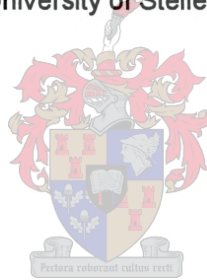


**MOLECULAR GENETICS OF *RHABDOMYS* SUBSPECIES BOUNDARIES:
PHYLOGEOGRAPHY OF MITOCHONDRIAL LINEAGES AND CHROMOSOMAL
FLUORESCENCE *IN SITU* HYBRIDIZATION**

RAMUGONDO VICTOR RAMBAU

PROMOTER: PROFESSOR T. J. ROBINSON

Dissertation presented for the Degree of Doctor of Philosophy (Zoology)
at the University of Stellenbosch



APRIL 2003

DECLARATION

I, the undersigned, hereby declare that the work contained in this dissertation is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

ABSTRACT

The geographic genetic population structure and evolutionary history of the African four-striped mouse, *Rhabdomys pumilio*, was investigated using mitochondrial (mtDNA) cytochrome *b* gene (1140 bp) and control region (994 bp) sequences and a combination of cytogenetic banding techniques (G- and C-banding), and fluorescence *in situ* hybridization. Two cytotypes ($2n = 46$ and $2n = 48$) were identified by cytogenetic analysis. No evidence of diploid number variation within populations was found nor were there differences in gross chromosome morphology, or subtle interchromosomal rearrangements at levels detected by ZOO-FISH. The comparative painting data (using the complete suite, $N = 20$, of *Mus musculus* chromosome specific painting probes) show that 10 mouse chromosomes have been retained as chromosomal arms, or intact chromosome blocks within the *R. pumilio* genome, six produced double signals, while the remaining four hybridized to three or more *R. pumilio* chromosomes. In total, the 20 mouse chromosome paints detected 40 segments of conserved synteny. Their analysis revealed eight *R. pumilio* specific contiguous segment associations, a further two that were shared by *R. pumilio* and other rodents for which comparable data are available, the Black (*Rattus rattus*) and Norwegian (*Rattus norvegicus*) rats, but not by the Chinese hamster, *Cricetulus griseus*. The results suggest that mouse chromosomes 1, 10, and 17 have undergone extensive rearrangements during genome evolution in the murids and may be useful markers for enhancing our understanding of the mode and tempo of chromosome evolution in rodents. Following initial studies using control region sequences, the phylogeographic appraisal of *R. pumilio* was done using cytochrome *b* gene sequences. Analyses based on a variety of analytical procedures resulted in the detection of two major mtDNA lineages that correspond roughly to the xeric and mesic biotic zones of southern Africa. One clade comprises specimens with $2n = 48$, and the other representatives of two cytotypes ($2n = 48$ and $2n = 46$). The mean sequence divergence (12.0%, range 8.3% -15.6%) separating the two mtDNA clades is comparable to among-species variation within murid genera suggesting their recognition as distinct species, the prior names for which would be *R. dilectus* and *R. pumilio*. Low sequence divergences and the diploid number dichotomy within the mesic lineage support the recognition of two subspecies corresponding to *R. d. dilectus* ($2n = 46$) and *R. d. chakae* ($2n = 48$). The data do not support subspecific division within the nominate, *R. pumilio*. Molecular dating places cladogenesis of the two putative species at less than 5 million years, a period characterised by extensive climatic oscillations which are thought to have resulted in habitat fragmentation throughout much of the species' range.

OPSOMMING

Die geografiese bevolkingsstruktuur en evolusionêre verwantskappe binne die Afrika streepmuis, *Rhabdoys pumilio*, is ondersoek deur middel van mitochondriale DNS volgordebepaling van die geenfragment sitochroom b (1140 basispare) en die reguleerstreek (994 bp) in kombinasie met sitogenetiese tegnieke (G- en C-bandkleuring en fluoreseerende *in situ* hibridisasie). Twee sitotipes ($2n = 46$ en $2n = 48$) is geïdentifiseer deur sitogenetiese analise. Geen bewys van variasie in die $2n$ chromosoomgetal binne bevolkings is gevind nie. Verder is daar ook geen verskil in die morfologiese struktuur van chromosome aanwesig binne bevolkings nie. Vergelykende data (verkry met behulp van die $N = 20$ *Mus musculus* chromosoomspesifieke peilers) dui daarop dat 10 muis chromosome behoud gebly het as chromosoomarms of chromosoomblokke binne die *R. pumilio* genoom. Ses peilers het dubbel seine gelewer terwyl die oorblywende vier peilers gehibridiseer het aan drie of meer *R. pumilio* chromosome. In totaal het die 20 muischromosoomverwe 40 konserwatiewe segmente geïdentifiseer. Die analise dui agt *R. pumilio* spesifieke aaneenlopende segmentassosiasies aan, met 'n addisionele twee wat deur *R. pumilio* en ander muisagtiges vir wie vergelykende data beskikbaar is, byvoorbeeld die swart (*Rattus rattus*) en Noorweegse (*R. norvegicus*) rot maar nie die Chinese hamster, *Cricetulus griseus*, gedeel word. Die resultate stel voor dat muischromosoom 1, 10 en 17 ekstensiewe herrangskikkings ondergaan het gedurende die genoom evolusie binne die Muridae en dat hulle waarskynlik waardevolle merkers kan wees om beide die patroon en tempo van chromosome evolusie in muisagtiges verder te kan verstaan. Die filogeografiese verwantskappe binne *R. pumilio* is ondersoek deur middel van DNS volgordebepalings van die reguleerstreek asook sitochroom b. Die resultate van hierdie studie het twee divergente mitochondriale DNS eenhede ontdek wat gekorreleer kan word met xeriese en mesiese klimaatsones binne suidelike Afrika. Een groep bestaan uit diere met $2n = 48$, terwyl die ander genetiese groep twee sitotipes ($2n = 46$ en $2n = 48$) insluit. 'n Gemiddelde genetiese divergensie van 12.0% (varieer tussen 8.3% - 15.5%) verdeel die twee mtDNS-groepe en is vergelykbaar met tussenspesievariasie binne ander muisagtige genera, wat moontlik daarop dui dat twee verskillende spesies teenwoordig is; die voorgestelde name is *R. dilectus* en *R. pumilio*. Lae genetiese divergensie binne die mesiese groep versterk die moontlike teenwoordigheid van twee subspesies, *R. d. dilectus* ($2n = 46$) en *R. d. chakae* ($2n = 48$). Die data verleen egter nie steun aan die divisie binne *R. pumilio* nie. Molekulêre datering van die twee spesies dui daarop dat die divergensie ten minste 5 miljoen jaar gelede plaasgevind het. Die periode was gekarakteriseer deur

ekstensiewe klimaatswisselings, wat gelyk het tot habitat fragmentasie in die spesie se verspreidingsgebied.

ACKNOWLEDGEMENTS

This study would not have been possible without the various valuable contributions of several people and the financial contributions of several institutions. First, my sincere gratitude goes to my supervisor and mentor, Prof. T. J. Robinson for suggesting the topic of this study and providing unwavering support throughout the study period. Prof. Robinson's intellectual input, erudite discussions and suggestions formed an integral part of this thesis and the two papers that have so far emanated from it. Second, several people assisted with collection of material and their contribution is gratefully acknowledged. These are Christia Newbury, Connie Crug, Prof. Jan Nel, Dr. Barbara Cook, Neels Coetzee, Dr. Peter Taylor, Dr. Nanu Mahida, Mike Campbell, Ute Kryger, Johan Watson, Dr. Erik van der Straeten, Dr. Neville Pillay, Professors Mike Kerzner and Alicia Linzey. Nanu Mahida, Mike Campbell and Peter Taylor kindly donated some of the samples included in their previous publication.

Instruction in and the establishment of FISH protocol in our laboratory were offered by world class cytogeneticists, Mr. Wilbur Harrison and Drs. Roscoe Stanyon and Fengtang Yang. Their suggestions were useful for troubleshooting and obtaining good quality FISH preparations. Part of the data used in this thesis were generated in Dr. R. Stanyons' laboratory in the Laboratory for Genomic Diversity, National Cancer Institute, Maryland, USA. Drs. Conrad Matthee and Bettine Jansen van Vuuren provided useful suggestions with regard to analysis and interpretation of molecular sequencing data. Many thanks for the moral support from fellow laboratory colleagues, Amanda Pardini, Gavin Gouws, Krystal Tolley, Sandy Willows-Munro, Savel Daniels and Peter Teske.

My heartfelt thanks for financial assistance in the form of bursaries and grants which were received from Mellon Foundation (through the University of Pretoria), National Research Foundation (NRF) and Harry Crossley Merit Bursary. Supplementary financial support for conference participation was provided by the University of Stellenbosch. Both the University of Pretoria (where the study was initiated) and Stellenbosch University are thanked for providing well equipped laboratories for carrying out the research conducted for this study. Last but not least I would like to thank my fellow siblings, Tshikhipha, Maganu, Mudzunga, Tshililo and Ntshengedzeni, for their consistent support, understanding and encouragement throughout my studies. This dissertation is dedicated to my mother, Semakatjo who was a pillar of strength throughout.

TABLE OF CONTENTS

	Page
ABSTRACT	iii
OPSOMMING	iv
ACKNOWLEDGEMENTS	vi
TABLE OF CONTENTS	vii
LIST OF TABLES	x
LIST OF FIGURES	xi
CHAPTER 1: GENERAL INTRODUCTION	1
Preamble	1
Higher order rodent phylogeny	2
“<i>Arvicanthis</i> division” rodents	3
Life history characteristics of <i>R. pumilio</i>	4
Medical, economic and ecological role of <i>R. pumilio</i>	5
Systematics of <i>R. pumilio</i>	5
Classical subspecific delimitation of <i>R. pumilio</i>	5
Species and subspecies controversy	7
Allozyme, cytogenetics and behavioural differentiation in <i>R. pumilio</i>	9
Distribution of <i>R. pumilio</i>	10
Molecular analysis	12
Mitochondrial DNA (mtDNA)	12
Rate heterogeneity and molecular clock	14
MtDNA genetic distances and taxonomic inference	15
Comparative cytogenetics	16
FISH	17
Phylogenomics	18
Role of Robertsonian translocations in speciation	19
Robertsonian translocations	19
Polarity of Robertsonian translocations	20
Aims	21
Objectives	21

CHAPTER 2: MTDNA PHYLOGEOGRAPHY AND KARYOTYPIC ANALYSIS BY FISH	23
Introduction	23
Materials and methods	25
Samples	25
Cytogenetic analysis	25
<i>Fibroblast tissue culture</i>	25
<i>Bone marrow preparations</i>	28
<i>Chromosome banding</i>	28
Fluorescence <i>in situ</i> hybridization (FISH)	29
<i>Probe mixture</i>	29
<i>Slide pretreatment</i>	29
<i>Denaturing</i>	29
<i>Detection</i>	30
<i>Microscopy and image capture</i>	30
Chromosome nomenclature	30
Molecular analysis	31
<i>DNA amplification and sequencing</i>	31
<i>Sequence alignment and saturation</i>	31
<i>Data analysis</i>	32
Results	33
Karyotypic and FISH analysis	33
Sequence data	38
Phylogenetic analysis	41
Minimum spanning tree	45
Discussion	47
Divergence times	47
Ancestral karyotype	48
Biogeographic interpretation	48
Taxonomic considerations	50
CHAPTER 3: PHYLOGENOMIC ANALYSIS OF <i>R. PUMILIO</i>	52
Introduction	52
Methods	55
Samples and spleen cultures	55

