Chapter 2

The *Micaelamys namaquensis* (Rodentia: Muridae) species complex from southern Africa: Patterns of mitochondrial DNA versus morphological diversity

Abstract

Intraspecific variation has been determined in only a few southern African small mammals and research on the diverse rodent fauna of the subregion is especially limited. Here we report the genetic differentiation among populations of the Namaqua rock mouse, *Micaelamys namaquensis* Smith, 1834 a murine rodent from southern Africa, based on mitochondrial DNA (mtDNA) cytochrome *b* (cyt *b*) sequences. Phylogenetic analyses revealed 14 genetically distinct lineages of which several show strong geographic association with particular vegetation biomes or bioregions. The diversity and differentiation detected within *M. namaquensis* in the present study are much more complex than suggested by previous morphological assessments and strongly support earlier views that this taxon represents a species complex. Divergence times between lineages varied between 7.26 MYA and 2.70 MYA, suggesting a Late Miocene to Pliocene radiation. The identification of several regions of sympatry of distinct lineages offers future opportunities for the elucidation of the underlying speciation processes in the species complex. *Micaelamys namaquensis* lineages show contrasting patterns of phylogeographic structure but the majority of the diversification events date to the Late Miocene and Early Pliocene which coincide with major periods of aridification in southern Africa.

1. Introduction

Small mammal biodiversity in the southern African subregion is underestimated. Several recent studies provided evidence of cryptic species and species complexes (Jacobs et al., 2006; Lecompte et al., 2005; Smit et al., 2007). Few southern African species have been assessed for intraspecific variation and this is especially noteworthy for the local rodents, one of the most diverse groups of small mammals in the region (Lecompte et al., 2008). Most of the earlier studies were based on traditional morphometrics and/or qualitative morphology which include studies on species within the genera *Acomys* (Dippenaar and Rautenbach, 1986), *Saccostomus* (Ellison et al., 1993), *Otomys* (Taylor and Meester, 1993), *Aethomys* Thomas, 1915, (Chimimba et al., 1998; Chimimba 2000; Chimimba, 2001b) and *Micaelamys* Ellerman, 1941, (formerly designated to the genus *Aethomys*; Chimimba, 2001a; Chimimba and Bennett, 2005). Recently, however, the focus has shifted to a more molecular-based approach; examples include *Pedetes* (Matthee and Robinson, 1997), *Otomys* (Maree, 2002), *Micaelamys* (Russo, 2003), *Rhabdomys* (Rambau et al., 2003), *Cryptomys* (Ingrim et al., 2004), *Xerus* (Herron et al., 2005) *Aethomys* (Russo et al., 2006) and *Saccostomus* (Maputla, 2007). The molecular based studies reveal higher levels of intraspecific diversity and thus may be more sensitive than the more traditional approaches. These higher levels of diversity may be indicative of a species within a complex that may belong to sibling species categories (Meester et al., 1986; Russo et al., 2006; Taylor, 2000). Small mammals would be greatly affected by environmental variables and potential changes thereof, such as those seen across the African continent.

African climatic and vegetation changes have had a major impact on the evolutionary history of the African mammalian fauna (deMenocal, 2004). Large scale shifts in climate have altered the ecological composition of the landscape (deMenocal, 2004), reflecting a long history of responses to habitat changes (Riddle, 1996). In this respect, recent phylogenetic studies highlight the impact of aridification and vegetation changes on the diversification of African murine rodents (e.g., Lecompte et al., 2008). In general, small mammals such as rodents have restricted dispersal abilities (Fedorov et al., 2008; Lidicker, 1975; Mares and Lacher, 1987) and many display patchy distributions. Smaller rodents, in particular, show adaptation to specific micro-habitats and would likely be more sensitive to environmental changes (Fedorov et al., 2008, Nicolas et al., 2008). Indeed, habitat selection and inter-specific competition are proposed to be amongst the most important factors influencing the co-existence of species (Mares and Lacher, 1987; Ricklefs and Schluter, 1993). In addition, studies involving several rodent species implicate karyotypic changes in speciation (*Otomys*: Taylor et al., 2009 and *Rhabdomys*: Rambau et al., 2003).

The focus of the present study is the Namaqua rock mouse, *Micaelamys namaquensis* Smith, 1834, originally described from the Northern Cape Province (Witwater, Little Namaqualand) of South Africa (Shortridge, 1942). The genus *Aethomys* Thomas, 1915 includes a radiation of endemic African murid rodents and was formerly subdivided into two subgenera, *Micaelamys* and *Aethomys* (Chimimba and Bennett, 2005). Recent molecular studies (Castiglia et al., 2003; Ducroz et al., 2001; Russo, 2003) reported the paraphyly of the genus and the two subgenera have since been elevated to full generic rank. The genus *Micaelamys* Ellerman, 1941, includes *M. namaquensis* and *M. granti* Wroughton, 1908, while *Aethomys* includes the remaining nine species. The close relationship between *M. namaquensis* and *M. granti* has been documented in other studies using dental morphology (Ellerman et al., 1953), karyology (Visser and Robinson, 1986), gross sperm and bacular morphology (Visser and Robinson, 1986) and phenetic analysis (Chimimba et al., 1999).

Fossil species of *Aethomys* have been recorded from Langebaanweg (Western Cape Province, South Africa; Pocock, 1987). It is noteworthy that the smaller of the two fossil species closely resembles the extant *M. namaquensis*, which may be indicative of the long term presence of this species in the region (Pocock, 1987). The oldest known representatives of the genus, *A. adamanticola* and *A. modernis*, were recently recorded from South Africa and date to between the Late Miocene and Early Pleistocene (Denys, 1990a, b).

Micaelamys namaquensis is widely distributed in southern Africa (south of the Zambezi/Cunene Rivers), but has also been recorded to the north of the subregion in Angola, Malawi and northern Mozambique (Chimimba and Bennett, 2005). They are catholic in their habitat requirements, but where there are rocky outcrops or hillsides, they will use these in preference to any other type of habitat (Chimimba and Bennett, 2005). The species is nocturnal, communal, terrestrial and to some extent arboreal. Colonies live in rock crevices, in or under fallen logs or in holes in trees, collecting grass stems and other debris to form huge piles over the entrance to their shelters (Chimimba and Bennett, 2005). They mostly feed on the seeds of grass and other plants with a small percentage of their diet consisting of insects (Chimimba and Bennett, 2005). Gravid females are normally recorded from September to May with a peak in March/April (Smithers, 1971). No signs of breeding have been observed during the colder winter months. They are known for unstable population cycles associated with high mortality and high reproductive potential (Withers et al., 1980).

The Namaqua rock mouse shows considerable geographic variation as seen in pelage colouration, tail length, and body size throughout its distributional range (De Graaff, 1981; Musser and Carleton, 2005; Smithers, 1971). This variation suggested that *M. namaquensis* may reflect either a complex of species (Musser and Carleton, 2005) or subspecies (De Graaff, 1981; Smithers, 1971). Earlier reports (e.g. Meester et al., 1964; Roberts, 1951) recognised 16 subspecies within *M. namaquensis* (Fig. 2.1). However, these distinctions were primarily based on a limited number of geographically restricted samples (Smithers, 1971; Smithers and Wilson, 1979), with little or no assessment of patterns of geographic variation over the entire distributional range of the species.

Prior to an intraspecific morphometric study within *M. namaquensis* from southern Africa (Chimimba, 2001a), the nature and extent of geographic variation within the species remained virtually unknown. The morphometric study based on a more comprehensive geographical coverage of the species' distribution, suggested the recognition of four subspecies, namely *M. n. namaquensis* Smith, 1834; *M. n. lehocla* Smith, 1836; *M. n. alborarius* Peters, 1852 and *M. n. monticularis* Jameson, 1909 which differed in both cranial size and shape (see inset Fig. 2.1). This study also suggested that the geographical limits of the proposed subspecies broadly coincide with the major phytogeographical zones of southern Africa (Low and Rebelo, 1996; Fig. 2.1).

The subspecies *namaquensis* was shown to be largely associated with a combination of the Succulent Karoo, Fynbos and the southern coastal Savanna/Grassland region of the Eastern Cape, KwaZulu-Natal and eastern Mpumalanga Provinces of South Africa, while the subspecies *alborarius* and *lehocla* were shown to be associated with the Savanna and Upper/Lower Karoo biomes, respectively (Chimimba, 2001a). The subspecies *monticularis* was largely confined to the Grassland biome of southern Africa (Chimimba, 2001a).

Our initial assessment of mitochondrial DNA cyt *b* differentiation among 16 localities (Russo, 2003) confirmed that the species is polytypic but revealed higher levels of variation than previously detected by morphometrics, qualitative cranial and external morphology. The molecular study showed some support for three of the four morphometrically-defined subspecies (Chimimba, 2001a): 1) a lineage widely distributed across the Upper/Lower Karoo; 2) a lineage found across the grasslands of the North-West, Limpopo, Free State and Mpumalanga Provinces; and 3) a lineage found in the Limpopo valley and Botswana corresponding to the Savanna biome of southern Africa. However, several additional unique and well-supported lineages defined by the molecular data were not concordant with the morphometrically-defined subspecies (Chimimba, 2001a). In addition, while some lineages showed considerable molecular sequence variation across the geographic area sampled, other lineages showed very little differentiation. Uncorrected HKY85 sequence divergence values between 37 maternal haplotypes identified ranged between 0.18% and 6.22%. These results lend support to earlier suggestions for the presence of a species complex within *M. namaquensis* from southern Africa.

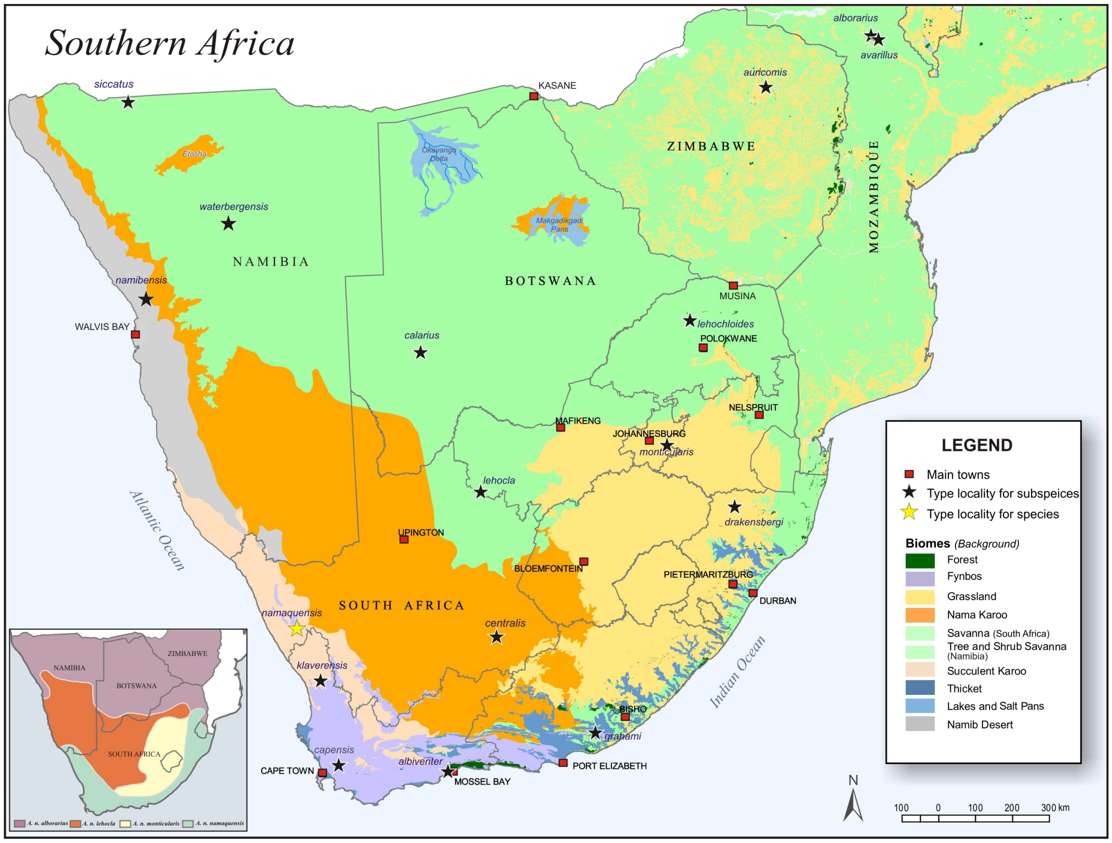

Figure 2.1 Major biomes of southern Africa (Low and Rebelo, 1996). Background colours for the eight major biomes in southern Africa are indicated in the legend; major lakes and saltpans are also included. The yellow star indicates the type locality for the species *Micaelamys namaquensis* and the blue stars indicate the type localities for the 16 previously described subspecies. The inset shows the geographic distribution of the phenetic diversity within *Micaelamys namaquensis* from southern Africa (after Chimimba, 2001a). The purple, orange, yellow and green zones correspond with *M. n. alborarius*, *A. n. lehocla*, *A. n. monticularis* and *A. n. namaquensis*, respectively.

In the present study we extend our earlier research (Russo, 2003) through an analysis of mtDNA variation within *M. namaquensis* over an extensive geographic scope in southern Africa and address the following specific questions: 1) What is the nature and extent of mtDNA variation within *M. namaquensis* over a much broader geographic area in southern Africa than previously covered, and with reference to previously proposed subspecies?; 2) Is there any phylogeographic structuring within some of the lineages within *M. namaquensis*?; and 3) What are the ages of the different lineages identified within the species?

2. Materials and Methods

2.1 Study area and sampling

Samples (*N* = 360) representative of the four previously proposed morphometrically-defined subspecies (Chimimba, 2001a), from 95 localities spanning the four major phytogeographical zones in southern Africa, were collected from South Africa, Namibia, Swaziland and Botswana (Fig. 2.2; Appendix 2.1). Only six of the originally described subspecies (Fig. 2.1: *waterbergensis* Roberts, 1938, *calarius* Thomas, 1926, *siccatus* Thomas, 1926, *auricornis* De Winton, 1897, *alborarius* Peters, 1852 and *avarillus*, Thomas and Wroughton, 1908) from northern Namibia, western Botswana, Zimbabwe and Mozambique, were not sampled (Figs. 2.1 and 2.2). Animals were live-trapped using Sherman traps (H.B. Sherman Traps Inc. Florida, U.S.A.) and handled under the guidelines of the American Society of Mammalogists (ASM; Animal Care and Use Committee, 1998; (http://www.mammalogy.org/committees/index.asp) as approved by the Animal Ethics Committee of the University of Pretoria (Project number: EC 010417-004). Animals were collected under the permit numbers as indicated in Appendix 2.2. Some animals were sacrificed by halothane inhalation, and ear clips from the rest were either frozen at -20°C, stored in 70% EtOH, or in Tissue/Blood Storage Buffer (100 mM Tris, 40 mM EDTA, 1 M NaCl and 0.5 % SDS). Voucher specimens were prepared using standard natural history museum procedures for mammals and were deposited in the mammal reference collection of the Transvaal Museum (TM) of the Northern Flagship Institution, Pretoria, South Africa.

2.2 DNA extraction, Polymerase Chain Reaction (PCR) amplification and sequencing

Total genomic DNA was extracted from tissue using either a standard phenol/chloroform protocol (Sambrook et al., 1989) or a Sigma GenElute™ Mammalian Genomic DNA Miniprep Kit (Sigma Aldrich). Some problem samples were extracted using a Genomic DNA Mini Kit I Multi (Animal/Plant) (Koma Biotech Inc.).

A mouse-specific primer (H15309) was designed in the tRNA-Thr from a *Mus musculus* sequence (GenBank J01420; Bibb et al., 1981). This primer is a *M. musculus* version of H15915 (Irwin et al., 1991) and was used in combination with the shortened universal vertebrate primer L14724 (Pääbo et al., 1988) that anneals in tRNA-Glu, to amplify the cyt *b* gene of two *M. namaquensis* individuals. An *Aethomys chrysophilus* De Winton, 1897, sequence (GenBank AF004587; Ducroz et al., 1998), together with these *M. namaquensis* sequences were aligned in Clustal X (Thompson et al., 1997) and used to design an internal species-specific *M. namaquensis* primer (H14769, 5' GTCTGCGTCTGAATTTAG 3'). H14769 was used in combination with the shortened L14724, or L14841 of Kocher et al. (1989), to amplify the 5' end of the cyt *b* gene for all individuals in our study. A preliminary analysis showed that the 5' end of the cyt *b* gene yielded considerable levels of variation within *M. namaquensis* and a 631 bp region was used for subsequent analyses.

