Patterns and processes underlying genetic diversity in the Namaqua rock mouse *Micaelamys namaquensis* Smith, 1834 (Rodentia: Muridae) from southern Africa

By

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Submitted in partial fulfilment of the requirements for the degree Philosophiae Doctor in the Faculty of Natural and Agricultural Sciences

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November 2009

Declaration

I, the undersigned, hereby declare that this thesis, which I hereby submit for the degree Philosophiae Doctor at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tetiary institution.

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SUMMARY

African rock rats of the genera *Aethomys* Thomas, 1915 and *Micaelamys* Ellerman, 1941, are endemic to East, Central and southern Africa but extend marginally into West Africa. In the past 16 subspecies have been described in the Namaqua rock mouse *M. namaquensis* Smith, 1834. Recent morphometric and morphological patterns of intraspecific variation suggested the recognition of only four subspecies: *M. n. namaquensis*, *M. n. alborarius*, *M. n. monticularis* and *M. n. lehocla*, of which the distributions appeared to coincide with the major phytogeographical zones of southern Africa. In the present study earlier analyses of mitochondrial DNA (mtDNA) cytochrome *b* (cyt *b*) gene variation were extended. Taken together these results show that *M. namaquensis* represents a polytypic species complex but with much more diversity than detected using morphology. Phylogenetic and phylogeographic analyses revealed 14 genetically distinct lineages of which several show strong geographic association with particular vegetation biomes or bioregions. The distributional ranges of eight of these lineages showed some correspondence with the type localities of previously described subspecies of *M. namaquensis*. Some clades displayed considerable within-lineage variation indicating possible fine-scale population structuring, while others showed very little differentiation. Divergence times between lineages varied between 7.26 MYA and 2.70 MYA, corresponding to a Late Miocene to Pliocene radiation. Cytochrome *b* sequences alone do not fully resolve the evolutionary relationships among the lineages and the phylogenetic analysis was thus supplemented with nuclear Recombination Activating Gene 1 (RAG1) sequences. This gene was successfully sequenced for 11 of the identified lineages. Independent analyses of the two genes were not congruent possibly as a result of incomplete lineage sorting of the nuclear gene. The combined dataset yielded good support for six of the lineages. Finally, a more detailed phylogeographic analysis was conducted among ten localities of the Eastern Kalahari Bushveld lineage based on mitochondrial cyt *b* sequences to elucidate processes underlying diversification in this species complex. A genetic pattern of phylogenetic continuity with a lack of spatial separation was observed. The mismatch distribution analysis suggests that the lineage has expanded its population size and the geographical expansion may have followed environmental changes in the recent past. Estimates of female gene flow indicate connectivity among localities but not to the extent expected for a panmictic population. Instead a combination of a stepping-stone model and metapopulation dynamics may be applicable to this lineage. Examination of

type material of described subspecies is needed to resolve the identity of the unique lineages which will allow us to better understand the phylogeography and mode of speciation in *M. namaquensis*. In addition, localities of sympatry (lineages in sympatry) should also be studied in more detail in order to help resolve the current taxonomic uncertainties within *M. namaquensis*. Future research should therefore include a multidisciplinary approach, such as cytogenetics, morphology and more gene regions.

For my sister, Rethana Russo

Thank you for your support, encouragement and unconditional love

ACKNOWLEDGEMENTS

I would firstly like to thank my supervisors Paulette Bloomer and Chris Chimimba for giving me this invaluable opportunity. Thank you both for all your advice and expertise, which made the completion of this thesis possible. Paulette, a special thank you for your support and understanding especially during the difficult times over that last two years, and for all the hours that you have invested in our manuscript. Chris, thank you for the fast turn-around time with the chapter edits and for your very useful comments on the earlier drafts of this thesis. An added thank you for the emotional support when I really needed it, it really is appreciated. I am also grateful to both of you for giving me the freedom to test my thoughts and ideas and in the end making it my own.

Secondly I would like to thank Duncan MacFadyen (E. Oppenheimer & Son), Nico Avenant (National Museum, Bloemfontein) and Johan Watson (Free State Department of Environmental Affairs and Tourism) for providing tissue samples. Special thanks also go to Sam Ranthakwe and Jackson Kone at the Transvaal Museum for their hard work in sample preparation. To Alicia Linzey, Michael Kesner and Arthur Hulse, I appreciate all the time that you have spent with me in the field, which in the end, greatly helped in filling gaps in my sampling efforts.

My sincere appreciation goes to all the farmers across southern Africa who showed such generous hospitality, friendliness, and assistance in the field. Not only did the farmers open up their homes to me with regard to accommodation and fantastic home-cooked food, “regte boere kos,” but they also showed great interest in what I was trying to achieve. My thanks also go to the various owners and managers of game farms and nature reserves, who allowed me to sample on their land.

I am especially grateful to the following people for field assistance: Rethana Russo, Sandrina dos Santos, Carel Oosthuizen, Wayne Delport, Ernst Swartz and Ingrid Stermann. Although some of them were more willing and enthusiastic than others, every little bit of help was greatly appreciated. I will always remember the fun times we had in the field.

To all my friends and colleagues from the Molecular Ecology and Evolution Programme (MEEP), thank you for all the laughs and tears. I would particularly like to thank Carel Oosthuizen, Sandrina dos Santos and Amanda Maswanganye for their helpful advice and companionship over these past years. Alexandra Jansen van Rensberg is thanked for helping with the long and tedious job of formatting the data for analyses in Chapter 2.

Ouma Minique de Castro, Vinet Coetzee and Tyron Grant, you have all been instrumental in my life in some way or other. Minique and Vinet thank you for all the laughs, cups of coffee and chats when I needed them most. Tyron, you will remain one of my closest friends, and for this I am eternally grateful. You phoned when I needed an ear to listen, you were always keen to have beer when I needed to relax and were always encouraging and positive when I was at my lowest, this is what friendship is all about, good and bad weather friends. I would like to extend a special thank you to my "omgeegroupie" over the last two years, your support and faith in me has been unwavering.

To somebody who without fail has been amazing in his support and understanding as I finished this thesis. Clarke Scholtz, thank you for providing me with the time and facilities to finish my thesis while still in your employment, your support is appreciated more than words can say. You are an example to us all.

To Catherine Sole thank you for your help with the data analyses and invaluable understanding of the processes involved behind them. I would like to extend a very personal thanks as you've not only been a role model in terms of science and perseverance in the field but you've also become one of my closest friends. Not only did you read through many of the chapters of this thesis, without complaint, but were always willing to RE-READ and comment on the newer versions as the thesis progressed. I'll miss all our endless discussions on the grass next to the Zoology building next year but will definitely visit when I am back in South Africa.

To Emilie Biossin, Thierry Hoareau and Christian Pirk, you came from far corners of the world to South Africa and even though we have only known each other for a very short time period, your friendship has been invaluable to me over the last couple of months. Emilie and Thierry through countless discussions you have provided me added insight that I would never otherwise have gained. Hope to see you in France next year! Christian your

support, understanding and advice through the difficulties involved in this thesis is greatly appreciated. A special thank you goes to Tim (Timothy) Bray who helped with editing of our manuscript, aside for this Tim you helped pull me through these emotional and stressful times. In your own way you have taught me many life lessons which made me look at life through different eyes and in a much more positive way. Your jokes, funny text messages and laughs have kept me sane over the last eight or so months. When passing through Wales stop by at Issie's doorstep for, weather permitting, a hot chocolate or an ice-cream!

To my parents, I would like to express my gratitude for the financial assistance and for your love. I know at times you wished this thesis would just finish itself and you found it difficult to understand that a thesis could take this long but thanks for your unwavering support. Dad, you can now as a very proud father refer to me as "Dr. Russo". Mom and dad, I greatly appreciate your monetary assistance in getting me to this point in my academic career, where I have the freedom to make decisions that will benefit me in my career, without the difficulty of having debt. Thank you also for making it possible for me to have gone to South America, I really needed the break emotionally and mentally. "Dankie" Oom Sakkie Nel, for all the break-aways which helped refocus my attention on my thesis. To Erika Thompson, through supporting my father you have given me the support I need, and for this I thank you.

To my sister, Rethana Russo, thank you so much for taking over all the household responsibilities during my busiest times. Thanks also to you and Victor Enslin for all the little things that you helped me with, they may have seemed small and insignificant at the time, but without your assistance, I don't think this thesis would have ever been completed. Victor to late nights and patience with regard to checking the references of this thesis, I thank you. Rethana, thank you for all of your love and support and for not only being my sister but also one of the best friends any one could ask for.

Finally, I would like to thank God.

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Chapter 1

General Introduction

1. General

The evolutionary history of species and populations is the product of processes occurring over two time scales: evolutionary time - being based on broad-scale changes under specific environmental conditions with associated selective pressures and ecological time - over which population processes (e.g., demographic changes, migration, local extinction and colonisation) occur (Martin and Simon, 1990; Carroll et al., 2007). Evolutionary biology aims at unraveling these interactions and assessing the importance of the processes. Understanding evolutionary processes is brought about through the study of closely related taxa (Martin and Simon, 1990). Genetic structure of a population can therefore be correlated with both biogeographical factors as well as ecological and demographic processes (Carisio et al., 2004). Our understanding of species formation is based on population level comparisons; by examining the variation among these populations, their historical associations and the processes of genetic restructuring that may have lead to speciation can often be revealed (Knowles 2004; Knowles and Maddison 2002; Wright, 1931).

Biological diversity can be meaningfully divided into least common evolutionary denominators, namely, the 'species' (Hendry et al., 2000). Delineating distinct species is often problematic but most biologists agree with Mayr (1957) that "the living world comprises more or less distinct entities that we call species". Taxonomic groups have historically been identified using morphological criteria but over the last couple of decades molecular techniques have provided a powerful tool for evaluating the validity of taxonomic units (Avise and Walker, 1999). Avise and Walker (1999) used patterns of mitochondrial DNA (mtDNA) variation to argue that mtDNA discontinuities and traditional taxonomic designations tend to converge which in turn may reveal real biotic units.

Speciation has been described as the evolutionary process by which new biological species arise (Mayr, 1942). Geographic models of speciation in nature have been described based on the extent to which populations are geographically isolated: allopatric (physical barrier separates populations), peripatric (species are formed in isolated, small peripheral populations that are prevented from exchanging genes with the main population),

parapatric (zones of two diverging populations are separate but do overlap) and sympatric (population sharing a geographic location is forced by environmental factors to diverge). The large genetic and phenotypic diversity observed within species is necessary for evolution to create new reproductively isolated species (Härdling et al., 2009). Although most biologist believe that reproductive isolation is the driving force behind species, it has been shown that reproductive isolation alone is not sufficient to permit coexistence of two species at the same locality (Mayr, 1949). Species should also be different in their ecological requirements to avoid competition (Crombie, 1947). Speciation then means the evolution of reproductive isolation as well as of ecological differentiation between populations. To this day, processes and mechanisms involved in speciation are still much debated.

There are many species concepts such as the phylogenetic species concept (PSC), the biological species concept (BSC), the evolutionary species concept (ESC), the cohesion species concept (CSC), the ecological species concept (ESC), the genetics species concept (GSC) and many others (Cracraft, 1989; Dobzhansky, 1940; Mayr, 1940; Mayr, 1942; Simpson, 1961; Schluter, 1998; Schluter, 2001; Templeton, 1989; Wiley, 1978). Regardless of the species concept (reviewed by De Queiroz, 2007; Mayden, 1997) chosen, biologists are confronted with the question of how much difference (or amount of isolation) defines a species (Hendry et al., 2000). The BSC states that "species are groups of interbreeding populations which are reproductively isolated from other such groups" (Mayr, 1940). If 100% reproductive isolation is used as a criterion for applying the BSC, then the identification of species would be relatively straight-forward (Hendry et al., 2000). On the other hand, if the BSC was universally adopted, many of the current taxonomic species would no longer be recognised due to hybridisation and introgression (see Petit and Excoffier, 2009) in populations in the wild (Hendry et al., 2000; Niemiller et al., 2008; Nosil, 2008).

While the analysis of geographic variation in widely distributed species may lead to the recognition of distinct aggregates of local populations, a problem arises in deciding whether such aggregates represent species or subspecies (Mayr, 1997; Mayr and Ashlock, 1991). The problem is exacerbated by the numerous definitions that have been proposed to define species as mentioned above. For example, the BSC defines species as "interbreeding natural populations that are reproductively isolated form other such

populations" (Dobzhansky, 1940; Mayr, 1942), while the PSC (Cracraft, 1989) considers a species as "a cluster of organisms, diagnosably distinct from other such clusters". The cohesion species concept (CSC) on the other hand, defines species as "the most inclusive population of individuals having the potential for phenotypic cohesion through intrinsic cohesion mechanisms" (Templeton, 1989). Ecological speciation states that natural selection on traits between populations in different environments leads to the evolution of reproductive isolation and as a consequence species (Schluter, 2001). The genetic species concept defines a genetic species as a group of genetically compatible interbreeding natural populations that is genetically isolated from other groups (Baker and Bradley, 2006). The GSC differs from the BSC that the focus is rather on generic isolation than reproductive isolation (Baker and Baker, 2006). The BSC and its variations, is the most widely used in mammals and are thus followed in this study. Some of the variation observed in the present study was explained using the ESC and GSC.

Biologists should avoid the notion of discrete categories into which organisms should be allocated and rather emphasise the level of variation within and among groups of organisms (Hendry et al., 2000). Species descriptions should thus be based on the geographic distribution of genetic diversity (Buth and Mayden, 1981; Johns and Avise, 1998). In addition, multidisciplinary approaches should preferentially be used in the verification/identification of valid biological species such as a combination of cytogenetics, classical morphology and morphometrics together with multi-gene molecular data (Huchon et al., 2005; Taylor et al., 2009).

Similarly, several problems exist in defining subspecies because of different criteria that have been proposed (Moritz et al., 1987; O'Brien and Mayr, 1991; Ryder, 1986). These range from the typological subspecies definition to those identifying distinct populations based on the conservation biology concepts of Evolutionarily Significant Units (ESUs) and Management Units (MUs) (reviewed by Fraser and Bernatchez 2001; Moritz, 1994b; Ryder, 1986; Ryder et al., 1988). The role of ESUs and MUs in biological classification has generally been limited to recognising intraspecific diversity, which is useful in the conservation and management of endangered and exploited species (Moritz, 1994a).

For the present study, Lidicker's (1962) subspecies definition was followed. It defines a subspecies as "a relatively homogenous and genetically distinct portion of a species which

represents a separately evolving, recently evolved lineage with its own evolutionary tendencies, inhabits a definite geographical area, is usually at least partially isolated, and may integrate with adjacent subspecies". Linked to the BSC, this subspecies definition may be useful in cases where it is difficult to test for reproductive isolation (Lidicker, 1962). Diversification at specific and subspecific levels is driven by a combination of intrinsic and extrinsic processes (deLong, 1967; Martens, 1997; Rogers and Bernatchez, 2007).

Detailed paleo-climatic records, extraordinary fossil discoveries and advanced analysis of extant fossil data have focused on the possible role that changes in African climate may have had in the evolutionary history of African mammalian fauna (deMenocal, 2004). Biological traits may evolve under the influence of a variety of selective forces such as environmental constraints (Ryan and Brenowitz, 1985). Large scale shifts in climate have altered the ecological composition of the landscape which, in turn, manifested specific faunal adaptation or speciation pressures leading to genetic selection (deMenocal, 2004) reflecting a long history of responses to habitat changes (Riddle, 1996). Small mammals would be greatly affected by environmental variables and climatic changes, such as those seen across the African continent and thus represent good models to understand the evolutionary past and to make predictions about potential future changes in the face of natural and anthropogenic environmental changes.

More specifically, the present study focuses on the southern African subregion which consists of a wide range of biomes. Although other geographical features like altitude and precipitation may play a role in the diversification between *M. namaquensis* lineages, I used biomes/bioregions for comparison with previous work (Chimimba, 2001a). The relatively moist, mostly winter-rainfall region, includes the Fynbos biome in the west. The drier Succulent Karoo biome forms the smallest of the world's six floristic kingdoms (Cox, 2001) and is distributed across the sandy lowlands of the south-western Cape (Mucina and Rutherford, 2006). It also occurs in the Richtersveld, Namaqualand and the Little Karoo. Savanna from the summer-rainfall region on the north and east of the subregion represents the southern extension of the largest biome in Africa (Mucina and Rutherford, 2006). The summer-rainfall Grassland biome occurs on the cooler, elevated interior of South Africa (Low and Rebelo, 1996). The mostly summer-rainfall Nama-Karoo biome is possibly the least species-rich and is confined to the western parts of the subregion. Desert occupies the

north-western parts of southern Africa. Thicket represents an unusual structural, floristic and evolutionary ancient biome on the subcontinent (Low and Rebelo, 1996). Afrotemperate forest in southern Africa are highly distinctive and are characterised by their small and patchy occurrence over the wetter parts of both the winter and summer-rainfall areas of the region (Mucina and Rutherford, 2006). A biome map is presented in Chapter 2 (see Fig. 2.1 for details). Both biomes and bioregions were considered for this study. A biome is therefore the largest land community unit recognised at a continental or sub-continental scale. Bioregions are focussed on plant diversity within biomes (i.e. a finer scale of resolution of each biome). Since some of the lineages corresponded to bioregions at a finer scale, I included both biomes and bioregions to explain the relationships between lineages and biomes/bioregions.

Uplifts affected African topography during the late Neogene (8 to 2 MYA) of which the most important topographic structure is the East African Rift System (Sepulchre et al., 2006; Axelrod and Raven, 1978). The western branch of the East African Rift System started to develop during the middle-late Miocene. This event initiated the formation of the central Tanganyika Basin at about 12 to 10 MYA (Cohan et al., 1993). More recent uplifts between 5 and 2 MYA were active in the Tanganyika and Malawi rifts (Ebinger et al., 1993). The major Tanzanian escarpments were present at 3 MYA (Foster et al., 1997). The Karoo plateau in South Africa has been raised during the past 5 million years. These uplifts have not only contributed to changes in climate but have also contributed to two major episodes of rapid speciation in South Africa (Axelrod and Raven, 1978). Uplifts gave rise to strong aridification and paleoenvironmental changes (Sepulchre et al., 2006).

Other extrinsic barriers that may influence genetic diversity are physical barriers like rivers and mountains. It has been suggested that the Orange River/Holgat River may act as a barrier to dispersal and consequently result in the genetic differentiation of insect populations (Sole et al., 2005). More specifically, rivers have also been cited as factors affecting the distribution of African mammal species (Quérouil et al., 2003; Telfer et al., 2003). Likewise, from a topographic perspective, the Drakensberg mountain range, may act as a barrier to gene flow between populations of small mammals on the eastern and western sides of the escarpment (Chimimba, 2001a).

2. African Rock Rats

2.1 General Background

African rock rats of the genera *Aethomys* Thomas, 1915a, and *Micaelamys* Ellerman, 1941, are long-tailed murid rodents of medium to large size (Chimimba and Bennett, 2005; Meester et al., 1986). The name *Aethomys* is derived from the Greek word *aithos*, meaning sunburnt and *mys*, meaning mouse (De Graaff, 1981; Rosevear, 1969). These genera represent a diverse group of rodents endemic to East, Central and southern Africa, with a marginal extension into West Africa (Chimimba and Bennett, 2005; Musser and Carleton, 2005). Originally, the genera *Aethomys* and *Micaelamys* were considered subgenera within the genus *Aethomys* (Chimimba and Bennett, 2005). Although *A. namaquensis* Smith, 1834, and *A. granti* Wroughton, 1908, have traditionally been allocated to the subgenus *Micaelamys*, recent molecular studies (Castiglia et al., 2003; Ducroz et al., 2001; Russo, 2003) and data on albumin fixation (Watts and Baverstock, 1995) have provided evidence for the paraphyly of the genus. As a consequence, the subgenera *Micaelamys* and *Aethomys* have been elevated to full generic rank (Chimimba and Bennett, 2005). The genus *Micaelamys* includes *M. namaquensis* and *M. granti*. The genus *Aethomys* is currently considered to include nine species, namely *A. silindensis* Roberts, 1938, *A. chrysophilus* De Winton, 1897, *A. ineptus* Thomas and Wroughton, 1908, *A. nyikae* Thomas, 1897, *A. bocagei* Thomas, 1904, *A. hindei* Thomas, 1902, *A. stannarius* Thomas, 1913, *A. thomasi* De Winton, 1897, and *A. kaiseri* Noack, 1887, (Chimimba et al., 1999; Musser and Carleton, 2005).

Although there is little biological data available, members of these genera are nocturnal, terrestrial and to some extent arboreal, living either in pairs or in small family units (Chimimba and Bennett, 2005). They utilise a wide variety of covered habitats, ranging from rock crevices, rocky terrains and outcrops, excavated burrows, tree trunks, grassland with some form of cover, to open savanna woodland (Chimimba and Bennett, 2005). Some species are well adapted to hot, arid environments while some aestivate during drier months (Withers et al., 1980). While they do not generally associate with humans to the same extent as for example the house mouse (*Mus musculus*), they may do so in agriculturally developed areas (Chimimba and Bennett, 2005). Little is known about their feeding biology, but all species currently included in the two genera are considered to be

omnivorous with a preference for seed, fruit and grain (Chimimba and Bennett, 2005; Watson, 1987; Woodall and Mackie, 1987). *Micaelamys namaquensis* (formerly known as *A. namaquensis*) is also known to forage on flowering heads of *Protea amplexicaulis* and *P. humiflora*, with a preference for their nectar and in the process act as pollinators for these plants (Johnson et al., 1999). *Aethomys chrysophilus* on the other hand, has a preference for grain crops and legumes such as sorghum, peas and beans (Chimimba and Bennett, 2005).

Some members may breed throughout the year with an average of three offspring per litter, while others show no evidence of breeding during colder months (Chimimba and Bennett, 2005; Rautenbach, 1978). They have a short generation time (0.16 to 0.33 years) producing as many as four litters by a single female (C.T. Chimimba pers. comm.). They also tend to have unstable population cycles associated with high mortality and high reproductive potential (Withers et al., 1980), resulting in population eruptions that have been implicated in causing epidemiological problems (Gear et al., 1966; Hallet et al., 1970; Swanepoel et al., 1978) and extensive damage to crops and stored grain (De Graaff, 1981; Smithers, 1971; Wilson, 1970, 1975).

2.2 Systematics

Taxonomically, the genus *Aethomys* has undergone a number of nomenclatural changes (Rosevear, 1969). Thomas (1915a) proposed *Aethomys* as a subgenus of *Rattus* and later elevated it to full generic rank (Thomas, 1915b). Ever since the genus was proposed, the erection of subgenera, the taxonomic allocation of species and the description of numerous subspecies have been the source of systematic uncertainty, necessitating a revision of the genus. This taxonomic uncertainty has been exacerbated by morphological conservatism but a high degree of chromosomal diversity within the genus (Visser and Robinson, 1986; 1987). For example, *A. chrysophilus*, traditionally considered a single species, was shown to contain two electrophoretically distinct cytotypes ($2n = 50$ and "slow" haemoglobin; $2n = 44$ and "fast" haemoglobin) (Baker et al., 1988; Gordon and Rautenbach, 1980; Gordon and Watson, 1986; Visser and Robinson, 1986, 1987). The two cytotypes have also been shown to differ in gross sperm and bacular morphology (Breed et al., 1988). Given the lack of evidence of hybrids, these studies strongly suggested the presence of two sibling species.

Subsequently, Chimimba et al. (1999) undertook an analysis of both morphometric and morphological patterns of inter- and intraspecific variation within the genus (*Aethomys senso lato*) across a more comprehensive geographical coverage in southern Africa than has previously been considered. Chimimba (2005) also undertook a phylogenetic appraisal of the then 11 recognised species. These studies recognised *A. namaquensis*, *A. granti* and *A. silindensis* as valid species. Furthermore, the two cytotypes within *A. chrysophilus* were formally allocated to the nominate species, *A. chrysophilus* for the $2n = 50$ cytotype and a newly recognised *A. ineptus* for the $2n = 44$ cytotype. Subsequently, Russo et al. (2006) examined mitochondrial DNA (mtDNA) cytochrome *b* (cyt *b*) genetic variation in these cryptic murid rodent species. Phylogenetic and phylogeographic analyses showed reciprocal monophyly between populations of the two species in southern Africa, but no support for monophyly of *A. chrysophilus* from southern and eastern Africa (Russo et al., 2006). This suggested that the analysis of mtDNA can be used to distinguish these sister species in southern Africa (Russo et al., 2006).

The above mentioned phylogenetic analysis (Chimimba, 1997, 1998) suggested *Aethomys* to be monophyletic with the retention of *Aethomys* and *Micaelamys* as subgenera, the latter consisting of *A. namaquensis* and *A. granti* and all remaining species allocated to the nominate subgenus *Aethomys* (Chimimba, 1998; Chimimba et al., 1999). Ducroz et al. (2001), however, recently suggested that *Aethomys* may be paraphyletic, with *A. namaquensis* as the most basal member of a clade that would combine arvicanthine rodents and the other African murines. Other molecular studies have also suggested that the genus *Aethomys* may be paraphyletic (Castiglia et al., 2003; Lecompte et al., 2008; Russo, 2003). Worth noting is that *M. namaquensis* (formerly known as *A. namaquensis*) was previously placed in the genus *Thallomys* (Ellerman, 1941).

Earlier reports (e.g., Meester et al., 1964; Roberts, 1951) recognised 16 subspecies within *A. namaquensis.* These subspecies designations were made with little or no assessment of patterns of geographic variation over the entire distributional range of the species in southern Africa. The intraspecific designations were also based on non-statistical comparisons of type material and/or small, geographically restricted samples (Smithers, 1971; Smithers and Wilson, 1979).

Prior to an intraspecific morphometric study within *A. namaquensis* (Chimimba, 2001a), the nature and extent of geographic variation within the species remained unknown. The morphometric analysis of intraspecific variation within *A. namaquensis* suggested the recognition of only four subspecies: *A. n. namaquensis*, Smith, 1834, *A. n. lehocla* Smith, 1836, *A. n. monticularis* Jameson, 1909 and *A. n. alborarius* Peters, 1852 (Chimimba, 2001a). The morphological discontinuities of these suggested subspecies broadly coincided with the major biomes of southern Africa (Chimimba, 2001a). 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, *monticularis* with Grassland, *alborarius* with Savanna and *lehocla* with Nama-Karoo (Chimimba 2001a. Morphometric variation within *A. chrysophilus* on the other hand, suggested the recognition of two subspecies, *A. c. chrysophilus* Thomas and Wroughton, 1908, and *A. c. imago* Thomas, 1927, whose distributions coincided with an altitudinal limit in the eastern parts of southern Africa (Chimimba, 2000). Geographic variation within *A. ineptus* and *A. granti* were shown to be clinal where cranial size within *A. ineptus* was positively and significantly correlated with longitude, while *A. granti* showed a southwestern-northeasterly clinal pattern of variation (Chimimba, 2001b; Chimimba et al., 1998).

A recent molecular study among 16 localities of *A. namaquensis* (currently known as *M. namaquensis*; Russo, 2003) confirmed that the species is polytypic but higher levels of variation than previously detected were revealed. This study showed some support for three of the four morphometrically-defined subspecies from the Chimimba (2001a) study: 1) a lineage found in the Limpopo valley and Botswana corresponding to the Savanna biome of southern Africa; 2) a lineage widely distributed across the Upper/Lower Karoo and 3) a lineage found across the Grassland biome of southern Africa. In addition, several unique and well-supported lineages defined by the molecular data were not concordant with the morphometrically-defined subspecies (Chimimba, 2001a). Furthermore, while some lineages showed considerable molecular sequence variation across the geographic area sampled, other lineages showed very little differentiation. These results lend support to earlier suggestions for the presence of a species complex within *M. namaquensis* from southern Africa.