LIST OF TABLES

		Page
Table 1	Correspondence among the various subspecific taxonomies suggested for <i>R. pumilio</i> by Ellerman (N=14; 1941), Roberts (N=20; 1951), and Meester <i>et al.</i> (N=7; 1986). A cross indicates recognition of the subspecies and a dash the converse.	6
Table 2	Geographic location of samples used in this investigation. Asterisks indicate localities for which cross-species chromosome painting was applied. One animal per locality was analysed using this approach.	27
Table 3	Distribution of variable and invariable amino acid residues in the three functional domains of the 1140 bp of <i>cyt b</i> gene.	38
Table 4	Uncorrected (p-) distance matrix for <i>cyt b</i> (1140bp) for all <i>R. pumilio</i> .	43
Table 5	Uncorrected (p-) distance matrix for the control region (871 bp).	77
Table 6	Uncorrected (p-) distance matrix for <i>cyt b</i> (1140 bp) for the reduced data.	77

LIST OF FIGURES

	Page
Figure 1	11
The distribution of <i>Rhabdomys pumilio</i> in Africa (redrawn from Mahida <i>et al.</i> 1999).	
Figure 2	26
Southern African localities of <i>R. pumilio</i> analysed in this investigation. Numbers indicate collection localities (see Table 2 for names and coordinates of these localities). Symbols next to the numbers show the mtDNA subgroup affinity as suggested by phylogenetic analysis: ▲ = subgroup A (<i>R. d. dilectus</i>), ■ = subgroup B (<i>R. d. chakae</i>), and ♦ = Clade 2 (<i>R. pumilio</i>).	
Figure 3	34
Standard GTG banded karyotype of <i>R. pumilio</i> from Irene (2n = 46, male).	
Figure 4	35
C-banding patterns of a female <i>R. pumilio</i> from Irene (2n=46) showing the distribution of constitutive heterochromatin in the pericentromeric regions of most autosomes. Interstitial and pericentric C-bands (arrows) were noted in two chromosome pairs. The insert shows the Y which is almost entirely heterochromatic	
Figure 5	37
FISH of MMU9, 13 and 18 on the metaphases from 2n = 46 (A-C) and 2n = 48 (D-F). The central panel shows the side-by-side G band comparisons of the metacentric Robertsonian fusion chromosome (RPU4) characteristic of 2n = 46, and the unfused acrocentric chromosomes "a" and "b" of the 2n = 48 cytotype. (A) Hybridization of MMU18 to RPU4p in 2n = 46 cytotype. (B) Hybridization of MMU9 to the proximal regions of RPU4q and RPU16, and entire RPU22 in the 2n = 46 cytotype. (C) Hybridization of MMU13 to the distal RPU4q and entire RPU11 in the 2n = 46 cytotype (this is a partial spread containing only one homologue of RPU11). (D) Hybridization of mouse chromosome 18 to the acrocentric homologues of pair "a" in 2n = 48. (E) Mouse chromosome 9 painting	

probe showing hybridization to the proximal regions of autosomal pair "b", the greater part of pair RPU16, and the entire RPU22 in the 2n = 48 cytotype. (F) Hybridization of mouse chromosome 13 to distal third of "b" and the whole of RPU11 in the 2n = 48 cytotype.

- Figure 6** Saturation plots for the first (A), second (B), and third (C) codon positions. (D) shows the transition versus transversion plots. The Y-axis is the number of substitutions while the X-axis is the uncorrected p-distances: \diamond = transitions for ingroup; \blacklozenge = transition for outgroup; \square = transversions for ingroup \blacksquare = transversions for outgroup. 39
- Figure 7** Neighbour joining tree showing the two major mitochondrial lineages which are separated by mean sequence divergence of 12%. Clade 1 contains specimens with both 2n = 46 and 48 while Clade 2 comprises specimens with 2n = 48. Representatives of Clade 1 are suggested to represent a single species, *R. dilectus*, which can be subdivided into two subspecies, *R. d. dilectus* (subgroup A) and *R. d. chakae* (subgroup B) while Clade 2, represents *R. pumilio*. Numbers at the nodes show bootstrap values for NJ (above nodes) and equal weighting parsimony (below nodes). The numbers in parenthesis indicate locality numbers (corresponding to those in Fig 1 and Table 1) while a and b refer to multiple specimens from the same locality. Single and double arrowheads distinguish the two competing evolutionary scenarios that can be invoked to explain the patterns suggested by the combination of cytogenetic and mtDNA data (see text for details). Single arrowhead on the branch leading to subgroup A indicates the Robertsonian fusion that was fixed in the ancestor of this lineage (first hypotheses). Double arrowheads on the branch leading to Clade 1 indicates the fixation of the 2n = 46 characterizing the 2.9 MYA divergence from Clade 2. Double arrowheads on the branch leading subgroup B indicate the reversal (fission) of the fusion that changed the diploid number back to the 2n = 48 found in representatives of this lineage. 42

- Figure 8** An unrooted minimum spanning tree constructed for the 35 haplotypes (numbered 1 – 35) detected in this investigation. Numbers of mutational steps joining the haplotypes are indicated along the connecting branches. Clades 1 and 2 also retrieved by NJ and MP are separated by 95 mutational steps, while the two cytotype specific subgroups (A and B) of Clade 1 are separated by 53 mutational steps. Correspondence between haplotype numbers and their geographic origins are given in Table 1. **46**
- Figure 9** Homology map summarising hybridization patterns produced by FISH using mouse specific painting probes onto *R. pumilio* metaphase chromosomes. Chromosome numbering (below the chromosomes) of the *R. pumilio* half-karyotype follows Ducroz *et al.* (1999; see Figure 3 in Chapter 2). Vertical lines show the extent of banding homology between *R. pumilio* and *M. musculus*. Numbers beside the vertical lines correspond to *M. musculus* chromosomes. Arrows indicate the position of the centromeres **57**
- Figure 10** FISH results with all *M. musculus* chromosome paints on *R. pumilio* metaphase spreads. The paints used are indicated in the left corner of each plate while the hybridized chromosomes are indicated by arrows. Green color indicates detection using FITC-avidin (1mg/ml; Amersham) for biotinylated probes and FITC-anti-digoxigenin (200mg/ml; Roche) for digoxigenin labelled probes and red staining indicates detection with CY3-avidin conjugate. Metaphase spreads were counterstained with DAPI. **58**
- Figure 11** G-banding comparisons between *M. musculus* and *R. pumilio* chromosomes that were identified as homologous by FISH. *Rhabdomys pumilio* chromosome numbers are preceded by R while *M. musculus* chromosome numbers are preceded by M. The centromere positions of all acrocentric chromosomes are at the tip with the exception of MMU 18 (corresponding to RPU4p), 6 (corresponding to RPU 10) **63**

and 13 (corresponding to *R. pumilio* 15) which have been inverted to emphasize banding homology. Vertical lines indicate the extent of banding homology between chromosomes of *R. pumilio* and *M. musculus*.

- Figure 12** Alignment of the *R. pumilio* consensus sequence to the Conserved homologues in the CSB1-3 (a-c) of human, chimpanzee, mouse, rat, rabbit, dolphin, cattle, seal, opossum. Gaps were introduced to emphasize regions of alignment. 71
- Figure 13** Putative secondary structures in *R. pumilio* obtained with the three conserved sequences (a) TAS, (b) CSB1, (c) CSB2 and (d) CSB3. Lines show the primary sequences of these regions. 72
- Figure 14** Neighbour joining tree obtained from the control region sequences (871 bp). Three subgroups (A-C) were retrieved. The strict consensus was based on three trees (length = 489 steps; CI = 0.824; RI = 0.563; HI = 0.176). The three subgroups represent the two species which were identified by the larger *cyt b* data, *R. dilectus* (subgroup A = *R. d. dilectus* ; subgroup B = *R. d. chakaë*) and *R. pumilio* (subgroup C). Numbers at the node indicate bootstrap support for NJ (above nodes) and equal weighting parsimony (below nodes). 75
- Figure 15** Neighbour joining tree topology obtained from the complete *cyt b* (1140 bp) showing the three subgroups. Two equally parsimonious trees were obtained (length = 533; CI = 0.76; RI = 0.69; HI = 0.23). 76

CHAPTER 1

GENERAL INTRODUCTION

Preamble

Like elsewhere in the world the African rodents occupy diverse habitats including forests (e.g. African soft-haired rats, genus *Praomys*; Lecompte *et al.* 2001), xeric environments (e.g. jirds, genus *Psammomys* and gerbils, genus *Gerbillus*; Shenbrot & Krasnov 2001), and predominantly mesic habitats (e.g. the water rats, genus *Dasymys*; Carleton & Martinez 1991). On the other hand, there are many more rodents which have a tolerance for more diverse habitats, the most common being the unstriped grass rats or Nile rats (*Arvicanthis*; Ducroz *et al.* 1997), the Namaqua rock mouse (*Aethomys namaquensis*; Chimimba 2001) and the four-striped mouse (*Rhabdomys pumilio*; Roberts 1951). In the latter group, most species display uniform phenotypic characteristics (pelage colouration) and are at best recognizable as species complexes which require in-depth taxonomic revisions using multiple independent data sets. The four-striped mouse is a good example since it has a uniform pelage pattern (four stripes), and an almost continuous distribution stretching from east Africa to southern Africa.

This study addresses the evolutionary genetics and systematics of *R. pumilio*. It employs a suite of techniques and molecular markers that previously have not been brought to bear on this problematic taxon. Although the ecology of this species is well documented, the level of geographic differentiation has never been thoroughly investigated. The results obtained using a variety of markers (the mitochondrial DNA cytochrome *b* gene (cyt *b*) and control region or d-loop, and molecular cytogenetics using fluorescence *in situ* hybridization or FISH) have been generated over a period of three years and certain aspects of the study have been presented at the national Second Conference of the Southern African Society for Systematic Biology (KwaZulu-Natal, South Africa, 10-14 July 2000) and the 14th International Chromosome Conference (Wurzburg, Germany, 4-8 September 2001). Furthermore, the data emanating from this study have formed the substance of two full-length scientific articles that have been accepted for publication: (1) Chromosome painting in the African four-striped mouse *R. pumilio*: Detection of possible murid specific contiguous segment combinations. *Chromosome Research*, and (2) Molecular genetics of *Rhabdomys pumilio* subspecies boundaries: mtDNA phylogeography and karyotypic analysis by fluorescence *in situ* hybridization (FISH). *Molecular Phylogenetics and Evolution*.

Higher order rodent phylogeny

The order Rodentia represents the most speciose mammalian group and is comprised of some 1,814 species and 29 families (Musser & Carlton 1993). Based on characteristics such as the shape of the skull, positioning of cranial muscles and the angle of the lower jaw, the 29 families have been classified into three sub-orders: Sciuromorpha (squirrels-like rodents), Myomorpha (rats and mice) and Hystricomorpha (porcupine-like rodents, Brandt 1855; Simpson 1945), or two suborders: Sciurignathi and Hystricognathi (Tullberg 1899). The distinction between these two classifications is based mainly on morphological characters associated with size and shape of the infraorbital foramen, the attachments and development of the masseter muscles and the position of the angular process relative to the plane of the incisor. Evolutionary relationships among these orders is unclear leading to the debate over whether rodents are monophyletic, as evidenced by morphological data (Locket & Hartenberger 1993), or polyphyletic, based on molecular data (Graur *et al.* 1991; Allard & Miyamoto 1991; Li *et al.* 1992; Hasegawa *et al.* 1992; Martignetti & Brosius 1993; Cao *et al.* 1994; D'Erchia *et al.* 1996; Huchon *et al.* 1999; Cao *et al.* 1997; Reyes *et al.* 2000).