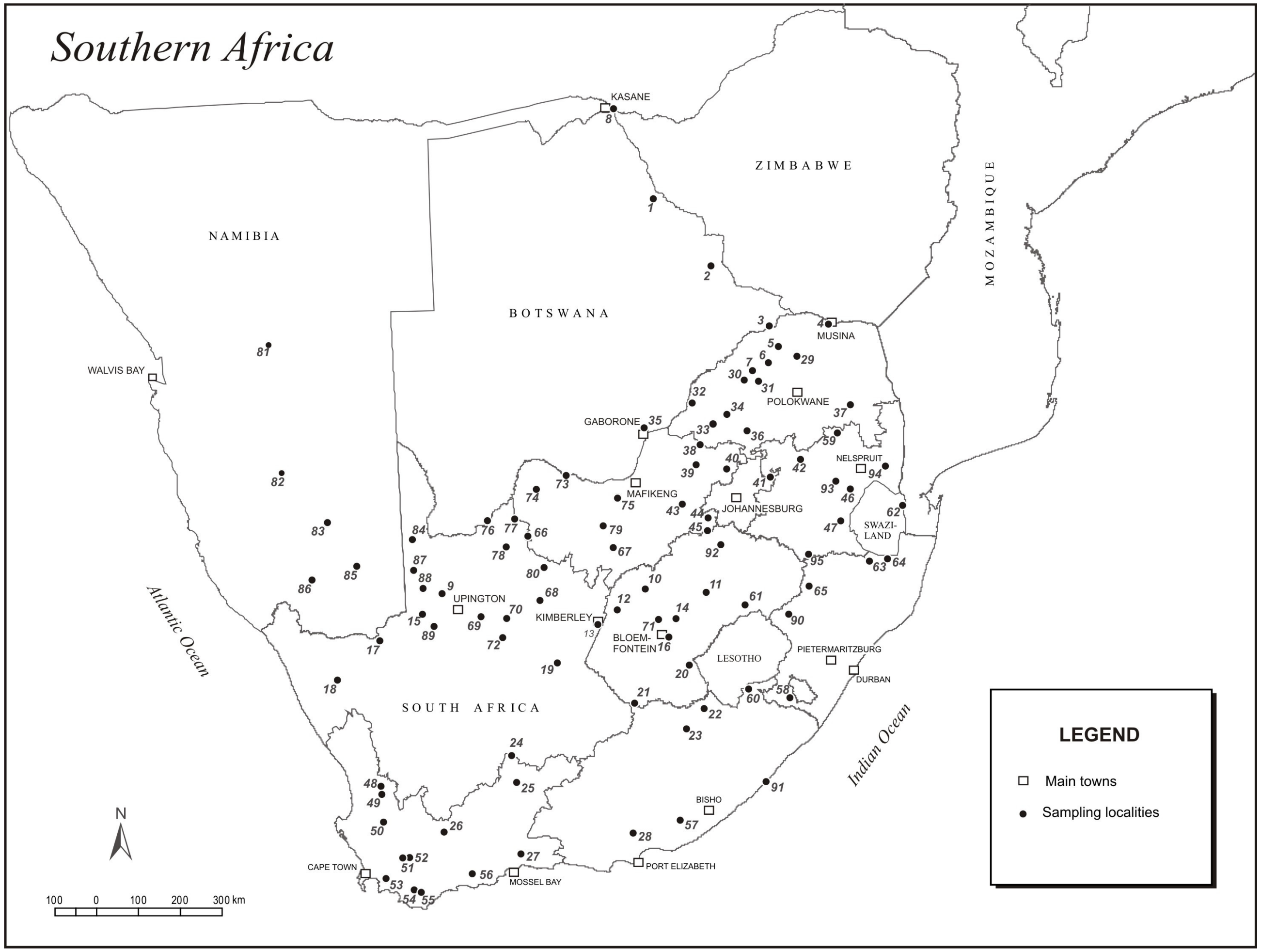

Figure 2.2 Collecting localities of samples of *Micaelamys namaquensis* from southern Africa. Numbers correspond to the locality numbers and names in Appendix 2.1.

Polymerase chain reactions (PCR; Saiki et al., 1988) were performed in a total volume of either 50 µl or 25 µl. Reactions contained approximately 50 - 100 ng genomic DNA template, 1 x buffer, 2.5 mM $MgCl_2$, 0.2 mM of each of the four nucleotides (Promega), 2.5 - 5 picamol of each primer and 0.15 U of Super-Therm® DNA polymerase (Southern Cross Biotechnology). PCR conditions were as follow: denaturing at 94° C for 5 min, 35 cycles of the following: 94° C for 30 seconds, primer annealing at 52° C for 30 seconds and elongation at 72° C for 45 seconds. This was followed by an extended elongation step for 7 min at 72° C in a Geneamp® PCR System 9700 (Applied Biosystems). The PCR products were purified using the High Pure™ PCR Product Purification Kit (Roche Diagnostics). Dye-terminator cycle sequencing was performed for both the light and heavy strands using the ABI PRISM Big Dye™ Terminator, version 3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystems). Cycle sequencing products were subsequently precipitated using a NaAc salt method (Applied Biosystems). Nucleotide sequences were determined using an ABI 3130 automated sequencer (Applied Biosystems).

2.3 *Sequencing analysis*

The quality of the raw sequence data was evaluated in either Sequencing Analysis, version 3 (Applied Biosystems) or BioEdit (Ibis Biosciences), and a consensus sequence for each individual from forward and reverse sequences was determined in either Sequence Navigator, version 1.01 (Applied Biosystems) or Vector NTI Advance 10 (Invitrogen). All sequences were deposited in GenBank under accession numbers GQ471959 to GQ472095. These accessions represent all unique haplotypes identified in the present study, including geographical information. Consensus sequences of all individuals were aligned in Clustal X (Thompson et al., 1997), followed by phylogeographic and phylogenetic analyses.

2.4 *Phylogeographic analyses*

The minimum number of mutational steps between *M. namaquensis* haplotypes was determined from a distance matrix using MINSPNET (Excoffier and Smouse, 1994) and TCS, version 1.21 (Clement et al., 2000). Frequencies and geographic distributions of different haplotypes were used to depict geographical and potential ancestor-descendant relationships among identified haplotype sequences. To illustrate contrasting patterns of intra-clade structuring, TCS, version 1.21 and MINSPNET were also used to produce minimum-spanning networks for the Nama-Karoo and Grassland groups, respectively.

Genetic diversity was estimated for the different groups as identified in the minimum-spanning network. Diversity indices such as haplotype diversity (Nei and Tajima, 1981) and nucleotide diversity, π (Nei, 1987) were calculated for the whole sample excluding alignment gaps or missing data using DnaSP, version 4.10.9 (Rozas et al., 2003).

A Mantel test as implemented in Mantel Nonparametric Test Calculator, version 2.0 (Mantel, 1967) was used to test for isolation by distance within identified clades. The test uses a permutation procedure (1 000 permutations) to determine the significance of the correlation between genetic versus geographic distances. Some of the groups could not be subjected to the Mantel test, as too few individuals were sampled.

2.5 Phylogenetic analyses

The selection of possible outgroups for phylogenetic analyses within *M. namaquensis* was problematic due to several proposed hypotheses of evolutionary relationships between *Micaelamys* and other murids (Chimimba, 2005 and references therein, Lecompte et al., 2008). In a preliminary analysis *Aethomys chrysophilus*, *A. ineptus* Thomas and Wroughton, 1908, *Parotomys brantsi*, *Dasymys incomtus*, *Rattus rattus*, *M. musculus*, *Rhabdomys pumilio* and *Arvicanthis somalicus* were used as outgroups. The two *Aethomys* species that were previously treated as congeneric with species within *Micaelamys* (Chimimba, 2005; Chimimba et al., 1999) and recently shown to be sister taxa to *Micaelamys* (Russo, 2003), were selected as outgroups in further analyses. Sequences from the other species were too distant when compared to the ingroup taxa. In contrast, *R. rattus* and *M. musculus* were used in the BEAST analysis as outgroups in the estimation of rates of evolution and dates of divergence between different lineages, since a divergence date for these species estimated at 12 MYA based on fossil records was available (see section on molecular clocks below).

A likelihood ratio test as implemented in Modeltest, version 3.06 (Posada and Crandall, 1998) was used to determine the best-fit model of DNA substitution for the 631 bp cyt *b* sequences under the Akaike Information Criterion (AIC). Parameters such as base frequencies, the shape parameter of the gamma distribution of rates among sites (Yang, 1996; Yang et al., 1994) and the proportion of invariable sites (I) were also estimated. The chosen model based on only 43 sequences (a subset of individuals representing the diversity within *M. namaquensis*) was subsequently used in maximum likelihood (ML; Felsenstein, 1973, 1981) and Bayesian Inference (Ronquist and Huelsenbeck, 2003) phylogenetic analyses. ML as implemented in PAUP, version 4.0b10 (Swofford, 2003) was conducted using 100 random addition replicates and was based on a heuristic search using the tree bisection-reconnection (TBR) option. Support values for internal nodes were determined using bootstrap analysis (Felsenstein, 1985) with 1 000 iterations performed on a computer cluster. The Bayesian analysis was conducted using MRBAYES, version 3.1.2 (Ronquist and Huelsenbeck, 2003). Four chains were run for 5×10^6 generations using random starting trees and flat priors. Trees and parameters were recorded every 100th generation. Two runs were performed simultaneously and split frequencies were compared every 100th generation to ensure convergence of the runs. All runs used the default heating and swap parameters. The first 5 000 generations (10%) were excluded as the burn-in. A 10% burn-in was sufficient to ensure that trees were only sampled from the region of stationarity.

2.6 Molecular dating

The use of molecular clocks is extensively debated (Bandelt, 2008; Bromham and Penny, 2003; Graur and Martin, 2004; Hedges and Kumar, 2003; Ho and Larsen, 2006; Howell and Howell, 2008). We acknowledge the uncertainties surrounding calibration points and estimated times of divergence, however, our aim was to obtain tentative estimates for the timing of key events in the diversification of *M. namaquensis* lineages. To estimate a rate of evolution and dates of divergence between these lineages, a log-normal relaxed-clock analysis was performed as implemented in BEAST, version 1.4.7 (Drummond and Rambaut, 2007). This analysis was performed on the same subset of individuals ($N = 43$) that were used in the ML and Bayesian analyses. A specific rate of change calibrated for murid rodents was determined since murid mtDNA has been shown to evolve at a faster rate than other rodents (Catzeflis et al., 1992). As a calibration point, sequence data from *R. rattus* and *M. musculus*, with a divergence date estimated at 12 MYA based on fossil records, was used (Jacobs and Downs, 1994). In contrast, other divergence dates have been suggested for *Rattus* and *Mus* (Adkins et al., 2001; Kumar and Hedges, 1998; Smith and Patton, 1999). For example, divergence dates of 10 MYA (Smith and Patton, 1999), 23 MYA (Adkins et al., 2001) and 41 MYA (Kumar and Hedges, 1998) have been suggested. The divergence date of 12 MYA was followed since it is based on the fossil record and this dating also provided a rodent-specific calibration. The use of a non-rodent divergence date as a calibration point results in divergence times much older than the paleontological record (Kumar and Hedges, 1998).

The best-fit model was determined to be the General-Time-Reversible (GTR) model of substitution with a gamma correction (Gu and Zhang, 1997), and a proportion of invariable sites (GTR + Γ + I). Posterior distributions of parameters were approximated by Monte Carlo Markov Chain (MCMC; Drummond et al., 2002) sampling, with samples drawn every 1 000th iteration over a total of 20×10^6 generations, excluding the first 4000 generations as the burn-in (Drummond and Rambaut, 2007). Three independent analyses were run and the results were combined using LogCombiner, version 1.4.7 (Drummond and Rambaut, 2007). Mixing and convergence to the stationary distribution were evaluated and the Bayesian skyline plot (Drummond et al., 2005) was calculated using Tracer, version 1.4 (Rambaut and Drummond, 2007). Posterior estimates for rate and divergence date estimates were similar between runs. The final tree created from the three independent runs was viewed in FigTree, version 1.2.2 (Drummond and Rambaut, 2007).

3. Results

3.1 Sequence statistics

The 5’ end of the cyt *b* gene (631 bp) was sequenced for 360 *M. namaquensis* individuals. All sequences obtained were of the mitochondrial cyt *b* gene with no stop codons found and the nucleotide sequences corresponded to the expected 210 amino acids (Esposti et al., 1993). This was also confirmed by a BLAST search (results not shown). The four nucleotides did not occur in equal frequencies and base composition was similar to that of other previously reported mammalian cyt *b* sequences. The strong bias in base composition showed a marked under-representation of guanine at second (15.7%) and especially third (2.7%) codon positions. There was a higher representation of adenine at third (45.3%) and thymine at second (41.2%) codon positions. In addition, first and second codon positions showed less variability than third codon positions (Irwin et al., 1991; Martin et al., 2000). Consequently, most of the substitutions were silent, with only 36 recorded variable amino acid sites.

3.2 Phylogeographic analyses

The 176 variable positions defined 137 maternal haplotypes (Appendix 2.3 and 2.4). Haplotypes NH112, NH014, NH114 and NH100 were the most widespread, being recorded from eight (N = 36), seven (N = 34), six (N = 24) and four (N = 21) localities, respectively. Most of the other haplotypes were locality-specific. All 360 sequences were considered as one population and the overall nucleotide diversity was estimated at 2.99% (SD = 0.01), while the haplotype diversity value of 0.91 (SD = 0.01) corresponded to those reported for other rodents.

The latter was confirmed by a minimum-spanning network of all individuals which showed 14 distinct *M. namaquensis* haplogroups/clades (lineages A-M); individual haplotypes connected with between one and 13 mutational steps (network not shown). The relationships between the clades were assessed using phylogenetic analyses (see section 3.3). The minimum-spanning networks of two of these groups (Nama-Karoo, Fig. 2.3A and Grassland, Fig. 2.3B) are illustrated separately to emphasise the different patterns observed within these groups. A 95% confidence connection limit of 10 steps was obtained when allele networks were drawn in TCS. The Nama-Karoo group was characterised by 31 closely related haplotypes over a wide geographical area, with haplotypes differing by one to 13 mutational steps (Fig. 2.3A). Seven of the haplotypes were each shared among 2 - 6 localities For example, haplotype NH014 was shared between six localities covering a geographical distance of more than a 1 000 km. In contrast, more differentiation was detected within the Grassland group where up to 28 mutational steps were detected between the most divergent haplotypes, indicating fine-scale population structuring (Fig. 2.3B). Six haplotypes were each shared among localities over large geographic distances. The Grassland biome appears to comprise at least six distinct sub-clades and the relationship between these need future in-depth phylogeographic and population genetic investigation.

A Mantel nonparametric test revealed no isolation by distance within any of the tested clades. In all analyses, the standard normal variate (g) was smaller than the critical value of 2.575 at $P \leq 0.005$ (Table 2.1), indicating that the null-hypothesis (no association between elements in the two matrices) could not be rejected. A Mantel test was only performed on eight of the 14 lineages that were identified since some lineages were represented by too few individuals.

Table 2.1 Mantel test results (mitochondrial DNA (mtDNA) cytochrome *b* (cyt *b*)) for the different groups/lineages within *Micaelamys namaquensis* from southern Africa as defined by a minimum-spanning network and phylogenetic analyses. In all analyses, the standard normal variate (*g*) was smaller than the critical value of 2.575. See Figs. 2.4 and 2.5 for the genetic and geographic distinction of the lineages.