Although the findings in the recent revision (Chimimba, 2000, 2001a, b, 2005) may be valid, these need to be independently tested using additional character sets before robust systematic conclusions can be drawn. It is clear in modern systematics that the resolution of taxonomic uncertainties is best achieved by using a multidisciplinary approach (Ducroz et al., 2001). To this end, the present study independently tests the findings of the morphologically based systematic revision by Chimimba et al. (1999) and intraspecific hypotheses (Chimimba, 2001a; Meester et al., 1964; Roberts, 1951; Smithers, 1971; Smithers and Wilson, 1979) by using molecular data following both phylogenetic and phylogeographic approaches. The main focus of the present study is on *M. namaquensis* from southern Africa.

2.3 Palaeontology

Fossils representing *A. namaquensis* Smith, 1834 (currently known as *M. namaquensis*; see Chimimba and Bennett 2005), and *A. chrysophilus* (*senso lato*) De Winton, 1897, have been described from South Africa (Avery, 1981, 1982, 1985; De Graaff, 1960; 1961; Hendey, 1981; Pocock, 1987). Recently, two fossil species, a small-sized *A. modernis* and a large-sized *A. adamanticola*, the oldest known representatives of the genus in Africa, were reported from Langebaanweg, Western Cape Province, South Africa (Denys, 1990a, b).

Aethomys modernis is very similar to extant *A. chrysophilus* (*senso lato*), while *A. adamanticola* is different from any other known *Aethomys*, but shows characteristics reminiscent of *A. namaquensis* and *A. hindei* (Denys, 1990a, b). Denys (1990a; b) suggested that this species may represent an advanced stage of an Early Miocene lineage closely related to *Dasymys*. Other fossil records include two East African Plio-Pleistocene species, *A. lavocati* (Jaeger, 1976, 1979) from Lake Natron and *A. deheinzelini* (Wesselman, 1984) from Lake Turkana (Black and Krishtalka, 1986; Denys, 1987). There is, however, no close relationship between the East African species and those from South Africa, which would allow speculation on the origin and time of divergence of the genus *Micaelamys* (Denys, 1990a, b).

3. Molecular data and Phylogeny

The present investigation was largely based on the analysis of mtDNA data which are valuable for understanding evolutionary relationships among species, populations and individuals (Irwin et al., 1991). Animal mtDNA is a duplex, covalently closed circular molecule that replicates itself and transcribes protein-coding genes within the organelle (Avise and Lansman, 1983; Moritz et al., 1987). Its gene content appears to be conserved, with two ribosomal RNA (rRNA), 22 transfer RNA (tRNA) and 13 protein-coding genes (Moritz et al., 1987). A "control" region that lacks structural genes but contains sequences that initiate replication and transcription is present (Moritz et al., 1987). Since mtDNA is maternally inherited, the history recorded in this molecule is not a complete characterisation of the intraspecific phylogeny of a species because relationships may be obscured by gender bias such as in levels of dispersal (Zhang and Hewitt, 1996).

Despite potential limitations of the cyt *b* gene, it has proved useful in addressing questions about relationships among and within species for a range of taxa (Ducroz et al., 1998; Fumagalli et al., 1999; Jansen van Vuuren and Robinson, 1997; Nicolas et al., 2008a Nicolas et al., 2008b; Ohdachi et al., 2001; Smith, 1998). More specifically, the gene has successfully been used to investigate systematic relationships in a number of murid rodents (e.g., Ducroz et al., 1998; Ducroz et al., 2001; Galewski et al., 2006; Patton and Smith, 1992; Russo et al., 2006; Smith and Patton, 1993, 1999; Verheyen et al., 1995, 1996), in addition to which its time scale calibrations (Smith and Patton, 1993) and rate of evolution are also well documented (Irwin et al., 1991). More recently, the cyt b gene has successfully been used to investigate the phylogeographic structure of the genus *Acomys* (Nicolas et al., 2009).

The Recombination Activating Gene 1 (RAG1) gene was also used in the elucidation of phylogenetic relationships within southern African *M. namaquensis* in the present study. The protein encoded by the RAG1 gene is involved in the rearrangement and recombination of the genes of immunoglobulin and T cell receptor molecules during the process of V-D-J recombination (Wenhui et al., 2001). The cellular expression is restricted to lymphocytes during their developmental stages and the RAG1 gene is therefore essential to the generation of mature B and T lymphocytes, cell types that are important

components of the adaptive immune system. The RAG1 protein is fairly large such as the mouse RAG1 gene that contains 1040 amino acids (Abbas et al., 2003). The enzymatic activity of the RAG1 protein is largely concentrated in a core region between residues 384-1008 (Abbas et al., 2003). This core region contains three acidic residues (D_{600}, D_{708} and E_{962}) in what is called the DDE motif (Janeway et al., 2005). Previous studies have shown that this nuclear gene is useful for phylogenetic studies of rodents (Steppan et al., 2004a, b; Steppan et al., 2005; Suzuki et al., 2004).

The cyt *b* gene was used in all analyses in Chapter 2 and this was followed by a combined approach in Chapter 3 (i.e., a combination of a fragment of RAG1 and cyt *b*); these genes were used to infer the phylogeny of *M. namaquensis*. This approach was adopted for Chapter 3 as phylogenetic analyses of several short stretches from different genes show a better performance than analyses based on nearby sites from a single gene fragment (Cummings et al., 1995). In combining these genes, it was considered that the cyt *b* gene with its faster mutation rate would possibly resolve relationships near the terminal nodes relative to the slower nuclear RAG1 gene which is considered useful for resolving relationships at deeper nodes (Suzuki et al., 2004).

4. Phylogeography

The analysis of the cyt *b* gene in this study was also augmented by a phylogeographic approach which represents a "mtDNA bridge between population genetics and systematics" (Avise et al., 1987). It is concerned with patterns and processes governing geographic distributions of genealogical lineages among and within closely related species (Avise et al., 1987). Phylogeographic differentiation represents an interplay between vicariance and dispersal processes (Avise et al., 1987). Intraspecific population structure is therefore determined by the species' potential for gene flow and the environmental influences on that potential (Avise et al., 1987). Phylogeographic patterns can be expected to range from total phylogenetic discontinuity between populations due to zoogeographic barriers and limited dispersal abilities, to genetic continuity in species with high dispersal potential and/or in unfragmented environments (Avise, 2000; Avise et al., 1987).

In the first decade of phylogeographic research, analyses largely involved the qualitative description of geographic patterns (Avise, 2000), yet valuable insights were gained regarding the structuring of intraspecific variation (see review by Avise, 2000), especially in response to Quaternary climate changes (see amongst others Hewitt, 2000; Hewitt, 2004). The subsequent introduction of statistical approaches now enables testing of hypotheses to investigate the processes underlying the patterns of diversity (Knowles, 2004; Knowles and Maddison 2002). One of the first of these approaches was nested clade analysis (Templeton et al., 1987; Templeton and Singh, 1993). Nested clade phylogeographic analysis (NCPA) attempts to distinguish between different historical processes that might have influenced the geographic distribution of haplotypes relative to higher level clades (Petit, 2008), however, the approach is a subject of considerable debate (Garrick et al., 2008; Petit, 2008). Recently, Petit (2008) suggested that the method should no longer be used until it has been more thoroughly and critically evaluated, while Garrick et al. (2008) suggested that the method could still be useful in generating "plausible hypotheses" as corroborating evidence or lack thereof. In reaction to previous debates on the effectiveness of NCPA (e.g., Knowles and Maddison, 2002; Templeton, 2004), a new software program (ANeCA) was developed that automates the complex NCPA methodology (Panchal, 2007). While I acknowledge the potential limitations of the approach, I adopted it along with other measures of population history (e.g., mismatch distribution analysis as implemented in Arlequin, version 3.0 (Excoffier et al., 2005)) to estimate population expansion and MIGRATE, version 2.4 that was used to estimate effective population sizes and past migration rates between *n* number of populations assuming a migration matrix model (Beerli and Felsenstein, 2001).

Although *M. namaquensis* is widely distributed in southern Africa, it is essentially restricted to rocky habitats (Chimimba and Bennett, 2005). Species occupying such habitat islands often display patterns of phylogenetic discontinuity. Geographically distant samples of the deer mouse (*Peromyscus maniculatus*) reflect the impact of physical barriers on dispersal (Lansman et al., 1983). Similarly, this pattern is also shown by southern African rock dwelling small mammals such as the rock hyrax (*Procavia capensis*; Prinsloo, 1993), the red rock rabbits (*Pronolagus sp.*; Matthee, 1993; Matthee and Robinson, 1996) and the Namaqua rock mouse (*M. namaquensis*; Russo, 2003). Nicolas et al. (2009) provided evidence of restricted gene flow with isolation by distance and a signal of population expansion was detected within several clades of *Acomys*, a rock-dwelling

small mammal. This species shows a strong phylogeographic structure (Nicolas et al., 2009).

In contrast, habitat uniformity of the open plains occupied by the Springhare (*Pedetes capensis*) is reflected in its phylogeographic pattern, which shows a lack of genetic divergence among samples over a broad geographic range in South Africa (Matthee and Robinson, 1997). This phylogeographic pattern is also evident in the yellow mongoose from southern Africa (*Cynictis penicillata*; Jansen van Vuuren, 1995), the old field mouse (*Peromyscus polionotus*; Avise et al., 1983) and the woodrats of the eastern United States (*Neotoma*; Hayes and Harrison, 1992). *Aethomys chrysophilus* and *A. ineptus* from southern Africa also reflect a lack of genetic divergence among samples over a broad geographic range (Russo et al., 2006).

Other life history characteristics of *M. namaquensis* that are predicted to impact on phylogeographic structuring include body size, age at weaning, age of sexual maturity, age of fecundity, time to first sexual activity and first reproduction, duration of gestation, litter size and interbirth interval. These life history traits relating to growth and reproduction vary greatly among species, populations and individuals within populations. Egron et al. (2001) demonstrated that the immediate environment (which includes water and food availability, nesting/breeding space ect.) plays a major role in shaping the life history characteristics of small mammals.

5. Aims of Study

Given the above background, the aims of the present study are as follow:

1. To assess the nature and extent mtDNA variation within *M. namaquensis* from southern Africa based on cyt *b* sequence data and to compare this variation to the previously described subspecies.

2. To assess phylogenetic relationships between lineages within *M. namaquensis* based on cyt *b* and RAG1 data.

3. To elucidate the phylogeographic pattern among *M. namaquensis* populations from the Eastern Kalahari Bushveld and to infer past processes underlying the current diversity.

6. Research Questions

Research questions were specific to each chapter. Questions 1 - 3 relate to Chapter 2, question 4 to Chapter 3 and question 5 to Chapter 4. The following specific research questions are addressed in the present study:

1. What is the nature and extent of mtDNA variation within *M. namaquensis* over a broad geographic area in southern Africa?

2. What is the phylogeographic structure within some of the *M. namaquensis* lineages?

3. What are the divergence dates of different *M. namaquensis* lineages?

4. What are the relationships among *M. namaquensis* lineages based on a combined phylogenetic analysis of the mitochondrial cyt *b* and the nuclear RAG1 genes?

5. What are the finer scale phylogeographic structure and underlying processes within *M. namaquensis* based on a case study of populations from the Eastern Kalahari Bushveld?

7. Relevance of Study

In addition to a significant contribution to small mammal systematics and biodiversity research in Africa, the findings of the present study may have implications in epidemiological and agricultural research associated with problem rodents. Some members of the genus *Aethomys* and *Micaelamys* have been implicated in causing epidemiological problems (Gear et al., 1966; Hallet et al., 1970; Swanepoel et al., 1978) while others may cause extensive damage to crops and stored grain (De Graaff, 1981; Smithers, 1971;

Wilson, 1970, 1975). Consequently, the present study may assist health and agricultural authorities in gaining a better insight into these potentially problematic rodents.

In order to assist agricultural and health authorities it is important to assess the extent and nature of *M. namaquensis* diversity. High levels of genetic diversity within the species were evident in a previous study by Russo (2003). These levels of diversity should be taken into account when recommendations are made to the authorities given the balance between conservation of biodiversity and management of problem rodents are important. Furthermore, for the management of pest species it is essential to have some understanding of the life history traits of a species (i.e., movement patterns) with regard to the spread of diseases.

From an academic (scientific) point of view, small mammals such as these rodents are good models in evolutionary and population biology since they have short generation times, short gestation periods and large litter sizes (Chimimba and Bennett, 2005).

8. Thesis Outline

CHAPTER 2: The *Micaelamys namaquensis* (Rodentia: Muridae) species complex from southern Africa: Patterns of mitochondrial DNA versus morphological diversity

This chapter represents the main focus of the study which was to assess the nature and extent of the mtDNA variation, phylogeographic structuring within some lineages and estimated times of divergence for the lineages of interest identified within *M. namaquensis* from southern Africa. Phylogenetic analyses revealed 14 distinct lineages of which several show strong geographic association with particular vegetation types of southern Africa. The diversity and differentiation detected in the present study are much more complex than suggested by previous morphological assessments. An Early to Late Miocene time of divergence was suggested. *Micaelamys namaquensis* lineages show contrasting patterns of phylogeographic structure but the majority of the diversification events date to the Late Miocene and Pliocene that coincide with major periods of aridification in southern Africa.

CHAPTER 3: Phylogenetic relationships within *Micaelamys namaquensis* (Rodentia: Muridae) from southern Africa as inferred from mitochondrial and nuclear genes

This chapter extends the research reported in Chapter 2 by addressing the phylogenetic relationships among *M. namaquensis* lineages based on combined mitochondrial cyt *b* and nuclear RAG1 data. Incongruence was shown between the cyt *b*, the combined (cyt *b* and RAG1 genes) analyses and the independent RAG1 analysis possibly as a result of incomplete lineage sorting in the nuclear gene. The combined molecular data suggested that *Micaelamys* from southern Africa comprises 11 lineages of which six were well-supported with strong geographical associations to biomes/bioregions of southern Africa, while the remaining lineages were not associated with specific vegetation types.

CHAPTER 4: Phylogeography of *Micaelamys namaquensis* (Rodentia: Muridae) from the Eastern Kalahari Bushveld bioregion of South Africa

In order to refine our understanding of evolutionary and ecological processes underlying the remarkable diversity within *M. namaquensis*, this chapter presents a fine scale phylogeographic analysis of the mtDNA lineage from the Eastern Kalahari Bushveld bioregion. This bioregion has specifically been chosen since samples were distributed over a fairly small, well-defined geographic area. Secondly, since localities of sympatry have been identified in Chapter 2 for this region, it was thought that a fine scale phylogeographic analysis would give some insight in understanding the ecological processes underlying diversity. This bioregion was also represented by a fairly large sample size compared to some of the other biomes/bioregions. This fine scale analysis revealed a genetic pattern of phylogenetic continuity. Mismatch distribution analysis suggests that the lineage has experienced recent population growth following environmental changes associated with habitat modification over the past 3 000 to 10 000 years. Historical female gene flow does not appear to be equal amongst all localities.

CHAPTER 5: Thesis conclusion

The thesis concludes with a general synthesis of the major findings of this multidisciplinary study of *M. namaquensis* from southern Africa. In addition, some recommendations for future research are made.

9. General Notes

All chapters (except 1 and 5) were written as manuscripts that have been or will be submitted for publication in peer-reviewed journals. Chapter 2 has been submitted to BMC Evolutionary Biology. Given that it has been submitted for publication, I refer to the work being done by us (I.M. Russo, C.T. Chimimba and P. Bloomer). A reference list occurs at the end of each chapter rather than at the end of the thesis. Due to this format, there may be instances of duplication across chapters. Each chapter contains its own appendices. The introduction and the concluding chapter were tailored from the respective chapters, giving a general overview of this thesis and the overall concluding remarks and synthesis. For the purposes of this thesis the format of Molecular Phylogenetics and Evolution was followed and references in the text were listed in alphabetical rather than chronological order.

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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.

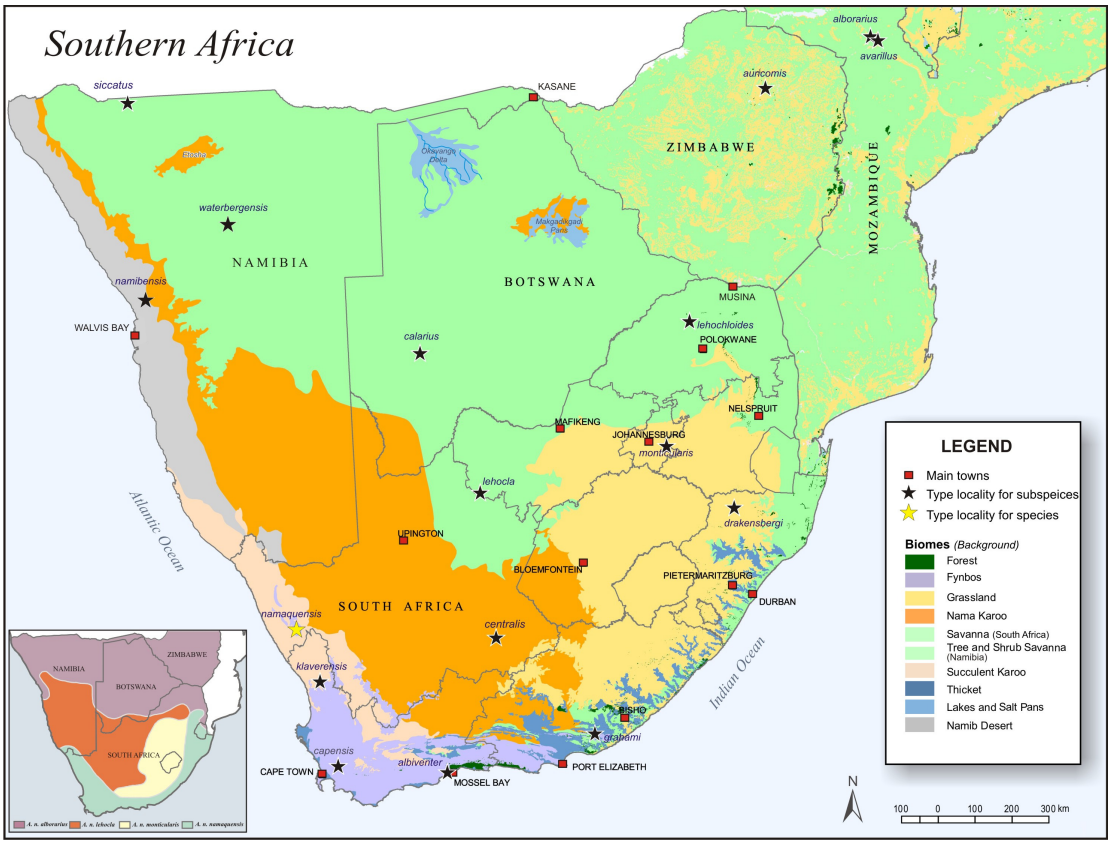

Figure 2.1 Major biomes of southern Africa (Low and Rebelo, 1996). Background colours for the eight major biomes in southern Africa are indicated in the legend; major lakes and saltpans are also included. The yellow star indicates the type locality for the species *Micaelamys namaquensis* and the blue 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.

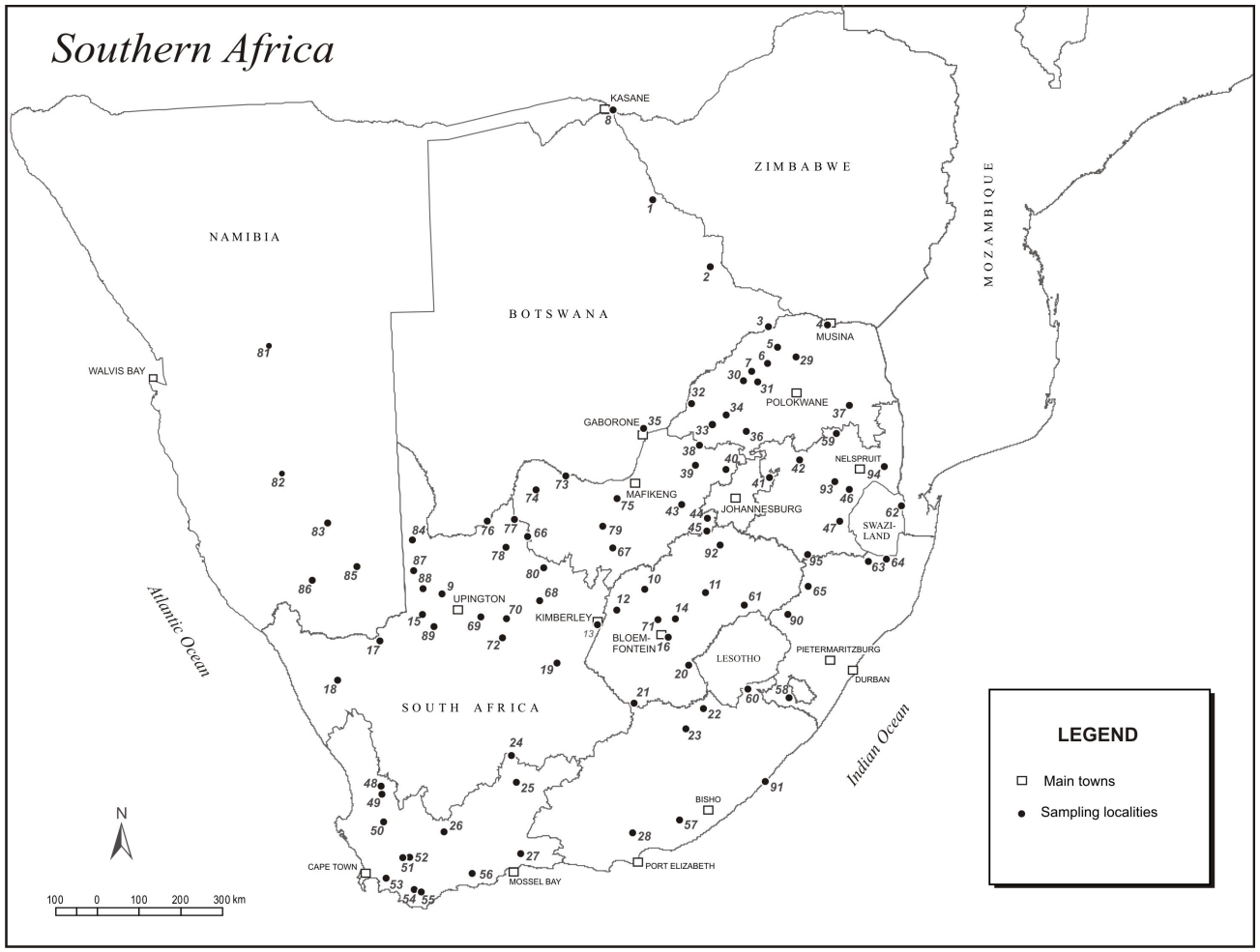

Figure 2.2 Collecting localities of samples of *Micaelamys namaquensis* from southern Africa. Numbers correspond to the locality numbers and names in Appendix 2.1.

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 100^{th} generation. Two runs were performed simultaneously and split frequencies were compared every 100^{th} 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.

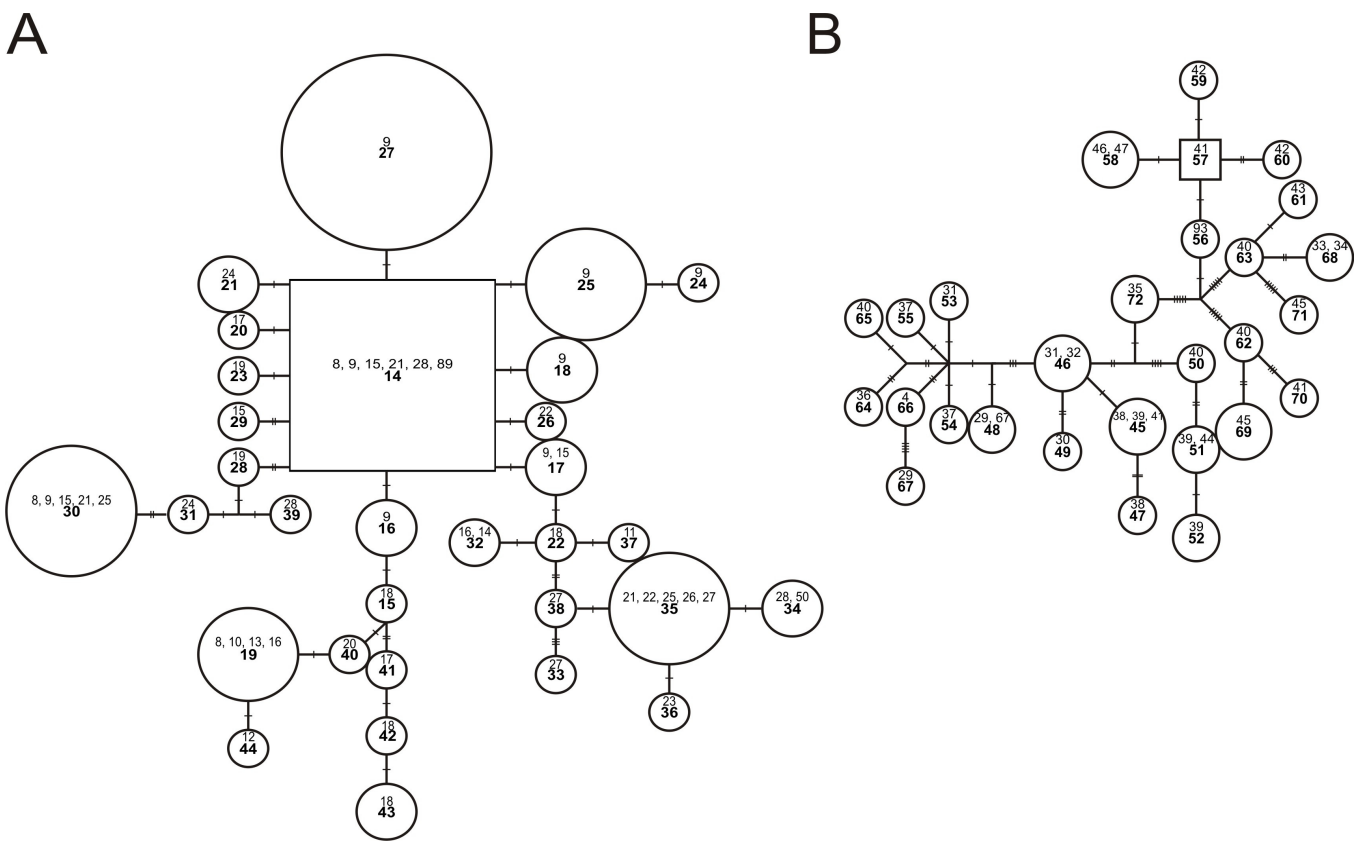

Figure 2.3 Minimum-spanning networks indicating the least number of mutational steps between composite mitochondrial DNA (mtDNA) cytochrome *b* (cyt *b*) haplotypes within two *Micaelamys namaquensis* clades from southern Africa. Sizes of the circles and squares represent haplotype frequencies while 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.

