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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