728. (Surrey Beatty & the Royal Zoological Society of New South Wales: Sydney.)

Rich, T. H. (1991). Monotremes, placentals, and marsupials: their record in Australia and its biases. In 'Vertebrate Paleontology of Australasia'. (Eds P. Vickers-Rich, J. M. Monaghan, R. F. Baird and T. Rich.) pp. 893–1004. (Pioneer Design Studios: Victoria.)

Saitou, N., and Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **4**, 406–425.

Schodde, R., and Calaby, J. H. (1972). The biogeography of the Australo-Papuan bird and mammal faunas in relation to Torres Strait. In 'Bridge and Barrier: the Natural and Cultural History of Torres Strait'. (Ed. D. Walker.) pp. 257–306. (Australian National University Press: Canberra.)

Smith, M. J. (1983). Western pygmy-possum. In 'The Australian Museum Complete Book of Australian Mammals'. (Ed. R. Strahan.) p. 162. (Reed Books: Sydney.)

Springer, M. S. (1997). Molecular clocks and the timing of the placental and marsupial radiations in relation to the Cretaceous–Tertiary boundary. *Journal of Mammalian Evolution* **4**, 285–302.

Strahan, R. (Ed.) (1995). 'The Australian Museum Complete Book of Australian Mammals.' (Reed Books: Sydney.)

Swofford, D. L. (2000). 'PAUP*. Phylogenetic Analysis Using Parsimony. Version 4.0b4a.' (Sinauer Associates: Sunderland, Massachusetts.)

Tamura, K., and Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of the mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* **10**, 512–526.

Tedford, W. D., Banks, M. R., Kemp, N. R., McDougall, I., and Sutherland, F. L. (1975). Recognition of the oldest known fossil marsupials from Australia. *Nature* (*London*) **225**, 141–142.

Troughton, E. Le G. (1957). 'Furred Animal of Australia.' 6th Edn. (Angus and Robertson: Sydney.)

Turnbull, W. D., and Schram, F. R. (1972). Broom cave *Cercartetus* with observations on pygmy possum dental morphology, variation and taxonomy. *Records of the Australian Museum* **28**, 437–461.

Turnbull, W. D., Rich, T. H. V., and Lundelius, E. L. (1987). Burramyids (Marsupialia: Burramyidae) of the Pliocene Hamilton Local Fauna, southwestern Victoria. In 'Possums and Opossums: Studies in Evolution'. (Ed. M. Archer.) pp. 729–739. (Surrey Beatty & the Royal Zoological Society of New South Wales: Sydney.)

Wakefield, N. A. (1963). The Australian pigmy-possums. *The Victorian Naturalist* **80**, 99–116.

Wakefield, N. A. (1967). Mammal bones in the Buchan district. *The Victorian Naturalist* **84**, 211–214.

Wakefield, N. A. (1970). Notes of Australian pigmy-possums (*Cercartetus*, Phalangeridae, Marsupialia). *The Victorian Naturalist* **87**, 1–8.

Woodburne, M. O., MacFadden, B. J., Case, J. A., Springer, M. S., Pledge, N. S., Power, J. D., Woodburne, J. M., and Springer, K. B. (1993). Land mammal biostratigraphy and magnetostratigraphy of the Etadunna Formation (late Oligocene) of South Australia. *Journal of Vertebrate Palaeontology* **13**, 483–515.

Manuscript received 8 October 2001; accepted 13 February 2002