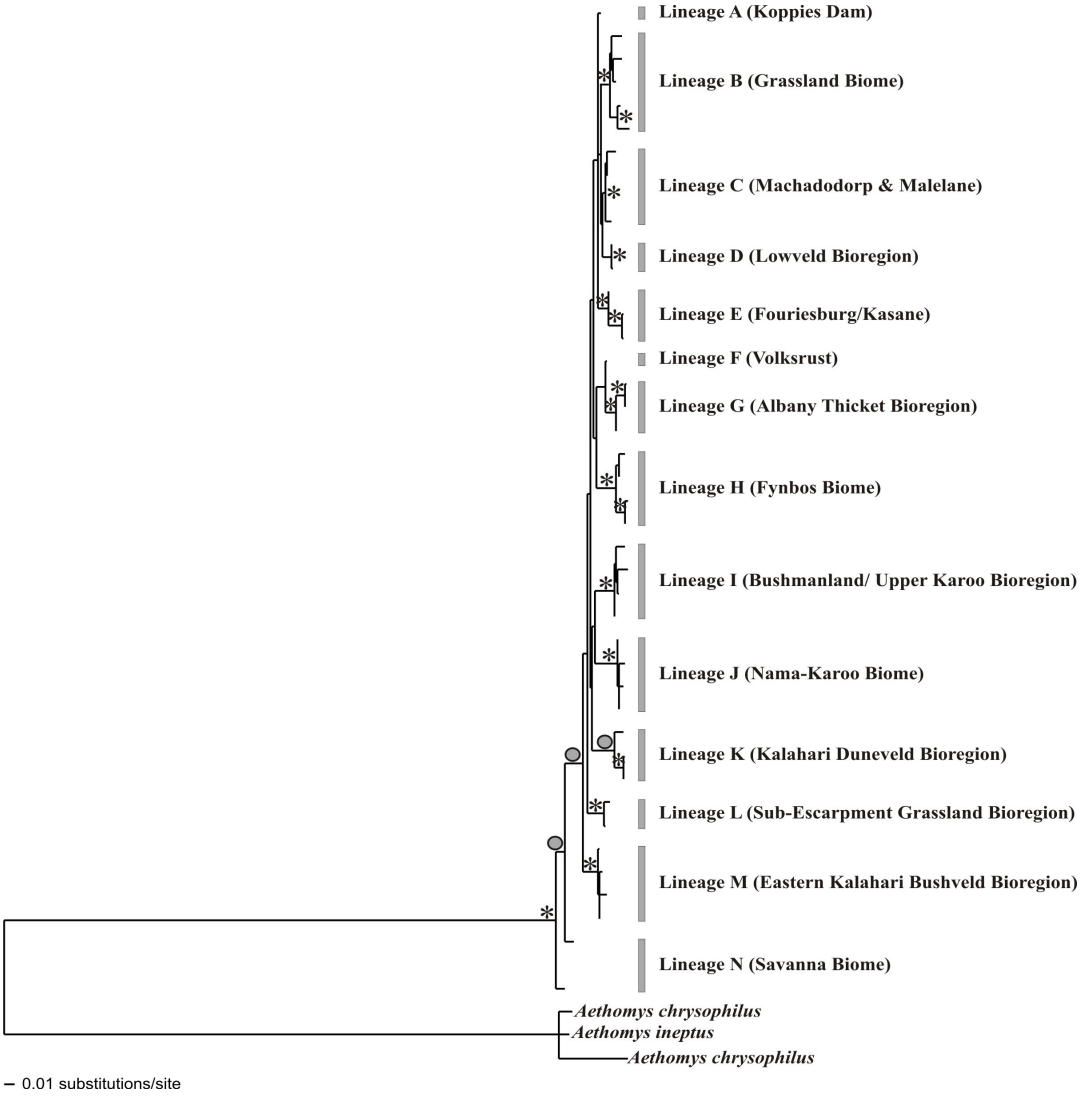

Figure 2.4 A maximum likelihood (ML) phylogram based on 631 bp of the mitochondrial DNA (mtDNA) cytochrome *b* (cyt *b*) gene of *Micaelamys namaquensis* from southern Africa. Bootstrap confidence limits (1 000 replicates) and Bayesian posterior probability values for internal nodes are given at each 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.

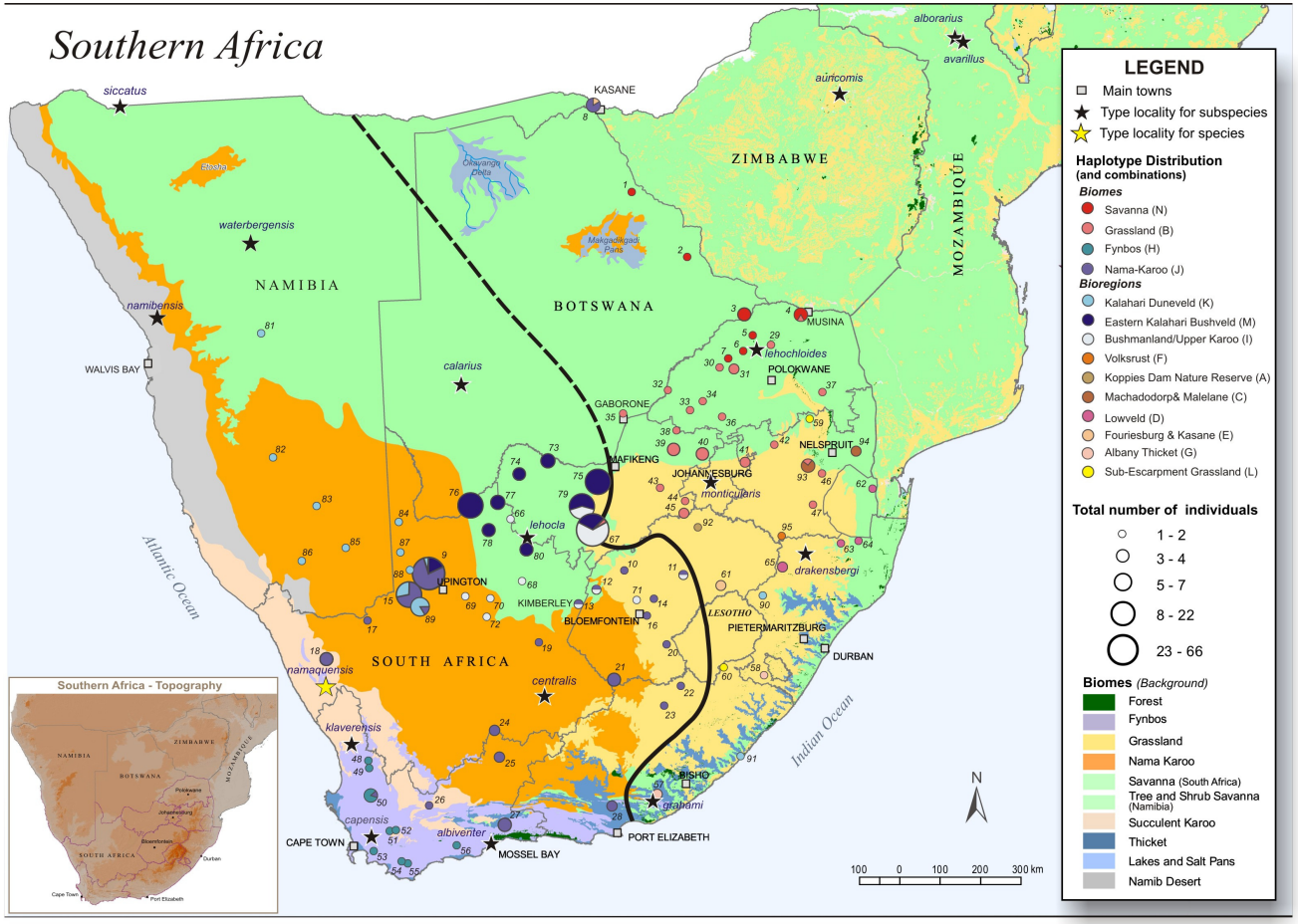

Figure 2.5 Geographic distribution of *Micaelamys namaquensis* mitochondrial DNA (mtDNA) cytochrome *b* (cyt *b*) lineages in the biomes/bioregions of southern Africa (see legend for colours). The sizes of circles indicate the number of individuals sampled at each locality (see legend for scale); the 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

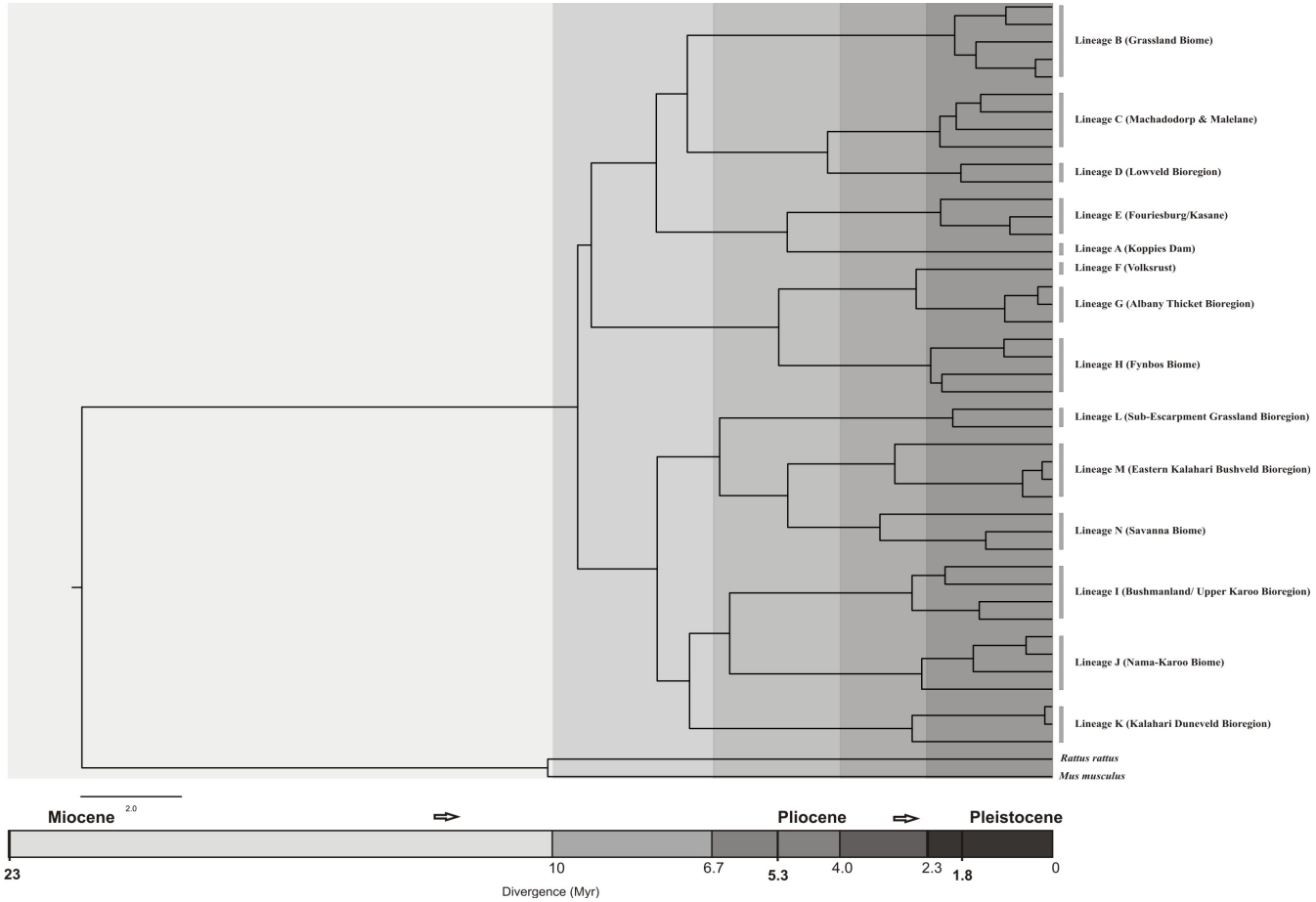

Figure 2.6 A phylogenetic tree for 43 representative mitochondrial DNA (mtDNA) cytochrome *b* (cyt *b*) haplotypes of *Micaelamys namaquensis* from southern Africa as obtained by BEAST analysis. Divergence dates (epochs) are indicated in the grey-scale key at the bottom of the figure. Lineages A - N 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 *Aethomys* (*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).

6. Acknowledgements

We are grateful to A.V. Linzey, M. Kesner (Indiana University of Pennsylvannia, USA), H. Smith (University of Stellenbosch), D.N. MacFayden (E. Oppenheimer & Son), J. Watson (Free State Department of Environmental Affairs and Tourism) and N. Avenant (National Museum, Bloemfontein, South Africa) for samples. We are also grateful to all the field trip assistance that helped with the collection of samples. Thanks also to Fourie Joubert and deepthought (http://deepthought.bi.up.ac.za), a 64-node cluster, for computational support. C.L. Sole and T.C. Bray are thanked for the constructive comments on earlier drafts of this manuscript. We also thank two anonymous reviewers for invaluable comments on the manuscript. This study was funded by the National Research Foundation grant to PB and CTC (Grant number: 2073181) and the University of Pretoria.

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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, Geluksprui t	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).

Haplotype Number	Variable positions within the 631 bp fragment of cytochrome *b* gene
	1111111111111111111122222222222222222222233333333333333333333333333344444444444444444444444455555555555555555555555555555566666666666660
	1111111122222233344455666777889900011222333444566778001111233333445788899000012222344445556677788899901222233345555667777788990011223334444555666667788888999990000011111122333
	5890123458901234703825846037258473925815157256145958489170469212457368625817136981378925891473558914706957036946883679581235836281469234780369238134783602458124780369456789010 1
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.....CT.....TC.....C.....C.AC.C.C.....AACCC
NH040	????????.....C....T.....A...G..T..C....A.....C.T..TT.TG.....CG.....T.....AT.....T.....T.T.....G.....C.....TC.....C.....C.AC...C.....?????????????
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
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Grassland Biome

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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??
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Fynbos Biome

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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.T............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.....????????????
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Albany Thicket Bioregion

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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??
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Sub-Escarpment Grassland Bioregion

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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.........?????????
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Fouriesburg/Kasane

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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......???????????
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Lowveld Bioregion

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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)

Chapter 3

Phylogenetic relationships within *Micaelamys namaquensis* (Rodentia: Muridae) from southern African as inferred from mitochondrial and nuclear genes

Abstract

Evolutionary relationships among members of the murid rodent genus *Micaelamys* (formerly allocated to the subgenus *Micaelamys* within the genus *Aethomys*) are poorly understood. Here I extend my research on the Namaqua rock mouse, *M. namaquensis* from southern Africa by combing existing partial mitochondrial DNA (mtDNA) cytochrome *b* (cyt *b*) sequences with the nuclear Recombination Activating Gene 1 (RAG1) gene to examine the relationships among 11 of 14 recently identified phylogroups. Cytochrome *b* sequence divergence values ranged between 0.32% and 7.68% while divergence values ranged between 0.08% and 1.44% for the RAG1 gene. Incongruence was shown between the cyt *b* and the combined analyses versus the independent RAG1 analysis possibly as a result of incomplete lineage sorting in the nuclear gene. The combined molecular data supports the polytypic nature of *M. namaquensis* and the fact that most lineages are associated with specific vegetation types of southern Africa.

1. Introduction

African rock rats of the genera *Aethomys* Thomas, 1915, and *Micaelamys* Ellerman, 1941 (see Chimimba and Bennett, 2005), are a diverse group of murid rodents endemic to East, Central and southern Africa and extending marginally into West Africa (Musser and Carleton, 2005). Phylogenetic relationships between these genera and other African murids are uncertain (Musser and Carleton, 2005). Characteristics of the genus (*Aethomys*, *senso lato*) overlap to some extent with *Rattus* and *Arvicanthis* (Ellerman, 1941) added to which the genus has also variously been considered to be closely related to *Mus*, *Mastomys*, *Thallomys*, *Zelotomys* (De Graaff, 1981), *Stochomys*, *Dephomys*, *Dasymys* and *Pelomys* (Bonhomme et al., 1985; Denys, 1990a; b).

Prior to the generic recognition of the genus *Micaelamys* (Chimimba and Bennet, 2005), a phylogenetic study of the genus *Aethomys* using cladistic analysis of all recognised species at that time, based on cranial data suggested the presence of three clades: 1) *A. bocagei* Thomas, 1904, *A. thomasi* De Winton, 1897, *A. silindensis* Roberts, 1938, *A. kaiseri* Noack, 1887, and *A. nyikae* Thomas, 1897; 2) *A. chrysophilus* De Winton, 1897, *A. ineptus* Thomas and Wroughton, 1908, and *A. hindei* Thomas, 1902; 3) *A. granti* Wroughton, 1908, *A. namaquensis* Smith, 1834, and *A. stannarius* Thomas, 1913 (Chimimba, 2005). This study also revealed a sister-taxon relationship between the two cryptic species, *A. chrysophilus* and *A. ineptus* (Chimimba, 2005). In contrast, molecular studies by Ducroz et al. (2001) and Lecompte et al. (2008) revealed no sister-taxon relationship between individuals from *Aethomys* and *Micaelamys*.

Attention was drawn to the close evolutionary relationship between *A. namaquensis* and *A. granti* (currently referred to as *M. namaquensis* and *M. granti*; see Chimimba and Bennett 2005). Similarly, this close relationship has been documented in other studies using dental morphology (Ellerman et al., 1953), karyology (Visser and Robinson, 1986), gross sperm and bacular morphology and their staining properties (Visser and Robinson, 1987) and phenetic analysis (Chimimba et al., 1999). *Micaelamys namaquensis* and *M. granti* were well separated from all other species of *Aethomys*, providing good grounds for the subgeneric separation of *Aethomys* and *Micaelamys* (Chimimba, 2005). Similarly, Davis

(1975) supported the view of a close affinity between *A. namaquensis* and *A. granti* and their inclusion within the subgenus *Micaelamys* (Ellerman et al., 1953).

Palaeontological data of some members of the genera *Aethomys* and *Micaelamys* have been recorded from South Africa (Avery, 1981, 1982, 1985; De Graaff, 1960, 1961; Hendey, 1981; Pocock, 1987) where two Pliocene fossil species, *A. adamanticola* and *A. modernis*, the oldest known representatives of the genus in Africa, have been described (Denys, 1990a, b). *Aethomys adamanticola* shows characteristics reminiscent of *A. namaquensis* (*Micaelamys namaquensis* as currently understood) while *A. modernis* is very similar to *A. chrysophilus* (*sensu lato*) (Denys, 1990a, b). *Aethomys adamanticola* may also represent an advanced stage of an Early Miocene lineage closely related, but not ancestral to *Dasymys* (Denys, 1990a, b). Other palaeontological records include two East African Plio-Pleistocene fossil species, *A. lavocati* (Jaeger, 1976, 1979) and *A. deheinzelini* (Black and Krishtalka, 1986; Denys, 1987; Wesselman, 1984).

Earlier reports (e.g., Roberts, 1951; Meester et al., 1964) recognised 16 subspecies within *M. namaquensis.* Prior to an intraspecific morphometric study within *M. namaquensis* (Chimimba, 2001), the extent of geographic variation within the species remained unknown. Chimimba (2001) suggested the recognition of only four subspecies within *M. namaquensis*: *M. n. namaquensis*, Smith, 1834, *M. n. lehocla* Smith, 1836, *M. n. monticularis* Jameson, 1909 and *M. n. alborarius* Peters, 1852, each associated with one of four of the major biomes of southern Africa (Chimimba, 2001). My subsequent analysis of mitochondrial DNA (mtDNA) cytochrome *b* (cyt *b*) diversity across the distributional range of the species (Russo, 2003; Chapter 2) confirmed that *M. namaquensis* is polytypic. Fourteen distinct evolutionary lineages were described and most appeared to be associated with major biomes/bioregions of southern Africa. The phylogenetic analysis could only resolve a few of the deeper nodes based on this single mtDNA marker. Coalescent-based dating suggested a major radiation over a relatively short evolutionary period at the end of the Miocene and the beginning of the Pliocene.

In the present study, I utilised a fragment of the Recombination Activating Gene 1 (RAG1) in combination with the protein coding cyt *b* gene in an attempt to obtain a more resolved phylogeny. These genes were used in the present study as phylogenetic analyses of several short stretches from different genes, on average, show a better performance than analyses

based on nearby sites from a single gene fragment (Cummings et al., 1995). Previous studies have shown the RAG1 gene to be useful for phylogenetic analysis of rodents (e.g., Steppan et al., 2004a, b; Steppan et al., 2005; Suzuki et al., 2004). The protein encoded by this gene is involved in activation of immunoglobulin V-D-J recombination (Wenhui et al., 2001). Similarly cyt *b* sequences, when used carefully, have contributed to the investigation of the phylogenetic relationships among murids (e.g., Ducroz et al., 2001; Galewski et al., 2006; Verheyen et al., 1995; Verheyen et al., 1996). In combining RAG1 and cyt *b* characters, it was considered that the latter gene with its faster mutation rate would resolve relationships near the terminal nodes relative to the slower nuclear RAG1 characters which may resolve deeper nodes due to lesser effects of saturation and consequent homoplasy (Suzuki et al., 2004).

A lack of resolution in a phylogenetic tree is represented as a polytomy (i.e., uncertainty about relationships between lineages/groups) in which three or more lineages/groups diverge from a single node. In general, systematics requires resolved trees, which may yield stronger inferences about character evolution and the relationships and biogeography of the species under investigation (McCracken and Sorenson, 2005). The resolution of a polytomy at the species level can be complicated by the incongruence of individual gene trees and the species tree (i.e., the true history of diversification). This lack of resolution in phylogenetic analyses could be as a result of incomplete lineage sorting where the lineage sorting will take longer (3-4 times longer) in any nuclear gene compared to lineage sorting in a mitochondrial gene due to differences in the effective population sizes of the respective genomes (McCracken and Sorenson, 2005; Moore, 1997; Palumbi et al., 2001).

The present study, therefore, represents a phylogenetic analysis using independent cyt *b* and RAG1 sequence data and a combination of both sets of data, based on material from southern Africa and addresses the following questions: 1) What are the phylogenetic relationships among lineages of the southern African *M. namaquensis* (as identified in Chapter 2) based on molecular and nuclear data?; and 2) Does the nuclear RAG1 gene contribute to the overall resolution of the phylogenetic relationships among lineages within *M. namaquensis*?

2. Materials and Methods

2.1 Study area and sampling

Thirty-five individuals representative of 11 of the newly identified lineages of *M. namaquensis* from southern Africa were selected for the phylogenetic analysis (Fig. 3.1; Appendix 3.1). Some of the RAG1 amplifications were unsuccessful and I therefore only included 35 samples. Individuals for the remaining lineages (lineage A, F and L) were not included since, as mentioned before, the RAG1 amplifications were unsuccessful. For sampling protocols, permit numbers and detailed locality information refer to Chapter 2.

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

The cyt *b* sequences described in Chapter 2 were re - analysed in the present study. To amplify and sequence the targeted fragment in the RAG1 gene, the primers S70 (5' TCC GAG TGG AAA TTT AAG MTG TT 3'; modified from R13 of Groth and Barrowclough, 1999) and S73 (5' GAG GAA GGT RTT GAC ACG GAT G 3'; Steppan et al., 2004b) were used to amplify the region in five individuals. These *M. namaquensis* sequences were aligned in Clustal X (Thompson et al., 1997) and used to design internal species-specific *M. namaquensis* primers, RAGNam IL (5' GCG TAG GCT CAG CAG CAA GGA 3') and RAGNam IH (5' GAT TTC ACA AAG TGT GCA GGG 3'). The targeted fragment of the remaining individuals was amplified using primers S70/RAGNam IH and S73/RAGNam IL.

Polymerase Chain Reactions (PCR; Saiki et al., 1988) of the RAG1 gene consisted of denaturing at 94° C for 5 min, 35 cycles of the following: 94° C for 30 seconds, primer annealing at 53.5° C (S70/RAGNam IH) and 57° C (S73/RAGNam IL) for 30 seconds and elongation at 72° C for 45 seconds. This was followed by an extended elongation step for 7 minutes at 72° C in a Geneamp® PCR System 9700 (Applied Biosystems). PCR products were purified using the High Pure™ PCR Product Purification Kit (Roche Diagnostics) as prescribed by the manufacturers.

Dye-terminator cycle sequencing was performed with S70/RAGNam IH and S73/RAGNam IL to obtain a 1309 bp fragment of the RAG1 gene. Nucleotide sequences

were determined using an ABI 3130 automated sequencer (Applied Biosystems). Cycle sequencing products were subsequently precipitated using a NaAc salt method.

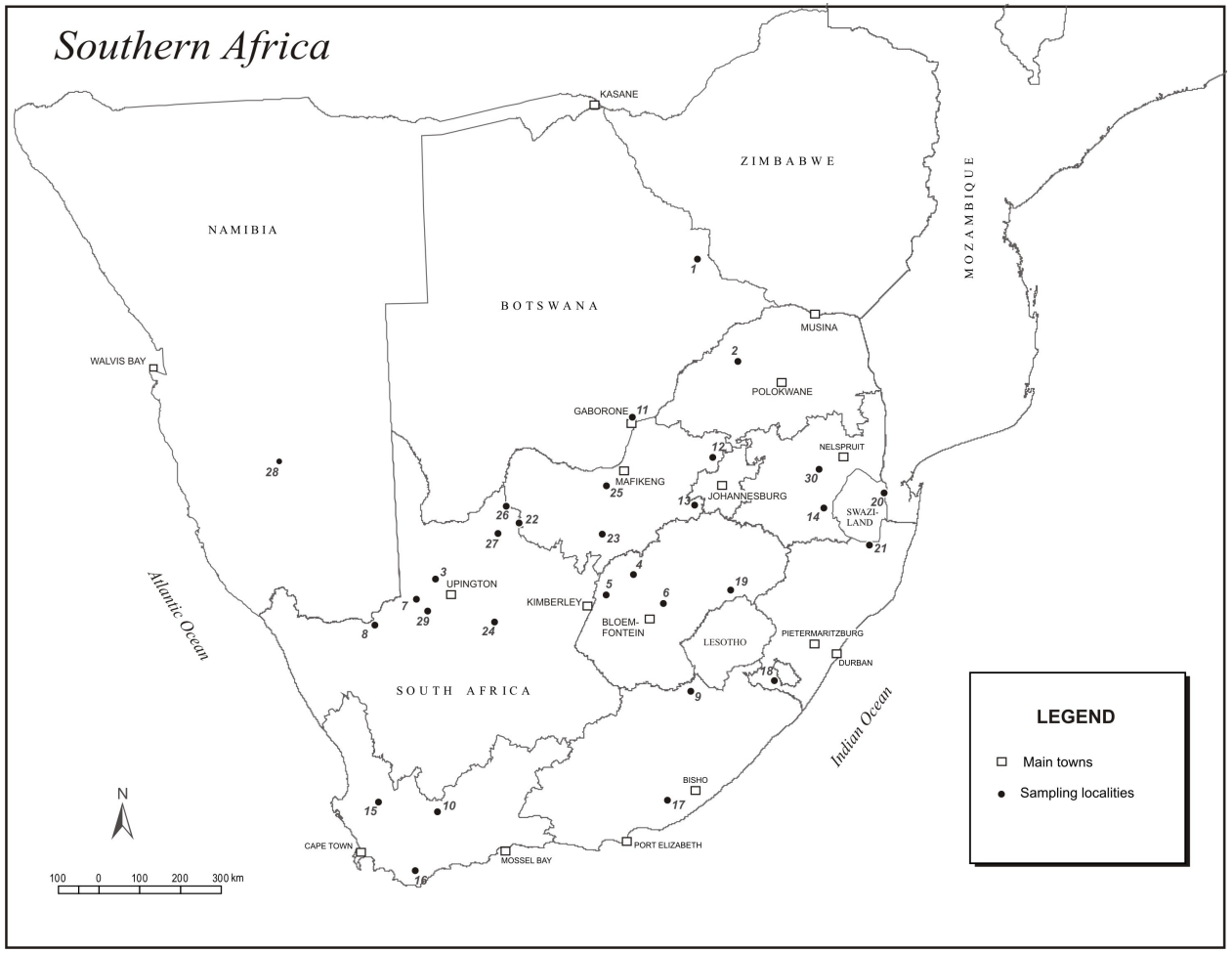

Figure 3.1 Collecting localities of *Micaelamys namaquensis* in South Africa, Swaziland, Botswana and Namibia, representing individuals with sequences for both the mitochondrial DNA (mtDNA) cytochrome *b* (cyt *b*) gene and the nuclear Recombination Activating Gene 1 (RAG1) gene. Numbers correspond to the locality numbers in Appendix 3.1. Also see Appendix 3.1 for locality names.