GROUPS/LINEAGES AS DEFINED BY A MINIMUM-SPANNING NETWORK AND PHYLOGENETIC ANALYSES	**STANDARD NORMAL VARIATE (*G*)**	**CORRELATION COEFFICIENT (R)**	***P*-VALUE**
Savanna Biome (lineage N)	-0.4748	-0.1074	0.18
Nama-Karoo Biome (lineage J)	-0.0548	-0.0081	0.40
Grassland Biome (lineage B)	-0.0782	0.0077	0.46
Fynbos Biome (lineage H)	1.3640	0.3076	0.21
Lowveld Bioregion (lineage D)	0.2562	0.1451	0.43
Bushmanland/Upper Karoo Bioregion (lineage I)	-0.4188	-0.0715	0.40
Eastern Kalahari Bushveld Bioregion (lineage M)	-1.1448	-0.2029	0.14
Kalahari Duneveld Bioregion (lineage K)	-0.2154	-0.0465	0.48

3.3 Phylogenetic analyses

A subset of 43 haplotypes representing the full spectrum of *M. namaquensis* diversity (most divergent individuals) was used in all phylogenetic analyses. The best-fit Tamura-Nei (TrN+ Γ (1.55) +I (0.60)) model of substitution was applied in all phylogenetic analyses excluding the BEAST analysis where the GTR+ Γ + I (General-Time-Reversible) model of evolution with a gamma shape parameter of 2.4 and the proportion of invariable sites as 0.70 was used. This model was used in the BEAST analysis since a HKY or a GTR model of substitution are the only defined models in BEAST.

The ML phylogram (Fig. 2.4) depicts the relationships between the 14 lineages and is characterised by short internal branches that have also been reported for other rodent species. Eleven well-supported phylogenetic lineages (Fig. 2.4) with clear geographical patterns were identified, of which most were associated with different vegetation types in southern Africa (Fig. 2.5): 1) B was associated with Grassland; 2) D with the Lowveld bioregion; 3) G with Albany Thicket; 4) H with the western Fynbos; 5) I with the Bushmanland/Upper Karoo bioregion; 6) J with the Nama-Karoo; 7) K with the Kalahari Duneveld; 8) L with the Sub-Escarpment Grassland bioregion; and 9) M with the Eastern Kalahari Bushveld. In contrast, none of the localities from the two remaining well-supported lineages (lineages C and E) were associated with any specific biome/bioregion (vegetation type). Lineage C represented individuals from Machadodorp and Malelane in the Mpumalanga Province of South Africa, while lineage E included individuals from Fouriesburg (Free State Province) and Kasane (Botswana) which are approximately 1 000 km apart. While lineage N appeared to be associated with the Savanna, this node had no bootstrap/Bayesian Inference support (Fig. 2.4). Similarly, lineages A and F were not well-supported and were only recorded from single localities that included Koppies Dam Nature Reserve (Free State Province) and Volksrust (Mpumalanga Province), respectively.

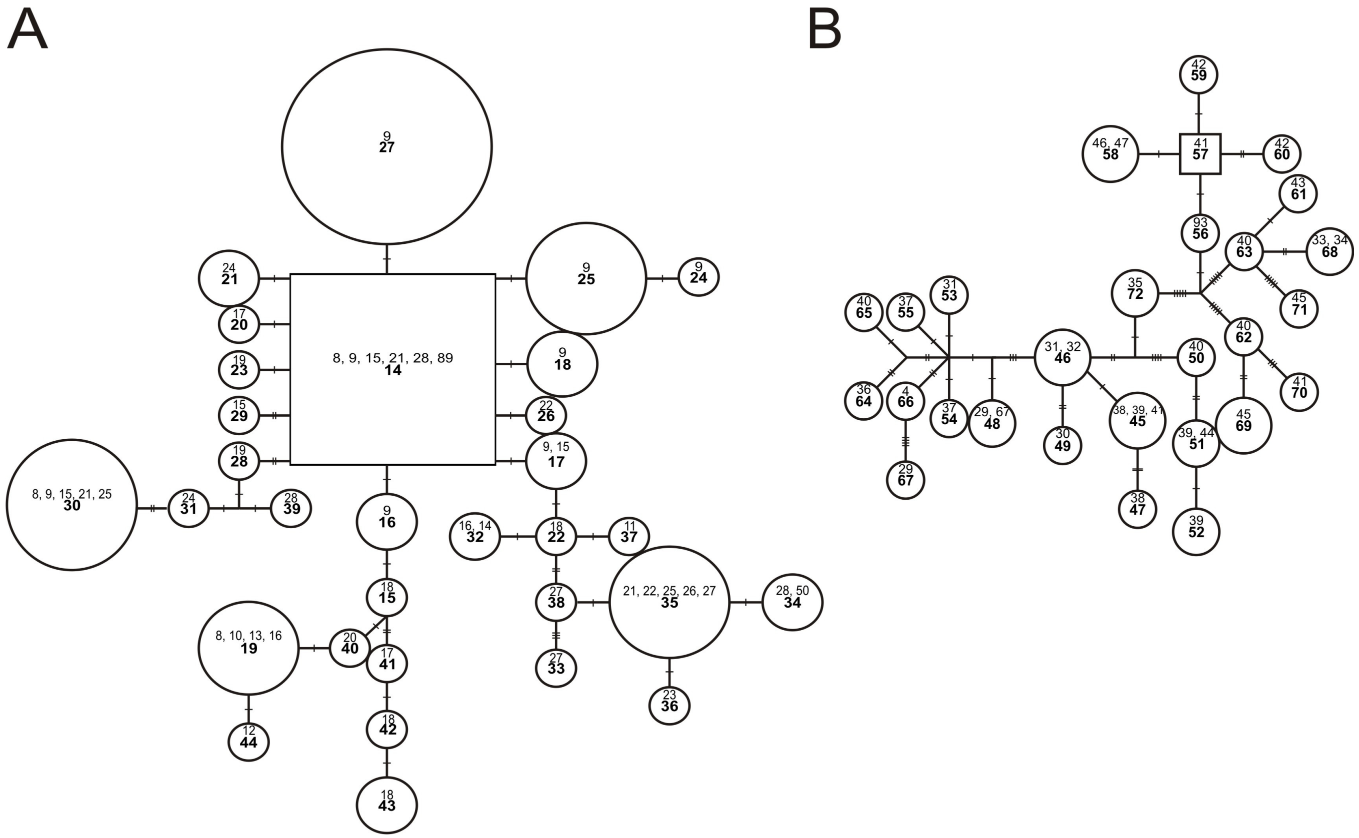

Figure 2.3 Minimum-spanning networks indicating the least number of mutational steps between composite mitochondrial DNA (mtDNA) cytochrome *b* (cyt *b*) haplotypes within two *Micaelamys namaquensis* clades from southern Africa. Sizes of the circles and squares represent haplotype frequencies while cross-hatching along branches designates the number of detected changes (TCS analysis connected all haplotypes at the 95% confidence limit). Numbers in bold inside circles represent haplotype designations (Appendix 2.3 and 2.4) and numbers not in bold correspond to locality numbers (Appendix 2.1). This corresponds to the information in Appendices 2.3 and 2.4. Squares represent potential ancestral haplotypes as identified by the TCS analysis. (A) Nama-Karoo (lineage J, Fig. 2.4) and (B) Grassland (lineage B, Fig. 2.4) group.

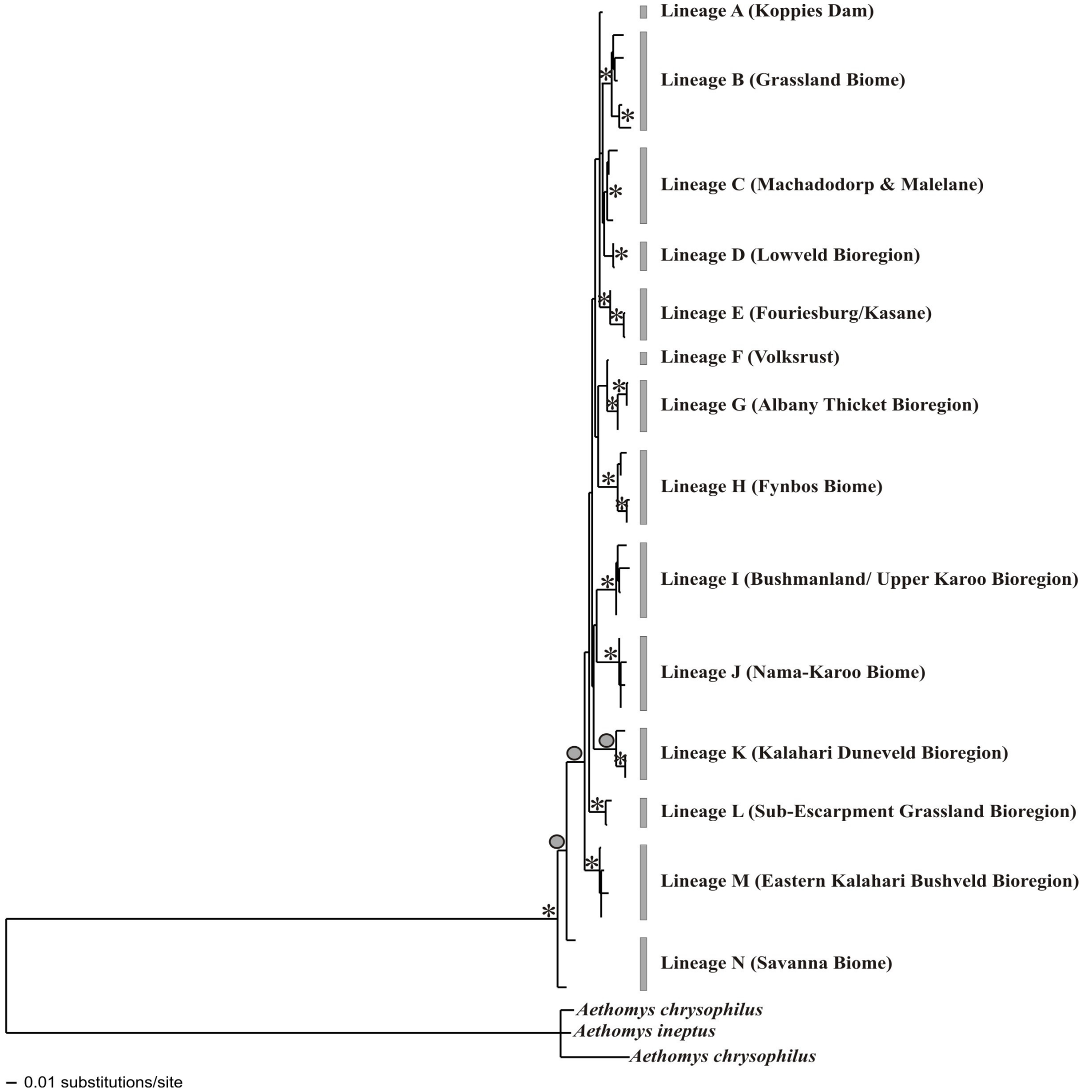

Figure 2.4 A maximum likelihood (ML) phylogram based on 631 bp of the mitochondrial DNA (mtDNA) cytochrome *b* (cyt *b*) gene of *Micaelamys namaquensis* from southern Africa. Bootstrap confidence limits (1 000 replicates) and Bayesian posterior probability values for internal nodes are given at each node with either an asterisk (*) or a circle (•). Circles indicate bootstrap support values above 70% and asterisks indicate bootstrap support above 70% as well as Bayesian posterior probability values of ≥ 0.95. *Aethomys chrysophilus* and *A. ineptus* were used as outgroups. Lineages A - N mainly correspond to different biomes or bioregions of southern Africa (Fig. 2.5) indicated in parentheses (see text for some minor exceptions).

Most lineages appear to form separate geographical units displaying an allopatric/parapatric pattern of distribution. In contrast, several of the lineages were found in sympatry with others (Fig. 2.5), for example, lineages J, I and B were recorded with several other lineages. Lineages E and K exhibit disjunctive geographical distributions and future research should distinguish between historical gene flow or the retention of ancestral polymorphisms as the potential underlying process generating these patterns.

Figure 2.5 further allows a comparison between the geographic distribution of previously recognised subspecies and the distribution of mtDNA cyt *b* diversity. Four of the well-supported mtDNA lineages (lineages B, H, J and N) broadly correspond with the biome-related distributional patterns of the subspecies *monticularis*, *namaquensis*, *lehocla* and *alborarius*, respectively proposed by Chimimba (2001a) (see also inset Fig. 2.1). However, there appears to be a better correspondence to the formerly described subspecies (Meester et al., 1964; Roberts, 1951; see Fig. 2.1). The type localities of eight of the ten sampled subspecies were located within the geographic regions of the mtDNA lineages (Fig. 2.5): *namibensis* Roberts, 1946, in the Kalahari Duneveld (K), *lehocla* in the Eastern Kalahari Bushveld (M), *centralis* Schwann, 1906, in the Nama-Karoo (J), *capensis* Roberts, 1926, in the western Fynbos (H), *grahami* Roberts, 1915, in the Albany Thicket (G), *monticularis* in the Grassland (B), *drakensbergi* Roberts, 1926, in the Lowveld (D) and *lehochloides* Roberts, 1926, in the Savanna (N). The affinities of *albiventer* Jentinck, 1910, and *klaverensis* Roberts, 1926, remain unclear.

The GTR + Γ + I (General-Time-Reversible) model was only used to determine sequence divergence values with a gamma correction of 1.3 and a value of 0.6 for the proportion of invariable sites. The GTR + Γ + I sequence divergence values between the 137 maternal haplotypes ranged from 0.16% to 11.06% (pairwise divergences not shown; see Table 2.2 for within and between lineage comparisons). Percentage nucleotide diversity for the different groups as identified in the minimum-spanning network and phylogenetic analyses is reported in Table 2.2. The suggested heterogeneity within lineage B (Fig. 2.3B) is mirrored by the high nucleotide diversity recorded for this lineage (1.44%). The highest sequence divergence value was recorded between haplotypes NH033 from the Nama-Karoo group (lineage J) and NH075 from the Fynbos group (lineage N). Uncorrected GTR sequence divergence values differed from 18.10% to 24.12% between the ingroup and outgroup taxa.

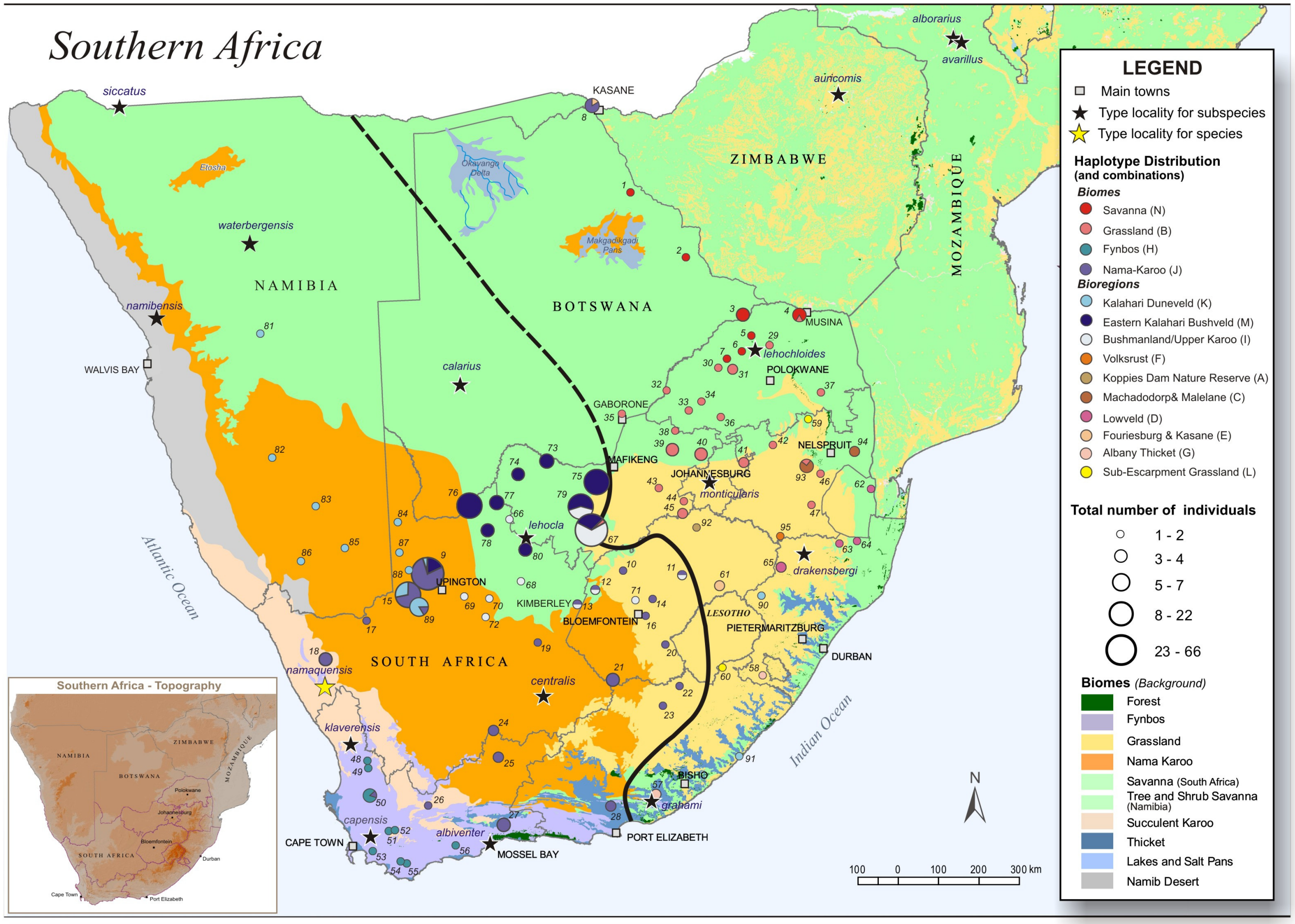

Figure 2.5 Geographic distribution of *Micaelamys namaquensis* mitochondrial DNA (mtDNA) cytochrome *b* (cyt *b*) lineages in the biomes/bioregions of southern Africa (see legend for colours). The sizes of circles indicate the number of individuals sampled at each locality (see legend for scale); the size of the pie charts represents haplotype frequencies. Stars indicate the type localities of the species/subspecies. A split between the arid (west) and mesic (east) region of South Africa is indicated by the solid black line; the 500 mm isohyet is projected for the rest of southern Africa, indicated by the dotted line. The inset shows a topographical map of southern Africa.