Central to the debate of rodent monophyly/polyphyly are a number of issues (for review see Huchon *et al.* 1999) such as the method used in retrieving phylogenetic information. For instance, maximum parsimony analysis which supports rodent monophyly (Graur *et al.* 1991) has been criticized for the inherent error of long branch attraction (Felsenstein 1981), while maximum likelihood, which suggests rodent polyphyly, is not immune to producing inconsistent estimations of phylogeny when the assumptions are oversimplified (Sullivan & Swofford 1997). Huchon *et al.* (1999) concluded that taxon representation is often critical, and that mouse and rat, which are frequently used to reconstruct rodent phylogenies, do not adequately represent rodent diversity, or the fast evolutionary rate of murids (Wu & Li 1985; Li *et al.* 1990; Catzeflis *et al.* 1992). Further, it turns out that most studies that supported rodent paraphyly have been based on saturated mtDNA data which minimised phylogenetic signal (Philippe 1997; Springer *et al.* 2001). Indeed, recent studies, based on a large number of nuclear markers totalling approximately 16, 000 bp (Murphy *et al.* 2001a, b; Madsen *et al.* 2001; Eizirik *et al.* 2001) and a broader taxonomic sampling representative of the diverse rodent families (Huchon *et al.* 2002) retrieved a robust support for rodent monophyly.

"Arvicanthis Division" rodents

The Family Muridae is the largest in the order Rodentia and is comprised of approximately 1,336 species distributed into 16 subfamilies (Nowak 1999) most of which belong to the subfamily Murinae. As with the higher order controversies, phylogenetic relationships within and between these subfamilies are unclear due, in large part, to convergent evolution (De Graaff 1981). For example, the single synapomorphic dental character based on fossils (Catzeflis *et al.* 1992) and the repetitive Lx element (Pascale *et al.* 1990) support the monophyly of murine genera. In contrast, nuclear gene (LCAT; Michaux & Catzeflis 2000) and mitochondrial DNA (mtDNA *cyt b*, 12 S rRNA, 16 S rRNA; Ducroz *et al.* 2001) phylogenies show that the Otomyinae are nested within the subfamily Murinae, suggesting that the Otomyinae should rather be relegated to a tribe (the Otomyinii) within the Murinae. Further, these two markers, mtDNA (*cyt b*, 12S rRNA and ND4; Verheyen *et al.* 1995; Conroy & Cook 1999; Chevret *et al.* 2001) and the nuclear gene LCAT (Chevret *et al.* 2001) indicate that the genus *Acomys* (previously placed within the Murinae) should be accorded distinct subfamilial status, the Acomyinae.

Included within the Murinae is a grouping of species described by Missone (1969) as the "Arvicanthis Division" of rodents. This monophyletic assemblage is comprised of 13 genera: *Aethomys*, *Arvicanthis*, *Dasymys*, *Dephomys*, *Desmomys*, *Golunda*, *Hadromys*, *Hybomys*, *Lemniscomys*, *Mylomys*, *Pelomys*, *Stochomys* and *Rhabdomys*. Members of the clade share similarities such as grassland habitat, herbivorous/ insectivorous diet, diurnal activity, skull morphology (e.g. inflated bullae) and presence of black dorsal longitudinal stripes which vary between species (Musser 1987; Musser & Carleton 1993). Further, diagnostic characters differentiating members of the "Arvicanthis Division" include dentition patterns (grooved /ungrooved upper incisors), number of mammae and vestigial fifth digit of the hind foot.

Recently it was shown that the monophyly of this grouping is supported by mtDNA sequences (Ducroz *et al.* 2001). However, the level of variation and the number of species/ subspecies within genera require further investigation as has been done in detail for the genus *Arvicanthis* (Ducroz *et al.* 1997, 1998). The wide distribution of members of the "Arvicanthis Division", some of which display distinct geographic pelage colouration differences, raises questions about possible cryptic speciation within the group. This also holds true for the monotypic *R. pumilio*, which is estimated to have diverged, together with *Desmomys harightoni* (its sister taxa), from the Arvicanthine

rodents some 7 million years; this was followed by the radiation of the remaining "Arvicanthine Division" rodents approximately 2-3 million years later (Ducroz *et al.* 2001).

Consistent with its early separation, *R. pumilio* lacks most of the diagnostic characters defining the "Arvicanthis Division". As currently understood *R. pumilio* differs from its close allies primarily by having a reduced fifth digit on the forefeet, and the presence of the four stripes on the dorsal side of the body (Roberts 1951). It is the latter characteristic from which various colloquial names were derived, namely, the Cape striped field mouse, four-striped grass mouse, striped field mouse and field mouse or four-striped mouse.

Life history characteristics of *R. pumilio*

Rhabdomys pumilio is a diurnal grassland rodent feeding largely on a vegetarian diet (Kingdon 1974; Christian 1977). This diet is supplemented by gnawing at tree barks (Taylor & Perrin 1996), feeding on dicotyledonous seeds and stems, and invertebrate fauna such as isopterans (Rourke & Wiens 1977; Churchfield 1985; Rowe-Rowe 1986). Moreover, by feeding on floral parts of *Protea* species (family Proteacea) during and prior to anthesis, *R. pumilio* (together with the Namaqua rock mouse, *Aethomys namaquensis*, Edward's elephant shrew, *Elephantulus edwardsii* and pygmy mouse, *Mus minutoides*) facilitates cross pollination in this important fynbos taxon (Rourke & Wiens 1977; Van Tets & Nicolson 2001). The xylose-rich nectar of *Protea* is particularly important in rodent winter diets when their energy requirements are high (Van Tets & Nicolson 2001). Due to this varied diet *R. pumilio* is often called an opportunistic omnivore species (Churchfield 1985).

Food availability, which is largely determined by occurrence of rainfall, plays a critical role in the reproductive cycle of *R. pumilio*. Reproductive onset is triggered by summer rains when primary production is high (Christian 1979a, b). Desert dwelling *R. pumilio* reproduce seasonally, whereas in areas where rainfall is even throughout the year, it breeds in both summer and spring (Rowe-Rowe & Meester 1982a). Mean gestation period is 25 days and litter sizes average six offspring. Altricial offspring are produced which reach sexual maturity at two months. While *R. pumilio* is a social rodent, and has a dominance hierarchy, this is only apparent during the breeding season when population densities increase. As observed in other rodents (e.g. multimammate mouse, *Mastomys natalensis*, Taylor 1998) breeding females are territorial and enforce the hierarchy (Choate 1972; Brooks 1974).

Medical, economic and ecological role of *R. pumilio*

Rhabdomys pumilio is a medically important species because it serves as intermediate host to disease-transmitting larvae of the ticks *Hyalomma truncatum* and *H. marginatum rufipes* (Magano *et al.* 2000). These ticks play an important role in transmission of Crimean-Congo Haemorrhagic Fever virus to humans (Hoogstraal 1979; Swanepoel *et al.* 1983). Like most rodents, *R. pumilio* also harbors a number of zoonotic bacterial pathogens of man such as the often lethal *Salmonella* species and *Yersinia enterocolitica* which cause, among other symptoms, acute gastroenteritis (Shepherd & Lema 1985). Although *R. pumilio* does not co-habit human dwellings (Taylor 1998), their granivorous habit often leads them to seed stores on farms which may pose a potential health hazard to humans (De Graaff 1981).

Feeding habits of *R. pumilio* in the form of tree gnawing (especially in winter when food is scarce) has resulted in huge financial losses for the forestry industry (Taylor & Perrin 1996). Willan (1992) estimates that tree damage caused by *R. pumilio* and other rodents amounts to R50 million per annum or 7% losses to the forestry industry in South Africa. Population densities of *R. pumilio* can reach 800 kg/km² which is substantial when compared to the 1000 kg/km² biomass of ungulates in Kruger National Park (David 1979). Therefore, like most rodents (see Happold 2001), *R. pumilio* plays an important ecological and economical role in a variety of ecosystems owing to their cosmopolitan nature.

Systematics of *R. pumilio*

Classical subspecific delimitation of *R. pumilio*

Despite clear pelage difference between geographic forms of *R. pumilio* there is no consensus regarding the number of subspecies. Ellerman (1941) listed 14 subspecies, while Roberts (1951) described 20 of which Meester *et al.* (1986) retained seven (Table 1). In sharp contrast, De Graaff (1981) recognized only two southern African subspecies: an eastern and western form. Characters used to differentiate the subspecies listed by Roberts (1951) include tail length relative to body size, pelage colouration, head and body length, and a variety of skull measurements. Many of these subspecies show marked phenotypic differences which is perhaps indicative of adaptive

Table 1: Correspondence among the various subspecific taxonomies suggested for *R. pumilio* by Ellerman (N=14; 1941), Roberts (N=20; 1951), and Meester *et al.* (N=7; 1986). A cross indicates recognition of the subspecies and a dash the converse.

Ellerman (1941)	Roberts (1951)	Meester <i>et al.</i> (1986)
1. <i>pumilio</i>	X	X
2. <i>meridionalis</i>	--	
3. <i>chakae</i>	X	
4. <i>moshesh</i>	X	
5. <i>intermedius</i>	X	X
6. <i>cinereus</i>	X	X
7. <i>qriquae</i>	--	X
8. <i>bechuanae</i>	X	X
9. <i>deserti</i>	X	
10. <i>namibensis</i>	X	
11. <i>nyasae</i>	--	
12. <i>dilectus</i>	X	X
13. <i>angolae</i>	--	
14. <i>diminutus</i>	--	
15. --	<i>vittatus</i>	
16. --	<i>orangiae</i>	
17. --	<i>namaquensis</i>	
18. --	<i>prieskae</i>	
19. --	<i>fourie</i>	X
20. --	<i>vaalensis</i>	
21. --	<i>bethulanesis</i>	
22. --	<i>cradockensis</i>	
23. --	<i>griquoides</i>	
24. --	<i>algoae</i>	

response to local climatic conditions (Mayr & Ashlock 1991), or limited gene flow between demes (Avice 2000). For example, *R. p. fouriei* is the whitest of all the *R. pumilio* subspecies, has faint dorsal stripes and it occurs in northern Namibia, while *R. p. cinereus* has grey pelage colouration and occurs in northern Cape Province of South Africa.

Species and subspecies controversy

Species are the basic units of biodiversity and are important for systematists who seek to provide accurate descriptions and phylogenetic relationships between them (Hillis *et al.* 1996). This concept is obviously also important for conservation biologists in order to identify biodiversity hotspots (Myers *et al.* 2000) and to provide conservation guidelines on the best way to protect and manage biodiversity (Ryder 1986; Moritz 1994; Bulte & Van Kooten 1999; Crandal *et al.* 2000; Gullison *et al.* 2000; Myers *et al.* 2000; Cincotta *et al.* 2000). Unfortunately, despite several centuries of debate there is no consensus as to what constitutes a species. Currently, almost 20 species definitions are recognized but these are variants of the two main divergent species concepts, biological species concept (BSC; Mayr 1963) and phylogenetic species concept (PSC; Cracraft 1983). The BSC defines species as "a group of interbreeding natural population that is reproductively isolated from other such groups "(Mayr 1963). Opponents of this definition argue that it underestimates the number of diagnosable evolutionary distinct units and hence may mislead systematists and conservation biologists by underestimating biodiversity (Cracraft 1997). On the other hand, PSC is defined as "the smallest population or group of populations within which there is a parental pattern of ancestry and descent which is diagnosable by unique combinations of character states" (Cracraft 1997). The main drawback of this concept is that defining each individual organism as the smallest diagnosable distinct population leads to the proliferation of species (see Avice & Wollenburg 1997).

There are also difficulties with testing these concepts. For instance, the BSC does not provide for the formation of the reproductively viable hybrids of the barking deer, genus *Muntiacus* (Liming *et al.* 1980) among others, while the PSC may not be able to address sufficient sampling which is often required in phylogenetic analysis. Avice & Wollenburg (1997) take cognizance of the flaws of these divergent species concepts and argue that they are not mutually exclusive. By retaining the desirable properties of the BSC such as reproductive isolation and reformulating the PSC to emphasize the details of

pedigrees and lineage sorting at macroevolutionary scales would effectively resolve the conflict between these species concepts. Avise & Wollenburg (1997) emphasize that "historical descent and reproductive ties are related aspects of phylogeny and jointly illuminate biotic discontinuity". With the advent of molecular techniques, and availability of comparable data sets for different taxa (specifically allozymes and *cyt b*, Avise & Aquadro 1982; Johns & Avise 1998), the debate has now turned to discussions on the relevance of genetic distances in inferring species status (Bradley & Baker 2001; Ferguson 2002).