2.3 Sequencing analysis

The individual RAG1 sequences were imported into either Sequence Navigator, version 1.01 (PE Applied Biosystems) or Vector NTI Advance 10 (Invitrogen). A consensus sequence of each individual was computed by aligning the forward and reverse sequences. The GenBank accession numbers of the relevant samples from Chapter 2 and the newly acquired RAG1 sequences are given in Appendix 3.2. Consensus sequences of all individuals were aligned in Clustal X (Thompson et al., 1997) and sequences were subsequently imported into PAUP, version 4.0b10 (Swofford, 2003) and MRBAYES, version 3.1.2 (Ronquist and Huelsenbeck, 2003) for phylogenetic analyses. Sequences were also imported into MacClade, version 3.0 (Maddison and Maddison, 1992) to translate the cyt *b* and RAG1 nucleotide sequences into amino acids. Amino acid sequences were examined for irregularities (e.g., stop codons in the reading frame) while the transition:transversion (Ti:Tv) ratio was also estimated using MacClade, version 3.0 (Maddison and Maddison, 1992).

2.4 Outgroup choice

The selection of possible outgroups for *M. namaquensis* was difficult because of the previous evolutionary relationships that have been proposed between *Micaelamys* and numerous other murids (see Chimimba, 2005 and references therein). In a preliminary analysis *A. chrysophilus*, *A. ineptus*, *Parotomys brantsi*, *Dasymys incomtus*, *Rattus rattus*, *M. musculus*, *Rhabdomys pumilio* and *Arvicanthis somalicus* were used as outgroups. *Rhabdomys pumilio* was used as outgroup in the PAUP, version 4.0b10 and MRBAYES, version 3.1.2 (Ronquist and Huelsenbeck, 2003) analyses. Sequences for both the cyt *b* and RAG1 genes were available for *R. pumilio* and the species has been shown to be closely related to *M. namaquensis*.

2.5 Phylogenetic analysis

Since the data included sequences from one mtDNA gene and one nuclear gene, two analytical strategies were possible for the treatment of these data due to the controversy surrounding the merits of these two approaches (De Queiroz et al., 1995; Huelsenbeck et al., 1996). The first approach was to analyse the different datasets separately and to construct a consensus tree from these separate analyses, but this approach (Adams, 1972) is considered to be more appropriate if the datasets are heterogeneous. The second approach was to perform the analysis directly using the combined data. This “combined

approach" is considered to often provide phylogenies that are more resolved than consensus trees from separately analysed data (De Queiroz, 1993). The data in the present study were analysed separately and combined.

Modeltest, version 3.06 (Posada and Crandall, 1998) was used to determine optimal substitution models identified by the Akaike Information Criterion (AIC) for the combined and separate datasets. Parameters such as 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 36 sequences (a subset of individuals representing the diversity within *M. namaquensis,* including the outgroup) was subsequently used in maximum likelihood (ML; Felsenstein, 1973; 1981) analyses as implemented in PAUP, version 4.0b10 (Swofford, 2003) and Bayesian Inference (BI; Ronquist and Huelsenbeck, 2003) phylogenetic analyses. Base frequencies were also estimated in PAUP, version 4.0b10 (Swofford, 2003).

Three independent ML analyses (the two genes as independent datasets and a combined analysis) were conducted using PAUP, version 4.0b10 (Swofford, 2003). The ML analyses were conducted using 100 random addition replicates and were based on a heuristic search using the tree bisection-reconnection (TBR) option with nucleotides as unordered characters. Tree nodal support was assessed by 1 000 bootstrap replicates (Felsenstein, 1985) performed on a computer cluster located at the University of Pretoria. Three independent BI analyses were conducted. Analyses with four chains were run for 5×10^6 generations using random starting trees. Trees and parameters were recorded every 100 generations. Two runs were performed simultaneously and split frequencies were compared every 100^{th} generation to ensure convergence of the runs. All runs used the default heating and swap parameters. The first 5 000 generations (10% burn-in) were excluded as the "burn-in". A 10% burn-in was sufficient to ensure that trees were only sampled from the region of stationarity.

A maximum parsimony tree (Kluge and Farris, 1969; Farris et al., 1970) was generated in PAUP for the combined data, using nucleotides as unordered characters and the tree bisection-reconnection (TBR) method were used to construct the shortest tree. A strict consensus tree was constructed if more than one minimum length tree were obtained. The following were reported: tree-length, consistency index (CI; Kluge and Farris, 1969),

retention index (RI; Farris, 1989), and rescaled consistency index (RC; Archie, 1989; Meier et al., 1991). Support values for internal nodes were determined using bootstrap analysis with 1 000 iterations (Felsenstein 1985).

3. Results

3.1 Sequence statistics

A total of 631 bp of the 5' end of the cyt *b* gene and 1309 bp of the 5' end of the RAG1 gene were analysed. Sequences obtained were either from the mitochondrial cyt *b* or the RAG1 gene since no stop codons were found when sequences were translated into the expected 210 and 436 amino acids, respectively. Of the 112 variable sites in the cyt *b* gene, 78 were phylogenetically informative (Table 3.1). A total of 12 individuals were heterozygous for sites in the RAG1 gene, with a maximum of 8 polymorphic sites in individuals NNH03 and NNH09. Polymorphic sites were included in all analyses and were treated as heterozygous sites. Of the 29 variable positions in the RAG1 gene, only 15 were phylogenetically informative (Table 3.1). The number of invariant site detected in the present study was noticeably higher. Most of the substitutions were silent with four variable amino acid sites between *M. namaquensis* individuals and 13 variable amino acids between the ingroup and the outgroup (*Rhabdomys pumilio*) individual for the cyt *b* gene. Six variable amino acids were detected between *M. namaquensis* individuals and 12 variable amino acids were detected between the ingroup taxa and the outgroup individual for the RAG1 gene.

Mean base compositions at the three different codon positions of the cyt *b* gene and for the RAG1 gene fragment for the whole sample examined are provided in Table 3.1 (excluding the outgroup individual). The overall base composition of cyt *b* showed that the four nucleotides do not occur in equal frequencies, and is similar to that of other mammalian cyt *b* sequences reported in the literature (Ducroz et al., 1998; Irwin et al., 1991). This strong bias in base composition showed an under-representation of guanine at both second (15.70%) and third (2.72%) codon positions. Similar to the sequence statistics in the larger dataset (Chapters 2), a higher representation of adenine at the third codon positions (44.96%) and thymine at the second codon positions (41.12%) was also observed in the present study. RAG1 has a nearly equal average base composition (see overall base

composition in Table 3.1). This nearly equal base composition is partially due to differing composition bias across codon position and gene regions that balance each other (i.e., the divergent versus the conserved regions).

Nucleotide divergence estimates were reported as Tamura-Nei distances with a gamma correction (Gu and Zang, 1997), and a proportion of invariable sites (TrN + Γ + I) for both the cyt *b* and RAG1 genes. Pairwise estimates of the nucleotide sequence divergence for the cyt *b* and the RAG1 gene are indicated in Table 3.2. Corrected cyt *b* Tamura-Nei sequence divergence values ranged between 0.00% and 8.11% within *Micaelamys* and sequence divergence values ranged between 0.08% and 1.44% for the RAG1 gene. Therefore, the RAG1 gene showed less variability as would be expected. Interestingly, some pairwise comparisons between individuals showed no sequence divergence based on the cyt *b* gene but when using the RAG1 gene, differences were detected.

Table 3.1 Variable sites and the average percentage base composition in a 631 bp fragment of the mitochondrial DNA (mtDNA) cytochrome *b* (cyt *b*) gene (A) and in the 1309 bp fragment of the nuclear Recombination Activating Gene 1 (RAG1) gene (B) within *Micaelamys namaquensis* from southern Africa.

A

CYTOCHROME *B*:	VARIABLE SITES	PHYLOGENTIC INFORMATVE	A	G	C	T
First codon position	13	9	28.87	23.31	17.55	30.27
Second codon position	4	2	18.34	15.70	24.84	41.12
Third codon position	95	67	44.96	2.72	32.98	19.33
Overall	112	78	30.72	13.92	25.12	30.24

B

RAG1:	VARIABLE SITES	PHYLOGENTIC INFORMATIVE	A	G	C	T
First codon position	9	2	31.26	25.37	25.69	17.68
Second codon position	9	1	33.99	22.46	18.31	25.24
Third codon position	44	12	21.05	26.15	31.27	21.53
Overall	62	15	28.77	24.66	25.08	21.49

Table 3.2 Pairwise estimates of percent Tamura-Nei (TrN) + Γ (1.97) + I (0.66) cytochrome *b* (cyt *b*) sequence divergence (below the diagonal) and percent Tamura-Nei (TrN) + Γ (0.87) + I (0.83) Recombination Activating Gene 1 (RAG1) sequence divergence (above the diagonal) between 35 *Micaelamys namaquensis* individuals from southern Africa. Outgroup individual: *Rhab* = *Rhabdomys pumilio*. Names follow that of the RAG1 alleles (Appendix 3.2). Corrected cyt *b* sequence divergence values for the outgroup are not given since undefined distances were obtained.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
1	NNH01	-	0.55	0.08	0.24	0.08	0.32	0.33	0.31	0.32	0.24	0.08	0.33	0.32	0.42	0.42	0.47	0.00	0.24	0.24	0.57	0.40	0.34	0.41	0.43	0.24	0.24	0.16	0.16	0.16	0.33	0.23	0.08	0.16	0.18	0.65	4.50
2	NNH02	2.12	-	0.94	0.95	0.82	1.16	1.04	1.04	1.16	0.94	0.94	0.88	1.03	1.16	1.16	0.68	0.74	0.96	0.95	1.11	1.06	1.16	1.26	0.95	0.93	0.93	0.85	0.85	0.75	1.16	0.93	0.93	0.75	0.75	1.38	5.86
3	NNH03	5.96	4.97	-	0.24	0.24	0.49	0.24	0.48	0.50	0.41	0.16	0.54	0.41	0.25	0.25	0.48	0.08	0.50	0.52	0.89	0.57	0.56	0.59	0.44	0.24	0.24	0.24	0.32	0.24	0.52	0.32	0.16	0.32	0.27	0.45	4.63
4	NNH04	5.95	4.97	0.00	-	0.24	0.49	0.08	0.48	0.49	0.41	0.16	0.52	0.32	0.33	0.33	0.18	0.16	0.49	0.51	0.88	0.57	0.54	0.58	0.34	0.00	0.00	0.24	0.32	0.24	0.51	0.24	0.08	0.32	0.27	0.56	4.45
5	NNH05	5.99	5.00	0.00	0.00	-	0.32	0.24	0.16	0.33	0.16	0.08	0.35	0.32	0.59	0.58	0.47	0.08	0.08	0.08	0.36	0.24	0.36	0.41	0.51	0.24	0.24	0.16	0.08	0.08	0.34	0.23	0.08	0.08	0.19	0.85	4.73
6	NNH06	5.99	4.97	0.32	0.33	0.33	-	0.58	0.16	0.00	0.08	0.32	0.00	0.00	0.88	0.87	0.59	0.41	0.33	0.34	0.68	0.40	0.00	0.08	0.71	0.48	0.48	0.16	0.16	0.16	0.00	0.32	0.32	0.32	0.44	1.18	5.48
7	NNH07	6.54	5.50	0.34	0.34	0.34	0.67	-	0.58	0.59	0.50	0.24	0.62	0.40	0.33	0.33	0.27	0.24	0.60	0.61	0.88	0.68	0.64	0.67	0.43	0.08	0.08	0.32	0.41	0.32	0.60	0.32	0.16	0.41	0.36	0.55	4.77
8	NNH08	6.22	5.20	0.16	0.16	0.16	0.49	0.50	-	0.16	0.00	0.32	0.17	0.16	0.87	0.86	0.56	0.40	0.08	0.08	0.36	0.16	0.17	0.24	0.52	0.48	0.48	0.16	0.08	0.08	0.16	0.32	0.32	0.24	0.44	1.16	5.28
9	NNH09	6.01	4.98	0.32	0.33	0.32	0.33	0.67	0.49	-	0.08	0.33	0.00	0.00	0.88	0.87	0.59	0.41	0.33	0.34	0.68	0.41	0.00	0.08	0.71	0.49	0.49	0.16	0.16	0.16	0.00	0.32	0.32	0.33	0.44	1.18	5.60
10	NNH10	6.82	5.70	0.67	0.67	0.49	0.49	1.03	0.84	0.66	-	0.24	0.08	0.08	0.78	0.77	0.47	0.33	0.08	0.08	0.37	0.24	0.08	0.16	0.52	0.41	0.41	0.08	0.08	0.08	0.08	0.24	0.24	0.16	0.35	1.05	5.33
11	NNH11	7.09	6.01	0.67	0.67	0.67	0.66	1.03	0.84	0.67	1.00	-	0.34	0.24	0.42	0.41	0.36	0.08	0.34	0.34	0.68	0.42	0.35	0.41	0.52	0.16	0.16	0.08	0.16	0.08	0.33	0.16	0.00	0.16	0.09	0.64	4.65
12	NNH12	6.23	5.21	0.49	0.50	0.50	0.16	0.85	0.65	0.51	0.65	0.84	-	0.00	0.93	0.93	0.59	0.44	0.36	0.35	0.70	0.45	0.00	0.08	0.75	0.52	0.52	0.17	0.17	0.17	0.00	0.25	0.33	0.35	0.36	1.22	5.98
13	NNH13	7.32	7.07	5.59	5.47	5.43	6.12	6.14	5.88	5.67	6.22	6.62	6.37	-	0.69	0.69	0.37	0.33	0.32	0.33	0.67	0.40	0.00	0.08	0.52	0.32	0.32	0.16	0.16	0.16	0.00	0.24	0.24	0.24	0.33	0.94	5.31
14	NNH14	7.04	6.21	6.05	6.03	6.04	6.04	6.29	6.32	6.04	6.56	7.25	6.34	2.56	-	0.00	0.58	0.33	0.90	0.89	1.22	0.97	0.96	0.97	0.35	0.16	0.16	0.59	0.69	0.59	0.89	0.60	0.33	0.68	0.55	0.18	5.02
15	NNH15	6.71	5.92	5.80	5.79	5.85	5.78	6.02	6.05	5.80	6.57	6.93	6.06	2.54	0.00	-	0.58	0.33	0.89	0.89	1.22	0.96	0.96	0.96	0.35	0.16	0.16	0.59	0.68	0.59	0.89	0.59	0.33	0.68	0.55	0.18	4.94
16	NNH16	6.54	6.00	5.08	5.07	5.08	5.09	5.55	5.34	5.09	5.59	6.19	5.36	2.06	1.50	1.49	-	0.37	0.58	0.58	0.92	0.58	0.59	0.69	0.38	0.17	0.17	0.28	0.38	0.28	0.59	0.17	0.26	0.38	0.38	0.78	5.72
17	NNH17	6.31	6.05	5.03	5.04	5.05	5.08	5.63	5.28	5.10	5.86	6.09	5.28	1.05	1.92	2.04	1.64	-	0.33	0.34	0.69	0.49	0.46	0.50	0.34	0.16	0.16	0.16	0.24	0.16	0.43	0.24	0.08	0.16	0.18	0.54	4.45
18	NNH18	6.55	5.65	6.42	6.38	6.39	6.47	6.88	6.73	6.48	7.01	7.68	6.74	5.11	4.81	4.77	4.65	4.51	-	0.00	0.28	0.16	0.37	0.42	0.52	0.49	0.49	0.16	0.00	0.00	0.35	0.32	0.33	0.16	0.46	1.18	5.44
19	NNH19	6.09	5.15	5.96	5.93	5.94	5.95	6.18	6.25	5.96	6.17	7.21	6.27	5.77	5.29	5.30	5.03	4.86	0.38	-	0.28	0.17	0.37	0.42	0.52	0.51	0.51	0.17	0.00	0.00	0.34	0.34	0.34	0.17	0.46	1.18	5.59
20	NNH20	6.40	5.21	5.82	5.87	5.82	5.88	6.49	6.10	5.88	5.77	7.02	6.12	5.00	4.92	4.81	4.54	4.40	0.54	0.37	-	0.38	0.68	0.78	0.88	0.88	0.88	0.48	0.28	0.28	0.68	0.60	0.68	0.49	0.79	1.44	6.52
21	NNH21	7.05	5.26	4.50	4.50	4.52	4.96	5.03	4.74	4.56	5.48	5.50	5.18	4.92	5.19	4.99	4.34	4.88	3.41	3.92	3.17	-	0.36	0.50	0.61	0.57	0.57	0.24	0.16	0.16	0.43	0.40	0.40	0.32	0.46	1.18	4.92
22	NNH22	7.86	5.86	5.41	5.40	5.42	5.42	5.97	5.66	5.43	6.18	6.46	5.67	5.16	5.18	5.00	4.78	4.76	3.38	3.87	3.15	1.01	-	0.09	0.75	0.54	0.54	0.18	0.18	0.18	0.00	0.34	0.34	0.37	0.46	1.18	6.36
23	NNH23	6.19	5.67	6.20	6.19	6.23	6.27	6.82	6.50	6.29	7.15	7.40	6.50	4.71	4.70	4.51	4.36	3.83	4.48	5.08	4.15	3.80	3.78	-	0.80	0.58	0.58	0.25	0.25	0.25	0.08	0.41	0.41	0.41	0.54	1.28	5.79
24	NNH24	5.52	4.05	3.72	3.71	3.74	3.71	3.93	3.94	4.21	4.16	4.72	3.95	3.88	3.09	3.00	2.89	3.28	3.71	3.94	3.26	3.21	3.21	3.73	-	0.32	0.32	0.43	0.43	0.34	0.71	0.42	0.42	0.52	0.65	0.56	5.48
25	NNH25	5.91	5.36	5.00	4.98	4.73	5.06	5.44	5.26	5.50	5.24	6.09	5.27	3.36	3.21	3.30	2.93	3.26	3.05	3.49	2.88	4.13	4.14	3.24	1.30	-	0.00	0.24	0.32	0.24	0.51	0.24	0.08	0.32	0.27	0.34	4.50
26	NNH26	5.78	5.23	4.87	4.85	4.85	4.93	5.32	5.14	5.38	5.41	5.97	5.15	3.40	2.75	2.72	2.49	2.89	3.46	3.96	3.34	3.58	3.58	3.12	0.74	0.70	-	0.24	0.32	0.24	0.51	0.24	0.08	0.32	0.27	0.34	4.50
27	NNH27	7.34	6.11	3.94	3.80	3.79	4.38	4.33	4.18	3.99	4.21	4.76	4.60	3.95	5.49	5.30	4.60	4.17	4.68	4.76	3.80	3.57	3.97	5.03	4.21	4.39	4.27	-	0.00	0.00	0.17	0.08	0.08	0.08	0.17	0.85	4.79
28	NNH28	8.11	6.75	4.72	4.71	4.71	5.22	5.16	4.98	4.76	4.91	5.79	5.48	4.83	5.78	5.70	5.37	4.81	4.88	4.98	4.18	4.41	4.36	5.52	4.57	4.49	4.52	0.53	-	0.00	0.17	0.16	0.16	0.08	0.26	0.96	4.93
29	NNH29	7.52	6.31	4.13	4.00	4.00	4.57	4.52	4.36	4.17	4.39	4.95	4.79	4.14	6.29	6.03	5.31	4.74	4.42	4.47	3.57	3.75	4.20	5.24	4.43	4.15	4.50	0.51	0.71	-	0.17	0.08	0.08	0.00	0.17	0.86	4.79
30	NNH30	7.76	6.81	4.78	4.78	4.80	5.29	5.06	5.05	4.83	5.26	5.88	5.05	5.53	6.61	6.43	5.24	5.44	5.92	5.82	5.05	4.75	5.44	6.14	4.68	5.05	4.91	1.25	1.49	1.06	-	0.33	0.33	0.34	0.45	1.18	5.89
31	NNH31	5.26	4.72	4.60	4.47	4.24	5.08	4.99	4.85	5.08	4.88	5.47	5.32	5.91	6.34	6.64	5.58	5.82	6.31	6.21	5.79	5.83	6.81	6.94	4.49	4.62	4.78	4.90	5.18	5.11	5.51	-	0.16	0.16	0.17	0.74	5.02
32	NNH32	5.90	4.89	4.69	4.67	4.47	5.17	4.88	4.93	5.19	5.12	5.72	5.42	6.28	6.36	6.66	5.66	6.07	6.33	6.18	6.07	6.05	7.08	7.18	4.49	4.69	4.83	5.20	5.27	5.40	5.57	0.72	-	0.16	0.09	0.54	4.52
33	NNH33	5.24	4.70	4.61	4.47	4.24	5.06	4.98	4.84	5.08	4.87	5.45	5.30	5.91	6.34	6.62	5.58	5.80	6.31	6.21	5.79	5.81	6.80	6.93	4.47	4.61	4.78	4.90	5.18	5.09	5.51	0.00	0.72	-	0.26	0.96	4.91
34	NNH34	6.74	6.85	5.19	5.17	4.89	5.29	5.77	5.47	5.69	4.86	5.96	5.48	6.50	5.80	5.83	5.45	5.85	6.88	6.32	5.76	6.94	7.44	5.76	4.87	4.56	4.70	5.48	6.18	5.66	7.01	6.20	6.40	6.18	-	0.75	5.33
35	NNH35	4.92	4.49	4.11	4.10	4.10	4.14	4.35	4.09	4.60	4.61	5.15	4.37	3.98	3.20	3.17	3.01	3.21	3.55	3.64	3.38	3.62	3.62	3.65	1.75	1.88	1.53	4.32	4.63	4.56	5.36	4.07	4.10	4.07	4.77	-	5.89
36	*Rhab*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

3.2 Phylogenetic analyses

The results of saturation analyses for both cyt *b* and RAG1 are summarised in Figs. 3.2 and 3.3, respectively. First and second position changes accumulated slowly for both genes. The rate of transversional substitutions was lower than that of transitions (Ti:Tv ratio = 13:1 and 2:1 for the cyt *b* and RAG1 genes, respectively). Transitions normally outnumber transversions but this high Ti:Tv ratio in the cyt *b* gene is rather unusual and has also been reported in vlei rats of the genus *Otomys* (Maree, 2002). Transversions and transitions at all positions were not saturated (Figs. 3.2 and 3.3). This result has also been reported for other rodents (Lecompte et al., 2002).

Tamura-Nei sequence divergence (α-value for the gamma shape parameter and a value for the proportion of invariable sites) estimates were used to infer relationships among the 35 *M. namaquensis* individuals for the cyt *b* and RAG1 genes. Data for the outgroup were also included in these analyses. The average separation between *M. namaquensis* and the outgroup was estimated to be between 16.48% and 18.70% based on uncorrected p-distances (Tamura-Nei + Γ + I resulted in undefined distances) for the cyt *b* gene, while the average separation between *M. namaquensis* and the outgroup was estimated at 6.05% (range 4.45% - 6.52%) for the RAG1 gene.

The BI phylograms (Figs. 3.4, 3.5 and 3.6) depict the relationships between 11 of the 14 lineages (as identified in Chapter 2) and is characterised by short internal branches possibly due to a rapid radiation of lineages that have also been reported for other rodents. Individuals from lineage A (Koppies Dam), lineage F (Volksrust) and lineage L (Sub-Escarpment Grassland bioregion) were not included in the BI analyses (see Chapter 2). Six well-supported (posterior probability values of ≥ 0.95) lineages with clear geographical patterns were identified in the combined (cyt *b* and RAG1) analysis (Fig. 3.6): 1) B, Grassland; 2) G, Albany Thicket; 3) H, western Fynbos; 4) I, Bushmanland/Upper Karoo bioregion; 5) J, Nama-Karoo; and 6) M, Eastern Kalahari Bushveld. While lineages D and N appeared to be associated with the Lowveld bioregion and the Savanna biome, respectively, these nodes had no support. Lineages C, E, and K were only represented by one individual each. It was also evident from the combined analysis that there may be a clade comprising lineages B (Grassland biome), C (Machadodorp and Malelane) and D (Lowveld bioregion) although lineages C (with only one individual) and D were not supported (Figs. 3.6 and 3.7). Likewise, there was support (posterior probability value of ≥

0.90) for an association between lineages E (Fouriesburg), G (Albany Thicket bioregion) and H (Fynbos biome) (Figs. 3.6 and 3.7). Some of the associations between lineages and biome/bioregions were not apparent (lineage B - Grassland biome and lineage G - Albany Thicket bioregion). The independent BI analysis using cyt *b* sequences (Fig. 3.4) gave the same topology as the combined (cyt *b* and RAG1) BI phylogram (Fig. 3.6). The independent RAG1 BI tree had a different topology and some lineages were not monophyletic (Fig. 3.5). More nodes were supported using the combined approach (14 nodes compared to nine and four nodes for cyt *b* and RAG1, respectively). Most lineages showed good support (posterior probability values of $\geq$ 0.95) in the combined analysis (Fig. 3.6) and this was also evident in the ML analysis (not shown). The ML analyses gave the same topologies as their respective BI phylograms (not shown). The combined parsimony analysis gave the same topology as the combined BI tree (Figs. 3.6). The following parsimony statistics were obtained: 102 parsimony informative characters; tree length = 252; CI = 0.46; RI = 0.74; RC = 0.34. These nodes showed high bootstrap support (indicated in squares in Fig. 3.6). The parsimony analysis did not support the two groupings that were identified in the combined BI analysis: 1) Lineages B, C, and D and 2) Lineages E, G and H. Lineage N (Savanna biome) was not supported in the combined BI analysis, while a support of 71% was detected in the maximum parsimony analysis.

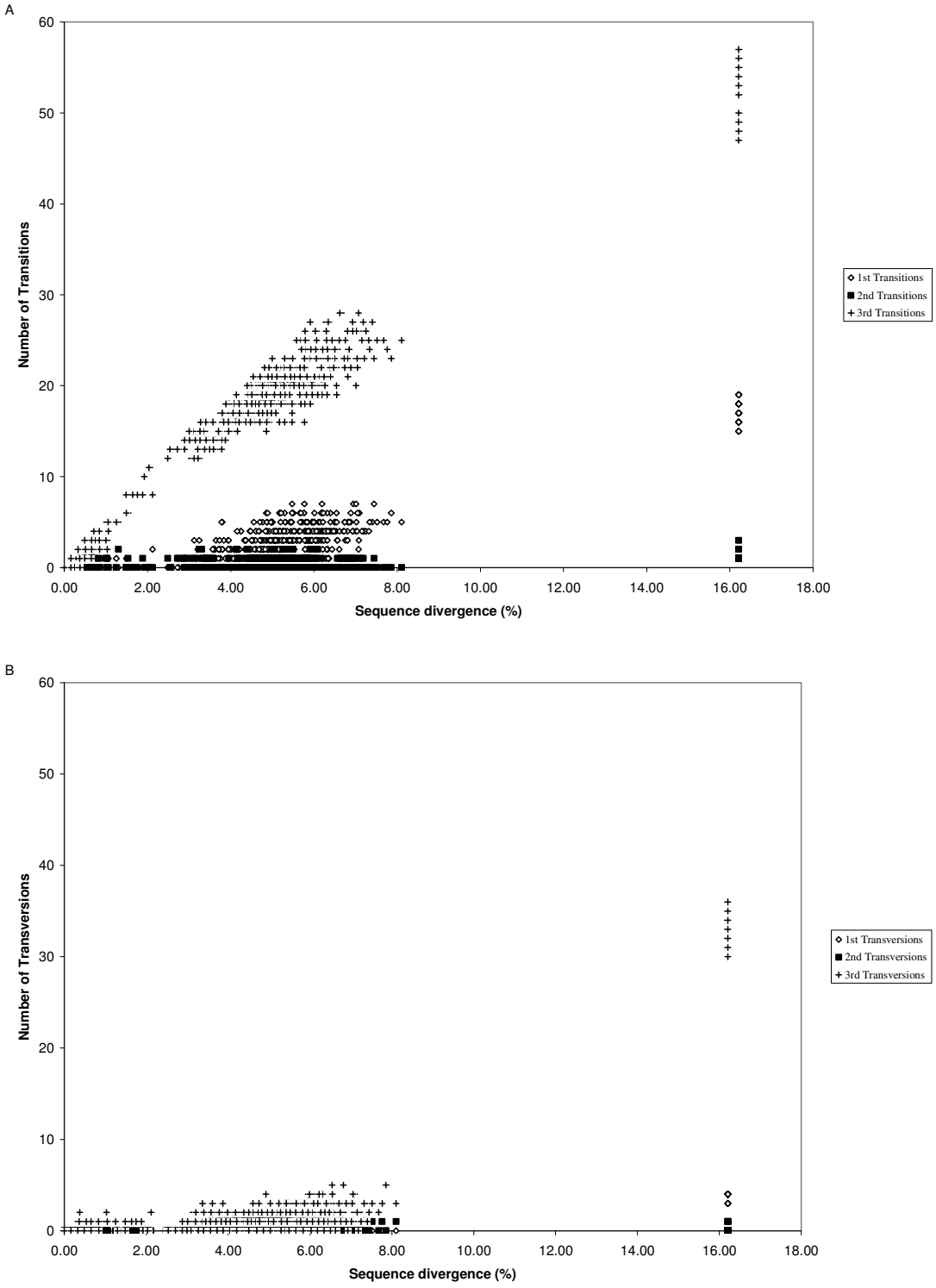

Figure 3.2 The number of transitions (A) and transversions (B) plotted against cytochrome *b* Tamura-Nei + Γ (1.97) + I (0.66) sequence divergence (%) estimates between 35 individuals of *Micaelamys namaquensis* and one outgroup (*Rhabdomys pumilio*) from southern Africa. (◊) = First codon positions, (■) = second codon positions and, (+) = third codon positions.