Figure 2.6 represents estimated times of divergence within and between *M. namaquensis* lineages. Within lineage divergences differed from 940 000 years (lineage G, Albany Thicket bioregion) to 3.42 MYA (lineage N, Savanna biome). The divergence time separating different lineages differed from 2.70 MYA (between lineages F and G) to 7.26 MYA (between lineages B, C and D). Time to the most recent common ancestor for *M. namaquensis* was estimated at 9.44 MYA. Two groups subsequently diverged: 1) a group more confined to the mesic (wetter) habitats of southern Africa (lineages A - H), and 2) a group found in the more arid (drier) habitats of southern Africa (lineages I - N). The major diversification within this species-group appears to have occurred during the Late Miocene to Early Pliocene, between 7.87 MYA and 5.30 MYA, resulting in the lack of resolution in the non-ultrametric phylogenetic analyses (Fig. 2.4).

Patterns of divergence revealed by minimum-spanning networks for the Grassland and the Nama-Karoo groups were also evident from the BEAST analysis. The divergence within lineage B (Grassland biome) was estimated to have occurred 1.93 MYA, compared to lineage J (Nama-Karoo biome) where the divergence time was estimated at 2.59 MYA. Despite this fairly recent radiation within lineage J, its origin lays much deeper (~6.5 MYA) within the arid *Micaelamys* radiation.

Table 2.2 Mitochondrial DNA (mtDNA) cytochrome *b* (cyt *b*) percent sequence divergence between and within groups/lineages as identified by a minimum-spanning network and phylogenetic analyses, as well as nucleotide diversity values for each group/lineage within *Micaelamys namaquensis* from southern Africa.

GROUPS/LINEAGES AS DEFINED BY A MINIMUM-SPANNING NETWORK AND PHYLOGENETIC ANALYSES	**GTR + Γ + I WITHIN SEQUENCE DIVERGENCE**	**GTR + Γ + I BETWEEN SEQUENCE DIVERGENCE**	**PERCENTAGE NUCLEOTIDE DIVERSITY**
Koppies Dam (lineage A)	–	1.15 – 5.32%	–
Grassland Biome (lineage B)	0.16 – 3.13 %	1.21 – 8.13 %	1.44
Macadodorp/Malelane (lineage C)	0.17 – 1.56%	0.92 – 6.56%	0.62
Lowveld Bioregion (lineage D)	0.18 – 1.29 %	0.92 – 6.51 %	0.46
Fouriesburg and Kasane (lineage E)	0.35 – 1.73 %	1.43 – 7.92 %	0.89
Volksrust (lineage F)	–	1.25 – 5.38%	–
Albany Thicket Bioregion (lineage G)	0.16 – 1.11 %	1.25 – 7.51 %	0.56
Fynbos Biome (lineage H)	0.16 – 3.70 %	2.61 – 11.06 %	0.73
Bushmanland/Upper Karoo Bioregion (lineage I)	0.16 – 2.25 %	2.61 – 8.23 %	0.57
Nama-Karoo Biome (lineage J)	0.16 – 1.92 %	2.74 – 11.06 %	0.37
Kalahari Duneveld Bioregion (lineage K)	0.16 – 3.33 %	2.42 – 9.53 %	0.99
Sub-Escarpment Grassland Bioregion (lineage L)	0.67%	2.19 – 6.57 %	0.45
Eastern Kalahari Bushveld Bioregion (lineage M)	0.16 – 1.28 %	2.29 – 8.81 %	0.35
Savanna Biome (lineage N)	0.17 – 2.30 %	3.45 – 8.77 %	0.63

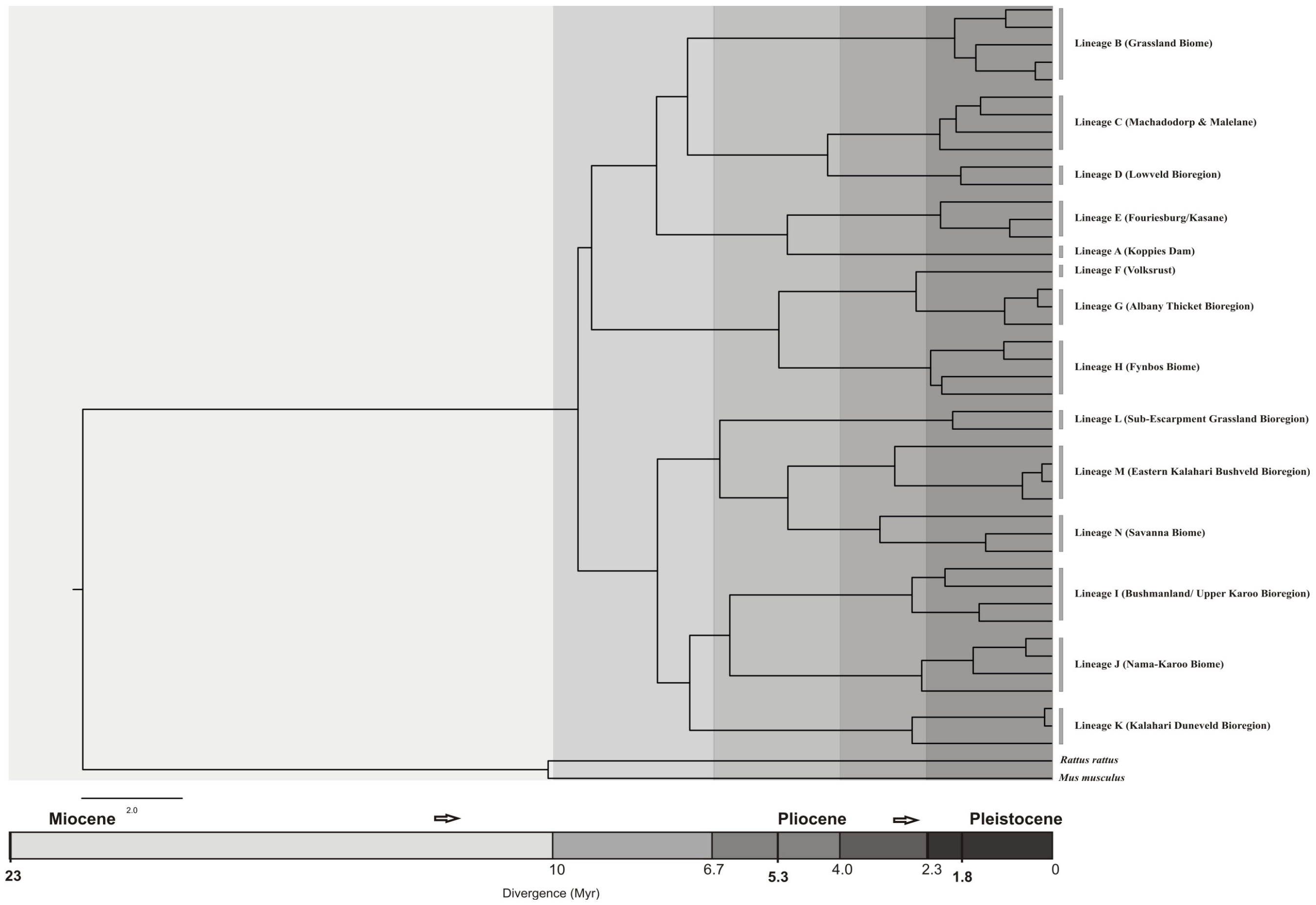

Figure 2.6 A phylogenetic tree for 43 representative mitochondrial DNA (mtDNA) cytochrome *b* (cyt *b*) haplotypes of *Micaelamys namaquensis* from southern Africa as obtained by BEAST analysis. Divergence dates (epochs) are indicated in the grey-scale key at the bottom of the figure. Lineages A - N mainly correspond to different biomes or bioregions of southern Africa (see also Figs. 2.4 and 2.5; see text for some minor exceptions).

4. Discussion

4.1 Diversification between M. namaquensis *lineages*

The analysis of a 631 bp fragment of the mtDNA cyt *b* gene in the present study supports previous suggestions that *M. namaquensis* from southern Africa is polytypic (Chimimba, 2001a; Meester et al., 1964; Roberts, 1951; Russo, 2003). The present analysis, however, reveal the presence of a much higher degree of variation than detected by a morphometric study that proposed the recognition of only four subspecies within *M. namaquensis* (Chimimba, 2001a). Of particular significance in the present study is the lack of resolution at the deeper nodes of the *M. namaquensis* phylogeny. The mtDNA cyt *b* gene alone is not informative enough at this level, and combined analyses with nuclear genes and morphological data will be required to fully resolve species-level diversity and the biogeographic history of this species complex.

The degree of differentiation between the lineages within *M. namaquensis* is comparable to those between other mammalian species (Castiglia et al., 2008; Colangelo et al., 2005; Ducroz et al., 1998; Yu et al., 2006). For example, Yu et al. (2006) reported sequence divergence values of 8.1 - 9.1% between two giant flying squirrel species (*Petaurista*). Of significance in the present study is that the GRT + Γ + I sequence divergence estimates between lineages of *M. namaquensis* ranged between 0.16 to 11.06%. The detection of partial overlap between intraspecific and interspecific divergences suggests the need of a combination of DNA sequences and other types of data (e.g., morphology, ecology and chromosomes). These patterns of variation are comparable to those of other within-population or within-species comparisons in muroid rodents such as those reported within Andean mice of the genus *Akodon* where sequence divergence values of up to 11.7% were detected (Patton and Smith, 1992). Likewise, sequence divergence values between 10% and 18% were also evident between *Mastomys* species (Dobigny et al., 2008; Lecompte et al., 2002, 2005).

In rodents, sequence divergence values of 4% have been reported to reflect inter-specific differences (Hayes and Harrison, 1992). It has also been suggested that sequence divergence values in the mtDNA cyt *b* gene above 11% may be indicative of valid species (Bradley and Baker, 2001). It is significant that some of the lineages identified in the present study have previously been proposed to be recognised as subspecies on morphometric grounds (Chimimba, 2001a). In contrast, a sequence divergence value of only 2.70% (range = 1.69% - 3.78%; corrected net within-species divergence (Nei and Li, 1979) = 1.64%) was detected between two sibling murid rodent species within the genus *Aethomys*, *A. chrysophilus* De Winton, 1897 and *A. ineptus* Thomas and Wroughton, 1908 (Russo et al., 2006).

Overall nucleotide diversity based on 360 individuals was estimated at 2.99%, while the haplotype diversity value of 0.91 corresponded to those reported for other rodents (Avise et al., 1989). This may reflect either the high incidence of locality-specific haplotypes or it may be indicative of a population bottleneck followed by rapid population growth (Grant and Bowen, 1998; Avise, 2000). In contrast, nucleotide diversity estimates of only 1.10% to 1.54% have been reported for a Patagonian rodent (Kim et al., 1998) and values of 0.33% to 1.45% for the Norwegian lemming (Fedorov and Stenseth, 2001). Similarly, nucleotide diversity estimates of 0.54% to 1.5% were reported for the Yellow-necked field mouse (Michaux et al., 2004). These lower values reported for nucleotide diversity may be indicative of the presence of shared haplotypes between localities and limited population structure (Fedorov and Stenseth, 2001). In contrast, the relatively high nucleotide diversity value reported in this study may be indicative of deep phylogenetic divergences without excluding the possibilities mentioned earlier.

The first step in testing the biological species concept within *M. namaquensis* would be to narrow the distributional boundaries of the identified lineages and mapping their areas of overlap through further sampling and genetic analyses. In areas of either sympatry (localities 4, 8, 9, 11, 12, 13, 16, 50, 67, 79, 89 and 93; see Fig. 2.5) or parapatry, the presence or absence of hybrids (identified with diagnostic nuclear or cytogenetic characters) could be used to test the breeding integrity of these groups because lack of hybrids could indicate the existence of biological species. For example, breeding isolation has been shown between two sibling species, *A. chrysophilus* and *A. ineptus* using karyology and sperm and bacular morphology (Breed et al., 1988; Gordon and Rautenbach, 1980; Gordon and Watson, 1986; Visser and Robinson, 1986). Males of *A. ineptus* have sperm heads with a disc-shaped nucleus and a large acrosome with a huge apical segment, with the chromatin not fully condensed (Breed 1997; Breed et al., 1988). In contrast, males of *A. chrysophilus* have sperm heads with a typical apical hook, a perforatorium and fully condensed chromatin, and significantly longer sperm tails (Breed 1997; Breed et al., 1988). These results suggest that a considerable morphological change in the sperm nucleus, acrosome, and subacrosomal space can evolve even between two closely related species resulting in reproductive isolation (Breed et al., 1988).

The remarkable karyotypic diversity found in mammals suggests that speciation may be associated with karyotypic changes (Robinson et al., 1986). This is certainly true for some rodents that include variation in diploid numbers within the North American genera, *Spermophilus* and *Marmota* (Nadler and Hoffmann, 1970) and the two cryptic species, *A. chrysophilus* and A. ineptus (Gordon and Rautenbach, 1980; Visser and Robinson, 1986), and heterochromatic differences between South African ground squirrels (Robinson et al., 1986). Similarly, the diploid numbers of hamsters of the genus *Cricetulus* ranged from $2n = 20$ to $2n = 24$ (Romanenko et al., 2007). Other examples include southern African rodent species within the genera *Aethomy*s (*senso lato*), *Mastomys*, *Saccostomus*, *Rhabdomys* and *Otomys* where morphologically similar but genetically distinct species complexes have been identified using karyotypes and/or protein electromorph mobility (Gordon and Watson, 1986; Rambau et al., 2003; Taylor, 2000).

Individuals of *A. chrysophilus* (*senso lato*) were characterised by $2n = 50$ and $2n = 44$ (Gordon and Rautenbach, 1980; Visser and Robinson, 1986). In addition, no intermediate chromosomal forms have been detected in sympatric populations, suggesting the lack of gene flow leading to the recognition of two sympatric species, the nominate *A. chrysophilus* ($2n = 50$) and *A. ineptus* ($2n = 44$) (see Chimimba 1998; Chimimba et al., 1999). These species also show differences in haemoglobin mobility, gross sperm and bacular morphology, and in cranial morphometrics (Baker et al., 1988; Breed 1997; Chimimba et al., 1999; Gordon and Watson, 1986).