The subspecies designation is used to account for geographical differences within species (Mayr & Ashlock 1991). Roberts (1951) defines subspecies of mammals as "...aggregates from different geographical areas, which differ from one another in minor degree, such as shades of colour, body size, length of ears, feet or tail, and even in cranial or dental characters, but link up in these characters between the geographical areas". Ryder (1986) defines mammalian subspecies as geographical forms, or representatives of polytypic species, which may have minor cranial and external morphological differences such as pelage colouration. Although these definitions recognize the genetic distinctiveness of species, the recognition of subspecies status lies with workers who may either be "splitters" or "lumpers" (Simpson 1945). Further problems with the subspecies designation seem to have been compromised either by using geographic distribution as the sole criteria, or by only taking into consideration "minor cranial and external morphological differences such as pelage (or feather in case of birds) with no statistical treatment of the data to test their validity" (Ryder *et al.* 1988). This inconsistency is particularly evident in the treatment of *R. pumilio* where the number of subspecies ranges from 2 to 20 (see above).

In order to maintain genetic diversity contained by subspecies for conservation purposes (i. e. evolutionary significant units, ESUs, Ryder (1986) and management units, MUs, Moritz (1994)) or for clarifying borderline cases in systematics, Ryder *et al.* (1988) recommend that different data sets (biochemical, ethological, cytogenetic and mtDNA) should be integrated before assigning taxonomic rank (Corbet 1997). Accordingly, Corbet (1997) recognizes three instances indicative of support for subspecies status: (1) "Diagnosable parapatric forms with substantial hybridization or intergradation, e.g. European house mouse, *M. musculus* (2) Parapatric forms with minimal differences and some hybridization e.g. mole rat *Nannospalax ehrenbergi* which are diagnosed by karyotypic difference (3) Allopatric forms diagnosable on the basis of differences of a kind that are commonly found within interbreeding populations e.g. voles, *Microtus*

arvalis orcadensis and *Clethrionomys glareolus skomerensis*." It is noteworthy that in each of these cases a genetic distinction is critical.

Allozyme, cytogenetics and behavioural differentiation in *R. pumilio*

In an attempt to shed light on the subspecific delimitations of *R. pumilio* an allozyme study (Mahida *et al.* 1999) drawing on specimens of the species from geographically diverse localities (e.g. Malawi and Cape Point in South Africa) revealed that the taxon follows an isolation by distance model (Wright 1943). The UPGMA phylogeny produced two groupings of which one clusters samples from Malawi and from Swartberg in Western Cape, South Africa together. The mean similarity value was 0.883. As expected for a generalist species with extreme habitat plasticity, *R. pumilio* was found to have high mean polymorphism (16.1%, compared with the mean for mammals = 14.7%; Nevo, 1978) and heterozygosity values (0.073 compared with the mammalian mean heterozygosity = 0.039, Avise & Aquadro 1982). These allozyme indices place the genetic population structure of *R. pumilio* as intermediate on the Wrightian-panmictic scale (Mahida *et al.* 1999).

Two cytotypes, $2n = 46$ and 48 , have been detected within *R. pumilio* (Taylor 2000). Based on existing data, the distribution of the $2n = 46$ morph is restricted to the highveld of South Africa, Zimbabwe and Malawi, while the $2n = 48$ has a wider distribution in South Africa. The difference between the apparently parapatric cytotypes is attributed to a fixed Robertsonian fusion (Taylor 2000). Due to the lack of significant genotypic divergence between these cytotypes, the chromosomal divergence is thought to be a recent event (Taylor 2000).

Further attempts to shed more light on the geographic partitioning of *R. pumilio* populations were done using laboratory based breeding studies. Comparisons included specimens from three South African populations: Goegap Nature Reserve (Northern Cape Province) and Alice (Eastern Cape Province, both with $2n = 48$) and Irene (Gauteng Province, $2n = 46$; Pillay 2000a). These populations represent the extremes of the range of *R. pumilio* in South Africa. Control experiments (intrapopulation crosses) revealed high fecundity rate with 73% mating success compared to 53% between Goegap x Alice (Alice x Irene and Goegap x Irene had little or no breeding success). However, the 53% mating success rate produced a significantly reduced litter size compared to the control and, the hybrids suffered from impaired pre-weaning growth. Backcrosses involving these hybrids produced offspring that died prior to weaning. The

incidence of violence between *inter*-population breeding pairs suggests that pre-mating isolation would prevent hybridization under natural conditions (Pillay 2000a).

Moreover, mate-choice experiments involving females in oestrous showed that they consistently responded better to homotype (mates from same population) stimulus than to heterotype stimulus (Pillay 2000b). Based on these data Pillay (2000a, b) hypothesised that ethological divergence in *R. pumilio* occurred in allopatry, and that assortative mating developed allopatrically. Although cytogenetic and allozyme data provided baseline information on the genetic population structure of the species, they failed to provide clear geographic partitions which are central to the definition of subspecies.

Distribution of *R. pumilio*

Rhabdomys pumilio is a grassland species with a wide distribution that spans much of southern Africa (Figure 1). In east Africa its distribution becomes patchy and it is restricted to highlands due to competition for resources with *Arvicanthis* and *Lemniscomys* species (Kingdon 1974), all of which share a grassland habitat preference. This is particularly interesting since although *R. pumilio* co-exists in southern Africa with other rodents, competition is avoided through temporal partitioning of resources. For example, although *Acomys* occurs in sympatry with *R. pumilio* in some areas, the former is nocturnal, while the latter is largely diurnal (Choate 1972; Christian 1977; Bond *et al.* 1980; Rowe-Rowe & Meester 1982a, b).

The *R. pumilio* geographic distribution includes dry river beds in areas such as Gobabeb which is located on the edge of the Namib Desert (in Namibia) as well as pans in the Karoo in South Africa where the mean annual rainfall is less than 400 mm (Coetzee 1970). *Rhabdomys pumilio* is also abundant in mesic parts of southern Africa where its typical habitat (predominantly grasslands, savanna and bushland) receives an annual rainfall exceeding 1200 mm (Coetzee 1970; Skinner & Smithers 1990). Although laboratory based experiments suggested that *R. pumilio* can survive long periods without drinking water (Willan & Meester 1987), field observations indicate that *R. pumilio* does not have the typical physiological adaptations of other desert dwelling rodents. For example the species is unable to produce concentrated urine such as is found in gerbils for example (Christian 1977, Christian 1979a, b). As further evidence of its abilities to adapt to a broad range of environmental extremes, *R. pumilio* has been trapped at elevations exceeding 2700 m above sea level provided there is grass cover (Bond *et al.* 1980; Rowe-Rowe & Meester 1982a, b). Due to its wide distribution, and an ability to

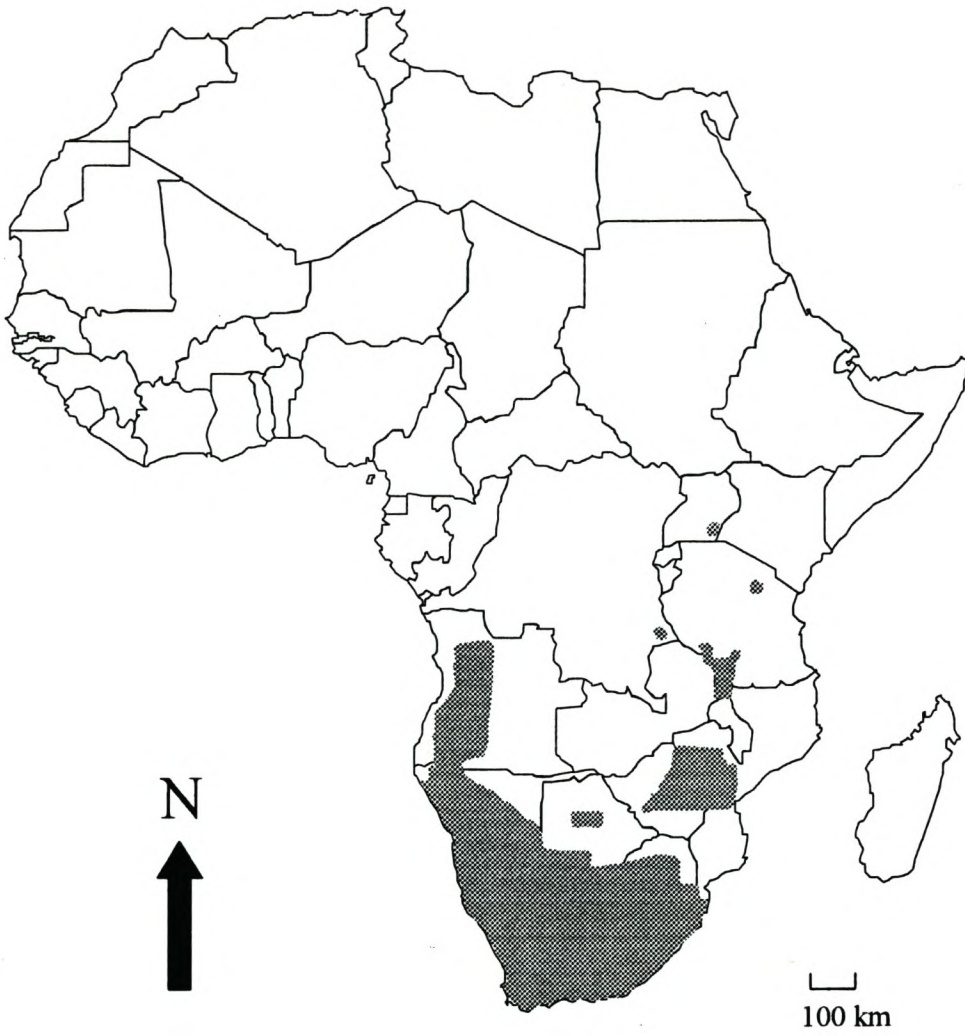


Figure 1: The distribution of *Rhabdomys pumilio* in Africa (redrawn from Mahida *et al.* 1999).

survive in markedly different habitat types (i.e. broad-niche species, Brooks 1974), *R. pumilio* provides a model species to test the effects of vicariance on geographic structure of southern Africa small mammal species. Given that small rodents generally have low or restricted vagility (Burda 2001), it is expected that *R. pumilio* will be affected by habitat fragmentation caused by climatic changes.

Molecular analysis

Mitochondrial DNA (mtDNA)

Genetic variation can be measured using chloroplast DNA in plants, mtDNA in animals and nuclear DNA in both groups. Various methods can be applied, among others allozymes, restriction fragment length polymorphism (RFLPs) and microsatellites. With the exception of allozymes, most of these techniques are based on DNA fragments generated from DNA isolates using cloning or the polymerase chain reaction (PCR, Mullis & Faloona 1987). Using sequences of oligonucleotides (primers) residing in the conserved regions of the mtDNA (universal primers; Kocher *et al.* 1989) it is possible to amplify specific DNA targets.

Several attributes render mtDNA useful for investigating phylogenetic and/ or phylogeographic patterns. First, mtDNA has a rapid evolutionary rate which among others is attributed to inefficient DNA repair mechanisms (Moritz *et al.* 1987). Second, mtDNA is maternally inherited (Giles *et al.* 1980), and does not undergo recombination. Although these mtDNA features have been accepted as dogma, recent evidence suggests that there are exceptions as evidenced by cases of heteroplasmy (existence of more than one extracellular mtDNA or chloroplast DNA in a cell) and paternal leakage (inheritance of DNA from the male gamete; for review see Wallis 1999; Hey 2000). Nonetheless, these reports do not detract from the utility of mtDNA to address systematic problems in a variety of taxa.