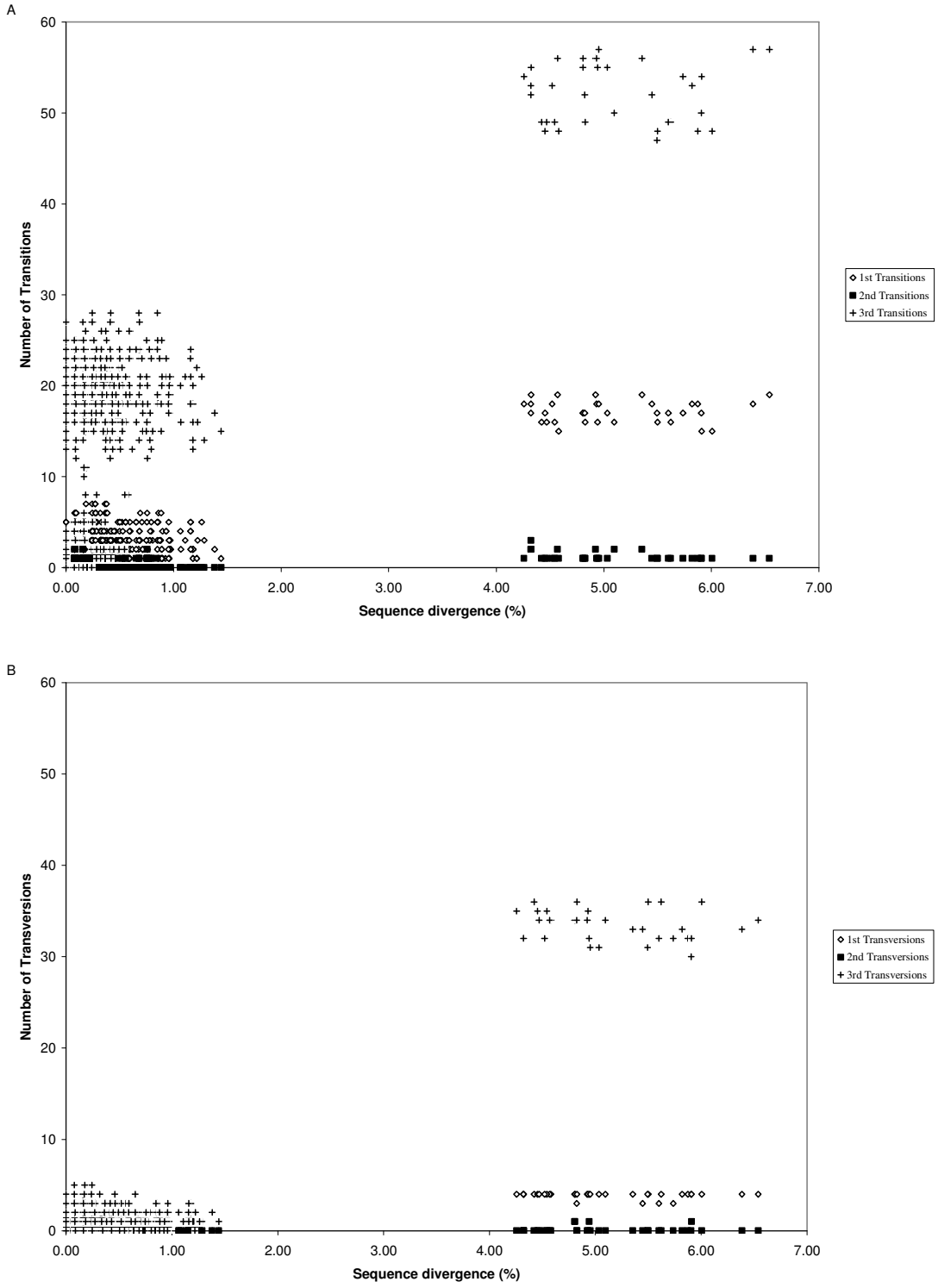

Figure 3.3 The number of transitions (A) and transversions (B) plotted against RAG1 Tamura-Nei + Γ (0.87) + I (0.83) sequence divergence (%) estimates between 35 individuals of *Micaelamys namaquensis* and one outgroup (*Rhabdomys pumilio*) from southern Africa. (◊) = First codon positions, (■) = second codon positions, and (+) = third codon positions.

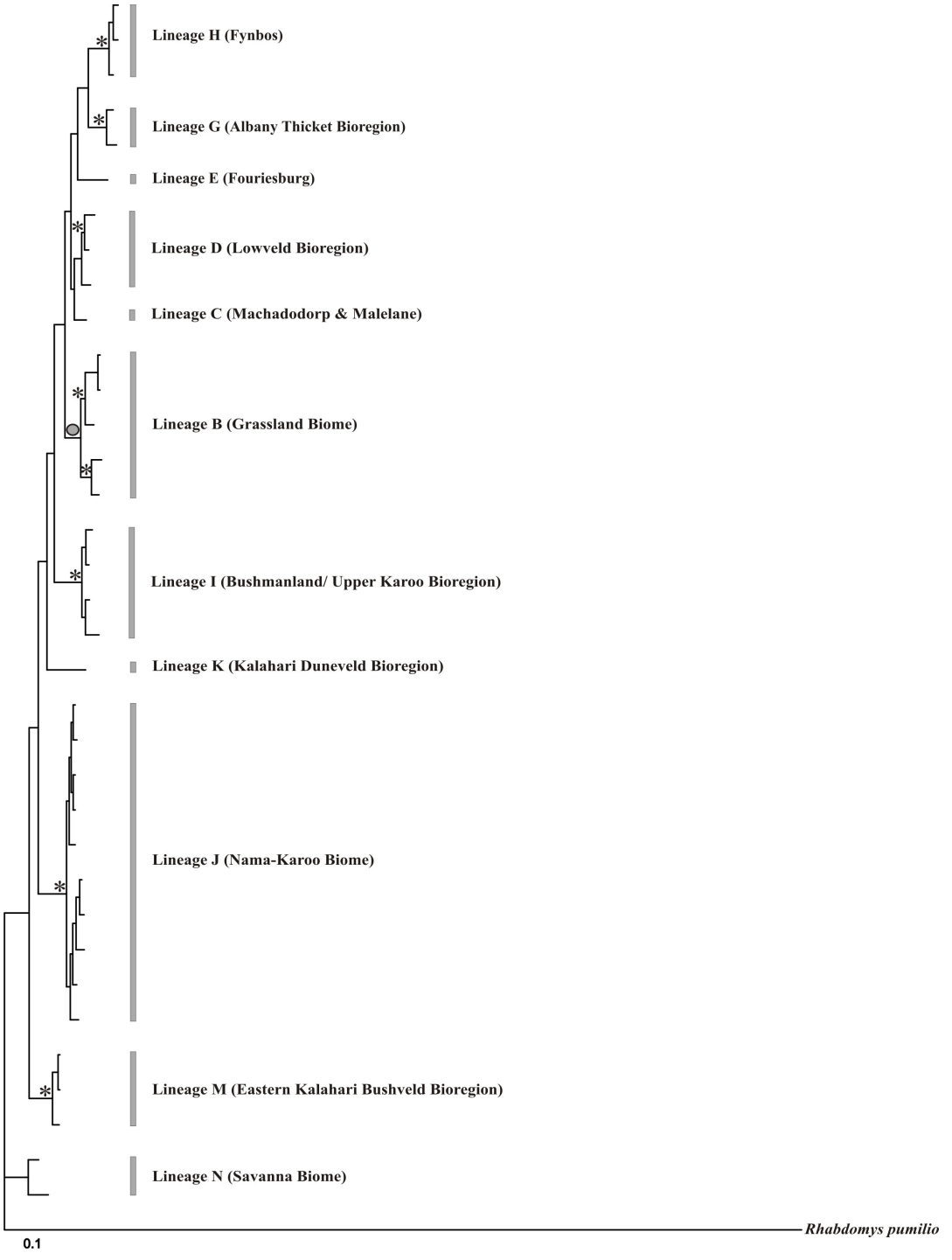

Figure 3.4 A Bayesian Inference (BI) tree derived from mitochondrial DNA (mtDNA) cytochrome *b* (cyt *b*) sequences of *Micaelamys namaquensis* from southern Africa. Different lineages correspond to the lineages identified in Chapter 2 (see Fig. 2.4). The BI posterior probability values for internal branches are given at each node with either an asterisk (*) or a circle (•) where asterisks indicate BI posterior probability values ≥ 0.95, while circles indicate BI posterior probability values ≥ 0.90. *Rhabdomys pumillio* was used as an outgroup.

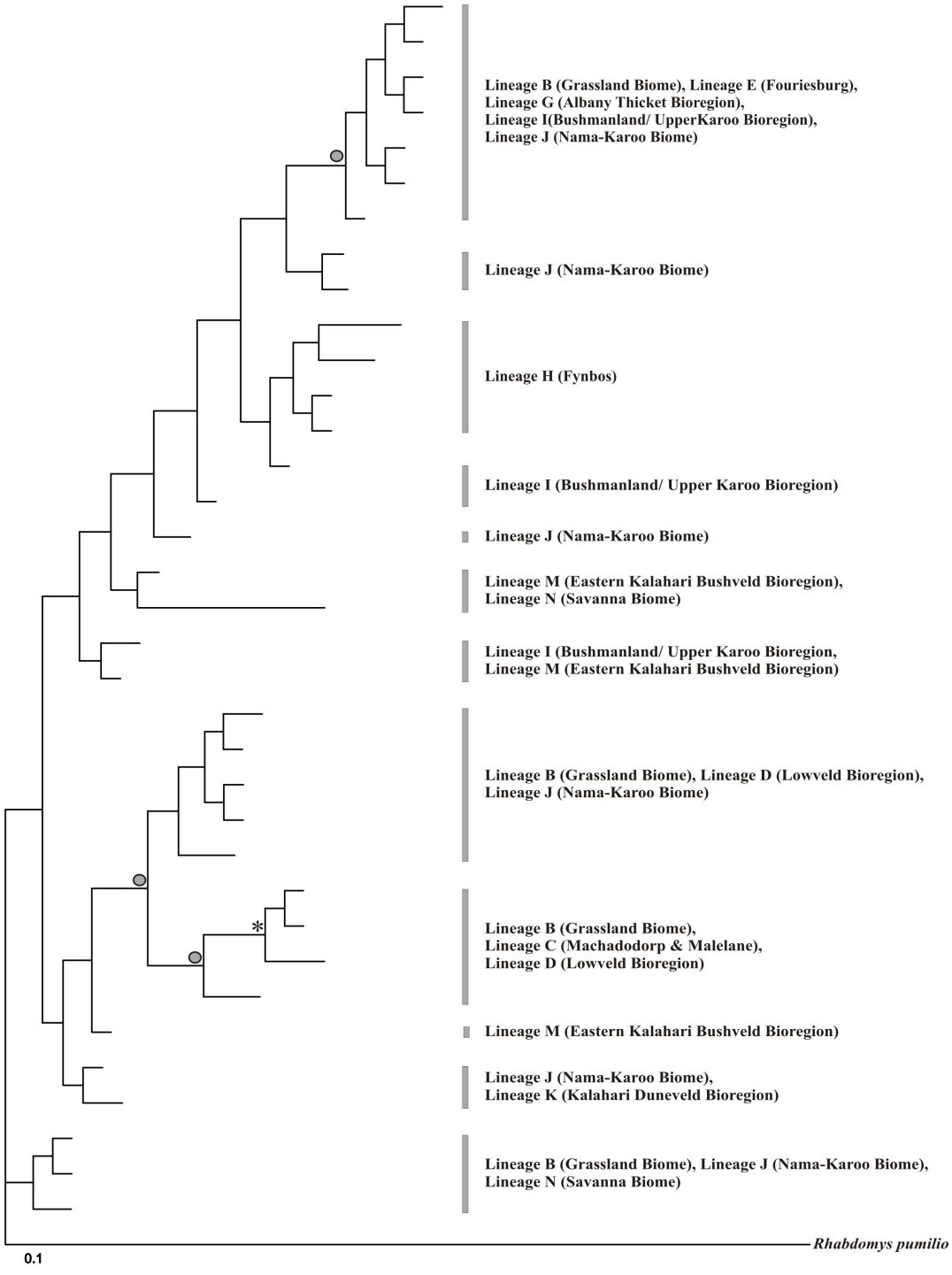

Figure 3.5 A Bayesian Inference (BI) tree derived from Recombination Activation Gene 1 (RAG1) sequences of *Micaelamys namaquensis* from southern Africa. Different lineages correspond to the lineages identified in Chapter 2 (Fig. 2.4). The BI posterior probability values for internal branches are given at each node with either an asterisk (*) or a circle (•) where asterisks indicate BI posterior probability values ≥ 0.95, while circles indicate BI posterior probability values ≥ 0.90. *Rhabdomys pumillio* was used as an outgroup.

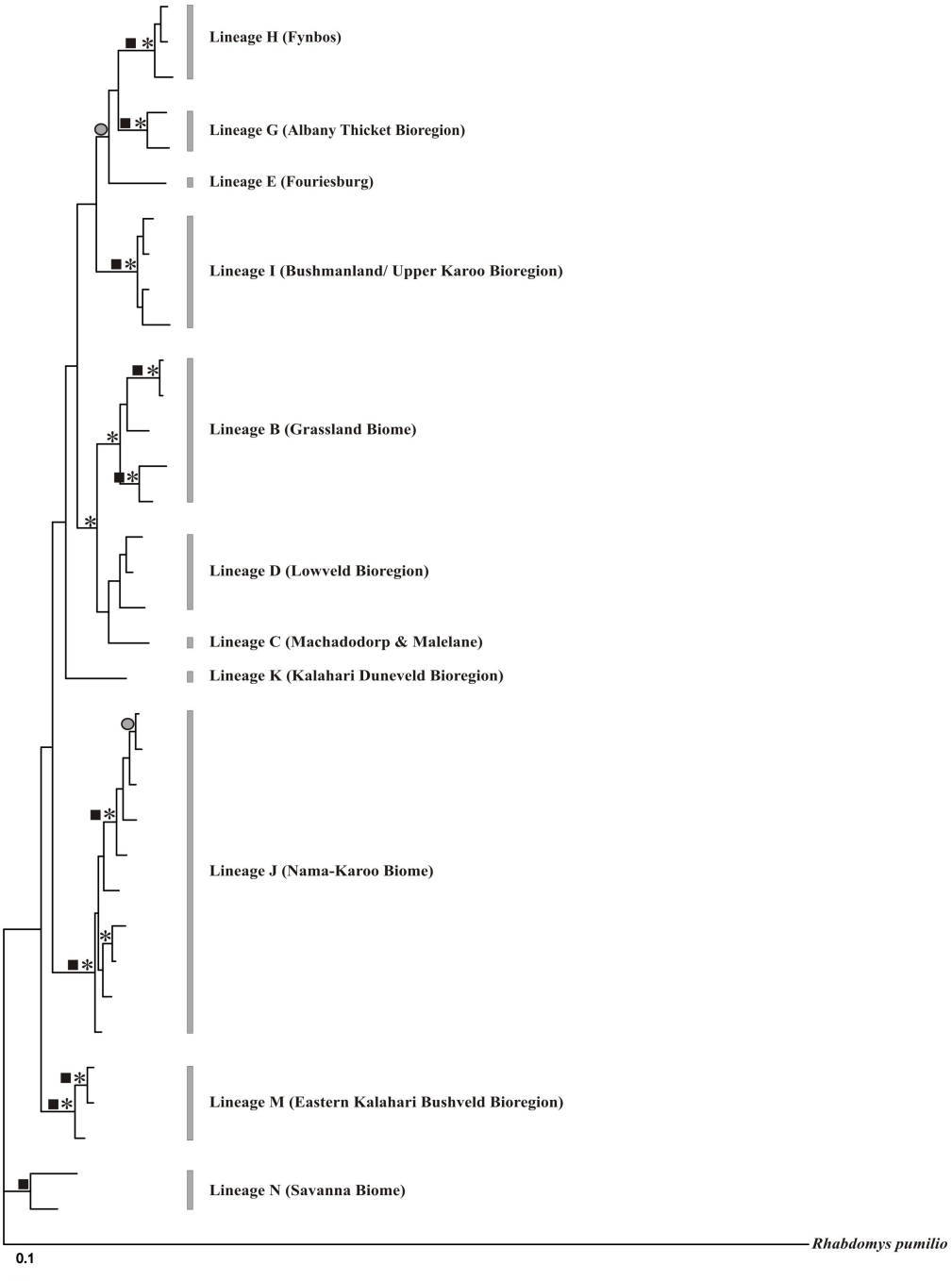

Figure 3.6 A Bayesian Inference (BI) tree derived from mitochondrial DNA (mtDNA) cytochrome *b* (cyt *b*) and Recombination Activation Gene 1 (RAG1) sequences of *Micaelamys namaquensis* from southern Africa. Different lineages correspond to the lineages identified in Chapter 2 (Fig. 2.4). The BI posterior probability values for internal branches are given at each node with either an asterisk (*) or a circle (•) where asterisks indicate BI posterior probability values ≥ 0.95, while circles indicate BI posterior probability values ≥ 0.90. Maximum parsimony bootstrap confidence limits (above 70% occurrence in 1 000 replicates) for internal branches are given at each node and indicated by squares (■). *Rhabdomys pumillio* was used as an outgroup.

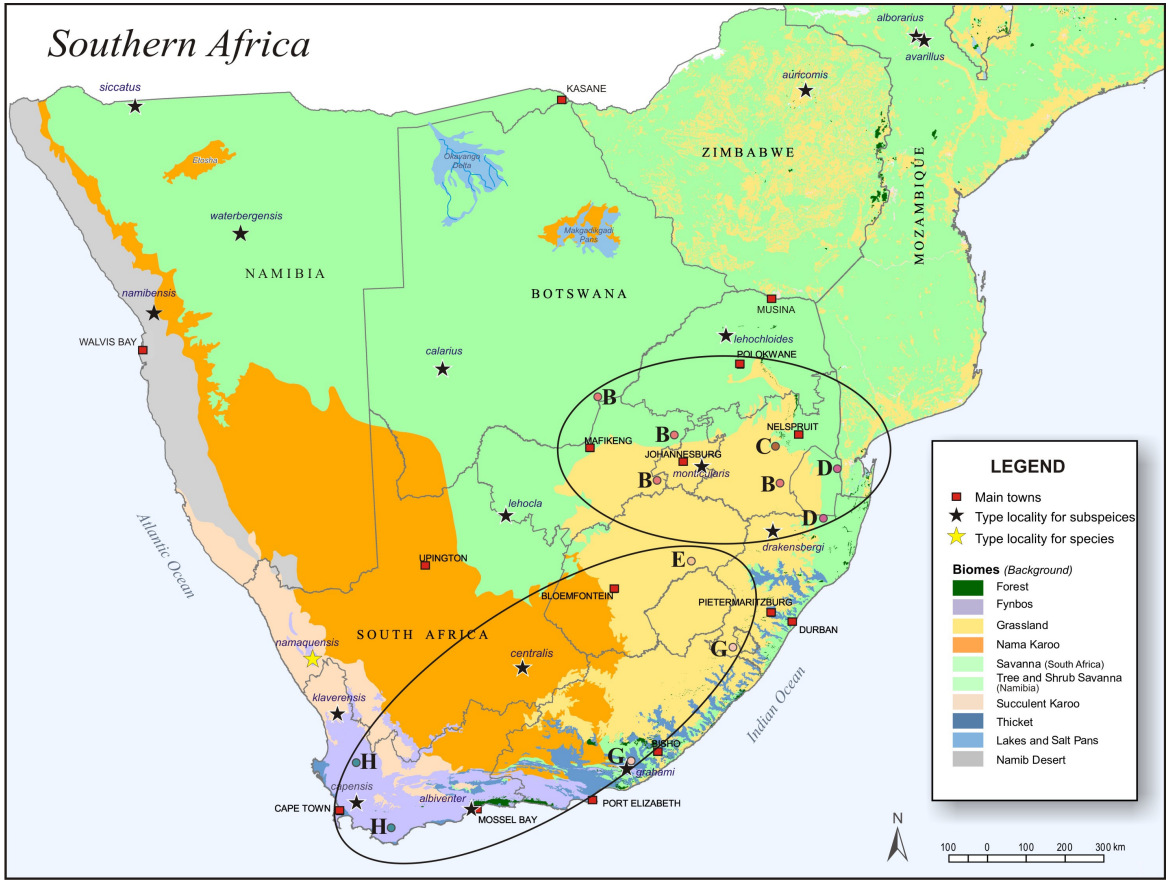

Figure 3.7 Major biomes of southern Africa (Low and Rebelo, 1996). Background colours for the major biomes are indicated in the legend; major lakes and saltpans are also included. The yellow star indicates the type locality for *Micaelamys namaquensis* and the blue stars indicate the type localities for its 16 previously described subspecies. Lineages B, C, D, E, G and H (see Chapter 2 for identified lineages) are indicated on the map (only well-supported associations between lineages are indicated). The encircled areas indicate the two groupings: (1) Lineage B, C and D and (2) Lineage E, G and H that were identified in the combined Bayesian Inference (BI) phylogram (Fig. 3.6).

4. Discussion

The phylogenetic analyses presented in the present study offer several insights into the evolutionary relationships within *M. namaquensis*. In this study, 35 individuals representing 11 of the 14 lineages from southern Africa as identified in Chapter 2 were analysed. The samples were subjected to molecular systematic analyses using the mitochondrial cyt *b* and nuclear RAG1 genes. Based on the independent cyt *b* and combined (cyt *b* and RAG1) ML and BI analyses, six lineages were recognised with strong geographical patterns showing an association with different vegetation types of southern Africa. Although the remaining lineages were not supported, they also were associated with biomes/bioregions (vegetation types) of southern Africa. These unsupported lineages were, in contrast, supported by the ML, BI and BEAST analyses in Chapter 2. It was not surprising that many of the relationships within the genus *Micaelamys* were not well-resolved, even using the combined approach. This lack of posterior support may well be a reflection of incomplete lineage sorting in the nuclear RAG1 gene (Belfiore et al., 2008).

A relationship between lineages B (Grassland biome), C (Machadodorp and Malelane) and D (Lowveld bioregion) was evident from the combined analysis although lineage C (represented by only one individual) and D were not supported (Figs. 3.6 and 3.7). Likewise, there was support (posterior probability value of ≥ 0.90) for some association between lineages E (Fouriesburg), G (Albany Thicket bioregion) and H (Fynbos biome) (Figs. 3.6 and 3.7). It was also evident that there is a geographic association between these lineages. Lineages B, C and D occur within the Grassland biome while lineage E, G and H are associated with the Great Escarpment. The geographic association of lineages may explain the groupings of these respective lineages. The sister-relationship between lineages B, C and D was also evident in the BEAST analysis but the relationship between lineages E, G and H was not supported by the BEAST analysis (see Chapter 2, Fig. 2.6). Some of the associations between lineages and biome/bioregions (lineage B - Grassland biome and lineage G - Albany Thicket bioregion) were not apparent; these discrepancies might be an indication that some lineages have been expanded in recent times. Associations between lineages might not hold considering more individuals representing each lineage. Results should therefore be interpreted with caution.

The incongruence between the independent RAG1 analysis and the independent cyt *b* and combined analysis may be due to incomplete lineage sorting within the nuclear RAG1 gene. Different historical and demographic scenarios have been described as being responsible for incomplete lineage sorting in rapidly radiating rodent species within the genus *Thomomys* (Thaeler, 1968). The different scenarios may be as follow: 1) some species have diverged recently through vicariant events, as a result of partial niche overlap, such that lineage sorting is incomplete but will become complete with time (Thaeler, 1968); 2) other species have arisen rapidly by peripheral isolation from the "parent" species, a portion of the genome of the new species is then identical to that of the "parent" species, without additional selective forces these lineages will not become completely sorted unless an extinction of one of the species occurs (Rogers, 1991a, b) and 3) species have diverged and become effectively reproductively isolated, but neither drift nor selection has been strong enough to eliminate the shared gene histories (Patton, 1990; Patton et al., 1972). *Micaelamys namaquensis* is currently classified as a single species and although the above scenarios have been described at a higher taxonomic level, the divergences that have been estimated within *M. namaquensis* are comparable with these higher level (between species) divergences (up to 7.68% divergence in cyt *b*, see Table 3.2).

Time elapsed since the dichotomy event should also be considered as a key factor in the evolution of lineage sorting. If enough time has passed then lineage sorting should be complete. In contrast, a signal of incomplete lineage sorting would be evident in species that have evolved in recent times. Divergences between *M. namaquensis* lineages are fairly old (if the estimated divergence dates presented in Chapter 2 is correct) and the result of incomplete lineage sorting is somewhat surprising. The incomplete lineage sorting detected within the species may be as a result of the selection specific to the gene (RAG1) and the mutation rate for this gene.

Mitochondrial DNA trees have a greater chance of being congruent with speciation history because its effective population size (N_e) is only one-quarter of any nuclear locus (McCracken and Sorenson, 2005; Moore, 1997). Therefore, complete lineage sorting will take longer in any nuclear gene. In general, a lack of complete lineage sorting would not have been evident without the use of multiple genes and more than one individual per taxon (Belfiore et al., 2008).

So far, only a few studies have compared the utility of mitochondrial and nuclear sequence data for phylogenetic analysis (e.g., Adkins et al., 2001; Matthee et al., 2001; McCracken and Sorenson, 2005). Most of these studies have been conducted on groups with older divergences where the slower rate of nuclear genes was more informative (Steppan et al., 2005). Steppan et al. (2005) showed that within recently evolved subfamilies, the mitochondrial DNA appears to be less informative at the deeper nodes. This was surprising because the basal node was only 12 MYA, an age younger than many of the mitochondrial phylogenetic studies that have been conducted on mammals (Catzeflis et al., 1995; Honeycutt et al., 1995; Irwin et al.; 1991; Yang and Yoder, 2003). Steppan et al. (2005) also demonstrated that the decline in bootstrap values for deeper nodes was as a result of the mtDNA whereas the nuclear genes showed no significant loss of robustness with increasing depth. The lower bootstrap support for the deeper mtDNA nodes may be due to the shorter deep branches in the mtDNA tree. Likewise, the mtDNA cyt *b* BI phylogram (Fig. 3.4) in the present study was characterised by short deep branches. In contrast, the RAG1 BI phylogram (Fig. 3.5) did not show such short deep branches. The poor resolution that was obtained for higher-level relationships (deeper nodes) made it impossible to ascertain the phylogenetic affinities between the *M. namaquensis* lineages. This may be due to the rapid radiation of rodents entering sub-Saharan Africa from Eurasia, possibly through the Middle East (Ducroz et al., 2001), and more samples and other DNA markers (e.g., more nuclear genes) may assist in resolving these relationships. The Murinae has therefore originated in Asia and colonised both Europe and Africa during dispersal events at about 11.8 MYA (Lecompte et al., 2008). Animals followed the establishment of a vegetation corridor across the Arabian peninsula connecting Africa and Asia. It has also been suggested that lineages moving into Africa were differentiated prior to their dispersal into Africa (Lecompte et al., 2008).