It is possible that similar scenarios as outlined above may also be the case within the genus *Micaelamys*. Visser and Robinson (1986) reported on a single diploid number ($2n = 24$; $N = 24$) in *M. namaquensis* from eight localities spanning the geographical ranges of lineages B, J and N identified in the present study (Figs. 2.4 and 2.5). As localities from the geographic ranges of the remaining 11 mtDNA lineages identified in the present study were not covered in Visser and Robinson's (1986) study, it may be premature to assume that there is no variation in diploid number and karyotypic structure within *M.*

namaquensis. Consequently, the species may require additional investigations that should include increased comprehensive sampling, and apart from cytogenetics, the use of other alternative data such as nuclear DNA markers, morphology and gross sperm and bacular morphology.

For *M. namaquensis*, several behavioural and/or physiological adaptations may be a result of adaptation to seasonal variability, food availability, climate and rainfall. For example, the smaller body size of *M. namaquensis* from the Nama-Karoo (Chimimba, 2001a) may represent a form of adaptation to this biome. Bergmann's and Allen's rules suggest that homeotherms from cold environments should be larger (Bergmann, 1847) and have smaller appendages (Allen, 1877) than those from warmer environments. These characteristics will in turn reduce the relative surface area from which heat is lost to the environment (Allaby, 1985). Studies on pouched mice of the genus *Saccostomus* have shown that temperature has little effect on morphology, as there is no clear correlation between the size of appendages and temperature (Ellison et al., 1993). However, geographic variation in body size was shown to be significantly correlated with latitude, temperature, and seasonality, largely due to a positive correlation between body size and rainfall (Ellison et al., 1993). The smaller body size of pouched mice in localities experiencing lower rainfall (such as the Nama-Karoo) and colder temperatures (such as the Nama-Karoo during winter) might represent an adaptation for conserving energy in areas where food supplies are limited and thermoregulatory costs are high (Ellison et al., 1993).

The species is also known to utter piercing calls when disturbed (De Graaff, 1997). Alarm vocalisations are often species-specific and can be used to distinguish between closely related species (Bradbury and Vehrencamp, 1998). Habitat dissimilarities, as is the case with *M. namaquensis*, could play an important role in the evolution of interspecific call variation (Brandbury and Vehrencamp, 1998). Differences in habitats uniquely shape the vocalisations of species that inhabit them (Morton, 1975; Ryan and Brenowitz, 1985). Morton (1975) proposed that vocalisations of animals in a densely vegetated area would be lower in frequency, with fewer rapid frequency modulations than those of animals in a more open environment. These proposed predictions are based on environmental factors, differing between habitats such as scattering by obstructions, echoes, background noise and irregular amplitude fluctuations (Morton 1975; Ryan and Brenowitz, 1985). It has been reported that related taxa do not only diverge in vocalisations but also in other characteristics (Gannon and Lawlor, 1989). Reproductive character displacement in alarm calls has been reported such as in vocal differences being enhanced at parapatric sites of some populations of Townsend's chipmunks of the genus *Eutamias* (Gannon and Lawlor, 1989).

In contrast, neither of the two species of whistling rats of the genus *Parotomys* showed reproductive character displacement in their alarm calls but consistent differences in their alarm whistles may be related to differences in microhabitat preferences (Le Roux et al., 2002). Calls of *P. littledalei* are lower in dominant frequency and wider in frequency bandwidth than those of *P. brantsii*, which supports Morton's (1975) hypothesis (Le Roux et al., 2002). This implies that species living in closed habitats emit calls that are less pure in tone than those living in open habitats (Le Roux et al., 2002) which is contrary to the predictions of Morton's (1975) acoustic adaptation hypothesis that was also contradicted by a similar study on marmots of the genus *Marmota* by Daniel and Blumstein (1998). The acoustic adaptation hypothesis is at least applicable only partially to rodent calls and it may explain the differences in call structure between the two whistling rat species (Le Roux et al., 2002). While it is not known if vocalisation plays a role in species recognition, mate-recognition, or mate-choice within *Micaelamys*, its occurrence in different vegetation types with different acoustic properties may require further investigations in order to test whether variation in vegetation plays a role in vocalisation and subsequently speciation events in the species.

The understanding of speciation processes is important in predicting changes in species number and the planning of conservation strategies (Moritz et al., 2002). Speciation is not an inevitable consequence of population differentiation and molecular evidence of reduced gene flow is needed to strengthen support for the incidence of ecological speciation (Magurran, 1998; Orr and Smith, 1998; Schluter 1998). Since there may be an association between the lineages identified in the present study and biomes/bioregions, ecological speciation may have played an important role in diversification within *M. namaquensis*. Ecological speciation occurs when divergent natural selection on traits between populations in different environments leads to the evolution of reproductive isolation (Schluter, 2001). Ecological speciation might occur in either allopatry or sympatry (Schluter, 2001), but within *M. namaquensis*, it may have occurred in both sympatry and allopatry. In addition, it is not clear whether the nature and extent of morphometric variation within *M. namaquensis* (Chimimba, 2001a) is a result of historical differentiation, ecological selection, or both.

In order to test for the occurrence of ecological speciation within *M. namaquensis*, it would be necessary to sample extensively on a micro-geographic scale in order to assess changes among lineages with reference to changes in vegetation. If ecological speciation occurred within *Micaelamys*, it may be expected to find sympatric species in areas with vegetation overlap. Consequently, more evidence is needed on the geographic distribution of the lineages identified in the present study in order to assess their association with vegetation types. Allopatric speciation alone seems unlikely to have occurred within *M. namaquensis*. From a topographic perspective, the Drakensberg mountain range, which is part of the Great Eastern Escarpment, may act as a barrier to gene flow between populations on the eastern and western sides of the escarpment (Chimimba, 2001a). The Drakensberg mountain range has crest elevations above 3 000 m, with the highest peak at 3 484 m (Butzer, 1973).

Diversification within *M. namaquensis* lineages varied from 940 000 years (lineage G) to 3.42 MYA (lineage N), but the most significant time period appeared to be the Late Miocene to Early Pliocene (Fig. 2.6). Divergence times between the lineages were estimated between 7.26 MYA and 2.70 MYA suggesting a Late Miocene to Pliocene time of divergence. The deepest split within *M. namaquensis* in the Late Miocene appeared to be between more arid versus mesic adapted lineages. The majority of diversification events date to the Late Miocene and Early Pliocene which coincide the time of changes in C4/C3 plants and opening of landscapes as invoked by Cerling (1999) and correspond to a major shift into arid climate that continued until 5 MYA. All other splits of lineages (at 9 MYA, 7 MYA, 5.3 Ma and 2.4 MYA correspond to cold climatic episodes) can be discussed at the favour of climatic events.

Considerable environmental changes particularly with regard to temperature have occurred during the last 50 million years, more especially in the last three million years and these changes may have influenced the evolution of *M. namaquensis*. Environmental changes had the effect of breaking up the African landmass into a series of discontinuous patches and thus serve as a mediator for allopatry (Brain, 1985). A decline in temperature from the Paleocene until the end of the Miocene was followed by a series of oscillations (Brain, 1985). Between 6.5 and 5 million years ago, a severe temperature plunge, the Terminal Miocene Event, resulted in a rapid and dramatic sea level drop of over 100 m worldwide (Brain, 1985). The temperature during the succeeding period appeared to have fluctuated until 2.6 - 2.5 million years ago (Brain, 1985). Since then, temperatures have oscillated, between glacial and interglacial conditions, at least 17 times during the last 1.7 million years and individual cycles had a mean duration of about 100 000 years (Brain, 1985). These temperature changes had a definite impact on vegetation and may have contributed to speciation in southern African mammals (Axelrod and Raven, 1978).

The Early Pleistocene and Pliocene were characterised by uplifts which raised the interior plateaus of South Africa more than 1 800 m above the Miocene level (Baker and Wohlenberg, 1971). There were also major land elevations during the later Tertiary that resulted in the elevation of the central Highveld (Mpumalanga and Gauteng Provinces of South Africa) and the out-ward tilting of the marginal regions (King, 1963). Uplift also occurred along a line outside the Great Eastern Escarpment, while the central Karoo plateau developed as a basin which led to the spread of aridity. Superimposed on these were minor uplifts (in the form of long ridge-like axes) and basins which led to the elevation of the central plateau above its former level (King, 1963). These deformations modified both the topography and the climate (temperature and rainfall) of the region (Axelrod and Raven, 1978).

The broad warping and uplift of Africa during the Plio-Pleistocene and Miocene contributed to two major episodes of rapid speciation in South Africa (Axelrod and Raven, 1978). The first episode of speciation commenced in the Miocene and the second burst of speciation was evident during the Plio-Pleistocene as a result of fluctuations in climate (Axelrod and Raven, 1978). Mountains were elevated and basins developed over the interior, the low areas became drier and the mountains moister, with erosion resulting in many new small basins (Axelrod and Raven, 1978). Climate differed between wet and dry so that populations were shifting continuously (Axelrod and Raven, 1978). Moist climate vegetation invaded the present semi-desert area, allowing for the return to their original areas as conditions became drier (Axelrod and Raven, 1978). These changes had an influence not only on the vegetation but also on the large mammal fauna as new habitat zones spread and became available to the animals (Axelrod and Raven, 1978). These changes in climate, vegetation and deformation of the African continent might have contributed to the differentiation within *M. namaquensis*. Many of these geological and climatic changes could have resulted in speciation, either through physical isolation (allopatric speciation) or changes in habitat (sympatric or ecological speciation).

The geographic distribution of lineage J serves as an illustration of the potential role of habitat changes in the diversification within *M. namaquensis*. Lineage J appears to be broadly associated with the Nama-Karoo biome (Fig. 2.5) with an annual rainfall of between only 70 - 500 mm (Mucina and Rutherford, 2006). The Nama-Karoo biome is a large, landlocked region on the central plateau of the western half of South Africa and extends marginally into Namibia (Mucina and Rutherford, 2006). It is flanked by six biomes: the Succculent Karoo to the south and west, Desert to the northwest, the arid Kalahari form of the Savanna biome to the north, Albany Thicket to the southeast, parts of Fynbos to the south and Grassland to the northeast (Mucina and Rutherford, 2006). On the periphery of the distribution of lineage J within *M. namaquensis*, several other genetic lineages were identified to occur in sympatry with this lineage. It is significant to note that the sample localities (Porterville, Augrabies, Kakamas, Upington, Kimberley, Boshof, Willem Pretorius Nature Reserve and Kasane) of these unique lineages represent areas where different semi-arid or arid vegetation types merge or are in close contact.

Some of these localities are not located within the Nama-Karoo biome but Karoo (semi-arid or arid) elements are still evident (Low and Rebelo, 1996) which may explain the occurrence of lineage J at these localities. Porterville, Upington, Kimberley, Boshof and Willem Pretorius Nature Reserve are situated in areas where two vegetation types are in close contact, which may explain the occurrence of divergent lineages. This may also apply to some of the localities where only lineage J occurs such as the Kalahari Duneveld, Eastern Kalahari Bushveld, Rainshadow Valley Karoo and the Mesic Highveld Grassland bioregions that represent vegetation types (bioregions) that are in close contact with the Nama-Karoo biome at these localities (Mucina and Rutherford, 2006).

Although the Mesic Highveld Grassland bioregion does not display characteristics of semi-arid or arid elements, it is difficult to map the border between the two biomes as there is a gradual transition from the one biome to the other (Mucina and Rutherford, 2006). This might explain the occurrence of the Nama-Karoo lineage at Willem Pretorius Nature Reserve situated within the Grassland biome. Porterville is also located in an area where two bioregions are in contact namely, the Rainshadow Valley Karoo bioregion and the Northwest Fynbos bioregion (Mucina and Rutherford, 2006). Fynbos burns at between 6 and 45 years of age, a process that sustains plant biodiversity. The majority of plant species rely on the predictability of fires and only regenerate after seeds escape their protective coatings and germinate (Low and Rebelo, 1996). If the vegetation does not burn frequently enough, the Fynbos might be replaced by Succulent Karoo that requires an annual rainfall of below 200 mm (Low and Rebelo, 1996).

The distributions of some of the lineages remain puzzling. For example, the occurrence of lineage K (Kalahari Duneveld bioregion; e.g., Bergville (locality 90) and Dwesa Nature Reserve (locality 91) may be a result of secondary contact between populations (Dowling and Hoeh, 1991) that in many cases is supported by behavioural observation (Moran and Kornfield, 1993). This may also be the case with the occurrence of lineage J at Kasane in Botswana. Although most of Botswana is within the Savanna biome, Kasane may exhibit elements of the arid Nama-Karoo. However, some studies (e.g., Avise et al., 1983; Neigel and Avise, 1986) have suggested mtDNA lineage sorting and the retention of ancestral polymorphisms as possible explanations. On the other hand, the presence of lineage K at Bergville and Dwesa Nature Reserve may be a result of recent Kalahari sandflows from north to south (Haacke, 1989; Lancaster, 1989). Vrba (1985) suggested that as a species' habitat expands or shifts, so does the distributional range of that species, this could therefore explain the presence of lineage K at Bergville and Dwesa Nature Reserve.

The correspondence between lineages and biomes/bioregions may be obscured by recent adaptation of populations. For example, lineage J initially differentiated in the arid Nama-Karoo but as populations within this lineage adapted to new ecological conditions a distribution not only confined to the Nama-Karoo biome might be observed. This extended distribution of some lineages may be as a result of biome limits that may have moved recently according to Quaternary fluctuations.

4.2 Within lineage phylogeographic patterns

In addition to the remarkable phylogenetic diversity in *M. namaquensis*, the independent lineages also displayed interesting patterns of intra-lineage diversity. The geographic extent of some of the lineages remains to be determined (e.g., lineages A, C, E, F, G and L). The remaining lineages were adequately represented in our sampling. There was no evidence of isolation by distance within any of these lineages (Table 2.1). Two of the lineages were chosen to illustrate contrasting patterns of phylogeographic structure (Fig. 2.3). There was more genetic differentiation within lineage B than within lineage J, despite the analysis of fewer samples over a geographically restricted area.

Some lineages such as the Nama-Karoo lineage are more homogeneous while others like the Grassland lineage are more complex with high levels of variation within the lineage. Haplotypes within lineage J were separated by only a few mutational steps, even between haplotypes that were geographically distant (Fig. 2.3A). Rare haplotypes are more likely to be mutational derivations of the common haplotypes found (Excoffier and Langaney, 1989). Therefore, the presence of the closely related haplotypes within almost all populations of lineage J (Appendix 2.3 and 2.4) might reflect close common female ancestry, probably due to recent range expansion from a source population(s). However, four maternal haplotypes were recorded in the six samples collected from Springbok suggesting either a relatively old, historically large population, or a recent colonisation event, in essence, lineage J exhibits less phylogeographic structure with a relatively low nucleotide diversity (0.34%; Table 2.2). Despite evidence for this recent expansion, lineage J has an ancient ancestry dating back to the Miocene.

All the individuals collected within the distributional range of the proposed subspecies *lehocla* (Chimimba, 2001a) grouped within lineage J. It is evident from the previous study by Chimimba (2001a) that the proposed subspecies *lehocla* is restricted to the Nama-Karoo but it seems from the present study that this subspecies may also occur in neighbouring semi-arid areas. However, not all of these localities fall within the recognised boundaries of the Nama-Karoo, Karoo elements, and/or where the invasion of the Nama-Karoo is evident.

The absence of correspondence between morphological and genetic variation would not be a surprise in the case of local adaptation. Constraints on the morphological/morphometric characters chosen by Chimimba (2001a) may also explain the higher levels of variation using molecular data compared to the morphological/morphometric data.

In contrast, the population history within lineage B may have been more complex with waves of colonisation or dispersal among regions. It is possible that large population sizes of lineage B are responsible for maintaining both the high nucleotide diversity (1.44%; Table 2.2) and the divergent haplotypes. This may also suggest that the populations have been separated from each other for a long period, thus allowing for more site changes to have accumulated. In one instance, a closer affinity was shown to haplotypes from other localities rather than to those from the same locality (NH059 and NH060). Six haplotypes were shared between populations and the presence of several unique haplotypes within populations may reflect evolutionarily old populations, which may have served as core regions of colonisation.