The mammalian mtDNA genome is a circular molecule consisting of 17 kilobases which is divided into coding (13 proteins, 2 rRNAs, 22 tRNAs) and a noncoding control region containing the displacement region or d-loop (Awise 1991). Coding genes evolve relatively slowly while non-coding regions evolve more rapidly. In other words, the rate of genetic change is generally negatively correlated with degree of functional constraint,

and "genes whose physiological functions are least disrupted by genetic alterations are those that typically evolve most rapidly" (Moritz *et al.* 1987).

The mammalian control region of most taxa is generally divided into three domains: the 5' left domain containing the terminally associated sequence (TAS, Doda *et al.* 1981), and the 3' right domain containing conserved sequence blocks (CSB, Walberg & Clayton 1981; Saccone *et al.* 1991) both of which flank the central conserved area (Brown *et al.* 1986; Saccone *et al.* 1987). While this structural integrity is generally maintained in mammals, there is base composition heterogeneity among these domains which is attributable to unique indels (insertions and deletions), and the rampant occurrence of repeat motifs that result in length differences between d-loop sequence of the same species (e.g. platypus, *Ornithorhynchus anatinus*, Gemmel *et al.* 1996; evening bat, *Nycticeius humeralis*, Wilkinson & Chapman 1991). However, the central conserved block is highly conserved across monotremes and therian mammals (Brown *et al.* 1986; Gemmell *et al.* 1996) mainly due to its functional role as a site for origin of replication (Brown *et al.* 1986 and references herein). The variation observed in the so-called hypervariable regions (5'end bordering tRNA_{pro} and 3'end bordering tRNA_{phe}) has been used to measure both intraspecific variation (e.g. shrews, genus *Sorex*, Stewart & Baker 1994a, b; Striped field mouse, *Apodemus agrarius*, Koh *et al.* 2000; Asian elephants, *Elephas maximus* Fernando *et al.* 2000) and interspecific differences between recently diverged species (e.g. masked shrew complex, *Sorex cinereus cinereus* and pygmy shrew, *S. hoyi*, Stewart & Baker 1994; house mouse, *Mus musculus* and *M. macedonicus*, Gunduz *et al.* 2000; red-backed voles, *Clethrionomys rufocanus* and *C. californicus*, Matson & Baker 2001). Furthermore, the variability observed in these regions has been useful to characterise the effect of natural radiation in man, *Homo sapiens* (Forster *et al.* 2002) and to determine conservation policy where genetic distinctness of taxa is uncertain (e.g. Robinson & Matthee 1999; Tolley *et al.* 1999; Fernando *et al.* 2000; Flagstad *et al.* 2000; Barber *et al.* 2002; Marshal & Ritland 2002).

Due to the high mutation rate, most studies have based their conclusions on partial sequences of this portion of the control region. For example, only partial sequences of the left domain have been used to determine the historical demographics of several taxa including man (Vigilant *et al.* 1991), the Norwegian lemming, *Lemmus lemmus* (Fedorov & Stethseth 2001), evening bats, *Nycticeius humeralis* (Wilkinson & Chapman 1991) as well as for describing genetic population structure in harbour porpoises, *Phocoena phoecona* (Tolley *et al.* 2001). Others have based their conclusions on complete sequences for example the house mouse, *M. domesticus* (Nachman *et al.* 1994;

Gunduz *et al.* 2000, 2001). However, there have been instances where the left domain has failed to provide resolution as evidenced in the North American collared lemming, *Dicrostonyx groenlandicus* (Ehrich *et al.* 2000), and wolverines, *Gulo gulo* (Walker *et al.* 2001). Given that the flanking regions may not provide sufficient variation, it is possible that analysis of partial sequences may result in gross underestimation of genetic variability (Stewart & Baker 1994a, b). To this end it may prove necessary to sequence the complete control region in order to evaluate the distribution of variable sites.

Contrary to the norm, several studies have used sequences of the supposedly slow evolving *cyt b* gene (instead of control region sequences) to investigate intraspecific variation, particularly in rodents. This is perhaps because among mammals, rodents display the highest rate of mtDNA nucleotide substitution both at the species and gene level (Catzeflis *et al.* 1987, 1992). However, there are caveats to its use since the gene has base compositional bias, displays rate variation between lineages, and is prone to saturation (Meyer 1994). The latter may result in limited phylogenetic information for deep phylogenies. Nonetheless, these limitations can be overcome by testing for the presence of saturated mutations (transitions vs transversions) in the *cyt b* and downweighting them (Griffiths 1997), and by testing for rate heterogeneity using relative rate tests (Robinson *et al.* 1998; Robinson-Rechavi *et al.* 2000). As a result, sequences of *cyt b* have been used with success for reconstructing the phylogeographic history and for dating the cladogenic events within murid species (Barome *et al.* 2001; Bradley *et al.* 2000; Geise *et al.* 2001; Fedorov & Stenseth 2001).

Rate heterogeneity and molecular clock

The molecular evolutionary clock hypothesis is based on the observation that the accumulation of amino acid differences and the time elapsed since common ancestry increase proportionally such that the protein being analysed evolves in a clock-like fashion (Margoliash 1963; Ayala 1997). Further, it is assumed that the evolution of the protein follows the neutrality theory which posits that mutations that are harmful to their carriers will be purged or kept at a low frequency by natural selection (Kimura 1968; Kimura & Ohta 1972). While the molecular clock provides a temporal scale for the interpretation of evolutionary divergence and biogeographic inferences, its application is subject to the influence of heterogeneity which results from differences in body size and metabolic rate, and generation time (see review in Avise 1994) although the latter has been shown not to be valid (see Schlotterer *et al.* 1991). Small bodied animals have a high metabolic rate that increases the concentration of mutagenic oxygen radicals in the

mitochondrion leading to a high nucleotide substitution rate in mtDNA (the converse is true; Martin & Palumbi 1993). It has been speculated that because of this, the rate of nucleotide substitution in rodents is 1.5 times higher than in Carnivora, Lagomorpha, Artiodactyla and Primates (Li *et al.* 1990). Therefore, prior to the application of the molecular clock it is crucial to assess rate differences among taxa (Sarich & Wilson 1973; Robinson *et al.* 1998; Robinson-Rechavi *et al.* 2000) since molecular clocks are applied on the premise that the evolutionary rate in each branch or lineage is uniform.

The calibration of mtDNA evolution in rodents is usually based on substitutions that accumulate slowly such as unsaturated third position transversions or nonsaturated substitutions for the complete data set (Smith & Patton 1999; Jolley *et al.* 2000; Ducroz *et al.* 2001). These are then correlated with paleontological data based on the 8 - 12 MYA split between mouse and rat (Jacobs & Pilbeam 1980). Given the high mutation rate in murids it is important to base calibrations on appropriate fossils data which, regrettably, in many cases is either incomplete or lacking (Avice 1994). As a result it is recommended that molecular clocks should be used with caution and that calibrations should be based on fossils of closely related taxa (Avice 1994; Ayala 1997).

MtDNA genetic distances and taxonomic inference

Taxonomic revisions based on mtDNA *cyt b* sequence data have become common among systematists (e.g. Smith & Patton 1993; Bradley *et al.* 2000; Harris *et al.* 2000) partly due to the level of variability of this marker, and the availability of comparable data from accessible repositories such as GenBank. Implementing Kimura's (1980) two parameter model (equal base frequency and transition bias) Johns & Avice (1998) observed that the mean sequence dissimilarity for the *cyt b* gene for confamilial and congeneric mammalian species ranged from 1 - 22%, for sister species the value was 1 - 16%, and <2% between subspecies. Recently it has been shown that this predictive pattern can be used to identify species. Using recognized species of four rodent genera (*Neotoma*, *Reinthonomys*, *Peromyscus*, and *Sigmodon*) and seven genera of bats (*Artibeus*, *Carollia*, *Chiroderma*, *Dermanura*, *Glossophaga*, *Rhinophyla*, and *Uroderma*) divergence values of >11% were found to be indicative of specific recognition, values between 2 and 11% had a high probability of being indicative of conspecific population differentiation, and values <2% were indicative of intraspecific variation (Bradley & Baker 2001). However, these authors note that several factors can affect the observed level of genetic distance including (1) breeding structure and reproductive history (migratory taxa will have greater divergent values than philopatric taxa); (2) lineage sorting and retention

of ancestral polymorphisms may increase sequence divergence; and (3) limited sampling of geographic extremes of a species range would lead to overestimation of genetic divergences.

While genetic distances may be useful they are not sufficient to identify separate species. Ferguson (2002) argues that identification of species based on genetic distance can be misleading since it is based on the erroneous assumption that the relationship between genetic distance and reproductive isolation (pre-mating or post-mating) is the consequence of cause-and-effect. By way of example Ferguson (2002) cites Hollocher *et al.* (1997a), Hollocher *et al.* (1997b), Kuramoto (1984), and Tilley *et al.* (1990) who demonstrated that populations of the fruit fly (*Drosophila*), tree frogs (*Hyla*) and salamander (*Desmognathus*) which have been assigned to respective single species *do* have pre-mating isolation. In conclusion, Ferguson (2002) argues that for identification of new species independent corroborating behavioural and ecological data reflecting mate recognition between the populations would be crucial. It is noteworthy that the suggested use of several independent data sets to identify taxonomic units is not new; it was originally suggested by Ryder *et al.* (1988, see above).

Comparative cytogenetics

Comparative cytogenetics allows karyotypic comparisons within and among closely related taxa and can provide additional information for phylogeographic studies. However, data collection, and interpretation of these comparisons, depend largely on the technique used. Early homogeneous staining only allowed gross descriptions of karyotypes (diploid number, number of chromosome arms) while subsequent -differential staining protocols (G-, Q-, R- and C-banding) allowed more detailed comparisons. Banding patterns (unique for each homologous chromosome pair) were instrumental for describing structural rearrangements underlying both human congenital diseases, and phylogenetic relationships between species (Mascarello *et al.* 1974). Although banding techniques are still being used effectively for closely related taxa (Volobouev *et al.* 2000), they may not be able to differentiate between true banding homology and false convergent banding patterns (see Stanyon *et al.* 1995 and for detailed discussion see Robinson 2001). These drawbacks were addressed by the development of the FISH protocol which provides for the annealing of DNA sequences to complementary sites (homologous regions) on the target DNA (see Chapter 2, Dyer & Meyne 1991).

FISH

Fluorescence *in situ* hybridization is a cytogenetic tool that provides an alternative for analysis of DNA at a macro-scale i.e. chromosome level. This technique was initially developed to use radioactively labeled probes for the localization of specific sequences of DNA, or RNA, in individual tissue sections, or in cells at various stages of mitotic division (Gall & Pardue 1969; Buongiorno-Nardeli & Amaldi 1969). The technique is applied in a variety of biomedical research fields including developmental biology, microbiology, virology, biological dosimetry and clinical cytogenetics (see Lichter & Ward 1990).

FISH uses nonradioactively labelled probes which can be divided into two classes based on size and the target complementary DNA: (1) Genomic DNA probes which are used for whole genomic hybridization (Houseal *et al.* 1995; Robinson *et al.* 1996), and (2) Probes that target structural domains on the chromosome such as telomere, centromere, chromosome band, specific loci and whole chromosome specific probes (Dyer & Myene 1991). The highly conserved telomeric probes can easily be generated by simple PCR (Lee *et al.* 1993). Subregional probes, and whole chromosome specific probes, can be derived by microdissection (Scalenghe *et al.* 1981) and/ or flow cytometry (see below; Gray *et al.* 1975).