It is also evident from Steppan et al. (2005) that mtDNA data deteriorates measurably for murine nodes older than about 6 MYA. Figure 2.6 in Chapter 2 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 well-supported nodes in the ML phylogram (Chapter 2, Fig. 2.4) may be a result of these younger divergences. 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. Subsequent divergence of two groups followed: 1) a group more confined to the mesic habitats of southern Africa (lineages A-H), and 2) a group found in the more arid habitats of southern Africa (lineages I-N). The major diversification within this species-group appears to have occurred during the Late Miocene, between 7.87 MYA and 5.30 MYA (see Chapter 2, Fig. 2.4). These deeper nodes were not well-supported in the ML phylogram (Chapter 2, Fig. 2.4) possibly due to ages older than 6 MYA. Therefore, the utility of mtDNA should be extended to more recent divergences (younger than 6 MYA). In contrast, mitochondrial DNA was still informative when divergence dates older than 6 MYA were used in the present study (see Chapter 2, Fig. 2.6).

Steppan et al. (2005) suggested that slower evolving nuclear exons should be used more often in phylogenetic studies even for relatively recent divergence dates (younger than 5 MYA). In addition, it has been shown that more promising results are coming from analyses of less rapidly evolving nuclear genes such as LCAT (Michaux and Catzeflis, 2000; Robinson et al., 1997), vWF (Jansa and Weksler, 2004; Michaux et al., 2001) and IRBP (deBry and Sagel, 2001; Jansa and Weksler, 2004; Suzuki et al., 2004). Similarily, intron sequences have been shown to be informative at a phylogenetic level (deBry and Seshadri, 2001; Robinson-Rechavi et al., 2000; Steppan et al., 2004a). An added advantange of intron sequences is that it provides an ideal source of nuclear non-coding DNA that are flanked by protein-coding regions that may allow for robust PCR primers (deBry and Seshadri, 2001).

It was also shown in the present study that fewer nodes in the independent cyt *b* and RAG1 analyses were supported. Once the datasets were combined the supported nodes were increased from nine (cyt *b*) and four (RAG1) to 14 in the combined analysis suggesting that combined approaches (multiple genes, mitochondrial and nuclear) may be useful for phylogenetic analysis. Cummings et al. (1995) noted that phylogenetic analyses of several short stretches from different genes show a better performance than analyses based on single gene fragments. Future research should include a multidisciplinary approach (both faster and slower evolving nuclear genes, chromosomes, morphology) on more samples.

5. Conclusion

Micaelamys namaquensis is a polytypic species with more variation than previously thought. The diversity detected within *M. namaquensis* appears to be indicative of a species complex. Only 11 lineages were included in the phylogenetic analyses that were supported in the BI analyses. Of these six lineages were well-supported with strong geographic patterns. Although the remaining lineages were not supported, they nevertheless are associated with biomes/bioregions (vegetation types) of southern Africa. This suggests that the taxonomic status of *M. namaquensis* needs further investigation and the species is in need of a taxonomic revision based on a multidisciplinary approach and extensive sampling.

6. Acknowledgements

I am grateful to all field assistance for helping with the collection of samples (M.J. Russo, T.J. Grant, C.J. Oosthuizen, S.M.R dos Santos, W. Delport, E.R. Swartz, H. Roos, F.J. Lötz, A.V. Linzey, A. Hulse, M. Kesner, I. Stermann, M. Cunningham, N. Maputla, V. Coetzee, M. de Castro, U. Kryger, H. Smit, S.L. Gardner). Thanks also to Albé van der Merwe for computational support. C.L. Sole is thanked for the constructive comments on earlier drafts of this chapter. This study was funded by the National Research Foundation grant to PB and CTC (Grant number: 2073181) and the University of Pretoria.

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Appendix 3.1 Geographic coordinates of all collecting localities of *Micaelamys namaquensis* in South Africa, Swaziland, Botswana and Namibia representing individuals with sequences for both the mitochondrial DNA (mtDNA) cytochrome *b* (cyt *b*) gene and the nuclear Recombination Activating Gene 1 (RAG1) gene that were analysed in the present study. Numbers 1 - 30 correspond to those in Fig. 3.1.

LOCALITY	COUNTRY	PROVINCE	GEOGRAPHIC COORDINATE
Savanna Biome			
1. Francistown, just outside town (municipal grounds)	Botswana		21°11'15"S 27°23'22"E
2. Farm: Goedgelegen, Baltimore	South Africa	Limpopo	23°26'27"S 28°23'02"E
Nama-Karoo Biome			
3. Farm: Steenkampsput, Upington	South Africa	Northern Cape	28°06'13"S 20°54'10"E
4. Farm: Warmhoek, Hoopstad	South Africa	Free State	28°10'08"S 25°49'11"E
5. Farm: Viljoenshof, Boshof	South Africa	Free State	28°34'45"S 25°04'33"E
6. Farm: Palmietfontein, Brandfort	South Africa	Free State	28°48'07"S 26°33'32"E
7. Farm: Tierkoppen, Augrabies	South Africa	Northern Cape	28°34'06"S 20°26'05"E
8. Farm: Boomrivier, Pofadder	South Africa	Northern Cape	29°04'33"S 19°18'24"E
9. Lady Grey, just outside town (municipal grounds)	South Africa	Eastern Cape	30°45'00"S 27°15'00"E
10. Matjiesfontein, just outside town (municipal grounds)	South Africa	Western Cape	33°15'00"S 20°34'48"E
Grassland Biome			
11. Gaborone, just outside town (municipal grounds)	Botswana		24°40'12"S 25°49'48"E
12. Brits Agricultural School, Brits	South Africa	North West	25°34'29"S 27°46'02"E
13. Farm: Ratzegaai, Ventersdorp	South Africa	North West	26°20'30"S 26°44'01"E
14. Farm: Uitspanning, Amsterdam	South Africa	Mpumalanga	26°39'56"S 30°31'26"E

Fynbos Biome			
15. Farm: Grootfontein, Porterville	South Africa	Western Cape	32°54'28"S 19°06'31"E
16. Farm: Fairfield, Napier	South Africa	Western Cape	34°27'27"S 19°45'10"E
Albany Thicket Bioregion			
17. Andries Vosloo Kudu Reserve, Grahamstown	South Africa	Eastern Cape	33°10'55"S 26°38'10"E
18. Mount Currie Nature Reserve, Kokstad	South Africa	KwaZulu-Natal	30°29'36"S 29°23'18"E
Fouriesburg/Kasane			
19. Wynford Guest Farm, Fouriesburg	South Africa	Free State	28°30'30"S 28°15'42"E
Lowveld Bioregion			
20. Mantenga Nature Reserve	Swaziland		26°26'37"S 31°10'22"E
21. Farm: Koedoesberg, Pongola	South Africa	KwaZulu-Natal	27°26'31"S 31°41'41"E
Bushmanland/Upper Karoo Bioregion			
22. Farm: Karlsrühe, Hotazel	South Africa	Northern Cape	26°58'34"S 22°59'57"E
23. Farm: Donkerpoort, Schweizer-Reneke	South Africa	North West	27°14'46"S 25°06'01"E
24. Farm: Rooidam, Groblershoop	South Africa	Northern Cape	29°08'33"S 22°19'34"E
Eastern Kalahari Bushveld Bioregion			
25. Farm: Rus en Vrede, Stella	South Africa	North West	26°10'23"S 25°13'27"E
26. Farm: Jones, Severn	South Africa	Northern Cape	26°35'22"S 22°41'46"E
27. Tswalu Kalahari Reserve, Sonstraal	South Africa	Northern Cape	27°12'51"S 22°'27'22"E
Kalahari Duneveld Bioregion			

28. Gibeon	Namibia		25°20'42"S 17°15'13"E
29. Farm: Zwartbooisberg, Kakamas	South Africa	Northern Cape	28°02'30"S 20°42'55"E
Machadodorp/Malelane			
30. Wathaba-Uitkomst, Machadodorp	South Africa	Mpumalanga	25°47'31"S 30°22'28"E

Appendix 3.2 *Micaelamys namaquensis* individuals (29 alleles for mitochondrial DNA and 35 for nuclear DNA) examined in this chapter and their GenBank accession numbers for both the mitochondrial DNA (mtDNA) cytochrome *b* (cyt *b*) and nuclear Recombination Activating Gene 1 (RAG1) genes. Note that the cytochrome *b* accession numbers correspond with those indicated in Chapter 2. Although some individuals shared the same cytochrome *b* allele, they had different RAG1 alleles.

CYT *B* ALLELE	CYT *B* ACCESSION NUM.	RAG1 ALLELE	RAG1 ACCESSION NUM
NH001	GQ471959	NNH01	GU139424
NH006	GQ471964	NNH02	GU139425
NH014	GQ471972	NNH03	GU139426
NH014	GQ471972	NNH04	GU139427
NH014	GQ471972	NNH05	GU139428
NH019	GQ471977	NNH06	GU139429
NH024	GQ471982	NNH07	GU139430
NH026	GQ471984	NNH08	GU139431
NH032	GQ471990	NNH09	GU139432
NH035	GQ471993	NNH10	GU139433
NH041	GQ471999	NNH11	GU139434
NH044	GQ472002	NNH12	GU139435
NH051	GQ472009	NNH13	GU139436
NH058	GQ472016	NNH14	GU139437
NH058	GQ472016	NNH15	GU139438
NH063	GQ472021	NNH16	GU139439
NH072	GQ472030	NNH17	GU139440
NH073	GQ472031	NNH18	GU139441
NH078	GQ472036	NNH19	GU139442
NH080	GQ472038	NNH20	GU139443
NH084	GQ472042	NNH21	GU139444
NH086	GQ472044	NNH22	GU139445
NH090	GQ472048	NNH23	GU139446
NH093	GQ472051	NNH24	GU139447

NH095	GQ472053	NNH25	GU139448
NH096	GQ472054	NNH26	GU139449
NH097	GQ472055	NNH27	GU139450
NH099	GQ472057	NNH28	GU139451
NH100	GQ472058	NNH29	GU139452
NH106	GQ472064	NNH30	GU139453
NH113	GQ472071	NNH31	GU139454
NH113	GQ472071	NNH32	GU139455
NH115	GQ472073	NNH33	GU139456
NH120	GQ472078	NNH34	GU139457
NH131	GQ472089	NNH35	GU139458

Chapter 4

Phylogeography of *Micaelamys namaquensis* (Rodentia: Muridae) from the Eastern Kalahari Bushveld bioregion of South Africa

Abstract

The Namaqua rock mouse *Micaelamys namaquensis* Smith, 1834 represents a species complex in southern Africa with several morphologically cryptic clades occupying distinct biomes and bioregions of southern Africa. Here I report a finer scale analysis of one of these clades, the Eastern Kalahari Bushveld bioregion of South Africa, based on mitochondrial DNA (mtDNA) cytochrome *b* (cyt *b*) sequences. Phylogeographic analysis reveals a genetic pattern of phylogenetic continuity with a lack of spatial separation. Mismatch distribution analysis suggests that the lineage has experienced recent population growth. The geographic expansion likely followed environmental changes associated with habitat modification over the past 3 000 to 10 000 years. Historical female gene flow does not appear to be equal amongst all localities and potential source and sink areas could be inferred. Metapopulation processes likely drive small mammal population dynamics in this arid region that is characterized by unpredictable climatic cycles.

1. Introduction

Recent mitochondrial DNA (mtDNA) cytochrome *b* (cyt *b*; Chapters 2 and 3) and Recombination activation gene (RAG1; Chapter 3) analyses revealed that the Namaqua rock mouse *Micaelamys namaquensis* Smith, 1834 represents a species complex in southern Africa. The majority of the 14 identified lineages appeared to be associated with specific southern African biomes or bioregions. In order to further explore evolutionary and ecological processes that shaped diversification in this species complex, I undertook a phylogeographic analysis of one of the phylogenetically and geographically well defined lineages from the Eastern Kalahari Bushveld bioregion of South Africa.

Phylogeography is the "mtDNA bridge between population genetics and systematics" (Avise et al., 1987). It thus focuses on processes underlying the geographic distributions of lineages (molecular variation of a species in space and time) among and within species (Avise, 2000). It also represents the interplay between vicariance and dispersal processes. Under vicariance scenarios, populations or taxa become separated when continuous ranges of ancestral forms are split by environmental changes, such as the rise of a mountain range. Under a dispersal scenario, active or passive dispersal from one or more ancestral origins, leads to the establishment of new populations. Consequently, population structure is affected by the potential of a species to disperse and successfully breed in a newly occupied area and the environmental influences that act on that potential (Avise et al., 1987). Many examples of phylogeographic studies on rodents using mtDNA data exist in the literature (e.g., Demastes *et al.,* 2002; Demboski and Sullivan, 2003; Grill *et al.,* 2009; Nicolas et al., 2008; Nicolas et al., 2009; Rajabi-Maham *et al.,* 2008; Riddle *et al.,* 2000; Yu *et al.,* 2004). These studies clearly show that phylogeographic structure within small mammals is influenced by both intrinsic (dispersal capabilities, reproductive strategies, current and historical demography and habitat specificity) and extrinsic (vegetation, geological and climatic effects) factors.

Habitat selection and inter-specific competition are amongst the most important factors that might influence the co-existence of species (Ricklefs and Schluter, 1993). The co-existence of species may therefore be explained by the amount of resources available and by the way in which species utilise these resources (Kotler and Brown, 1988). Fox (1982)

proposed a model suggesting that species enter a succession and colonise areas when habitat requirements are satisfied by changes in the environment that in turn alter the vegetation.

Limited gene flow and hence evolution in allopatry have influenced the development of specialised morphological, reproductive, and behavioural characteristics found in saxicolous (rock-dwelling) mammals (Mares and Lacher, 1987). Consequently, traits such as limited dispersal capabilities, strict habitat selection, strong territoriality, competition within and between species, patchiness of the environment and social structuring (communal versus solitary) may characterise African saxicolous mammals (Mares and Lacher, 1987). These traits would leave signatures on the structure of intraspecific genetic variation within these species, as is evident from several southern African small mammals: rock hyrax (*Procavia capensis* and *Heterohyrax brucei*; Prinsloo, 1993; Prinsloo and Robinson, 1992), rock rabbits of the genus *Pronolagus* (Matthee 1993; Matthee and Robinson, 1996), rock elephant-shrews (*Elephantulus edwardi*; Smith et al., 2007), and the Namaqua rock mouse species complex (Russo, 2003; Chapter 2).

Several biological characteristics of the Namaqua rock mouse are predicted to have an influence on its phylogeographic structure. Although the species is not a specialist, it prefers rocky habitats (Chimimba and Bennett, 2005) and is thus not continuously distributed. Small colonies live in rock crevices (Chimima and Bennett, 2005) but individuals from the Fynbos biome are believed to be solitary (T. Flemming pers. comm.). Members of the genus *Micaelamys* Ellerman, 1941, are believed to live between one to two years and have a short generation time producing as many as four litters by a single female (C.T. Chimimba pers. comm.). Thus the *M. namaquensis* population size is expected to be large due to its short generation time, with breeding occurring during nine months of the year (Smithers, 1971). The number of offspring ranges between one and seven (Rautenbach, 1978). Withers et al. (1980) reported that *M. namaquensis* tends to have unstable population cycles associated with high reproductive potential and high mortality rates. Dispersal is an important component in the regulation of populations with fluctuating sizes (Lidicker, 1975). Dispersal regulates densities below the level set by the food supply and it has been shown in voles that dispersal has the potential to alter population characteristics (Krebs 1971; Krebs et al., 1976). Likewise, dispersal is an

important factor in the determination of sociality, rates of genetic differentiation, as well as the generation and maintenance of species diversity (Lidicker 1975).

Despite dispersal being a crucial process within animal biology (Lidicker, 1975), most small mammal species appear to be organised into semi-isolated populations due to the availability and patchiness of suitable habitat (Patton et al., 1996). If the landscape between suitable habitat patches severely limits dispersal, maternal lineages should disperse more slowly and as a consequence have restricted geographic ranges (Kim et al., 1998). A habitat island of sufficient size and resources might over time reach equilibrium, even accumulating other closely related lineages (Kim et al., 1998). However, smaller habitat islands may not provide suitable resources and may be too variable so that mice living in these islands will become extinct from time to time and in turn be replaced by successful dispersers from nearby (Kim et al., 1998; Patton et al., 1996). This raises the possibility that local genetic diversity and genetic distances across geographic ranges could reflect dispersal power and demographic stability (Gaggiotti, 1996). In addition, this fine balance between habitat patchiness and dispersal ability will define the degree of population genetic structure and the level of local genetic diversity (Nunney and Campbell, 1993).

The dispersal of individuals can have drastic effects on the demographic and genetic structure of a population (Gaines and McClenaghan, 1980). Movement between populations not only facilitates gene flow but also helps maintain genetic variability (Gaines and McClenaghan, 1980). In mammals with short generation times, large and fluctuating population sizes, such as that seen in *M. namaquensis*, the new maternal lineages would disperse quickly from their points of origin creating genetic structuring in which the oldest lineages would have the broader geographic distribution (Neigel and Avise, 1993). Over time, such a species would therefore show genetic isolation by distance (Patton et al., 1996).

Extrinsic factors such as vegetation, which is directly impacted by climate variability, could have an influence in shaping phylogeographic structure within small mammals. This is clearly evident in the northern hemisphere where ice sheets directly influenced vegetation shifts and subsequent faunal responses (reviewed by Hewitt, 2000). Although only the highest mountains in southern Africa experienced periglacial conditions (Butzer,

1973), global climatic changes caused major vegetation changes in the region (Cerling et al., 1997; deMenocal, 2004). These changes likely not only influenced speciation (see Chapter 2) but also local differentiation within species. Rainfall on the other hand may increase seed production of grasses, shrubs and trees which in turn results in higher population numbers and would favour dispersal and migration would affect the genetic and phylogeographic structure of a population (Clobert et al., 2001).

The Savanna vegetation of South Africa (and Swaziland) constitutes the southern-most extension of this most widespread biome in Africa (Mucina and Rutherford, 2006). Two of the major macroclimatic elements characteristic of the Savanna biome include seasonal rainfall (alternation of wet summers and dry winters) and (sub) tropical temperatures with no or usually low incidence of frost (Mucina and Rutherford, 2006). In South Africa, savanna does not occur at high altitudes and is usually found below 1 500 m extending to about 1 800 m in parts of the highveld (Mucina and Rutherford, 2006). Savanna has an herbaceous ground layer dominated by grass species and a discontinuous to sometimes open upper layer of woody plants (Mucina and Rutherford, 2006; van Rooyen and Bredenkamp, 1996).

More specifically, the Eastern Kalahari Bushveld bioregion forms part of the greater Savanna biome. This bioregion occurs in an area where altitude ranges from sea level to about 1 800 m. It has an annual rainfall of between 235 to 1 000 millimetres (mm) and frost may occur from time to time. The region harbours almost every major geological and soil type. The average annual precipitation in the Eastern Kalahari Bushveld bioregion is 300 mm, which falls in summer and early autumn while temperatures vary between -9˚C and 42˚C. The vegetation is characterised by a well-developed tree stratum of Camel thorn and Shepherd's tree. The shrub layer is moderately developed, consisting of individuals of Black, Weeping Candle and Karoo thorn, with some grass cover depending on the amount of rainfall (van Rooyen and Bredenkamp, 1996).

This bioregion has specifically been chosen since samples were distributed over a fairly small, well-defined geographical area, localities of sympatry have been identified and this bioregion was also represented by a fairly large sample size compared to some of the other biomes/bioregions.

As molecular studies on rock-dwelling mammals have shown that population structure is often shaped by the island-like nature of their habitat (Kim et al., 1998; Patton et al., 1996), the present study reports the analysis of mtDNA cyt *b* variation within *M. namaquensis* from the Eastern Kalahari Bushveld bioregion from South Africa within a phylogeographic context. More specifically, the following research questions are addressed: 1) Is the mtDNA variation within *M. namaquensis* geographically structured?; 2) Is there gene flow between *M. namaquensis* populations from the Eastern Kalahari Bushveld bioregion?; and 3) What are the processes underlying the observed diversity?

2. Materials and Methods

2.1 Study area and sampling

Eighty-two individuals from 10 localities from the Eastern Kalahari Bushveld bioregion in South Africa were selected for the phylogeographic analysis (Fig. 4.1; Appendix 4.1). See Chapter 2 for sampling protocols. Animals were collected under the following permits: Northern Cape Province - 040/2001 and 0545/2004; North West Province - 000027 NW-06 (see Chapter 2; Appendix 2.2 for more details).

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

A fragment of the mitochondrial cyt *b* gene were amplified with primers and under reaction conditions described previously (Chapter 2). Amplification and sequencing strategy followed that outlined in Chapter 2.

2.3 Sequencing analysis

Unique maternal alleles (Chapter 2; GenBank accession numbers GQ472066 to GQ472077) were identified using 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.

A likelihood ratio test as implemented in Modeltest, version 3.06 (Posada and Crandall, 1998) was used to determine the model of DNA substitution that best fit the data at hand based on 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 was subsequently used to report on sequence divergence values using PAUP, version 4.0b10 (Swofford, 2003).

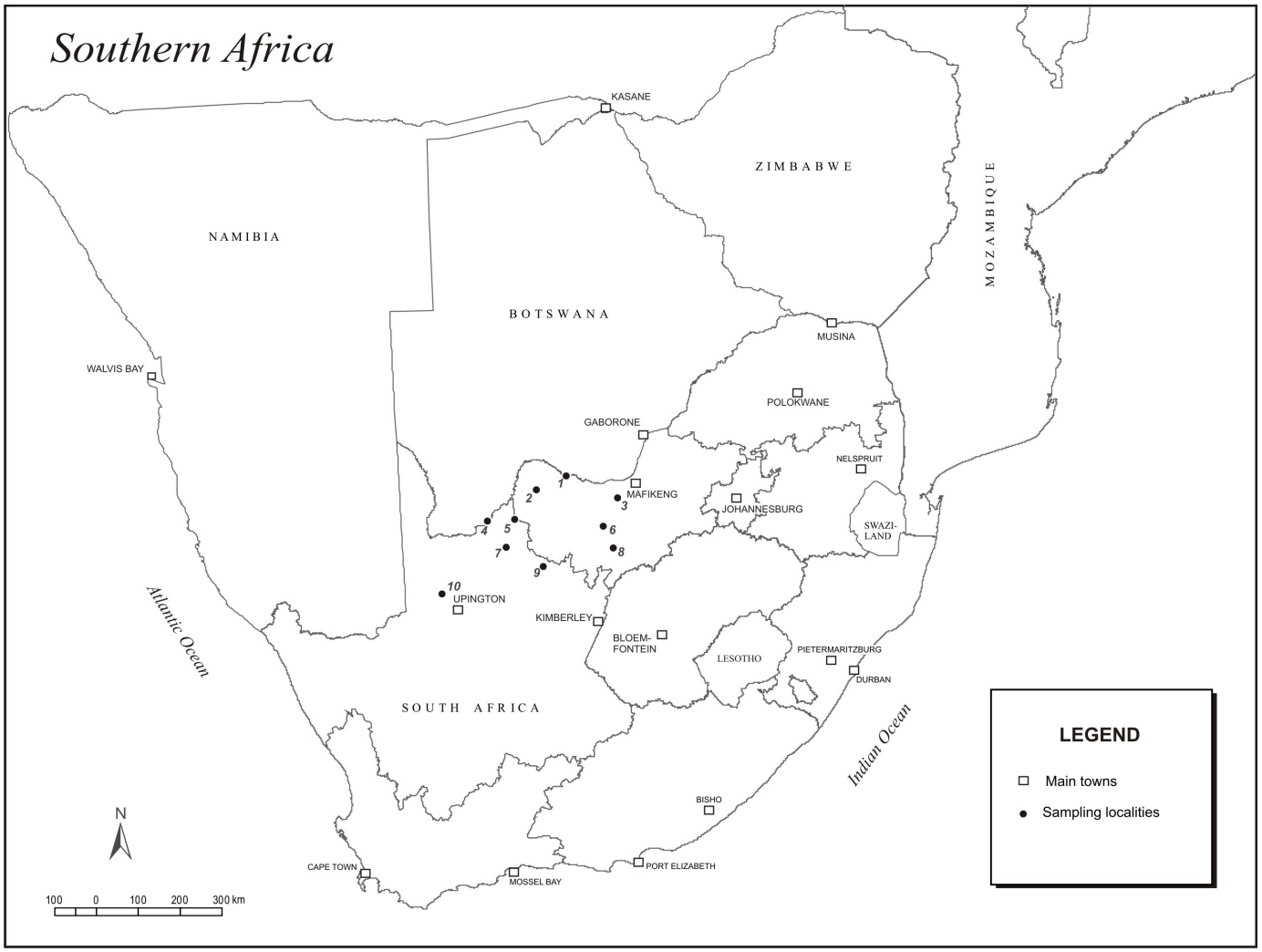

Figure 4.1 Collecting localities of samples of *Micaelamys namaquensis* from the Eastern Kalahari Bushveld bioregion in South Africa. Collecting locality numbers correspond to those in Appendix 4.1.

2.4 Molecular diversity and Phylogeographic analyses

Diversity indices such as haplotype diversity (the probability that two randomly chosen mtDNA sequences in the sample are different) (Nei and Tajima, 1981) and nucleotide diversity, π (the average number of nucleotide differences per site between two sequences) (Nei, 1987) were calculated for the entire sample 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. The test uses a permutation procedure (1 000 permutations) to determine the significance of the correlation between genetic versus geographic distances.

A spatial analysis of molecular variance (SAMOVA; Dupanloup et al., 2002) was conducted to maximise the proportion of genetic variance among *K* groups of populations. SAMOVA takes into account the geographic locations of samples and was run with different structures to determine the maximum value for F_{CT} (genetic variation due to differences between groups). Using *K* number of groups of populations as defined by SAMOVA, an analysis of molecular variance (AMOVA; Excoffier et al., 1992) as implemented in Arlequin, version 3.0 (Excoffier et al., 2005) was used to assess the extent of differentiation among populations (calculating ϕ_{ST}, ϕ_{CT} and ϕ_{SC}) using all haplotypes identified. Statistical significance of the different parameters was tested based on 10 000 non-parametric permutations as implemented in Arlequin, version 3.0 (Excoffier et al., 2005). A 5% level of missing data per site was allowed in all analyses. This method builds on the analysis of variance to compute molecular variance components at three different hierarchical levels. The total variance is partitioned into covariance components due to intra- and inter-individual differences and/or inter-population differences (Excoffier et al., 1992; Weir, 1996). Different population structures were identified for both SAMOVA and AMOVA analyses.

Three hierarchical structures defined for the AMOVA analyses are as follow: 1) All populations as one group; 2); Combining individuals from Tosca (locality 1, Fig. 4.1 and Appendix 4.1) and Stella (locality 3, Fig. 4.1 and Appendix 4.1) and the rest of the localities as one group; 3) Combining individuals from Vanzylsrus (locality 4, Fig. 4.1 and Appendix 4.1) and Severn (locality 5, Fig. 4.1 and Appendix 4.1) and the rest of the localities as individual groups. The groupings of localities in the AMOVA analysis were

based on Barrier, version 2.2 (Manni et al., 2004). Barrier tests for any association between genetic and geographic distance by using spatial autocorrelation and regression methods (Manni et al., 2004). These tests suggest the possible shape of the genetic landscape (Manni et al., 2004). A Monmonier's (1973) maximum difference algorithm was used to identify genetic barriers, while a significance test was implemented in the software by means of bootstrap matrices analysis (Manni et al., 2004). By combining the results from the significance test with the molecular data superimposed on a geographic map one can attempt to identify the significance of a geographic barrier and any potential patterns of variation associated with the genetic markers (Manni et al., 2004).