The geographically widespread lineage B is associated with Grassland, and broadly corresponds to the distributional range of the previously proposed subspecies *monticularis* (Chimimba, 2001a). It has been proposed that Grassland expanded during the glacial periods due to a colder climate that allowed for the formation of frost, an important factor in the distribution of the Grassland biome (Brain, 1985; Vrba, 1985). If the association between Grassland and winter frost together with the added effects of fire, is significant, then it may be plausible that when the area of winter cold expanded periodically in the past, it may have caused an expansion of Grassland at the expense of other vegetation types. There is evidence from Aliwal North and Florisbad that during the last glacial maximum, Grassland replaced other vegetation types (Coetzee, 1978; van Zinderen Bakker, 1957). If Grasslands expanded as a result of suitable climate, it may be assumed that animals with a preference for this kind of habitat such as the white-tailed rat (*Mastomys albicaudatus*) would have similarly expanded their range (Chimimba, 2001a). The expansion of Grassland during the last glacial maximum (LGM) might explain why the distribution of lineage B may have extended further north than expected. Some localities identified within lineage B represent areas recognised as Savanna with Grassland elements having changed both historically and naturally.

Lineage B (Fig. 2.3B) showed five distinct mtDNA groups, separated by four to six mutational steps. One of these mtDNA discontinuities coincides broadly with that of the rock hyrax, *Procavia capensis* (Prinsloo, 1993) and red rock rabbits, *P. rupestris* and *P. randensis* (Matthee and Robinson, 1996) in the Mpumalanga Province of South Africa. The discontinuity in the rock hyrax is considered to reflect dispersal along two routes corresponding to the two mtDNA clades: 1) along the Great Eastern Escarpment; and 2) the Soutpansberg-Magaliesberg axis (Prinsloo and Robinson, 1992). The south-eastern clade of *P. rupestris* may also have dispersed along the Great Eastern Escarpment, while the north-western assemblage was not constrained. It may be possible that the current observed patterns of genetic variation within this assemblage emanated from a combination of vicariance and dispersal events (Matthee and Robinson, 1996). Whether the association between the zone of contact in the two red rock rabbit species, the two mtDNA clades within *P. capensis*, and the two mtDNA groups within *M. namaquensis* is real or merely by chance, is subject to debate. Avise (1992) suggested that congruence in phylogeographic patterns in independent lineages might reflect similar vicariant events, a hypothesis that may also be true for many other mammals with similar habitat and dispersal capabilities.

4.3 Comparison with other rodents

The diversification within the *M. namaquensis* species complex coincides with the timeframe proposed for the rapid radiation within the African Murinae (Lecompte et al., 2008). Fossil evidence from South Africa suggests that *Aethomys* and *Micaelamys* representatives were present on the African continent since the Late Miocene, with one of these fossils closely resembles the extant *M. namaquensis* (Denys, 1990a, b; Pocock, 1987). The expansion and contraction of Savanna across parts of Africa during the Miocene and Pliocene have been linked to diversification in other rodent genera, for example *Tatera* (Colangelo et al., 2005), *Praomys* (Lecompte et al., 2005) and *Hylomyscus* (Nicolas et al., 2006). Based on the timing and pattern of diversification in *M. namaquensis* and these other broadly co-distributed rodents, it can be proposed that vicariance, dispersal and local adaptation shaped the diverse rodent fauna of the African continent.

5. Conclusion

Micaelamys namaquensis is a polytypic species with more variation, using molecular techniques, showing to exist than previously detected. Thus the diversity and differentiation detected within *M. namaquensis* appear to be indicative of a species complex. Given the geographic link of most of the 14 identified lineages to specific biomes or bioregions our future research will explore processes underlying ecological speciation in the group. The importance of conserving our evolutionary heritage for future generations and, simultaneously, the discovery of new species cannot be over emphasised. This can provide conservation authorities with crucial information relevant to the development of management action plans aimed at conserving our biodiversity (variation in the organic world which may be expressed in many ways, such as phylogenetics, molecular or phenotypic variation, Owens and Bennett, 2000).

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Appendix 2.1 Geographic coordinates of all collecting localities of *Micaelamys namaquensis* from southern Africa analysed in the present study. Numbers 1 - 95 correspond to those in Fig. 2.2. Biomes and bioregions correspond to the different groups that were identified in the phylogeographic analysis. Biomes and bioregions terminology follows that of Mucina and Rutherford (2006).

LOCALITY	COUNTRY	PROVINCE	GEOGRAPHIC COORDINATE
Savanna Biome			
1. Farm: Elephant Sands, Nata	Botswana		19°44’56”S 26°04’18”E
2. Francistown, just outside town (municipal grounds)	Botswana		21°11’15”S 27°23’22”E
3. Farm: Terrafou, south of Francistown	Botswana		22°27’29”S 28°45’32”E
4. Musina Nature Reserve, Musina	South Africa	Limpopo	22°24’45”S 30°03’01”E
5. Marula Lodge Safaris, Alldays	South Africa	Limpopo	22°35’10”S 29°10’08”E
6. Blouberg Nature Reserve, Vivo	South Africa	Limpopo	22°59’12”S 29°08’49”E
7. Farm: Goedgelegen, Baltimore	South Africa	Limpopo	23°26’27”S 28°23’02”E
Nama-Karoo Biome			
8. Kasane, just outside town (municipal grounds)	Botswana		17°47’07”S 25°10’59”E
9. Farm: Steenkampsput, Upington	South Africa	Northern Cape	28°06’13”S 20°54’10”E
10. Farm: Warmhoek, Hoopstad	South Africa	Free State	28°10’08”S 25°49’11”E
11. Willem Pretorius Nature Reserve, Winburg	South Africa	Free State	28°16’27”S 27°14’48”E
12. Farm: Viljoenshof, Boshof	South Africa	Free State	28°34’45”S 25°04’33”E
13. Langeberg Guest Farm, Kimberley	South Africa	Northern Cape	28°54’47”S 24°38’33”E
14. Farm: Palmietfontein, Brandfort	South Africa	Free State	28°48’07”S 26°33’32”E
15. Farm: Tierkoppen, Augrabies	South Africa	Northern Cape	28°34’06”S 20°26’05”E

16. Jacobsdal Agricultural School, Bloemfontein	South Africa	Free State	29°10’12”S 26°19’48”E
17. Farm: Boomrivier, Pofadder	South Africa	Northern Cape	29°04’33”S 19°18’24”E
18. Farm: Rietfontein, Springbok	South Africa	Northern Cape	29°51’40”S 18°11’10”E
19. Hopetown	South Africa	Northern Cape	29°44’45”S 23°37’30”E
20. Caledon Nature Reserve, Wepener	South Africa	Free State	29°49’30”S 26°53’16”E
21. Gariep Nature Reserve, Gariep Dam	South Africa	Free State	30°35’56”S 25°32’03”E
22. Lady Grey, just outside town (municipality)	South Africa	Eastern Cape	30°45’00”S 27°15’00”E
23. Farm: Klipfontein, Jamestown	South Africa	Eastern Cape	31°11’23”S 26°49’12”E
24. Farm: Rietpoort, Loxton	South Africa	Northern Cape	31°38’30”S 22°22’34”E
25. Karoo National Park, Beaufort West	South Africa	Northern Cape	32°15’00”S 22°30’00”E
26. Matjiesfontein, just outside town (municipality)	South Africa	Western Cape	33°15’00”S 20°34’48”E
27. Farm: Brakrivier, Oudtshoorn	South Africa	Western Cape	33°46’19”S 22°31’45”E
28. Kirkwood, just outside town (municipality)	South Africa	Eastern Cape	33°24’20”S 25°25’30”E
Grassland Biome			
29. Lajuma Mountain Retreat, Makhado	South Africa	Limpopo	23°02’02”S 29°26’27”E
30. Ellisras	South Africa	Limpopo	23°40’12”S 28°45’00”E
31. Lapalala Nature Reserve, Vaalwater	South Africa	Limpopo	23°52’04”S 28°19’55”E
32. Amanita Safaris, Rooibokkraal	South Africa	Limpopo	24°09’16”S 26°55’05”E
33. Ben Alberts Nature Reserve, Thabazimbi	South Africa	Limpopo	24°34’48”S 27°25’12”E
34. Farm: Waterval, Thabazimbi	South Africa	Limpopo	24°31’12”S 27°45’00”E
35. Gaborone, just outside town (municipality)	Botswana		24°40’12”S 25°49’48”E

36. Farm: Sunset Ranch, Bela-Bela	South Africa	Limpopo	24°45’00”S 28°15’00”E
37. Selati Nature Reserve, Hoedspruit	South Africa	Limpopo	24°09’30”S 30°40’50”E
38. Farm: Boskloof, Boshoek	South Africa	North West	25°28’43”S 27°03’39”E
39. Kgaswane Mountain Reserve, Rustenburg	South Africa	North West	25°44’20”S 27°12’56”E
40. Brits Agricultural School, Brits	South Africa	North West	25°34’29”S 27°46’02”E
41. Ezemvelo Nature Reserve, Bronkhorstspruit	South Africa	Gauteng	25°45’00”S 28°49’48”E
42. Kruisrivier Nature Reserve, Loskop Dam	South Africa	Mpumalanga	25°21’08”S 29°32’26”E
43. Farm: Rietfontein, Potchefstroom	South Africa	North West	26°38’36”S 27°21’48”E
44. Farm: Ratzegaai, Ventersdorp	South Africa	North West	26°20’30”S 26°44’01”E
45. Habula Lodge, Vredefort	South Africa	Free State	26°53’48”S 27°19’20”E
46. Josefsdal Nature Reserve, Barberton	South Africa	Mpumalanga	25°58’05”S 30°42’57”E
47. Farm: Uitspanning, Amsterdam	South Africa	Mpumalanga	26°39’56”S 30°31’26”E
Fynbos Biome			
48. Boscherberg, Algeria, Cederberg	South Africa	Western Cape	32°10’10”S 19°04’05”E
49. Jamaka, Algeria, Cederberg	South Africa	Western Cape	32°20’20”S 19°05’05”E
50. Farm: Grootfontein, Porterville	South Africa	Western Cape	32°54’28”S 19°06’31”E
51. Vrolijkheid Nature Reserve, Jonaskop	South Africa	Western Cape	33°45’10”S 19°30’10”E
52. Farm: Goederede, Robertson	South Africa	Western Cape	33°45’45”S 19°40’20”E
53. Farm: Mizpah, Grabouw	South Africa	Western Cape	34°10’10”S 19°02’15”E
54. Vrolijkheid Nature Reserve, Die Galg	South Africa	Western Cape	34°10’10”S 19°55’10”E
55. Farm: Fairfield, Napier	South Africa	Western Cape	34°27’27”S 19°45’10”E

56. Farm: Versig, Riversdale	South Africa	Western Cape	34°10'20"S 21°15'15"E
Albany Thicket Bioregion			
57. Andries Vosloo Kudu Reserve, Grahamstown	South Africa	Eastern Cape	33°10'55"S 26°38'10"E
58. Mount Currie Nature Reserve, Kokstad	South Africa	KwaZulu-Natal	30°29'36"S 29°23'18"E
Sub-Escarpment Grassland Bioregion			
59. Gethlane Lodge, Burgersfort	South Africa	Mpumalanga	24°45'51"S 30°23'11"E
60. Ongeluksnek Nature Reserve, Thaba Chitja	South Africa	Eastern Cape	30°20'05"S 28°21'17"E
Fouriesburg/Kasane			
61. Wynford Guest Farm, Fouriesburg	South Africa	Free State	28°30'30"S 28°15'42"E
Lowveld Bioregion			
62. Mantenga Nature Reserve	Swaziland		26°26'37"S 31°10'22"E
63. Ithala Nature Reserve, Louwsburg	South Africa	KwaZulu-Natal	27°30'10"S 31°15'10"E
64. Farm: Koedoesberg, Pongola	South Africa	KwaZulu-Natal	27°26'31"S 31°41'41"E
65. Newcastle	South Africa	KwaZulu-Natal	28°04'22"S 29°48'02"E
Bushmanland/Upper Karoo Bioregion			
66. Farm: Karlsrühe, Hotazel	South Africa	Northern Cape	26°58'34"S 22°59'57"E
67. Farm: Donkerpoort, Schweizer-Reneke	South Africa	North West	27°14'46"S 25°06'01"E
68. Farm: Tierkop, Postmasburg	South Africa	Northern Cape	28°21'33"S 23°14'33"E
69. Farm: Swemkuil, Grootdrink	South Africa	Northern Cape	28°39'07"S 21°47'54"E
70. Witsand Nature Reserve, Griekwastad	South Africa	Northern Cape	28°43'52"S 22°26'08"E
71. Soetdoring Nature Reserve, Bloemfontein	South Africa	Northern Cape	23°50'50"S 26°08'55"E

72. Farm: Rooidam, Groblershoop	South Africa	Northern Cape	29°08’33”S 22°19’34”E
Eastern Kalahari Bushveld Bioregion			
73. Farm: Welbedeur, Tosca	South Africa	North West	25°42’53”S 23°58’43”E
74. Farm: Arizona, Vorstershoop	South Africa	North West	25°57’00”S 23°13’55”E
75. Farm: Rus en Vrede, Stella	South Africa	North West	26°10’23”S 25°13’27”E
76. Farm: Loversleap, Vanzylsrus	South Africa	Northern Cape	26°38’20”S 22°01’4”E
77. Farm: Jones, Severn	South Africa	Northern Cape	26°35’22”S 22°41’46”E
78. Tswalu Kalahari Reserve, Sonstraal	South Africa	Northern Cape	27°12’51”S 22°’27’22”E
79. Farm: Waterloo & Vlakfontein, Vryburg	South Africa	North West	27°03’34”S 24°45’58”E
80. Farm: Strelley, Kuruman	South Africa	Northern Cape	27°39’48”S 23°23’04”E
Kalahari Duneveld Bioregion			
81. Windhoek	Namibia		22°35’32”S 17°10’26”E
82. Gibeon	Namibia		25°20’42”S 17°15’13”E
83. Quivertree Forest Rest Camp, Keetmanshoop	Namibia		26°28’56”S 18°14’39”E
84. Farm: Koppieskraal, Askham	South Africa	Northern Cape	26°56’18”S 20°13’38”E
85. Farm: Duurdrift, Karasburg	Namibia		27°26’10”S 18°53’17”E
86. Canon Lodge, Ais-Ais	Namibia		27°39’49”S 17°46’42”E
87. Farm: Witkoppen, Noenieput	South Africa	Northern Cape	27°35’44”S 20°13’49”E
88. Farm: Swartmodder, Gelukspruit	South Africa	Northern Cape	28°01’45”S 20°33’33”E
89. Farm: Zwartbooisberg, Kakamas	South Africa	Northern Cape	28°02’30”S 20°42’55”E
90. Farm: Meedwood, Bergville	South Africa	KwaZulu-Natal	28°42’44”S 29°19’25”E

91. Dwesa Nature Reserve, Dutywa	South Africa	Eastern Cape	32°18’02”S 28°49’40”E
Koppies Dam			
92. Koppies Dam Nature Reserve, Koppies	South Africa	Free State	27°13’27”S 27°40’29”E
Machadodorp/Malelane			
93. Wathaba-Uitkomst, Machadodorp	South Africa	Mpumalanga	25°47’31”S 30°22’28”E
94. Farm: Riverside, Malelane	South Africa	Mpumalanga	25°26’33”S 31°33’01”E
Volksrust			
95. Farm: Waterval, Volksrust	South Africa	Mpumalanga	27°22’55”S 29°45’31”E

Appendix 2.2 Permits and permit numbers for the nine provinces representing South Africa and permits for Botswana, Swaziland and Namibia.

PROVINCE	PERMIT NUMBER	PERMIT HOLDER
Free State	HK/P1/07106/001	Miss. I.M. Russo
Gauteng	1244	Miss. I.M. Russo
Mpumalanga	MPB. 5126	Miss. I.M. Russo
Eastern Cape	Letter with no permit number	Miss. I.M. Russo
Limpopo	CMP-004-00004	Miss. I.M. Russo
Kwa-Zulu Natal	3968/2004	Miss. I.M. Russo
Northern Cape	040/2001	Miss. I.M. Russo
Northern Cape	0545/2004	Miss. I.M. Russo
North West	000027 NW-06	Miss. I.M. Russo
Western Cape	378/2003	Miss. I.M. Russo
Cape Peninsula National Park	Letter with no permit number	Miss. I.M. Russo
Namibia	804/2004	Miss. I.M. Russo
Swaziland	Letter with on permit number	Miss. I.M. Russo
Botswana	13/1/1/30/1-86	Mr. N. Maputla

Appendix 2.3 Biome-related variable sites of 137 mitochondrial DNA cytochrome *b* haplotypes (631 base pairs) of *Micaelamys namaquensis* from southern Africa. Variable positions one and 631 correspond to positions 14139 and 14770 of *Mus musculus* (Bibb et al., 1981). Dots (.) indicate identity to the base in the references sequence NH001. Haplotype order corresponds to the different groups that were identified in the phylogeographic analysis. Biome terminology follows that of Mucina and Rutherford (2006).