Flow cytometry or fluorescence activated cell sorting (FACS) utilizes a dual laser beam to sort chromosomes by bivariate analysis using both chromosome size and base composition coupled to Hoetchst 33258 and chromomycin A3 staining (Ferguson-Smith *et al.* 1998). The chromosome sorts are amplified and labelled with biotin or digoxigenin haptens using degenerate oligonucleotide-primed PCR (DOP-PCR; Telenius *et al.* 1992). To date, whole chromosome flow sorts have been generated for more than 80 mammals including representatives of the orders Primates, Scandentia, Dermoptera, Lagomorpha, Rodentia, Carnivora, Certadiodactyla, Perissodactyla, Chiroptera, Eulipotyphla, Proboscidae, Macroscelidae (Murphy *et al.* 2001c) and representatives of divergent marsupial genera *Monodelphis*, *Macropus*, *Sminthopsis*, *Trichosurus* (see Rens *et al.* 2001 and references herein). The generation of whole chromosome painting probes using flow cytometry now makes it possible to compare taxa using reciprocal painting. This development is important as it allows a more detailed account of the subregional homologies between two species (Wienberg & Stanyon 1997).

The most widely used whole chromosome painting probes are those of man which have been mapped to other primate species (>25 species; Murphy *et al.* 2001c), equids (Raudsepp *et al.* 1996; Raudsepp *et al.* 1999), insectivores (Dixkens *et al.* 1998), ursids (Nash *et al.* 1998), bovines (Hayes 1995; Solina-Tolido *et al.* 1995; Iannuzzi *et al.* 1998; Fronicke & Wienberg 2001), pinnipeds (Fronicke *et al.* 1997), porcines (Fronicke *et al.* 1996; Goureau *et al.* 1996), cats (Wienberg *et al.* 1997), cetaceans (Scherthan *et al.* 1994; Bielec *et al.* 1998), lagomorphs (Korstanje *et al.* 1999; Robinson *et al.* 2002), even-toed ungulates (Scherthan *et al.* 1994 among others) and the Afrotherian Tubulidentata and Proboscidae (Yang *et al.* submitted). The data from these highly divergent taxa (some of which last shared an ancestor >50 MYA) suggest that the genomes of most mammals are highly conserved. Moreover, chromosome painting has revealed that information from hybridization patterns retrieved between different taxa can be useful for phylogenetic analysis especially because chromosome segments tend to be conserved as linkages which form stable chromosome segment associations.

Phylogenomics

The use of FISH in comparative cytogenetics has led to the development of "phylogenomics" where contiguous chromosome segment combinations are used as phylogenetic characters to infer evolutionary relationships (O'Brien & Stanyon 1999). Using this approach, karyotypes have been proposed for the hypothetical ancestral placental mammal (Chowdhary *et al.* 1998; Murphy *et al.* 2001; Yang *et al.* In press), and for particular groups of animals (e.g. Primates, Muller *et al.* 1999; O'Brien & Stanyon 1999; Carnivores, Graphodatsky *et al.* 2001; Nie *et al.* 2002; Marsupials, Marshall Graves 1998, Rens *et al.* 2001). The fundamental assumption is that chromosomal breaks are rare events that occur randomly (Rokas & Holland 2000) such that they constitute "phylogenomic markers" which can reliably be used to track evolutionary history of lineages (Murphy *et al.* 2001c). Using the phylogenomic approach, several chromosomal linkages (associations) derived from chromosome painting using human chromosome painting probes and gene mapping have been useful in documenting patterns of genome evolution. Chromosome segment associations of human chromosome 1/19p, 8/4, 3/21, 15/14, 10p/12pq/22q, 19q/16q and 12qdis/22qdis have been found to be retained in Carnivores, Cetartiodactyla, Chiropterans, Lagomorpha, Perissodactyls, Primates, Proboscidae, Rodentia, Scandentia and Tubulidentata (Murphy *et al.* 2001c; Yang *et al.* submitted). However, not all lineages have retained cytogenetic footprints that allow the reconstruction of phylogenies, especially where rapid radiation has been the norm (see Robinson *et al.* 2002).

Role of Robertsonian translocation in speciation

Robertsonian translocations

The two *R. pumilio* cytotypes ($2n = 46$ and 48) are distinguishable by a fixed Robertsonian rearrangement (whole arm translocation, Taylor 2000). This type of rearrangement is very common among mammals (King 1993) and may play a critical role in speciation processes (Searle 1998). Robertsonian rearrangements underpin chromosomal differences at the intra-specific level in a wide range of mammals including, for example, the geographic races of the house mouse, *M. musculus* (Capanna *et al.* 1976; Britton-Davidian *et al.* 2001), tuco-tucos, *Ctenomys minutus* (Gava & De Freitas 2002), the vlei rats, *Otomys irroratus* (Contrafatto *et al.* 1992; Rambau *et al.* 2001), short-tailed shrew, *Blarina carolensis* (Quimsiyeh *et al.* 1997), common shrew, *Sorex araneus* (Searle 1986) and large mammals (e.g. waterbuck, *Kobus ellipsiprymnus*, Kingswood 1998). They have also been found in sister-species of most taxa for example two species of the water rats, *Dasymys rufulus* and *D. incommutus* (Volobouev *et al.* 2000) and gerbils, *Taterillus pygargus* (*Taterillus* sp. 1 and sp. 2, Dobigny *et al.* 2002).

In their simplest form Robertsonian translocations involve fusion of a pair of acrocentric chromosomes which may occur naturally as balanced heterozygotes, or fixed as homozygous acrocentrics or homozygous metacentrics (Searle 1998; Gava & De Freitas 2002). Robertsonian translocations may also involve monobrachial fusions where acrocentrics of the same chromosome fuse to different partners (Baker & Bickam 1986). In the latter, Robertsonian heterozygotes are expected to have reduced reproductive output due to meiotic segregational difficulties which, in addition to normal balanced gametes, are likely to produce monosomic or trisomic gametes (King 1993). These may be negatively heterotic as evidenced in the mouse (Capanna *et al.* 1976; Baranov 1980; Harris *et al.* 1986), domestic animals (e.g. pig, *Sus scrofa*, Gustavsson 1988) and man (Page *et al.* 1996). When complex heterozygotes of this kind interbreed naturally they form complex meiotic chain or ring configurations leading to reproductive breakdown (Capanna *et al.* 1976; Baranov 1980; Britton-Davidian *et al.* 2000; Pialek *et al.* 2001). However, hybridization between cytotypes which differ as a result of a single fixed Robertsonian fusion (and excluding the presence of other rearrangements such as inversions, Noor *et al.* 2001) will form trivalents that segregate normally resulting in minimal reproductive impairment (King 1993). Consequently it seemed quite possible

that within the contact zone of the two *R. pumilio* cytotypes, floating polymorphisms with balanced heterozygotes may occur naturally (as in tuco-tucos, *C. minutus* Gava & De Freitas 2002). Currently, the parapatric distribution of the two cytotypes (Taylor 2000) makes it difficult to decide whether the homozygous metacentric or homozygous acrocentric state is ancestral or derived. This is further compounded by the fact that in lineages where Robertsonian translocations predominate it has been shown that they are often prone to reversals (Robinson 2001; Robinson *et al.* 2002).

Polarity of Robertsonian translocations

Certainly, the potential for reversals in Robertsonian translocations has added fuel to the debate regarding direction of chromosomal evolution in mammals. In the case of the house mouse, *M. musculus* (Garagna *et al.* 1995, 2001), the forest rodents of south America, genus *Akodon* (Fagundes *et al.* 1997), the Asian muntjac or barking deer, genus *Muntiacus* (Liming *et al.* 1980; Liming & Pathak 1981; Lin *et al.* 1991; Yang *et al.* 1997), and marsupials of the family Didelphidae (Svartman & Vianna-Morgante 1998), it has been demonstrated that chromosome evolution occurs through fusions of acrocentric chromosome elements (Wurster & Bernishke 1968). For these taxa the fusion theory assumes a parsimonious evolutionary pathway that involves the inactivation or loss of centromeres whose evolutionary presence may be indicated by residual interstitial heterochromatin in the derived low diploid numbers. In contrast, the fission theory as proposed by Todd (1970) suggests that complete acrocentric complements can be generated through chromosome fissions of metacentrics most recently reinterpreted through the "kinetochore reproduction theory" (Kolnicki 2000).

Kolnicki's hypothesis proposes that during gametogenesis centromeric DNA is duplicated in biarmed chromosomes followed by epigenetic formation of two pairs of kinetochores for the regulation of the dicentric chromatids. Subsequently, chromosome breakage between the kinetochore pairs generates two acrocentrics for each biarmed chromosome (Kolnicki 2000). However, the "kinetochore reproduction" theory is based on anecdotal experimental observations of abnormal chromosomal behaviour which have yet to be shown collectively in a particular organism or taxon. Instead, a credible scenario for the fission theory is provided by FISH experiments derived from studies on canids. A parsimonious evolutionary pathway that explains the distribution of conserved segments in the Raccoon dog (*Nectereus procyonoides viverrinus*), the Arctic fox (*Alopex lagopus*), the red fox (*Vulpes vulpes*) and the outgroup (man) suggests that the most recent ancestor of canid species had a low diploid number (Graphodatsky *et al.*

2001). To this extent it would appear that the mode of chromosome evolution (e.g. fusions in rodents and fissions in canids) is lineage specific, and not necessarily a "universal" phenomenon. Consequently, independent data would be crucial in resolving polarity (derived vs ancestral) with regard to direction of chromosomal evolution in *R. pumilio*, i.e. whether the transition between $2n = 46$ and $2n = 48$ is due to fission (Todd 1970) or fusion (Wurster & Bernishke 1968).

Aims

The aims of this study were three fold:

1. First, I set out to examine the phylogeography of *R. pumilio* in southern Africa by analysing complete sequences of the mtDNA *cyt b* gene and a segment of the control region from specimens drawn from localities throughout the species' range in South Africa. This was supplemented with limited extralimital sampling.
2. Second, I attempted to more clearly delimit the extent of the distribution of the two cytotypes in southern Africa by karyotyping specimens representative of the range of the species, and from areas where cytogenetic analyses had not previously been undertaken. Additionally, fluorescence *in situ* hybridization (FISH) using flow-sorted mouse chromosome specific painting probes and high resolution G banding were implemented in the hope of detecting subtle interchromosomal rearrangements in the genomes of the two cytotypes.
3. Third, a comparative phylogenomics approach was followed that utilized the *M. musculus/R. pumilio* FISH experiments to identify shared derived chromosomal states in those murids for which similar data are available. These were then placed in a broader phylogenetic context.

Objectives

The objectives of this study were to address the following key questions:

1. Is there evidence of genetic differentiation among the numerous subspecies of *R. pumilio* ?

2. Are there underpinning chromosomal rearrangements differentiating the two cytotypes?
3. Has *R. pumilio* retained chromosomal synteny that may be specific for the Murinae?

CHAPTER 2¹

MTDNA PHYLOGEOGRAPHY AND KARYOTYPIC ANALYSIS BY FISH

Introduction

Phylogeography deals with historical and phylogenetic components of the spatial distribution of gene lineages (Avice 2000). In so doing, it attempts to correlate cause and effect in explaining the current distribution of taxa, and uses historical distributions of microfossils of flora and fauna to infer past climatic changes (Werger 1978). Phylogeographic partitions are usually determined by vicariance and the dispersal capabilities of organisms. Vicariant phylogeographic separation occurs when taxa with continuous ranges are disrupted by environmental events, while range expansion and active dispersal can both lead to an organism exhibiting a continuous distribution (Avice 2000). Thus gene flow is a critical factor when considering phylogeographic interpretations. For instance, populations with extensive gene flow (which results in a strong homogenizing effect) will tend *not* to show any phylogenetic structure, while historically isolated populations show deep geographic structure due to divergent selection pressures, and lineage sorting. In other words, where the homogenizing effects of gene flow are minimal, structure may be anticipated.