2.5 Migrate analysis

The program MIGRATE, version 2.4 was used to estimate effective population sizes and past migration rates between *n* number of populations assuming a migration matrix model (Beerli and Felsenstein, 2001). The 10 sampling localities were treated as independent populations. Coalescence theory based maximum likelihood estimates for the migration rates among different populations were calculated using a Markov Chain Monte Carlo approach (Hastings, 1970). MIGRATE estimates for theta (per site) were interpreted as indicators of the extant effective population size with $N_e = \theta/2\mu$, with μ as the mutation rate per site per generation (Beerli and Felsenstein, 2001). A mutation rate of 0.176×10^{-7} was used as described in Nabholz et al. (2008). The MIGRATE analysis were run with 10 short chains (10 000 genealogies sampled, 500 recorded) and three long chains (100 000 genealogies sampled, 5 000 recorded) and a burn-in of 10 000 genealogies per chain (Beerli and Felsenstein, 2001).

2.6 Mismatch distribution

Inference of population history was assessed using mismatch distribution analysis under a sudden expansion model and a spatial expansion model assuming constant deme size (Roger and Harpending, 1992) as implemented in Arlequin, version 3.0 (Excoffier et al., 2005). The expansion null hypothesis was tested using the sum of squared deviation test of significance (P_{Ssd}; Schneider and Excoffier, 1999) and the raggedness index of significance (P_{Rag}; Harpending, 1994). The fit of the observed distribution of mismatches to a sudden model of expansion was tested using 10 000 permutations. The process of habitat expansion and/or contraction often implies demographic variation (Petit et al., 1999). It is therefore important to note that episodes of population growth and decline have

a strong effect on the pattern of genetic polymorphism, leaving characteristic signatures in the distribution of nucleotide site differences between individuals (Slatkin and Hudson, 1991). The distribution is usually unimodal for lineages that have undergone a recent bottleneck or population expansions, and a multimodal distribution for populations exhibiting equilibrium (Rogers and Harpending, 1992). The model of a sudden expansion is simple - it follows a scenario of an initial population with a female effective population size of N_0 that rapidly grows to a new size of N_1 (Rogers and Harpending, 1992). Parameters were estimated as follows: $\tau = 2\mu t$, $\theta_0 = 2N_0\mu$ and $\theta_1 = 2N_1\mu$, where τ is the time to the expansion; μ is the mutation rate per generation; t is the time of the expansion in generations (Harpending, 1994; Schneider and Excoffier, 1999). The effective population sizes before and after the expansion are indicated with θ_0 and θ_1, respectively (Harpending, 1994; Schneider and Excoffier, 1999). A generation time of 0.16 or 0.33 years were used, respectively (C.T. Chimimba pers. comm.). The raggedness statistic, r, which quantifies the smoothness of the observed mismatch distribution, was also estimated. A population having undergone expansion will usually generate a distribution that is smooth while populations that have remained constant in size generate distributions with very ragged peaks (Rogers and Harpending, 1992).

2.7 Nested Clade Phylogeographic Analysis (NCPA)

An allele network for the 82 individuals was estimated using statistical parsimony as implemented in TCS, version 1.21 (Clement et al., 2000). Since TCS excludes missing data, allele frequencies differed from those reported in Chapter 2. Nested clade phylogeographic analysis (NCPA) can discriminate between phylogeographic associations due to on-going restricted gene flow and historical events such as range expansion, fragmentation and colonisation (Templeton et al., 1995). Alleles in the derived cladogram were then grouped into hierarchical nesting levels from the tips to the interior of the cladogram following Templeton et al. (1987) and Templeton and Singh (1993). This was done by uniting haplotypes that were separated by a single mutational step (0-step clades); 0-step clade haplotypes were nested into 1-step clades. This procedure was repeated until the entire allele network was nested within a single clade. An exact contingency test was performed on each nested clade to test whether the null hypothesis of no association between clades or alleles and geographic location could be rejected. This test was performed without taking the geographic distances between localities into account (Templeton and Singh, 1993); the observed χ^2 values were compared to distributions of the

values generated from 10 000 random permutations in the program GEODIS, version 2.0 (Posada et al., 2000). All the above-mentioned procedures were undertaken using algorithms in the newly developed ANeCA that fully automates the complex NCPA methodology (Panchal, 2007). The NCPA was also undertaken by hand as is traditionally the case following the procedure developed by Templeton et al. (1987) and subsequently Templeton and Singh (1993).

In addition, geographic clade distances (D_c), nested clade distances (D_n), the average interior *versus* tip clade distances (IT_c), and the average interior *versus* tip nested clade distances (IT_n) were also calculated (Templeton et al., 1995). The clade distance is indicative of how geographically widespread individuals within a particular clade are (Templeton et al., 1995). The nested clade distance is a measure of the distance of individuals in a particular clade from all individuals within the nesting clade (Templeton et al., 1995). Geographic distances were calculated from latitudinal and longitudinal coordinates measured using a Garmin eTrex Global Positioning System™ (GPS) in the field. The statistical significance for all distances measured was also determined (Templeton et al., 1995). The inference key of Templeton (2008) was used to interpret the data.

3. Results

3.1 Sequence statistics

The pairwise GTR + Γ sequence divergence values for all individuals ranged between 0.12% and 1.71 %. The four nucleotides did not occur in equal frequencies, similar to that of other previously reported mammalian cyt *b* sequences. In addition, the first and second codon positions showed less variability than third codon positions (Irwin et al., 1991; Martin et al., 2000).

3.2 Molecular diversity

Based on a 631 bp fragment of the 5' end of the cyt *b* gene, 12 unique haplotypes were identified (Table 4.1) Haplotypes NH06 (N = 23) and NH08 (N = 24) were the most widespread, being recorded from seven and six localities, respectively. Most of the other

haplotypes were locality-specific or were recorded from two/three localities. Two localities (Vorstershoop and Tswalu; see Table 4.1) were represented by a single allele.

Overall nucleotide diversity based on 82 individuals was estimated at 0.35% (SD = 0.03%) while the haplotype diversity value of 0.69 (SD = 0.04) was lower than that reported for other rodents (Avise et al., 1989; Fedorov and Stenseth, 2001). Haplotype diversities differed from 0.33 to 0.89 within sampling localities and nucleotide diversities ranged between 0.23% to 0.61%.

Table 4.1 TCS based frequencies of mitochondrial DNA (mtDNA) cytochrome *b* (cyt *b*) alleles among 10 sampled localities of *Micaelamys namaquensis* from the Eastern Kalahari Bushveld bioregion in South Africa examined in the present study. Abbreviations of locality names, which correspond to those in Fig. 4.1 and Appendix 4.1 are as follow: TOS = Tosca; VOR = Vorstershoop; Ste = Stella; Van = Vanzylsrus; SEV = Severn; TSW = Tswalu; VRY = Vryburg; SCH = Schweizer-Reneke; KUR = Kuruman; and UPI = Upington.

ALLELE NUMBER	NUMBER OF INDIVIDUALS	TOS	VOR	STE	VAN	SEV	TSW	VRY	SCH	KUR	UPI
NH01	3	-	-	-	-	-	-	3	-	-	-
NH02	1	1	-	-	-	-	-	-	-	-	-
NH03	1	-	-	-	-	-	-	-	1	-	-
NH04	2	-	-	-		2	-	-	-	-	-
NH05	2	-	-	-	1	-	-	-	-	1	-
NH06	23	-	3	1	3	2	6	1	-	-	7
NH07	2	-	-	-	-	-	-	1	-	-	1
NH08	24	-	-	7	4	2	-	2	-	5	4
NH09	6	4		1				1			
NH10	1			1							
NH11	5			2	1			2			
NH12	12				6				6		
Total	**82**	**5**	**3**	**12**	**15**	**6**	**6**	**10**	**7**	**6**	**12**

The Mantel nonparametric test revealed no isolation by distance between localities from the Eastern Kalahari Bushveld bioregion. The standard normal variate (g) of -1.1448 was smaller than the critical value of 2.575 at $P \leq 0.005$ with a correlation coefficient of -0.2029. This indicated that the null-hypothesis (no association between elements in the two matrices) could not be rejected.

Dupanloup et al. (2002) reported that the largest mean ϕ_{CT} value is associated with the correct number of groups, suggesting that it has some power to retrieve the unknown number of groups. Based on this information, the largest mean ϕ_{CT} value in the SAMOVA analyses was 0.31 and 0.32 corresponding to two and nine groups, respectively (see Table 4.2). The specified structures (as a result of the Barrier analysis) were analysed in AMOVA in order to test for statistically significant genetic structuring among the samples. The AMOVA showed weak phylogeographic structuring of haplotypes. Twenty-eight percent of the total variance was among populations when all geographic regions were considered as one group (Table 4.2). It should be noted that the structures defined in SAMOVA were different from those defined in AMOVA. SAMOVA grouped the populations from Tosca (locality 1, Fig. 4.1 and Appendix 4.1), Kuruman (locality 9, Fig. 4.1 and Appendix 4.1) and Stella (locality 3, Fig. 4.1 and Appendix 4.1) in one group and the rest of the populations in another group when two groups were specified. In contrast, only individuals from Tosca (locality 1, Fig. 4.1 and Appendix 4.1) and Stella (locality 3, Fig. 4.1 and Appendix 4.1) were grouped based on Barrier, version 2.2 (Manni et al., 2004) and the rest of the localities were grouped in another group for the AMOVA analysis (see Fig. 4.2). Vanzylsrus (locality 4, Fig. 4.1 and Appendix 4.1) and Severn (locality 5, Fig. 4.1 and Appendix 4.1) were grouped when nine barriers were defined that resulted in nine groupings with the rest of the localities as individual groups (see Fig. 4.2). Similarly, Vanzylsrus (locality 4) and Severn (locality 5) were grouped in the SAMOVA analysis when nine groups were defined with the rest of the localities as individual groups.

Table 4.2 Hierarchical analysis of molecular variance (AMOVA) for *a priori*-defined groups and a spatial analysis of molecular variance (SAMOVA) of *Micaelamys namaquensis* from the Eastern Kalahari Bushveld bioregion in South Africa based on mitochondrial DNA (mtDNA) cytochrome *b* (cyt *b*) sequences. Statistical significance: * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

	AMOVA						SAMOVA					
Number of Groups	**Statistical Estimates**			**Percentage Variation**			**Statistical Estimates**			**Percentage Variation**		
	ϕ_{SC}	ϕ_{ST}	ϕ_{CT}	**Among**		**Within Population**	ϕ_{SC}	ϕ_{ST}	ϕ_{CT}	**Among**		**Within Population**
				Groups	**Populations**					**Groups**	**Populations**	
1	-	0.28***	-	-	27.98	72.02	-	-	-	-	-	-
2*	0.22***	0.36***	0.17	17.23	18.52	64.24	0.15***	0.42***	0.32***	32.04	10.11	57.85
9	0.19*	0.28***	0.12	11.92	16.32	71.76	-0.01	0.31***	0.31*	31.32	-0.65	69.33

3.3 Migration

The θ-estimators obtained in MIGRATE were very close to the values of nucleotide diversity (π) for the individual localities thus suggesting that the populations were in genetic/demographic equilibrium (Table 4.3). Most of the immigration and emigration estimates are very low (< 1 effective female migrant per generation; Table 4.3, Fig. 4.2). Some localities display a relative balance between immigration and emigration (Vanzylsrus, Severn, Tswalu, Kuruman, Tosca and Stella). Vryburg(locality 7) and Upington (locality 10) appear to be net receiving populations while Vorstershoop (locality 2) and Schweizer-Reneke (locality 8) show net emigration. Figure 4.2 highlights all the exchanges of more than 1 female migrant per generation between localities, with Vryburg (locality 7) showing the most immigration. It can be concluded that immigration/emigration was observed over large geographic areas indicating that these small mammals can likely travel over large distances. Using the mutation rate for mtDNA based on Nabholz et al. (2008) and the θ-values from MIGRATE, the effective female population sizes for each locality was calculated (see Table 4.3 in parenthesis). The effective female population sizes differed markedly between populations; the largest value was estimated for the Vryburg population.

Table 4.3 Estimates of migration rates in both directions among *Micaelamys namaquensis* localities from the Eastern Kalahari Bushveld bioregion in South Africa. Values in bold indicate more than one migrant per generation (N_fm) between populations. Migrants per generation were calculated by the following equation: Theta (θ) * M(m/mμ). + = receiving population. Locality numbers 1 - 10 correspond to those in Fig. 4.1 and Appendix 4.1. Estimated effective female population size (N_{fE}) is indicated in parentheses.

LOCALITY AND (N_{fE})	THETA	1;+	2;+	3;+	4;+	5;+	6;+	7;+	8;+	9;+	10;+
1. Tosca (82 571)	0.00289	-	0.60	0.05	0.05	0.49	0.44	0.49	0.22	0.27	0.55
2. Vorstershoop (171)	0.00000599	0.004	-	0.003	0.003	0	0.0006	0.0006	0.002	0.003	0.0003
3. Stella (10 857)	0.00104	0.70	0.61	-	0.40	0.51	0.91	0.71	0.51	0.20	0
4. Vanzylsrus (29 714)	0.00038	**1.57**	0.83	0.10	-	0	0.21	0	**1.25**	0.95	0
5. Severn (93 429)	0.00327	0.08	0.76	0.26	0	-	**1.19**	**1.78**	0.34	0.08	0.08
6. Vryburg (352 571)	0.01234	**1.62**	**2.23**	0.41	0.41	**1.22**	-	**2.23**	0.41	0.81	0.41
7. Tswalu (22 571)	0.00079	0.19	0.31	0.31	0.27	0.66	0.08	-	0.23	0.35	0
8. Schweizer-Reneke (26 286)	0.00092	0.07	0.09	0.05	0.09	0.17	0.03	0.07	-	0	0.10
9. Kuruman (40 000)	0.00140	0.17	0.14	0.25	0.22	0.03	0.11	0.11	0.06	-	0
10. Upington (65 429)	0.00229	0.14	0.20	0.14	0.14	0.20	0.07	0.30	0.20	0.07	-

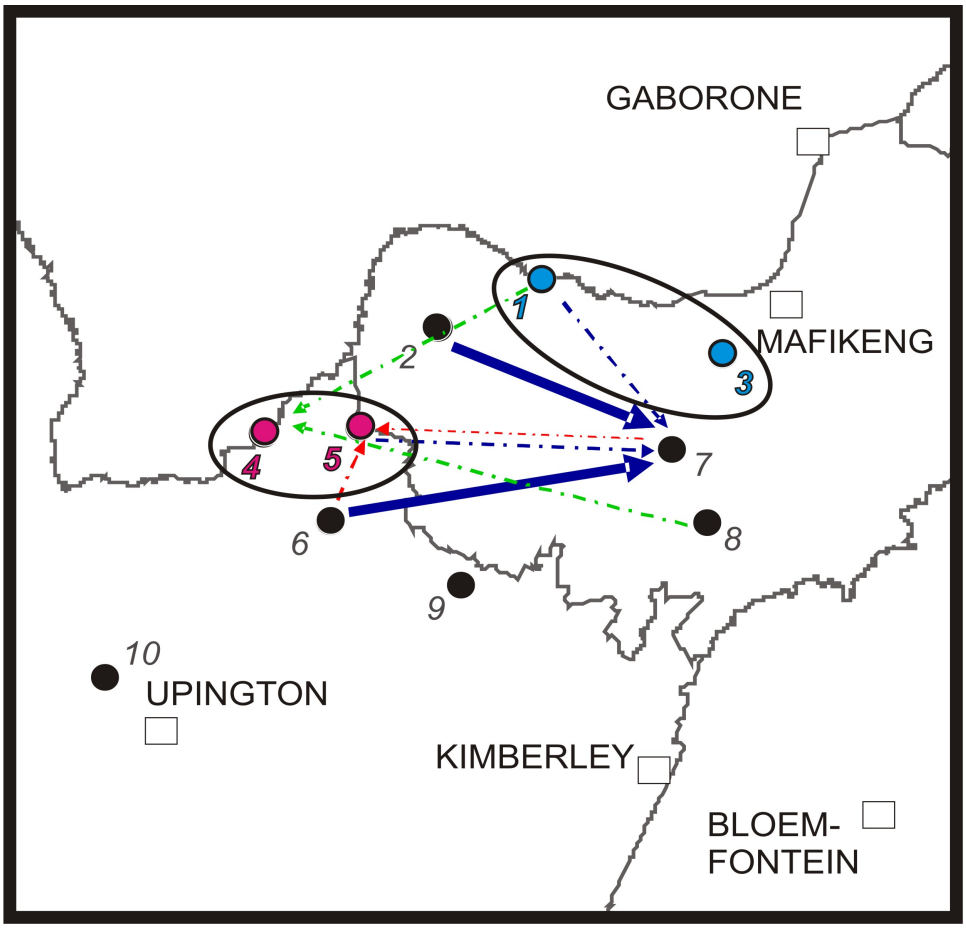

Figure 4.2 Clustering and migration between sampling localities of *Micaelamys namaquensis* from the Eastern Kalahari Bushveld bioregion from southern Africa. Locality numbers correspond to those in Fig. 4.1 and Appendix 4.1. Localities that are encircled were grouped according to Barrier, version 2.2 (Manni et al., 2004). Localities indicated in blue (Tosca; 1 and Stella; 3) were lumped as the first barrier when two groupings were defined. Localities in pink (Vanzylsrus; 4 and Severn; 5) were lumped when nine groupings were defined with the other localities as individual groups. Arrows indicate historical female migration between localities; solid arrows indicate more than two migrants per generation and dashed arrows more than one migrant per generation. Blue, red and green arrows indicate migration to Vryburg (7), Severn (5) and Vanzylsrus (4), respectively.

3.4 Mismatch distribution

The frequency distribution of pairwise nucleotide differences is illustrated in Fig. 4.3 and parameter estimates from the mismatch analyses are indicated in Table 4.4. Although the distributions under both models were multimodel, the sum of squared deviation under the sudden expansion model (Fig. 4.3A) was not statistically significant and thus the null hypothesis of a sudden population expansion could not be rejected. Similarly, the null hypothesis of a spatial expansion assuming constant deme size (Fig. 4.3B) could not be rejected. In contrast, the Harpending's raggedness index under the sudden expansion model (Fig. 4.3A) was statistically significant. Given a mutation rate of 1.76×10^{-7} (Nabholz *et al.,* 2008), the mutation rate per generation per haplotype for 631 bp of the mtDNA cyt *b* was estimated to be 1.1106×10^{-4}. This mutation rate was used to solve the equation $\tau = 2\mu t$ and the time of the expansion in generations, $t = \tau/2\mu$. The expansion time was estimated at 27 787 generations ago. This would therefore translate to 4 446 and 9 170 years ago if a generation time of 0.16 or 0.33 years (C.T. Chimimba pers. comm.) was used, respectively. Likewise, time to the spatial expansion assuming a constant deme size was estimated at 3 357 and 6 925 years ago depending on the use of a generation time of 0.16 or 0.33 years, respectively. Population size after the sudden expansion event was estimated at 26 459.

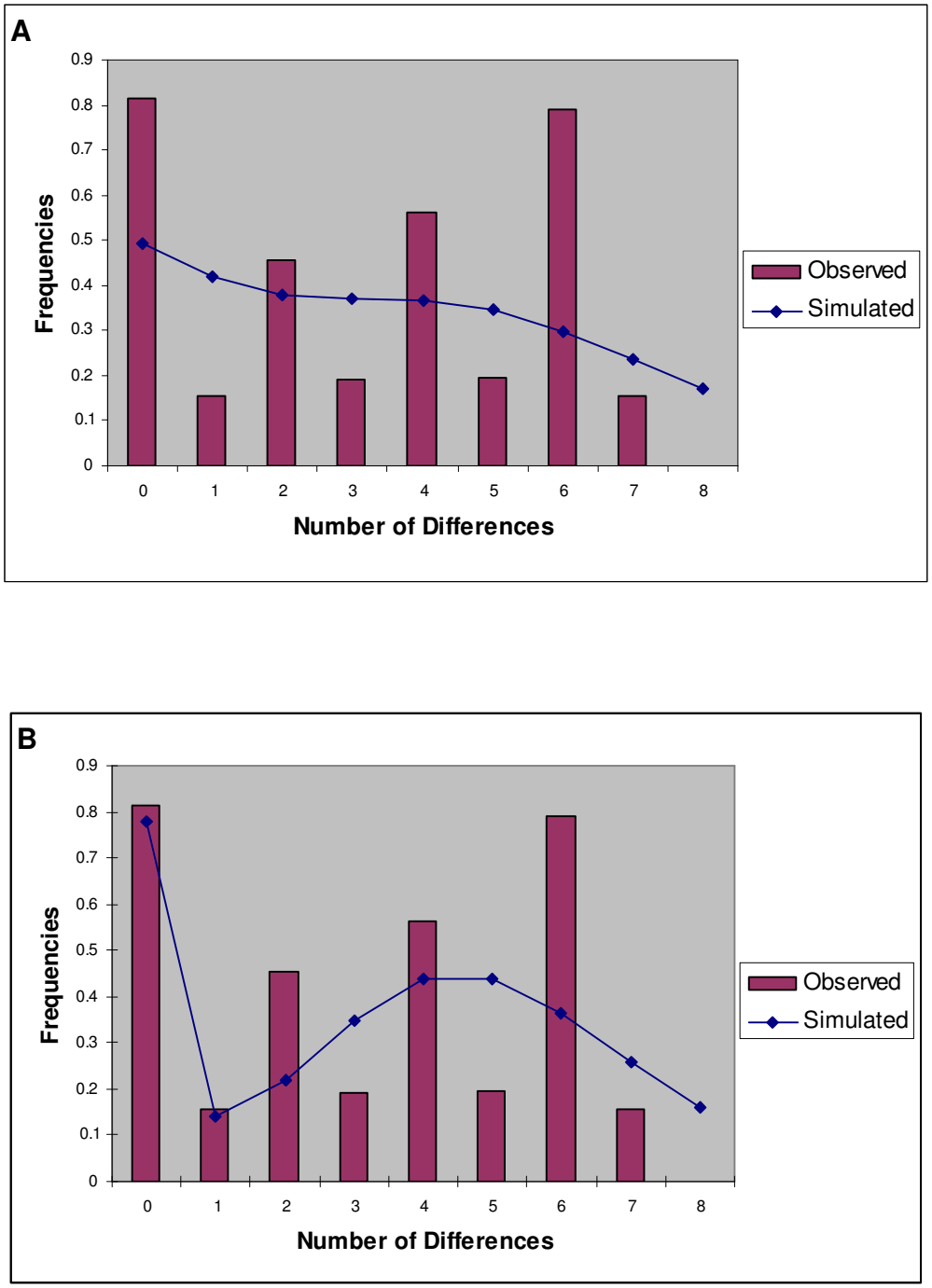

Figure 4.3 Frequency distributions of pairwise nucleotide differences between individuals of *Micaelamys namaquensis* from the Eastern Kalahari Bushveld bioregion in South Africa with (A) parameters estimated under a sudden expansion model (Sum of squared deviation = 0.05; $P = 0.07$; Harpending's raggedness index = 0.15; $P = 0.05$) and (B) parameters estimated under a spatial expansion model assuming constant deme size (Sum of squared deviation = 0.03; $P = 0.38$; Harpending's raggedness index = 0.15; $P = 0.45$).

Table 4.4 Mismatch distribution parameter results for *Micaelamys namaquensis* from the Eastern Kalahari Bushveld bioregion in South Africa based on mitochondrial DNA (mtDNA) cytochrome *b* (cyt *b*) sequences. Mismatch distribution analyses were performed in two ways: 1) under a sudden expansion model and 2) under a spatial expansion model assuming constant deme size. Demographic expansion parameters are expressed in units of mutational time.

TYPE OF ANALYSIS	OBS. MEAN*	*TAU*	THETA	$THETA_0$	$THETA_1$
Under sudden expansion	3.221 (5.781)	6.172	-	0.000	5.877
Under spatial expansion	3.221 (5.781)	4.661	0.344	-	-

*Mismatch observed variance given in parenthesis

3.5 Nested Clade Phylogeographic Analysis (NCPA)

Figure 4.4 depicts the nested design for the mtDNA haplotypes found within *M. namaquensis* from the Eastern Kalahari Bushveld bioregion following the rules of Templeton et al. (1987) and Templeton and Singh (1993) (see also Table 4.1 for allele frequencies). One ambiguous branch (between allele NH01 and NH12) was broken in order to keep branches elsewhere in the cladogram that connected mtDNA alleles with the least number of mutational steps (Fig. 4.4). The maximum number of mutational steps that were confirmed to be parsimonious with a probability of $P > 0.95$ was 10. Clades that only represented one locality were not tested for association between clades and their geographic distances.

Statistically significant associations between clades and the geographic locations were revealed at all clade levels that were tested in ANeCA (see Table 4.5; probability values in bold). This test indicated strong associations between clades and sampling localities for one 1-step clade (1-1; Table 4.5), one 2-step clade (2-2; Table 4.5) and one 3-step clade (3-1; Table 4.5).

The following clades were therefore tested: Clades 1-1, 1-5, 1-8, 2-2, 2-3 and 3-1. Results from the automated ANeCA program are shown in Table 4.6. Statistically significant associations between clades and sampling locations were revealed at all clade levels and evolutionary processes inferred from the NCPA inference key of Templeton (2008) are

shown in Table 4.6. Most of the clades had an inconclusive outcome due to inadequate geographical sampling. The evolutionary process for the total cladogram (clade 3-1) showed either fragmentation or isolation by distance.

The NCPA was also undertaken by hand following the rules of Templeton et al. (1987) and Templeton and Singh (1993) (results not shown). In this analysis, a statistically significant association between clade 2-1 and geographic distance was also tested. The evolutionary processes for most clades resulted in an inconclusive outcome. Clade 2-1 showed restricted gene flow/dispersal with some long distance dispersal and restricted gene flow with some isolation by distance was inferred for the total cladogram (3-1).

The inconclusive outcomes observed for most of the cladograms (Table 4.6) may be as a result of the limitations associated with this method. It is clear, based on the evolutionary processes inferred that more analyses using larger samples sizes in combination with other markers are required to investigate the genetic structure of this species in more detail.