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Haplotype                                  Variable positions within the 631 bp fragment of cytochrome b gene
 Number
                                             111111111111111111112222222222222222222223333333333333333333333333333444444444444444444444444455555555555555555555555555555555566666666666660
             11111111222222333444556667778899000112223334445667780011112333334457888990000122223444455566777888999012222333455556677777889900112233344445556666677888889999900001111112233
          58901234589012347038258460372584739258151572561459584891704692124573686258171369813789258914735589147069570369468836795812358362814692347803692381347836024581247803694567890101
Savanna Biome
NH001     CACATCCGAAACCCATTACTTCCTTCCGACCAAACTATTATTGATTTCTACCCCCTCCGCACACAGATAACATACAGTACACCTCACCAGGCTTCCCGATATACAATAAATACCGCACCTAACATAATAACATCCCTGACCCTTACTCTATCGTTTTACACCGCAACCAAC?????
NH002     ??????????????????....................................................................................G.......................................................T............AACAC
NH003     ????????......................................................................................................................................................T............AAC??
NH004     ??????????......C....T...T................A...................................................................................................................T.????????????????
NH005     ................C....T.................................C......G.....................T.....A...............C.....T.T........................................C.CT...A........AAC??
NH006     ................C....T.................................C......G.....................T.....A...............C.......T.....................C....................CT...A........?????
NH007     ................C....T.....A............................................................................................................................A.....T.????????????????
NH008     ................C....T....................................................................A..........C.......................................................T..........CAAC???
NH009     ................C....T....................................................................A.....................................G.............................T............AACAC
NH010     ???????????????.C....T.....A.......C......................................................A...................................................................T.......??????????
NH011     ................C....T.....A.........................................................................C........................................................T...??????????????
NH012     ................C....T....................................................................A..........C.......................................................T............AAC??
NH013     ................C....T.................G......................................................................................................................T.......??????????
Nama-Karoo Biome
NH014     ................C....T.........G..T..C....A............C.T..TT.TG............CG.....T.....AT.............T......T.T.....G......CG.......C......C......C.AC...C.............AACCC
NH015     ................C....T.....A...G..T..C....A............C.T..TT.TG............CG.....T.....AT.............T......T.T.....G......CG......TC......C......C.AC...C.............AAC??
NH016     ................C....T.....A...G..T..C....A............C.T..TT.TG............CG.....T.....AT.............T......T.T.....G......CG.......C......C......C.AC...C.............AACCC
NH017     .........G......C....T.........G..T..C....A............C.T..TT.TG............CG.....T.....AT.............T......T.T.....G......CG.......C......C......C.AC...C.............AACCC
NH018     ................C....T.........G..T..C....A............C.T..TT.TG............CG.....T.....AT.............T......T.T.....G......CG.......C......C......C.AC.C.C.............AACCC
NH019     ................C....T.........G..T..C....A............C.T..TT.TG............CG.....T.....AT.............T......T.T.....G......C.......TC......C......C.AC...C.............AACCC
NH020     ................C....T.........G..T..C....A............C.T..TT.TG............CG.....T.....AT...........T.T......T.T.....G......CG.......C......C......C.AC...C.............AACCC
NH021     ................C....T.........G..T..C....A............C.T..TT.TC............CG.....T.....AT.............T......T.T.....G......CG.......C......C......C.AC...C.............AACCC
NH022     ?????????????...C....T.........G..T..C....A............C.T..TT.TG............CG.....T.....AT.............T......T.T.....G......CG......TC......C......C.AC...C..????????????????
NH023     ................C....T.........G..T..C....A............C.T..TT.TG............CG.....T.....AT.......C.....T......T.T.....G......CG.......C......C......C.AC...C.............AAC??
NH024     ................C....T.........G..T..C....A............C.T..TT.TG............CG.....T.....AT.............T......T.T.....G......CG.....T.C......C......C.AC...C.......C??????????
NH025     ................C....T.........G..T..C....A............C.T..TT.TG............CG.....T.....AT.............T......T.T.....G......CG.....T.C......C......C.AC...C.............AACCC
NH026     ................C....T.........G..T..C....A............C.T..TT.TG............CG.....T.....AT.............T......T.TT....G......CG.......C......C......C.AC...C.............AACCC
NH027     ................C....T.........G..T..C....A............C.T..TT.TG............CG.....T.....AT.............T......T.T.....G......CG.......C.....CC......C.AC...C.............AACCC
NH028     ??????????......C....T.........G..T..C....A............C.T..TT.TG............CG.....T.....AT.............T......T.T.....G......CG...............C......C.AC...C.....T.......AAC??
NH029     ?...............C....T.........G..T..C....A............C.T..TT.TG.....T......CG.....T.....AT.............T......T.T.....G......CG.......C......C......C.AC...CT............AACCC
NH030     ................C....T.........G..T..C....A............C.T..TT.TG............CG.....T.....AT.............T......T.T.....G......CG.......C......C......C.AC...C........T.C.ACAA??
NH031     ??????????......C....T.........G..T..C....A............C.T..TT.TC............CG.....T.....AT.............T......T.T.....G......CG.......C......C......C.AC...C........T.C.ACACCC
NH032     ................C....T.........G..T..C....A............C.T..TT.T.............CG.....T.....AT.............T......T.T.....G......CG......TC......C......C.AC...C.............AACCC
NH033     T....A..........C....T.........G..T.GC....A............C.T..TT.TG............CG.....T.....AT.............T......T.T.....G......CT......TC......C......C.ACCC.C....??????????????
NH034     T...............C....T.........G..T..C....A.....C......C.T..TT.TG............CG.....T.....AT.............T......T.T.....G......CT......TC......C......C.AC.C.C.............AAC??
NH035     T...............C....T.........G..T..C....A............C.T..TT.TG............CG.....T.....AT.............T......T.T.....G......CT......TC......C......C.AC.C.C.............AACCC
NH036     ?????...........C....T.........G..T..C....A............C.T..TT.TG............CG.....T.....AT.............T......T.T.....GG.....CT......TC......C......C.AC.C.C.............AAC??
NH037     .......T........C....T.........G..T..C....A..C.........C.T..TT.TG............CG.....T.....AT.............T......T.T.....G...C..CG......TC......C......C.AC...C.............AACCC
NH038     ??????..........C....T.........G..T..C....A............C.T..TT.TG............CG.....T.....AT.............T......T.T.....G......CT......TC......C......C.AC.C.C...G??????????????
NH039     ?????A.CG.......C....T.........G..T..C....A............C.T..TT.TG............CG.....T.....AT.............T......T.T.....G......C.......TC......C......C.AC...C......????????????
NH040     ????????........C....T.....A...G..T..C....A............C.T..TT.TG............CG.....T.....AT.............T......T.T.....G......CG......TC...T..C......C.AC...C.............AAC??
NH041     ................C....T.....A...G..T..C....A............C.T..TT.TG..C.........CG.....T.....AT.............T......T.T.....G......CG......TC...T..C......C.AC...C.............AAC??
NH042     ???????????.....C....T.........G..T..C....A............C.T..TT.TG..C.........CG.....T.....AT.............T......T.T.....G......CG......TC...T..C......C.AC...C........??????????
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NH043   ................C....T........G..T..C....A...........C.T..TT.TG..C.........CG.....T.....AT............T......T.T...........CG......TC...T..C......C.AC...C............A????
NH044   ................C....T........G..T..C....A...........C.T..TT.TGA..........CG.....T.....AT............T......T.T.....G......C.......TC......C......C.AC...C............AACCC
 Grassland Biome
NH045   ..............CC.....C.........T............C.......T..TT.T....G........G.....T.....AT...T...............T.C....C.......C....C...C..T.T........C.A....CT...........AACAC
NH046   ..............CC.....C.........T....................T..TT.T....G........G.....T.....AT...T...............T.C....C.......C....C...C..T.T........C.A....CT...........AAC??
NH047   ..............CC.....C.........T............C.......T..TT.T....G........G.....T..T..AT...T...............T.C....C.......C....C...CA.T.T........C.A....CT.......?????????
NH048   ...............C.....C.....T...T....................T..TT.T...C.G........G.....T.....AT...T...............T.C....C.......C....C...C..T..........C.A....CT...........AAC??
NH049   ..............CC.....C.........T....................T..TT.T....GG.......G.....T.....AT...T...............T.C....C.......C....C...C..T.TC.......C.A....CT..........??????
NH050   ????????????????...C..C.........TC...................T..TT.T....G........G.....T.T.G.AT...T...............T.C...TC.......CG...C...C..T.T........C.A....CT.???????????????
NH051   ..............CC...C..C.........TC...................T..TT.T....G........G.....T.T.G.AT...T...........G...T.C....C.......CG...C...C..T.T........C.A....CT...........AACAC
NH052   ..............CC...C..C.........TC..........C.......T..TT.T....G........G.....T.T.G.AT...T...........G...T.C....C.......CG...C...C..T.T........C.A....CT...........AACAC
NH053   ...............C.....C.........T....................T..TT.T...C.G........G.....T...G.AT...T....G..........T.C....C.......C....C...C..T..........C.A....CT...........AAC??
NH054   ????????????????????...C.........T....................T..TT.T...C.G........G.....T...G.AT...T...............T.C....C.......C....C...C..T.......T..C.????????????????????????
NH055   ...............C....T.C.........T....................T..TT.T...C.G........G.....T...G.AT...T...............T.C....C.......C....C...C..T.........??????????????????????????
NH056   ..............CC.....C.........TC...........G.......T..TT.T....G..............T...G.AT...T..G.............T.T....C.....??????????????????????????????????????????????????
NH057   ??????????????????....C.........T............G.......T..TT.T....G..............T...G.AT...T..G.............T.T....C.......C....CT..C..T....G.....C.A....C??????????????????
NH058   ..............CC.....C.........T............G.......T..TT.T....G..............T...G.AT...T..G.............T.T....C.......C....CT..C..T....G..T..C.A....CT......???????????
NH059   ???????????????????????????????????...C.........G.......T..TT.T....G..............T...G.AT...T..G.............T.T....C.......C....CT..C..T....G.....C.A....CT...........AACAC
NH060   ..............CC.....C.........T............G.......T..TT.T...GG..............T...G.AT...T..G.............T.T....C...G...C....CT..C..T....G.....C.A....CT......???????????
NH061   .....A......T...C.....C.........T.........C....G.......T..TT.T..G..G.......CG.....T...G.AT...T.....C...........T.T....C.......C....C...C..T..........C.A....CT?????????????????
NH062   ?????????????????......CC........T............G.......T..TT.T..G..G........G.....T...G.AT...T.....C...........T.T....C.......C....C...C..T..........C.A....CT...........AAC??
NH063   ????????????????C.....C.........T.........C....G.......T..TT.T..G..G........G.....T...G.AT...T.....C...........T.T....C.......C....C...C..T..........C.A....CT.......??????????
NH064   ...............C.....C.........T..............T......T..TT.T....G..C.....GT....T...G.AT...T...............T.C....C.......C....C...C..T..........C.A....CT......???????????
NH065   ?????????......C.....C.........T..............T......T..TT.T....G......A.G.....T...G.AT...T...............T.C....C.......C....C...C..T..........C.A....CT...........AAC??
NH066   ...............C.....C.........T....................T..TT.T.A.C.G........G.....T...G.AT.C.T...............T.C....C.......C....C...C..T..........C.A....CTG????????????????
NH067   ...............C.....C.........T...........C.........T..TT.T.A.C.........G.....T...G.AT.C.T...............T.C....C.......CG...C...C..T..........C.A....CT...........AAC??
NH068   ??????????......C.....C.........T............G.......T..TT...G..G........G.....T...G.AT...T.....C...........T.T....C.......C....C...C..T..........C.A....CT.......??????????
NH069   .....A......T...C.....C.........T............G.......T..TT.T..G..G........G.....T...G.AT...T.....C......G...T.T....C.......C....C...C..T..........C.A....CT...........AAC??
NH070   .....A......T...C.....C.........T............G.......T..TT.T..G..GT.......G.....T...G.AT...T.....CG..........T.T....C.......C....C...C..T..........C.A....CT..???????????????
NH071   .....A......T..CC.....C.........T............G.......T..TT.T..G..G........G.....T...GAAT...T.....C........ACT.T....C.......C....C...C..T..C.......C.A....CT......???????????
NH072   ..T...........CC.....C.........TC...................T..TT.T....G........G.....T.....AT...T...............T.C....C.......C....C...C..T.T........C.A....CT.....T......AAC??
 Fynbos Biome
NH073   ?????????????????....T.........................T...T..T..TT.T..........G........T..T..AT...T......C.....G...TTT...........G...T.C...C..T..........C.A...CCT.......??????????
NH074   ?????????????????????????.......................T...T..T..TT............G........T..T..AT...T......G.....G...TTT...............T.C...C..T..........C.A...CCT......???????????
NH075   ?CATC.GA..C..ATCC....T.....A...........C........T...T..T..T..T...C...............T..TG.AT...T............G...TTC...............T.C...C..T..........C.A...CCT.......??????????
NH076   T..............C....T.........................T...T..T..T..T...C...............T..TG.AT...T............G...TTC...............T.C...C..T..........C.A...CCT.????????????????
NH077   T..............C....T.........................T...T..T..TT.T..........G........T..T..AT...T......G.....G...TTT...............T.C...C..T..........C.A...CCT.......??????????
NH078   T..............C....T.........................T...T..T..TT.T..........G.A......T..T..AT...T......G.....G...TTT...........G...T.C...C..T..........C.A...CCT..........???????
NH079   T..............C....T.........................T...T..T..TT.T..........G........T..T..AT...T......G.....G...TTT...........G...T.C...C..T..........C.A...CCT............AAC??
NH080   T..............C....T.........................T...T..T..TT.T..........G........T..T..AT...T......G.....G...TTT...............T.C...C..T..........C.A..CCCT............AAC??
NH081   ???????........C....T.........................T...T..T..TT.T..........G........T..T..AT...T......G.....G...TTT..........G....T.C...C..T..........C.A...CCT.....????????????
NH082   ????????????????????.T.........................T...T..T..TT.T..........G........T..T..AT...T......C.........TTT...........G...T.C...C..T..........C.A...CCT............AAC??
NH083   T..............C....T.........................T...T.....TT.T..........G........T..T..AT...T......G.....G...TTT...............T.C...C..T..........C.A...CCT.....????????????
 Albany Thicket Bioregion
NH084   ...............CG...T..........................T.T..C....TT.T..............G..T..T..TG.AT...T.............T..TTT............CG...C...C..T...C......C.A...CCT............AAC??
NH085   ...............CG...T..........G..C..............T.T..C.T..TT.T.................T..T..TG.AT...T.............T..TTT.............C....C...C..T...C......C.A...CCT............AACAC
NH086   ...............CG...T..........G..C..............T.T..C.T..TT.T.................T..T..TG.AT...TG............T..TTT.............C....C...C..T...C......C.A...CCT............AAC??
 Sub-Escarpment Grassland Bioregion
NH087   ...............C..A.T...TT...................T....T....T..T..TG..................T..T..AT...T.A...............T.T..........G.C........C..T..........C.A....CT.T......T...AACAC
NH088   ...............C....T...TT...................T....T....T..T..TG.........T.........T..T..AT...T.................T.T..........G.C........C..T..........C.A....CT........?????????
 Fouriesburg/Kasane
NH089   ??????????????????????????????...........A.............T..TT.TG........G....G....CT.....AT...T................T.T.G......G...C....C...C..?????????????????????????????????????
NH090   ...............C......C.....T.....C......A.......T.........CT.T.........G.......T.CT.....AT...T................T.T.G......G...C....C...C..T..........C.A...CCT............?????
NH091   ????????????????C......C.....T.....C......A.......T.........CT.T.........G....G..TTCT.....AT...T................T.T.G......G...C....C...C..T..........C.A...CCT......???????????
 Lowveld Bioregion
NH092   ????????????????C....T.C..T...........................C.T..TT.TG..................T...G.AT...T............G...T.T..T.........C....C...C.TT..........C.A...CCT....?????????????
NH093   ...............C....T.C..T.G.........................C.T..TT.TG..................T...G.AT...T................T.T..T.........C....C...C..T..........C??????????????????????????
NH094   ...............C....T.C..T...........................C.T..TT.TG..................T...G.AT...T............G...T.T..T.........C....C...C..T..........C.A...CCT............AACAC
NH095   T..............C......C..T.............C...............T..TT.TG..................T.....AT...T............G...T.T..T.........C....C...C..T..........C.A...CCT.......??????????
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NH096    ?????????.......C......C..T..............................T..TT.TG...................T...G.AT..TT................T.T..T.........C....C...C..T...........C.A...CCT.......??????????
 Bushmanland/Upper Karoo Bioregion
NH097    ................C....T.........G...C..........C..........T..TT.T.....G.......CG.G...T.TT..AT...T................T.T.......G....CG.......C..T....C......C.A..CCCT............AACAC
NH098    ?...............C....T.....A...G..TC..........C..........T..TT.T.....G.......CG.....T.TTG.AT...T........G.......T.T.......G....C?????????????????????????????????????????????????
NH099    ?????????.......C....T.........G...C..........C..........T..TT.T.....G.......C..G...T.TT..AT...T........G.......T.T...T...G....CG.......C..T....C......C.A..CCCT.......??????????
NH100    ................C....T.........G...C..........C..........T..TT.T.............CG.G...T.TT..AT...T........G...G...T.T.......G....CG.......C..T....C......C.A..CCCT............AACAC
NH101    ................C....T.C.......G...A..........C..........TA.TT.T.A...........CG.G...T.TT..AT...T........G.......T.T.......G....CG.......C..T....C......C.A..CCCT......???????????
NH102    ................C....T.........G...C..........C..........T..TT.T.............CG.G...T.TT..AT...T........G...G...T.T.......G....CGG......C..T....C......C.A..CCCT............AACAC
NH103    ???????????.....C....T.........G...C..........C..........T..TT.T.....G.......CG.....T.TTG.AT...T........G.......T.T.......G....CG.......C..T...........C.A....CT......???????????
NH104    ................C....T.....A...G...C......C...C..........T..TT.T.....G.......CG.....T.TTG.AT...T........G.......T.T.......G....CG.......C..T....C......C.A..C.CT............AACAC
NH105    ................C....T.....A...G...C.......G..C..........T..TT.T.....G.......CG.....T.TTG.AT...T........G.......T.T.......G....CG.......C..T....C......C.A..C.CT............AAC??
NH106    ................C....T.C.......G...A..........C..........T..T..T.A...........CG.G...T.TT..AT...T.....C..G.......T.T.......G....CG.......C..T....C......C.A..CC???????????????????
NH107    ................C....T.........G...C..........C..........T..TT.T.............CG.G...T.TT..AT...T........G...G...T.T.......G....CG.......C..T....C......C.A..CCCT............AACAT
 Eastern Kalahari Bushveld Bioregion
NH108    T...............C....TT.......................C..........T.TT..TG.............G.....T.....AT....................T.T...T........CG..G....C.G............C......CT.T.A........AACAC
NH109    T...............C....TT.......................C..........T.TT..TGA............G.....T.....AT...............G....T.T...T........CG..G....C.G............C.A....CT.T.A........AACAC
NH110    T...............C....TT.......................C..........T.TT..TG.............G.....T.....AT...............G....T.T...T........CG..G....C.G............C......CT.T..........AACAT
NH111    T...............C....TT.......................C..........T.TT..T..............G.....T.....AT...............G....T.T...T........CG..G......G............C......CT.T..........AACAC
NH112    T...............C.A..TT.......................C.........TT.TT..T..............G.....T.....AT...............G....T.T...T........CG..G....C.G............C......CT.T..........AACAC
NH113    T...............C....TT..........G............C..........T.TT..TG.............G.....T.....AT...............G....T.T...T........CG..G....C.G............C......CT.T..........AACAC
NH114    T...............C....TT..........G............C..........T.TT..TG.............G.....T.....AT...............G....T.T...T........CG..G....C.G............C.A....CT.T..........A????
NH115    T...............C....TT.......................C..........T.TT..TG...G.........G.....T......T...............G....T.T...T........CG..G....C..............C......CT.T..........AACAC
NH116    T...............C....TT.......................C..........T.TT..TG...G.........G.....T......T..............CG....T.T...T........CG..G....C..............C......CT.T..........AACAC
NH117    ???????????????CC....TT.......................C..........T.TT..TG...G.........G.....T......T...............G....T.T...T........CG..G....C..............C......CT.T..........AACAC
NH118    T...............C....TT.......................C..........T.TT..TG...G.........G..T..T......T...............G....T.T...T........CG..G....C..............C......CT.T..........AACAC
NH119    T...............C....TT.......................C..........T.TT..TG.............G.....T.....AT...............G....T.T...T........CG..G....C.G............C......CT.T..........AACAC
 Kalahari Duneveld Bioregion
NH120    T...............C..........A...G......C...A..........T...T..TT..G.............GT...C......AT...T................T.T............C........C..T......T.TC.C.AC.C.CT............AAC??
NH121    T...............C..........A...G..........A..............T..TT........T........T...C......AT...T................T.T.........C..CG.......C..T......T.T..C.AC.C.CT............AACAC
NH122    ?????????????...C..........A...G..........A..............T..TT..G.....T.......GT...C....G.AT...T...C............T.T.........C..CG.......C..T......T.T..C.AC.C.CT..........???????
NH123    T...............C..............G..........A..............T..TT........T........T...C......AT...T................T.T.........C..CG.......C..T......T.T..C.AC.C.CT............AACAC
NH124    ??????????????????????????.....G......C...A........A.T...T..TT..G.............GT...C......AT...T................T.T............C........C..T.......GT.GA?????????????????????????
NH125    T...............C..............G..........A..............T..TT........T........T...C....G.AT...T................T.T.........C..CG.......C..T......T.T..C.AC.C.CT............AACAC
NH126    T..........T....C..............G..........A..............T..TT........T........T...C......AT...T................T.T.........C..CG.......C..T......T.T..C.AC.C.CT............AACAC
NH127    T...............C..............G..........A..............T..TT........T........T..TC....G.AT...T................T.T.........C..CG.......C..T......T.T..C.AC.C.CT.....????????????
NH128    ??????..........C..............G..........A..............T..TT..G.....T.......GT...C....G.AT...T................T.T.........C..CG.......C..T......T.T..C.AC.C.CT............AACAC
NH129    T...............C..............G..........A..............T..TT........T........T.TTC....G.AT...T................T.T.........C..CG.......C..T......T.T..C.AC.C.CT............AACAC
NH130    ????????????....C..............G...C.C...CA..............T..TT..G......GC.....GT...C....G.AT....................T.T............CG.......C..T......T.T..C.AC.C.C??????????????????
 Koppies Dam
NH131    ??????..........C........................................T..TT.TG...................T...G.ATC..T................T.TA...........C....C...C..............CTA...CC??????????????????
 Macadodorp/Malelane
NH132    ???????????????????????????????....C.....................T..TT.TG.............G.....T.....AT...T................T.T............C....C..??????????????????????????????????????????
NH133    ................C......C................................TT..TT.TG...G...............T...G.AT...T............G....T.TA...........C....C...C..T...........C.A...CCT..T....??????????
NH134    ................C......C...A.............................T..TT.T....................TG..G.AT...T................T.TA...........C....C...C..T...........C.A...CCT..T??????????????
NH135    ??..............C......C.................................T..TT.TG...................T...G.AT...T................T.TA...........C....C...C..T...........C.A...CC...T.........AAC??
NH136    ??????????????????.....C.................................T..TT.TG...................T...G.AT...T................T.TA...........C....C...C..T...........C.A...CCT..T.........AACAC
 Volksrust
NH137    ????????????....CG...T............................T....C....TT.T..............G.....T.....AT....................T.T............CG...C...C..T....C......C.A...????????????????????
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Appendix 2.4 Biome-related frequencies and localities of 137 mitochondrial DNA cytochrome *b* haplotypes of *Micaelamys namaquensis* from southern Africa. Numbers in parentheses represent the number of individuals examined per locality. Haplotype order corresponds to the different groups that were identified in the phylogeographic analysis. Biome terminology follows that of Mucina and Rutherford (2006) while geographic coordinates of localities are indicated in Appendix 2.1.