Mitochondrial control region and *cyt b* sequences are frequently used for reconstructing the phylogeographic history at the population level (control region) and evolutionary relationships of species (*cyt b*; Hillis *et al.* 1996; Avice 2000). Since the control region has an elevated nucleotide substitution rate (4-5 times that of the rest of the mitochondrial genome; Taberlet 1996), it was selected as an appropriate marker in the analysis of *R. pumilio* population history. However, preliminary sequencing data of the 5' area of the control region using geographically divergent populations surprisingly resulted in few phylogenetically informative characters, indicating its limitations in this species. Detailed analyses of the *R. pumilio* mtDNA control region showed unexpected anomalies when compared to orthologous sequences from other mammalian taxa and

1. The data contained in this chapter form the substance of a paper that has been accepted for publication: Rambau R. V. & Robinson T. J. Molecular genetics of *Rhabdomys pumilio* subspecies boundaries: mtDNA phylogeography and karyotypic analysis by fluorescence *in situ* hybridization (FISH). *Molecular Phylogenetics and Evolution*

these data are presented separately in Chapter 4. In sharp contrast, however, the analysis of the *cyt b* data yielded high numbers of nucleotide substitutions many of which were parsimony informative allowing for fine scale resolution among the *R. pumilio* populations included in this investigation. Recently a number of studies have demonstrated the utility of the *cyt b* gene in reconstruction the phylogeographic patterns of species complexes within murid taxa. By correlating the data to past climatic changes it has been possible to infer historical processes that sculpted the evolutionary history of these species (Sullivan *et al.* 1997; Bradley *et al.* 2000; Harris *et al.* 2000; Riddle *et al.* 2000; Barome *et al.* 2001; Geise *et al.* 2001; Fedorov & Stenseth 2001)

With this in mind I set out to:

(1) Karyotype specimens that cover much of the species' range in South Africa in order to more clearly delimit the extent of the distribution of the two previously reported cytotypes ($2n = 46$ and 48 ; Ducroz *et al.* 1999; Taylor 2000). This included FISH analyses using flow sorted mouse chromosome specific painting probes (*M. musculus*) which was undertaken in the hope of detecting interchromosomal rearrangements in the genomes of the two cytotypes that may have been beyond the resolution of conventional cytogenetics. Preliminary investigations had shown the two cytotypes occur in close proximity (for example approximately 70 km separate the Irene and Suikerbosrand populations; see map Figure 2 and Table2) with no evidence of gene flow between them. Given the absence of negative heterosis normally associated with a single Robertsonian fusion (King 1993) it was considered possible that other more subtle interchromosomal rearrangements may underpin the dichotomy between them.

(2) Sequence the full length of the mtDNA *cyt b* gene in specimens drawn from localities throughout the species' range in South Africa so as to map the fine-scale phylogeography of *R. pumilio*. This was complemented with limited sampling from Malawi, Namibia, Uganda and Zimbabwe.

Materials and methods

Samples

A total of 35 specimens representing 15 South African locations, and one location each in Malawi, Uganda and Zimbabwe, and two in Namibia, were analysed (Table 2, Figure 2). All samples were karyotyped and sequenced (see details below). Two murine species, *Lemniscomys rosalia* and *Aethomys namaquensis* were selected as outgroups. These two genera, together with *Rhabdomys* and *Arvicanthis*, form part of a monophyletic cluster, the "Arvicanthis Division" (see Chapter 1) based on dentition (Musser 1987) and mtDNA sequence data (Ducroz *et al.* 2001).

Cytogenetic analysis

Fibroblast tissue culture

Conventional cytogenetic procedures were used to establish primary fibroblast tissue cultures which were propagated from tail clippings. Briefly, tail clips were sterilized by cleaning with 70% ethanol; they were subsequently placed in McCartney bottle containing Dulbecco's Modified Eagles Medium enriched with 15% (V/V) fetal calf serum. The tissue was left overnight at 37°C in order to monitor possible contamination. Following this, the tail tips were minced, placed in 25 cm² tissue culture flasks, covered with ± 1 ml of medium and incubated at 37°C in 5% CO₂. The cultures were allowed to grow to confluence, and subsequently passaged for analysis using standard techniques (Schwarzacher *et al.* 1974). Representative cultures were frozen in liquid nitrogen in fetal calf serum supplemented with 10% DMSO to serve as vouchers.

Cell division was arrested at metaphase using 50 µl Colcemid (10µg/ml; Gibco) for 1-3 hours. Cells were trypsinised and resuspended in pre-warmed hypotonic solution (0.075 M KCl) for 12 min. This was followed by fixation of the cells in modified Carnoy's fixative (3 parts methanol: 1 part glacial acetic acid; Schwarzacher *et al.* 1974). Slides were prepared by dropping cell suspension on fogged clean glass slides. The mitotic index was monitored using a phase contrast microscope.

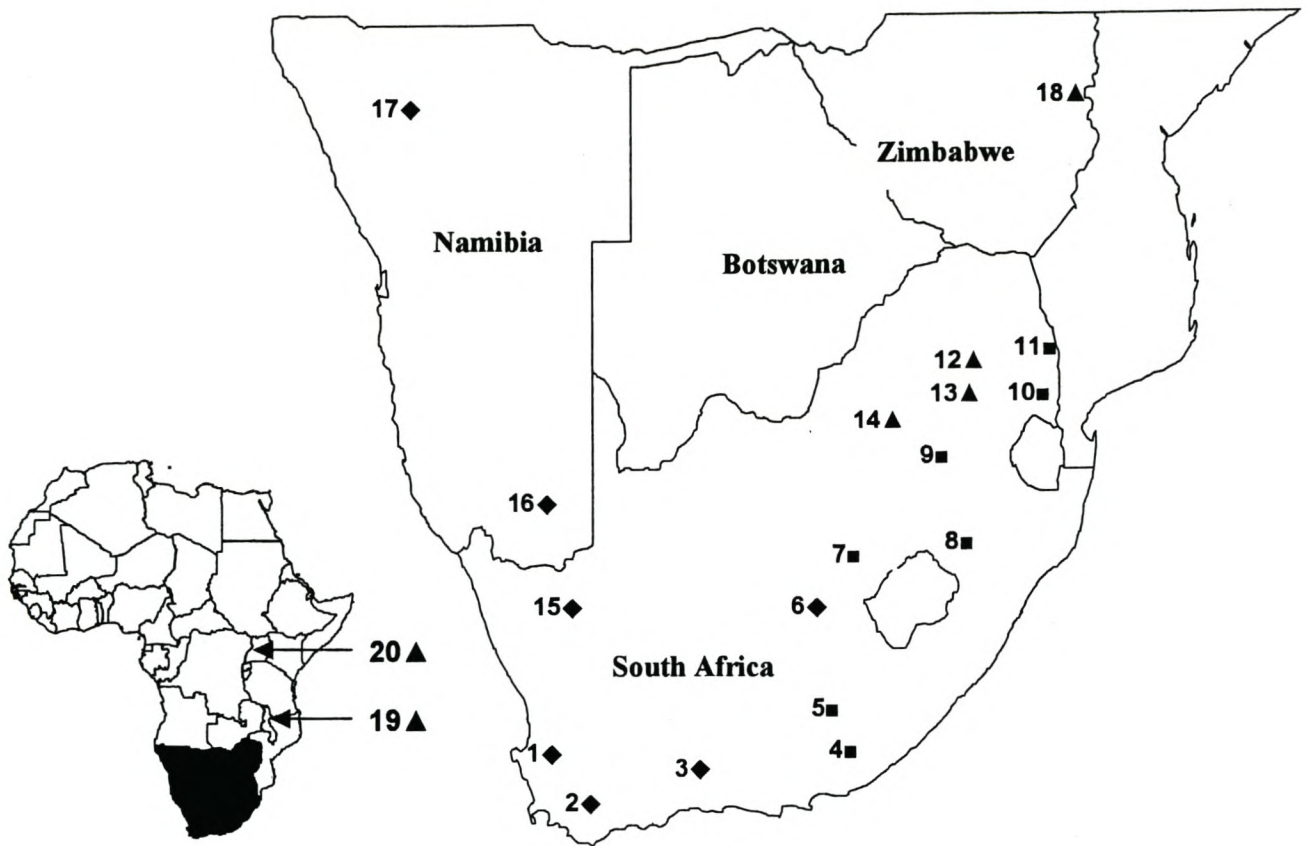


Figure 2: Southern African localities of *R. pumilio* analysed in this investigation. Numbers indicate collection localities (see Table 2 for names and coordinates of these localities). Symbols next to the numbers show the mtDNA subgroup affinity as suggested by phylogenetic analysis: ▲ = subgroup A (*R. d. dilectus*), ■ = subgroup B (*R. d. chakae*), and ◆ = Clade 2 (*R. pumilio*).

Table 2: Geographic location of samples used in this investigation (NR=Nature Reserve). Asterisks indicate localities for which cross-species chromosome painting was applied. One animal per locality was analysed using this approach.

Locality Number	Locality (<i>n</i>)	Geographic coordinates	Haplotype No.	2n
South Africa:				
1	Grotto Bay (<i>n</i> =2)	33°30'S, 18°18'E	15; 16	48
2	Bottelary (<i>n</i> =2)	33°59'S, 18°46'E	34; 35	48
3	Swartberg (<i>n</i> =2)	33°13'S, 22°03'E	7; 8	48
4	King William's Town (<i>n</i> =2)	32°53'S, 27°24'E	11; 12	48
5	Alice (<i>n</i> =1)	32°47'S, 26°50'E	6	48
6	Gariiep Dam (<i>n</i> =2)	25°46'S, 28°33'E	32; 33	48*
7	Willem Pretorius NR (<i>n</i> =2)	28°17'S, 27°15'E	13; 14	48*
8	Cathedral Peak (<i>n</i> =2)	28°55'S, 29°01'E	9; 10	48
9	Suikerbosrand NR(<i>n</i> =2)	26°30'S, 28°15'E	18; 19	48
10	Pilgrim's Rest (<i>n</i> =2)	24°51'S, 30°45'E	20; 21	48
11	Lydenburg (<i>n</i> =2)	25°06'S, 30°27'E	22; 23	48
12	Willem Prinsloo NR (<i>n</i> =1)	25°46'S, 28°33'E	1	48
13	Irene (<i>n</i> =1)	25°53'S, 28°18'E	31	46*
14	Potchefstroom (<i>n</i> =2)	26°42'S, 27°06'E	29; 30	46
15	Springbok (<i>n</i> =1)	29°40'S, 17°51'E	17	48*
Namibia:				
16	Keetmanshoop (<i>n</i> =2)	26°34'S, 18°18'E	4; 5	48
17	Swakopmund (<i>n</i> =2)	22°10'S, 14°14'E	2; 3	48
Zimbabwe:				
18	Inyanga (<i>n</i> =2)	18°12'S, 32°40'E	27, 28	46
Malawi:				
19	Nyika Plateau (<i>n</i> =2)	10°34'S, 33°48'E	24; 25	46
Uganda:				
20	Mountain Elgon (<i>n</i> =1)	1°11'N, 34°23'E	26	46

Bone marrow preparations

Bone marrow preparations were done using yeast stimulation following Lee & Elder (1980) with minor modifications. Yeast suspension were prepared by thoroughly mixing 2-3 g active dry yeast (Anchor Yeast, SA) and 5-6 g Dextrose in 25 ml water and incubated in 40°C until the yeast foamed vigorously. The suspension was administered subcutaneously in the dorsal region of the neck (0.5 ml of yeast suspension per 25 g of body weight). After 24 hours 0.1 ml of 10µg/ml ColcemidTM was administered into the peritoneum and then animals were sacrificed 1 hour later by Halothane overdose. Following dissection, bone marrow was extracted from pre-cleaned limb bones and resuspended in tissue culture medium. Mitotic cells were collected by centrifugation and resuspended in prewarmed (37°C) hypotonic solution for 10-15 minutes. Metaphase cells were fixed in freshly prepared Carnoy's fixative. Chromosome preparations were done as described above.