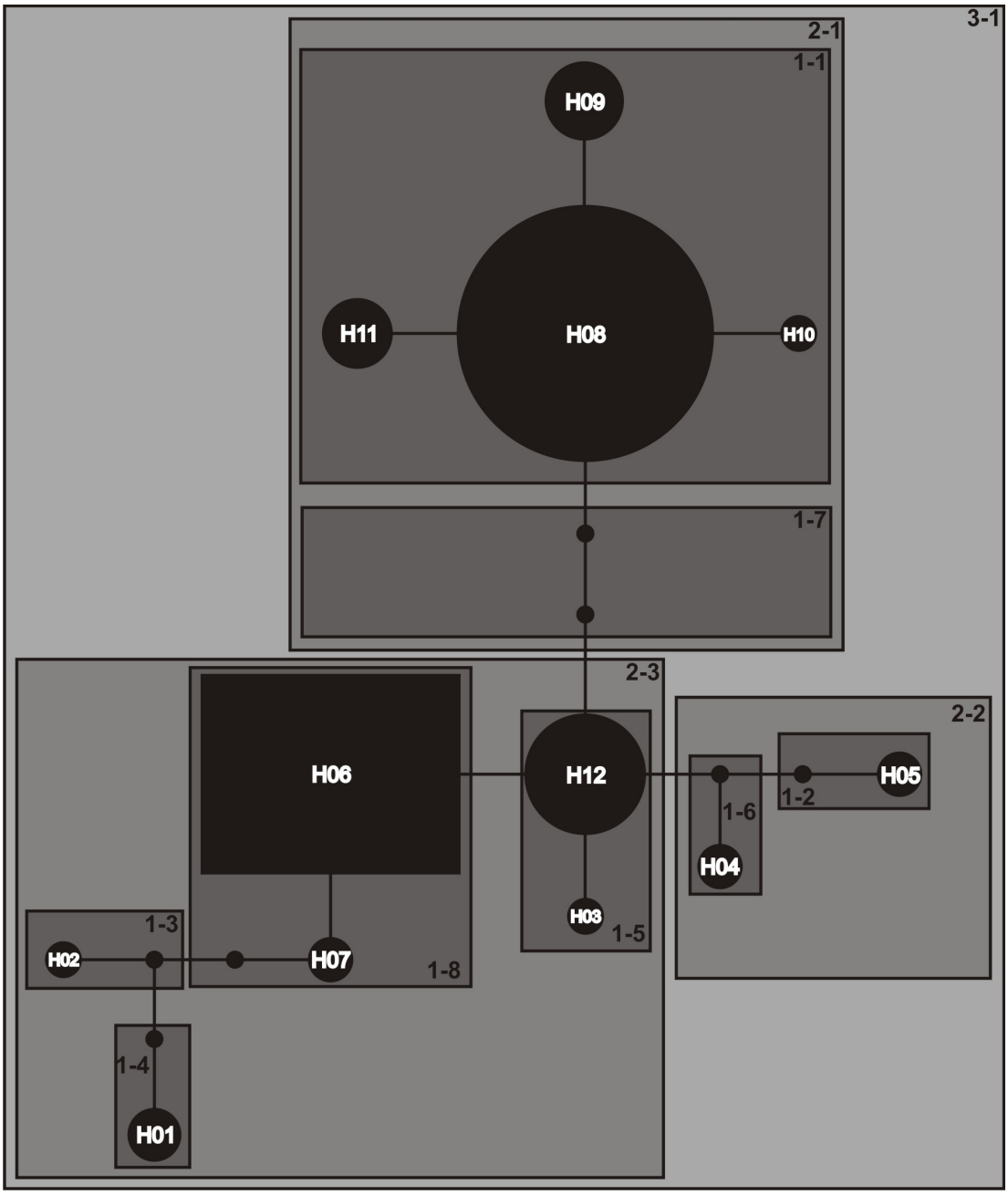

Figure 4.4 Automated nested clade design for 82 individuals of *Micaelamys namaquensis* from the Eastern Kalahari Bushveld bioregion in South Africa based on 631 bp of the mitochondrial DNA (mtDNA) cytochrome *b* (cyt *b*) gene region. The haplotype network was constructed with TCS, version 1.21 (Clement et al., 2000) as defined by a 95 % confidence limit. A square denotes an ancestral allele (NH06) as suggested by TCS. The size of circles indicates the frequency of the alleles (also see Table 4.1). Smaller circles that are not numbered indicate missing (unsampled/extinct) alleles.

Table 4.5 Nested contingency analysis of geographic associations between clades and sampling localities. Only clades with geographic variation are testable. The permutational probabilities were calculated by 10 000 random permutations. Statistically significant probabilities are indicated in bold.

CLADE	0BSERVED CHI-SQUARE STATISTICS	PROBABILITY
1-1	32.02	**0.022**
1-5	0.93	1.000
1-8	6.32	0.470
2-2	4.00	0.331
2-3	97.36	**0.000**
3-1	49.17	**0.000**

Table 4.6 Evolutionary processes as inferred from the inference key of Templeton (2008) as implemented in ANeCA. Clade designations are as derived from the automated nested clade phylogeographic analysis (ANeCA; see Fig. 4.4). Locality names are as indicated in Fig. 4.1 and Appendix 4.1. "No" in the table denotes the final step in the inference chain and leads to the conclusion of the evolutionary process.

CLADE	POPULATIONS	INFERENCE CHAIN	PROCESSES
1-1	Tosca, Stella, Vanzylsrus, Severn, Vryburg, Kuruman, Upington	1, 2, 3, 4, No	Inconclusive outcome
1-5	Vanzylsrus, Schweizer-Reneke	1, 2, 11, 17, No	Inconclusive outcome
1-8	Vorstershoop, Stella, Vanzylsrus, Severn, Tswalu, Vryburg, Upington	1, 2, No	Inconclusive outcome
2-2	Vanzylsrus, Severn, Kuruman	1, 19, 20, No	**Inadequate geographical sampling**
2-3	Tosca, Vorstershoop, Stella, Vanzylsrus, Severn, Tswalu, Vryburg, Schweizer-Reneke, Upington	1, 2, 11, 17, No	Inconclusive outcome
3-1	Tosca, Vorstershoop, Stella, Vanzylsrus, Severn, Tswalu, Vryburg, Schweizer-Reneke, Kuruman, Upington	1, 2, 3, 4, 9, 10, No	**Geographical sampling scheme inadequate to discriminate either fragmentation or isolation by distance**

4. Discussion

The *M. namaquensis* lineage from the Eastern Kalahari Bushveld bioregion is characterised by shallow phylogeographic structure. The latter is evident from several lines of analytical evidence based on mtDNA cyt *b* sequences, including low diversity indices, a star-like allele network and apparent demographic changes over recent evolutionary time. However, historical female migration levels are generally low and there are indications of potential metapopulation dynamics across the region.

The lineage has a low nucleotide diversity (0.35%), which corresponds to other studies done on small mammals; estimates of between 0.33% and 1.45% have been reported for the Norwegian lemming (Fedorov and Stenseth, 2001), while 0.54% and 1.5% have been reported for the Yellow-necked field mouse (Michaux et al., 2004). The haplotype diversity value of 0.69 is lower than the average reported for other rodents (Avise et al., 1989; Fedorov and Stenseth, 2001), probably as a result of the low incidence of locality-specific haplotypes (Avise, 2000). Most alleles are shared amongst two to five localities (see Table 4.1 and Fig. 4.4). This could either indicate a shared ancestry between these populations or on-going gene flow.

The lack of a statistically significant correlation between pairwise estimates of gene flow and geographic distance clearly indicate the absence of a pattern of isolation by distance. In addition, the AMOVA performed on localities assembled into groups according to the Barrier results did not show statistically significant apportionment of the genetic variance among regional groups (i.e., no apparent extrinsic barriers). Much of the genetic structuring observed could be explained through differentiation within localities.

Despite the apparent continuous pattern of genetic variation, the overall ϕ_{ST} value for *M. namaquensis* of 0.28 implies a moderate level of genetic heterogeneity among populations, suggesting a certain degree of isolation among samples (Apfelbaum et al., 1991). This value corresponds to an *Nm* larger than one which is above the minimum number of migrants per generation needed to minimise the chances of substantial local differentiation due to genetic drift ($Nm > 1$; Griswold and Baker, 2002; Hartl, 1980; Hutchison and Templeton, 1999; Slatkin, 1987), indicating either historical gene flow or recent

connectivity. The former is confirmed by the MIGRATE analysis which indicates that most populations were/are connected to each other via low to intermediate levels of female gene flow.

Evidence for both isolation and migration processes among the *M. namaquensis* localities from the Eastern Kalahari Bushveld bioregion suggest that populations within this bioregion share a relatively recent history. Shared alleles (such as NH06) indicate some level of movement between populations while unique alleles (such as NH01 from Vryburg, NH02 from Tosca, NH03 from Schweizer-Reneke, NH04 from Severn and NH10 form Stella) indicate that a certain level of isolation exists. Added to which the fairly low to intermediate migration detected may be as a result of the marker used, which excludes male-biased dispersal.

The exchange of migrants from Tswalu (locality 6), Severn (locality 5), Vorstershoop (locality 2) and Tosca (locality 1) to Vryburg (locality 7) is considered as large (greater than one). This locality (Vryburg) was one of the only localities that exhibited such a large number of immigrants per generation and it also received migrants from almost half of the other populations (possibly a sink populations). The θ-estimates further suggested that this region has the largest effective female population size which may be attributable to more favourable environmental conditions. It is well documented that population sizes of rodents such as *M. namaquensis* occurring in semi-arid to arid environments such as the Eastern Kalahari Bushveld bioregion fluctuate with annual rainfall (White et al., 1997). Rain increases seed production of grasses, shrubs and trees which in turn results in peaks in population numbers, favouring dispersal. Dispersal, in the form of migration or individual movement, affects the genetic structure of a population (Clobert et al., 2001), although it can also be influenced by landscape heterogeneity, resource distribution and population densities.

Gene flow may either constrain evolution by preventing adaptation to local conditions or promote evolution by spreading new genes throughout a species' distributional range (Slatkin, 1987). Continuously distributed species may be genetically structured if gene flow is either restricted or if they are under local selection (Congdon et al., 2000; Hudson et al., 1992). In contrast, some species are restricted to small spatial distributions due to their association with particular habitat conditions. This could be the case with the

Namaqua rock mouse, a species that is strongly associated with rocky koppies, outcrops and hillsides (Chimimba and Bennett, 2005). Migration most likely occurs between neighbouring populations, probably approximating a stepping stone model (Hutchison and Templeton, 1999; Slatkin and Barton, 1989). In addition, there are indications of potential metapopulation dynamics within *M. namaquensis* (sink and source populations).

A signature of sudden population size and spatial expansion could not be rejected given the demographic scenario with the mismatch distribution analysis (Fig. 4.3). This phenomenon of a population expansion was also detected in the star-like allele network from the TCS analysis (Fig. 4.4). A demographic scenario of a sudden population expansion could have been expected due to the favourable environmental conditions (i.e., good rain triggers an increase in food which in turn would result in an increase in population numbers) that these animals are exposed to from time to time. A record of past climate changes has been retained within the landforms of the 2.5 million km^2 of the Kalahari sedimentary basin (Deacon and Lancaster, 1988). For example, the last glacial maximum (LGM; 18 000 YBP) was characterised by colder and more arid conditions than at present (Shi et al., 1998) with dune-formation in central southern Africa. Dune-formation over the past 18 000 years to present have being inhibited by wetter conditions and strong winds than in the LGM (Thomas and Shaw, 2002). Cold upwellings from the Benguela current caused cold air coming in contact with warm air off the land to condense forming fog which provides a permanent water source (Pickford and Senut, 1999). This may have been the main environmental parameter that permitted dispersal into, and subsequent radiation, in areas that may have been previously inhospitable. Added to which these changes in climate would have had an influence in the vegetation and as more areas became suitable, individuals would have occupied them. During the last 2000 years, increased farming, burning and overgrazing reflect intensified human activity in the region leading to significantly altered landscapes.

The signature of sudden population expansion may have influenced habitat selection and inter-specific competition that are important factors in the co-existence of species (Ricklefs and Schluter, 1993). A rodent community study on rehabilitating dunes in the KwaZulu-Natal Province, South Africa showed that rodent densities decreased with an increase in habitat regeneration age, indicative of unsuitable environmental conditions (Ferreira and van Aarde, 1996). In comparison, negative correlation between population sizes of

sympatric small mammal species provided evidence that intra-specific competition could have consequences on population size and habitat use (Grant, 1972; Gurnell, 1985). Species may use the same habitat, but some species might either segregate among strata within a habitat or they can also segregate temporarily and be active at different times (Ziv and Kotler, 2003; Ziv et al., 1993) in either the presence or absence of competitors (other small mammals possible competing for the same food sources and space for shelter). Therefore, the spatial segregation (i.e., movement between populations) of *M. namaquensis* individuals into different habitats allows for the species to co-exist.

The phylogeographic analysis in the present study revealed no statistically significant geographic structuring of mtDNA variation among the 10 *M. namaquensis* localities from the Eastern Kalahari Bushveld bioregion in South Africa. At the highest clade level, the NCPA inference was that of past fragmentation or isolation by distance. Some form of past fragmentation seemed the more likely factor responsible for the observed spatial distribution of genetic variation given the lack of isolation by distance (see Mantel test results) and the unequal individual movement detected between *M. namaquensis* populations. Female-mediated gene flow between the 10 populations of *M. namaquensis* could thus not be refuted. Templeton et al. (1995) and Durand et al. (1999) emphasised that the various evolutionary processes shaping geographic associations of alleles are not mutually exclusive. In the present study, a pattern of gene flow superimposed on a signal of possible past fragmentation can be explained by relatively recent vicariance and subsequent secondary contact between populations.

The allele network contained one star-like phylogeny within clade 2-1 (Fig. 4.4). The central haplotype (NH08) was shared between six localities (24 individuals) with a geographic range in excess of 500 km. Although this allele was not identified as the ancestral allele by the TCS analysis (NH06 being identified as an ancestral allele; see Fig. 4.4), it can still be argued that such a star-like pattern with a central common allele independently connected with numerous fairly rare alleles at the tips can be regarded as a signal of recent population expansion (Avise, 2000). This finding was further corroborated by the mismatch distribution analysis where the Eastern Kalahari Bushveld populations (corresponding to clade 3-1) presumably experienced a sudden population size expansion between 3 000 to 10 000 years ago.

For clade 2-1 (consisting of individuals from Stella (locality 3), Vryburg (locality 7), Severn (locality 5), Kuruman (locality 9), Vanzylsrus (locality 4) and Tosca (locality 1); see Fig. 4.4), when using the traditional nested clade phylogeographic analysis, the evolutionary inference was that of restricted gene flow/dispersal with some long-distance dispersal. These results were also confirmed by the MIGRATE analysis that showed movement of individuals between localities with large geographic distances of over 300 km. To some extent, this clade exhibited restricted gene flow because allele NH10 was shown to be locality-specific to the Stella population (locality 3).

Comparisons with published data showed that *M. namaquensis* falls within the continuous end (category IV) of the spectrum of categories proposed by Avise et al. (1987) to classify phylogeographic structuring. Category IV entails phylogenetic continuity, lack of spatial separation and gene flow in the species not sub-divided by long-term zoogeographic barriers. The Springhare, *Pedetes capensis* occurs in a uniform habitat and this is reflected in its phylogeographic pattern which shows a lack of genetic divergence among samples over a broad geographic range (Matthee et al., 1997). The sibling red veld rats, *Aethomys chrysophilus* De Winton, 1897, and the Tete veld rat, *A. ineptus* Thomas and Wroughton, 1908, from southern Africa also showed similar patterns to that seen in the Springhare, reflecting a lack of genetic divergence among samples over a broad geographic range (Russo et al., 2006). This phylogeographic pattern is also evident in the yellow mongoose, *Cynictis penicillata* (Jansen van Vuuren, 1995) from southern Africa, the old field mouse, *Peromyscus polionotus* (Avise et al., 1983) and the woodrat from the eastern United States of America (*Neotoma*; Hayes and Harrison, 1992). In contrast to this, a pattern of phylogenetic discontinuity (category I) has been reported in other taxa showing discontinuous intra-specific mtDNA phylogenetic networks, with a strong geographic orientation such as the pocket gopher (*Geomys pinetis*; Avise et al., 1979) and deer mouse (*Peromyscus maniculatus*; Lansman et al., 1983). Category I pattern of phylogenetic discontinuity has also been detected in southern African small mammals such as the rock hyrax, *Procavia capensis* (Prinsloo, 1993), red rock rabbits of the genus *Pronogalus* (Matthee and Robinson, 1996), the scrub hare, *Lepus saxatilis* (Kryger, 2002), and within the Grassland lineage of the Namaqua rock mouse, *M. namaquensis* (Russo, 2003).

5. Conclusion

The genetic analysis of samples of *M. namaquensis* from the Eastern Kalahari Bushveld bioregion in South Africa, in the present study suggests that the species has recently expanded its population size, and that geographic expansion must have followed habitat modification associated with recent environmental changes. It was also evident from the present study that there was gene flow between populations and more so between some populations than others (possible sink and source populations). The description of the genetic structure of *M. namaquensis* is fundamental to understanding the history and evolutionary potential of the species.

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6. Acknowledgements

I am grateful to all field assistance for helping with the collection of samples (C.J. Oosthuizen, S.M.R dos Santos, W. Delport, E.R. Swartz, A.V. Linzey and A. Hulse). C.L. Sole is thanked for the constructive comments on earlier drafts of this chapter. This study was funded by the National Research Foundation grant to PB and CTC (Grant number: 2073181) and the University of Pretoria.

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Appendix 4.1 Geographic coordinates of all collecting localities of *Micaelamys namaquensis* from the Eastern Kalahari Bushveld bioregion, South Africa that were analysed in the present study. Locality numbers 1-10 correspond to those in Fig. 4.1. Bioregion terminology follows that of Mucina and Rutherford (2006).

LOCALITY	PROVINCE	GEOGRAPHIC COORDINATE
1. Farm: Welbedeur, Tosca	North West	25°42'53"S; 23°58'43"E
2. Farm: Arizona, Vorstershoop	North West	25°57'00"S; 23°13'55"E
3. Farm: Rus en Vrede, Stella	North West	26°10'23"S; 25°13'27"E
4. Farm: Loversleap, Vanzylsrus	Northern Cape	26°38'20"S; 22°01'04"E
5. Farm: Jones, Severn	Northern Cape	26°35'22"S; 22°41'46"E
6. Tswalu Kalahari Reserve, Sonstraal	Northern Cape	27°12'51"S; 22°'27'22"E
7. Farm: Waterloo & Vlakfontein, Vryburg	North West	27°03'34"S; 24°45'58"E
8. Farm: Donkerpoort, Schweizer-Reneke	North West	27°14'46"S; 25°06'01"E
9. Farm: Strelley, Kuruman	Northern Cape	27°39'48"S; 23°23'04"E
10. Farm: Steenkampsput, Upington	Northern Cape	28°06'13"S; 20°54'10"E

Chapter 5

General Conclusions

The main focus of the present study was elucidation of the nature and extent of diversity and processes involved in shaping diversity within *Micaelamys namaquensis* Smith, 1834 from southern Africa. This chapter presents the key findings of this investigation and recommends possible future research directions given these findings.

There is a general lack of congruence between the present molecular data and previous morphological/morphometric data. This study revealed more extensive variation and clear indications of a species complex. The latter is in agreement with earlier studies that recognised up to 16 subspecies (Meester et al., 1964; Roberts, 1951). Four of the statistically supported mtDNA lineages (lineages B, H, J and N) broadly correspond with the distributional patterns of subspecies proposed by Chimimba (2001): 1) *monticularis* Jameson, 1909 (Grassland biome); 2) *namaquensis* Smith, 1834 (Fynbos biome); 3) *lehocla* Smith, 1936 (Nama-Karoo biome); and 4) *alborarius* Peters, 1852, (Savanna biome), respectively. The type localities of eight of the previously described subspecies (indicated in yellow stars in Fig. 2.1) were located within the geographic regions of the mtDNA lineages. The associations were as follow: 1) *monticularis* in the Grassland biome (lineage B); 2) *capensis* Roberts, 1926 in the western Fynbos (lineage H); 3) *centralis* Schwann, 1906 in the Nama-Karoo (lineage J); 4) *lehochloides* Roberts, 1926 in the Savanna (lineage N); 5) *namibensis* Roberts, 1946 in the Kalahari Duneveld (lineage K); 6) *lehocla* in the Eastern Kalahari Bushveld bioregion (lineage M); 7) *grahami* Roberts, 1915 in the Albany Thicket (lineage G); and 8) *drakensbergi* Roberts, 1926 in the Lowveld (lineage D). A clear association between the geographical limits of each lineage and the biomes/bioregions of southern Africa was evident. Although individuals were also sampled from localities geographically close to the type localities for the subspecies *albiventer* Jentinck, 1910 and *klaverensis* Roberts, 1926 the affinities of these subspecies remain unclear. Although considerable genetic variation was detected between these lineages and molecular and former morphometric data were congruent, I suggest that these lineages should represent subspecies. It seems like some of the 16 previously described subspecies are valid.

In addition, the independent *M. namaquensis* lineages displayed unique patterns of within lineage diversity. Two lineages (lineage B and J) represented the extremes of heterogeneity and serve to illustrate overall differences in phylogeographic structure. More genetic differentiation was detected within lineage B (Grassland biome), although it is

geographically more restricted. Lineage J (Nama-Karoo biome) showed less differentiation with haplotypes only being separated by a few mutational steps, even between haplotypes that were geographically distant. Such star-like allele networks are often indicative of recent range expansions (Viñas et al., 2004). This would not be unexpected in a species such as the Namaqua rock mouse, as previous studies have reported extreme population cycles in response to climatic conditions and food availability (Withers et al., 1980). Haplotypes within lineage B were separated by up to six mutational steps. Although this lineage likely also experienced periods of population expansion and decline, other environmental and/or ecological processes may have promoted isolation between the geographical sub-regions that each harbour a unique group of alleles. This aspect requires further investigation.

Divergence times were estimated at 940 000 years ago (lineage G, Albany Thicket bioregion) to 3.42 MYA (lineage N, Savanna biome) within lineages, and between lineage divergences differed from 2.70 MYA (between lineages F and G) to 7.26 MYA (between lineages B and C & D). Time to the most recent common ancestor for *M. namaquensis* was estimated at about 9.44 MYA. In addition, this was followed by a divergence into two groups from this common ancestor as follows: 1) A group more confined to the mesic habitats of southern Africa (lineages A-H); and 2) A group found in the more arid habitats (lineages I-N). The major diversification within this species-group appears to have occurred between 7.87 MYA and 5.30 MYA, during the Late Miocene and Early Pliocene which loosely correspond with the major radiation of murine rodents in Africa (7 MYA to 9 MYA; Lecompte et al., 2008). This time period coincides with the timeframe following the first colonisation of murine rodents in Africa (10 MYA to 12 MYA) (Lecompte et al., 2008) and also agrees with the fossil record for *Aethomys* (Denys, 1990a, b; Pocock, 1987).

Major periods of aridification and expansion of Savanna habitats during the Late Miocene and Pliocene have also been implicated in the diversification within other rodents, such as *Tatera* (Colangelo et al., 2005), *Praomys* (Lecompte et al., 2005) and *Hylomyscus* (Nicolas et al., 2006). Likewise, these changes in the environment may have also influenced the diversification between and within *M. namaquensis* lineages. For example, as Savanna habitats expanded in the past so would the distributional range of the species/lineage (lineage N) specific to this habitat. This lineage may have originated from the Savanna

biome but the distribution as it is seem today have changed as more habitat became available. Likewise, lineage J initially differentiated in the arid Nama-Karoo biome but as more habitats became available (expansion of habitat according to Quaternary fluctuations) the distributional range of the lineage would have shifted as a consequence of the expansion of the habitat.

Eleven of the 14 lineages that were identified in Chapter 2 were further investigated in the phylogenetic analysis using a combined approach (i.e., combined cyt *b* and RAG1 gene data). Incongruence was detected between the independent cyt *b* and RAG1 analyses and may be explained by incomplete lineage sorting in the RAG1 gene as it takes on average three to four times longer for lineage sorting to occur in nuclear genes. Moore (1997) suggested that mitochondrial DNA haplotype trees are more likely to be congruent with the species tree than nuclear gene trees. It is important to assess intraspecific variation within a species, to understand the mating system of the species, to resolve the nuclear as well as the mitochondrial tree and to be cautions before concluding that any one of the gene trees is the species tree (Hoelzer, 1997). The combined analysis in the present study revealed six well supported lineages pointing towards the polytypic nature of the species. In addition, the combined analysis increased the number of supported nodes from nine (cyt *b*) and four (RAG1) to 14, emphasising the fact that multi-gene analysis holds merit.

A more in-depth analysis *M. namaquensis* from the Eastern Kalahari Bushveld bioregion in South Africa was undertaken to extend the more qualitative comparison of within-lineage phylogeographic patterns reported in Chapter 2. This geographically and phylogenetically well defined lineage exhibited shallow phylogeographic structure with a low incidence of locality-specific alleles. Summary statistics also reflected this trend. The TCS allele network was star-like with haplotypes being shared over large geographical distances. Such a star-like pattern can be regarded as a signal of recent population expansion (Avise, 2000). This was supported in the mismatch distribution analysis where the population was inferred to have experienced a sudden population size expansion over the past 3 000 to 10 000 years. This geographic expansion must have followed habitat modification associated with recent environmental changes.

Despite the apparent continuous pattern of genetic variation, the overall ϕ_{ST} value of 0.28 implies some genetic heterogeneity (Apfelbaum et al., 1991) and unequal gene exchange among localities was evident. Historical female gene flow does not appear to be equal amongst all localities and likely source and sink areas could be inferred. The large size of the Vryburg population was reflected by the larger estimates of θ in the MIGRATE analysis. This may be associated with Vryburg being a possible sink population where environmental conditions are more favourable leading to a fair amount of immigration into the area. The description of the genetic structure of *M. namaquensis* is fundamental to understanding the history and evolutionary potential of the species. In addition, the connectivity of different populations may also enhance the ability of individuals to move between populations. Sink populations should not necessarily be characterised by favourable environmental conditions but the connectivity (environmental conditions like vegetation cover, food and water resources) between populations should also be taken into consideration.

The understanding of speciation processes is important in predicting changes in species numbers and in the planning of conservation strategies (Moritz et al., 2000). 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 the 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, 2001) is a result of either historical differentiation, ecological selection, or both.

Ecological speciation seems to be the most likely explanation for the origin of diversity within *M. namaquensis*, since the geographically well-defined lineages corresponded broadly in their distribution with different biomes/bioregions of southern Africa as

described by Mucina and Rutherford (2006). The present study strongly suggests that the diversity and differentiation within *M. namaquensis* is more complex than previously thought and that the species represents a species complex. This suggests that the taxonomic status of *M. namaquensis* needs further investigation based on a multidisciplinary approach followed by a formal taxonomic revision. Consequently, further geographic sampling as well as the examination of type material of described subspecies is needed to resolve the identity of the unique lineages and to gain a better insight into the phylogeography and mode of speciation in this group of rodents. In addition, areas of sympatry should be studied at a finer scale, offering the opportunity to elucidate the underlying speciation processes in this species complex.

Apart from contributing to general small mammal studies and especially systematics in Africa, the present study may have implications in epidemiological, agricultural, and biological conservation research associated with potentially problematic rodents in the southern African sub-region and beyond. Consequently, the present study may assist health and agricultural authorities in gaining better insights into these potentially problematic rodents (the ability to disperse and the distance of dispersal).

In order to make recommendations to conservation authorities and understand the taxonomic status of this species, the nature and extent of diversity within *M. namaquensis* should be studied in more detail. More geographic sampling representative of the distributional range of the species is needed. Furthermore, a multidisciplinary approach (cytogenetics, morphology and more gene regions) should be adopted. The IRBP gene has recently been used by Lecompte et al. (2008) and gave good results at species level and above. I suggest that that the IRBP gene should be employed in further analyses. In order to test the biological species concept (BSC), I propose breeding experiments. Although well employed by Chimimba (2001), morphological (type specimen characters) and morphometric analyses (classical and geometric) of the molecularly typed specimens are needed. I would furthermore propose that the type/topotype specimens (deposited in mammal reference collections of museums) should be sequenced, employing an ancient DNA extractions method.

Lastly, I wish to propose a taxonomic hypothesis based on these findings. Following the biological species concept (BSC) I propose that the 14 lineages identified in this study

should be considered as subspecies and several of the original subspecies names should be retained: lineage B = *Micaelamys namaquensis monticularis*; lineage D = *M. n. drankensbergi*; lineage G = *M. n. grahami*; lineage H = *M. n. albiventer*; lineage I = *M. n. lehocla*; lineage J = *M. n. centralis*; lineage K = *M. n. namibensis*; lineage M = *M. n. calarius* if individuals collected from Central Botswana clusters within this lineage; lineage N = *M. n. lehochloides*. Lineages A, C, F and L should be formally named and described. *Micaelamys namaquensis capensis* and *klaverensis* are synonyms of *albiventer*. This hypothesis should be tested through more in-depth multi-disciplinary investigation, including conducting of breeding experiments.

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