HAPLOTYPE NUMBER	FREQUENCY OF HAPLOTYPE	LOCALITIES
Savanna Biome		**17**
NH001	1	Baltimore (1)
NH002	3	Alldays (1), Botswana: Terrafou (2)
NH003	1	Blouberg Nature Reserve (1)
NH004	1	Botswana: Terrafou (1)
NH005	1	Botswana: Elephant Sands (1)
NH006	1	Botswana: Francistown (1)
NH007	1	Musina Nature Reserve (1)
NH008	1	Musina Nature Reserve (1)
NH009	1	Botswana: Terrafou (1)
NH010	2	Botswana: Terrafou (2)
NH011	1	Botswana: Francistown (1)
NH012	1	Musina Nature Reserve (1)
NH013	2	Musina Nature Reserve (2)
Nama-Karoo Biome		**118**
NH014	24	Upington (10), Augrabies (9), Gariep Nature Reserve (1), Botswana: Kasane (1), Kirkwood (1), Kakamas (2)
NH015	1	Springbok (1)
NH016	3	Upington (3)

NH017	3	Upington (2), Augrabies (1)
NH018	4	Upington (4)
NH019	7	Kimberley (1), Hoopstad (2), Botswana: Kasane (3), Bloemfontein (1)
NH020	1	Pofadder (1)
NH021	3	Loxton (3)
NH022	1	Springbok (1)
NH023	1	Hopetown (1)
NH024	1	Upinton (1)
NH025	9	Upington (9)
NH026	1	Lady Grey (1)
NH027	18	Upington (18)
NH028	1	Hopetown (1)
NH029	2	Augrabies (2)
NH030	11	Gariep Nature Reserve (3), Karoo National Park (2), Upington (4), Augrabies (1), Botswana: Kasane (1)
NH031	1	Loxton (1)
NH032	2	Bloemfontein (1), Brandfort (1)
NH033	1	Oudtshoorn (1)
NH034	3	Kirkwood (2), Porterville (1)
NH035	9	Oudtshoorn (4), Karoo National Park (1), Gariep Nature Reserve (1), Lady Grey (1), Matjiesfontein (2)
NH036	1	Jamestown (1)
NH037	1	Willem Pretorius Nature Reserve (1)
NH038	1	Oudtshoorn (1)
NH039	1	Kirkwood (1)

NH040	1	Wepener (1)
NH041	1	Pofadder (1)
NH042	1	Springbok (1)
NH043	3	Springbok (3)
NH044	1	Boshof (1)
Grassland Biome		**41**
NH045	3	Ezemvelo Nature Reserve (1), Boshoek (1), Kgaswane Mountain Reserve (1)
NH046	3	Lapalala Nature Reserve (2), Rooibokkraal (1)
NH047	1	Boshoek (1)
NH048	2	Lajuma Mountain Retreat (1), Schweizer-Reneke (1)
NH049	1	Ellisras (1)
NH050	1	Brits (1)
NH051	2	Kgaswane Mountian Reserve (1), Ventersdorp (1)
NH052	2	Kgaswane Mountain Reserve (2)
NH053	1	Lapalala Nature Reserve (1)
NH054	1	Hoedspruit (1)
NH055	1	Hoedspruit (1)
NH056	1	Machadodorp (1)
NH057	1	Ezemvelo (1)
NH058	3	Amsterdam (2), Josefsdal Nature Reserve (1)
NH059	1	Kruisrivier Nature Reserve (1)
NH060	1	Kruisrivier Nature Reserve (1)
NH061	1	Potchefstroom (1)
NH062	1	Brits (1)
NH063	1	Brits (1)

NH064	1	Bela-Bela (1)
NH065	1	Brits (1)
NH066	1	Musina Nature Reserve (1)
NH067	1	Lajuma Mountain Retreat (1)
NH068	2	Thabazimbi: Waterval (1), Ben Alberts Nature Reserve (1)
NH069	3	Vredefort (3)
NH070	1	Ezemvelo Nature Reserve (1)
NH071	1	Vredefort (1)
NH072	2	Botswana: Gaborone (2)
Fynbos Biome		**17**
NH073	2	Porterville (2)
NH074	1	Robertson (1)
NH075	2	Cederberg: Jamaka (2)
NH076	1	Cederberg: Boscherberg (1)
NH077	3	Jonaskop (2), Die Galg (1)
NH078	2	Porterville (2)
NH079	1	Cederberg: Boscherberg (1)
NH080	2	Napier (2)
NH081	1	Riversdale (1)
NH082	1	Porterville (1)
NH083	1	Grabouw (1)
Albany Thicket Bioregion		**5**
NH084	2	Mount Currie Nature Reserve (2)
NH085	2	Andries Vosloo Kudu Reserve (2)
NH086	1	Andries Vosloo Kudu Reserve (1)

Sub-Escarpment Grassland Bioregion		**3**
NH087	2	Ongeluksnek Nature Reserve (2)
NH088	1	Burgersfort (1)
Fouriesburg/Kasane		**4**
NH089	1	Botswana: Kasane (1)
NH090	2	Fouriesburg (2)
NH091	1	Fouriesburg (1)
Lowveld Bioregion		**8**
NH092	1	Newcastle (1)
NH093	1	Pongola (1)
NH094	4	Ithala Nature Reserve (1), New Castle (3)
NH095	1	Swaziland: Matenga Nature Reserve (1)
NH096	1	Swaziland: Matenga Nature Reserve (1)
Bushmanland/Upper Karoo Bioregion		**33**
NH097	3	Postmasburg (1), Hotazel (1), Schweizer-Reneke (1)
NH098	1	Grootdrink (1)
NH099	1	Groblershoop (1)
NH100	20	Schweizer-Reneke (12), Vryburg (7), Willem Pretorius Nature Reserve (1)
NH101	1	Soetdoring Nature Reserve (1)
NH102	1	Schweizer-Reneke (1)
NH103	1	Groblershoop (1)
NH104	1	Griekwastad (1)
NH105	2	Kimberley (1),Griekwastad (1)
NH106	1	Boshof (1)
NH107	1	Schweizer-Reneke (1)

Eastern Kalahari Bushveld Bioregion		**82**
NH108	3	Vryburg (3)
NH109	1	Tosca (1)
NH110	2	Schweizer-Reneke (2)
NH111	2	Severn (2)
NH112	2	Vanzylsrus (1), Kuruman (1)
NH113	23	Upington (7), Tswalu Kalahari Reserve (6), Vorstershoop (3), Vanzylsrus (3), Vryburg (1), Severn (2), Stella (1)
NH114	2	Vryburg (1), Upington (1)
NH115	24	Upington (4), Stella (7), Severn (2), Kuruman (5), Vanzylsrus (4), Vryburg (2)
NH116	6	Tosca (4), Stella (1), Vryburg (1)
NH117	1	Stella (1)
NH118	5	Vanzylsrus (1), Stella (2), Vryburg (2)
NH119	11	Schweizer-Reneke (5), Vanzylsrus (6)
Kalahari Duneveld Bioregion		**25**
NH120	3	Gibeon (2), Bergville (1)
NH121	2	Gelukspruit (2)
NH122	2	Kakamas (2)
NH123	4	Kakamas (3), Augrabies (1)
NH124	1	Dwesa Nature Reserve (1)
NH125	5	Ais-Ais (1), Karasburg (2), Keetmanshoop (1), Noenieput (1)
NH126	1	Upington (1)
NH127	1	Askham (1)
NH128	1	Keetmanshoop (1)
NH129	4	Askham (1), Upington (2), Augrabies (1)

NH130	1	Windhoek (1)
Koppies Dam		**1**
NH131	1	Koppies Dam Nature Reserve (1)
Machadodorp/Malelane		**5**
NH132	1	Machadodorp (1)
NH133	1	Malelane (1)
NH134	1	Malelane (1)
NH135	1	Machadodorp (1)
NH136	1	Malelane (1)
Volksrust		**1**
NH137	1	Volksrust (1)