Chromosome banding

Chromosome banding was by trypsin digestion (GTG-banding; Seabright 1971) for identification of homologues, and by barium hydroxide (C-banding; Sumner 1972) for determining the position of repetitive DNA sequences located as heterochromatic regions in chromosomes. In the case of G banding, slides were aged by either baking at 90°C for 20 min, or by storing slides at 37°C for one to three weeks. This treatment yielded chromosomes with crisper bands. GTG-banding involved momentary treatment of the slides in Hanks' balanced salt solution (HBSS; Ca²⁺ and Mg²⁺ free) followed by 2-3 min digestion (depending on the age of the slides) in a 0.25% ENZAR-T trypsin/Hank's Balanced Salt Solution (HBSS, Ca²⁺ and Mg²⁺ free, pH = 7.0). Enzymatic digestion was stopped by immersion of slides in 80% (V/V) ethanol. Chromosome staining was done using a 2% (V/V) phosphate buffered Giemsa solution (pH = 6.8); staining time was approximately 4 minutes.

Fresh metaphase slides were used for C-banding. Slides were treated for 30 min in 0.2 N HCl solution, immersed for 90 min in pre-warmed (55°C) 5% (V/V) barium hydroxide, followed by 1 hour in 2xSSC (60°C). Slides were stained in 4% (V/V) phosphate buffered Giemsa solution.

Fluorescence *in situ* hybridization (FISH)

The FISH protocol described by Stanyon *et al.* (1999) was followed (see below) and appropriate conditions for slide aging and denaturation (necessary for good FISH preparations) were optimised following suggestions outlined by Henegariu *et al.* (2001).

Probe mixture

The complete suite (N = 20) of flow sorted commercial mouse chromosome specific painting probes (Cambio) were used for ZOO-FISH. The probe mixture (for total volume of 12-15µl) was comprised of 2-3 µl biotin labelled PCR product, 10-12 µl of hybridization buffer (50% formamide, 1xSSC, 10% Dextran Sulphate) and variable amounts of cot-1 DNA (GibcoBRL) depending on the amount of background hybridization. The probe mixture was denatured at 65-75°C for 10 min and repetitive DNA sequences were reannealed at 37°C for 30-60 min.

Slide pretreatment

Chromosomes were G banded and selected metaphases were photographed under oil using an Olympus BX60 microscope equipped with fluorescence. Oil was removed from the slides by two 5 min washes in Xylene, air dried and destained in 70% (V/V) ethanol. This was followed by a 3 min treatment in 3% (V/V) Formaldehyde solution, 2 min wash in 2xSSC and dehydration in ethanol series (70, 80, 90, 100% V/V).

Denaturing

Two methods were used for denaturing the probe mixture and metaphase chromosomes:

(a) Co-denaturation - The probe mixture was pipetted on the slide, covered with glass coverslip and sealed with rubber cement. The slide was placed on a heating block preset at 71-73°C for 3-4 min. Slides were incubated at 37°C for two days to allow for the probe to anneal to target DNA (hybridization).

(b) Separate denaturation - Metaphase slides were dehydrated 5 min in 100% ethanol, air-dried, treated for 5-10 min in 0.01% (V/V) Pepsin (prepared in 10 mM HCl) to remove cytoplasm and rinsed in 2xSSC. Slides were briefly dehydrated in ethanol series and the chromosomes aged by immersion in 100% ethanol at 65°C followed by

30 min baking in a 65°C oven. Slides were denatured in 70% formamide/2xSSC (V/V) for 1 min, and rapidly quenched in an ethanol series and air-dried. The reannealed probe mixture was then pipetted on the denatured metaphase slide, covered with a glass coverslip and sealed with rubber cement. Preparations were incubated in a 37°C chamber for 2 days. In order to prevent desiccation during *in situ* hybridization, slides were placed in 3x8 cm plastic containers lined with paper towels soaked in water.

Detection

After 48 h of incubation, coverslips were removed and the slides washed in 2xSSC, two passes through 50% formamide/ 2xSSC (V/V) which were followed by two more successive washes in 2xSSC; each wash was 5 min. Thereafter, 200µl of FITC-avidin/ CY3-avidin (1 mg/ml, Amersham) for biotinylated probes and/ or FITC-antidigoxigenin (200 mg/ml; Roche) for digoxigeninlabelled probes were pipetted on the slides. This was covered with parafilm and thereafter the slides were incubated in 37°C for 20-40 min. Subsequently, the preparations were taken through 3x5 min washes in 4xSSC containing 0.05% Tween-20 and mounted using 0.2mg/ml 4', 6-diamidino-2-phenylindole (DAPI) in Antifade.

Microscopy and image capture

Images were visualised with the Olympus BX60 fluorescence microscope fitted with appropriate excitation filters (Reichman 1998) and captured using a cooled CCD camera. Automated karyotyping and analysis were done using Cytovision® / Genus™ version 2.7 software (Applied Imaging). Hybridization signals were assigned to specific chromosomes, or chromosome regions, defined by G banding patterns that were previously photographed.

Chromosome nomenclature

Chromosome numbering in *R. pumilio* and the mouse, *M. musculus* followed the standards published by Ducroz *et al.* (1999) and Evans (1996). In discussions regarding comparisons between chromosomes of respective species, the ISCN (1978) nomenclature was adopted where *M. musculus* and *R. pumilio* were abbreviated to MMU and RPU respectively.

Molecular analysis

DNA amplification and sequencing

Genomic DNA extractions were derived from liver and/or muscle tissue following standard procedures (Maniatis *et al.* 1982). Tissue was digested using proteinase K (10 mg/ml)/ Amniocyte buffer (1M Tris, 1M NaCl, 0.5M Na-EDTA, 10% SDS and distilled water). After complete digestion impurities were removed by successive washes in calibrated phenol (pH = 7.0) followed by washes in Isopropyl-chloroform. DNA was precipitated overnight at -20°C using 10% (V/V) of 2 M Ammonium Acetate and 100% ethanol, and then pelleted by centrifugation. DNA was eluted in distilled water or 1xTE.

The mitochondrial *cyt b* gene was amplified by PCR with universal primers (L14724 and H15915; Paabo & Wilson 1988; Kocher *et al.* 1989; Irwin *et al.* 1991). Sequences obtained with these conserved primers were used to design two internal *R. pumilio* specific primers: a forward primer *Rhab* L2 (5'TTCTCAGTAGATAAAGCTAC 3') that corresponds to L500 in the *R. pumilio* *cyt b* sequence, and a reverse primer *Rhab* H2 (3'GTTGAGTGGGTTAGCTGG 5'), corresponding to position L772. Although the complete *cyt b* gene was amplified by PCR using L14724 and H15915 (and products were cleaned with the QIAquick PCR purification kit, Qiagen Ltd.), cycle sequencing was done using the two species-specific primers following recommendations of the manufacturer (Perkin-Elmer Applied Biosystems). Cycle sequencing products were purified with Cephadex spin columns (Princeton Separations), and analysed using an ABI 3100 automated sequencer.

Sequence alignment and saturation

Nucleotide sequences were checked in Sequence Navigator version 1.0.1 (Applied Biosystems, Inc., 1994) and then aligned by eye. MacClade version 3.04 (Maddison & Maddison 1992) was used to convert *cyt b* sequences into amino acids to check for stop codons (indicative of non-coding nuclear homologues; Arctander 1995), and to ascertain whether the nucleotide substitution pattern followed that of protein coding genes (Irwin *et al.* 1991). Sequences were deposited in GenBank under Accession numbers AF533083 – AF533116. The data were examined for saturation by plotting the total number of substitutions (transitions and transversions) against sequence divergence (p-) values, and by plotting the total number of transitions against transversions in order to check for

the effect of multiple substitutions on our phylogenetic analysis (e.g. Halanych *et al.* 1999).

Data analysis

Phylogenetic reconstruction was done in PAUP* version 4.0b6 (Swofford 2001) using parsimony and neighbour joining (NJ; Saitou & Nei 1987). Parsimony trees were obtained using both the empirical weighting computed in MacClade version 3.04 (Maddison & Maddison 1992), and equally weighted characters; heuristic searches were implemented using 100 replicates of random addition of taxa. The NJ tree was derived using the TrN+G model selected by the Modeltest version 3.06 using hierarchical likelihood ratio tests (Posada & Crandall 1998). The robustness of tree topologies was assessed by 1000 bootstrap resamplings (Felsenstein 1985). In order to apply a molecular clock, rate differences among lineages were tested by means of the RRtree programme version 1.1.11 (Robinson *et al.* 1998; Robinson-Rechavi & Huchon 2000) using the closely related *Aethomys namaquensis* as a reference taxon. Additionally, in order to investigate fine-scale differences among *R. pumilio* samples, the minimum spanning tree was constructed in Arlequin version 2 (Schneider *et al.* 2000).

Results

Karyotypic and FISH analysis

Analysis of the South African *R. pumilio* showed the populations to be fixed for either $2n = 46$ or $2n = 48$; no intra-population karyotypic variation was detected. Specimens collected at Irene (Gauteng Province) had $2n = 46$, while those sampled at the Suikerbosrand Nature Reserve had $2n = 48$. A diploid number of $2n = 48$ was obtained for animals occurring in all the remaining South African Provinces, namely, Eastern Cape (Alice), Free State (Gariiep Dam, Willem Pretorius Nature Reserve), Mpumalanga (Lydenburg and Pilgrim's Rest), Western Cape (Bottlelary, Grotto Bay) and Northern Cape (Springbok). Samples from the two Namibian localities (Swakopmund and Keetmanshoop) also had a diploid number of $2n = 48$ (see map, Figure 2). Our $2n = 46$ cytotype is identical to that reported by Ducroz *et al.* (1999) for a specimen collected at the Willem Prinsloo Nature Reserve (Gauteng Province). The karyotype contains six pairs of metacentric autosomes, two pairs of submetacentrics and 14 pairs of acrocentric autosomes (Figure 3). The two sex chromosomes are acrocentric, the Y being small and the X medium sized. Side by side G-band comparisons of the $2n = 46$ and $2n = 48$ karyotypes revealed no differences at this level of resolution (see page 36). The only exception is an additional acrocentric pair in $2n = 48$ karyotypes which is consistent with a single Robertsonian fusion delimiting the $2n = 46$ and 48 karyotypes ($N_{fa} = 60$). The C-banding results are illustrated in Figure 4 which show that heterochromatin is located on the centromeric regions of all chromosomes, and at interstitial positions in two pairs of autosomes. The Y chromosome was almost entirely heterochromatic.



Figure 3: Standard GTG banded karyotype of *R. pumilio* from Irene (2n = 46, male).



Figure 4: C-banding patterns of a female *R. pumilio* from Irene ($2n=46$) showing the distribution of constitutive heterochromatin in the pericentromeric regions of most autosomes. Interstitial and pericentromeric C-bands (arrows) were noted in two chromosome pairs. The insert shows the Y which is almost entirely heterochromatic.

Fluorescence *in situ* hybridization with mouse chromosome specific painting probes produced similar patterns for the two cytotypes revealing that no interchromosomal rearrangements (other than the centric fusion difference) distinguish the apparently parapatrically distributed cytotypes. The short arm (p arm) of the fusion chromosome (*R. pumilio* chromosome 4, RPU4; Ducroz *et al.* 1999) in the $2n = 46$ cytotype corresponds to mouse chromosome 18 (MMU18), while the proximal third and distal two thirds of the long arm (q arm) were hybridized by segments of MMU9 and MMU13 respectively (Figure 5). FISH signals on the $2n = 48$ cytotype were restricted entirely to acrocentric autosomes. MMU18 hybridises to an acrocentric pair ("a") that corresponds to the short arm of the fusion chromosome (RPU4) in the $2n = 46$ karyotype. The remaining portion of the fusion chromosome is painted by MMU9 and MMU13 (Figure 5). In addition to the fusion chromosome, MMU9 also anneals to parts of two chromosomes not involved in the fusion product, RPU22 and proximal two thirds of RPU16, while MMU13 paints to distal RPU11. Obviously the molecular cytogenetic analysis does not provide insights to intrachromosomal rearrangements (gene order), and consequently small inversions and transpositions that may distinguish the cytotypes escape detection.

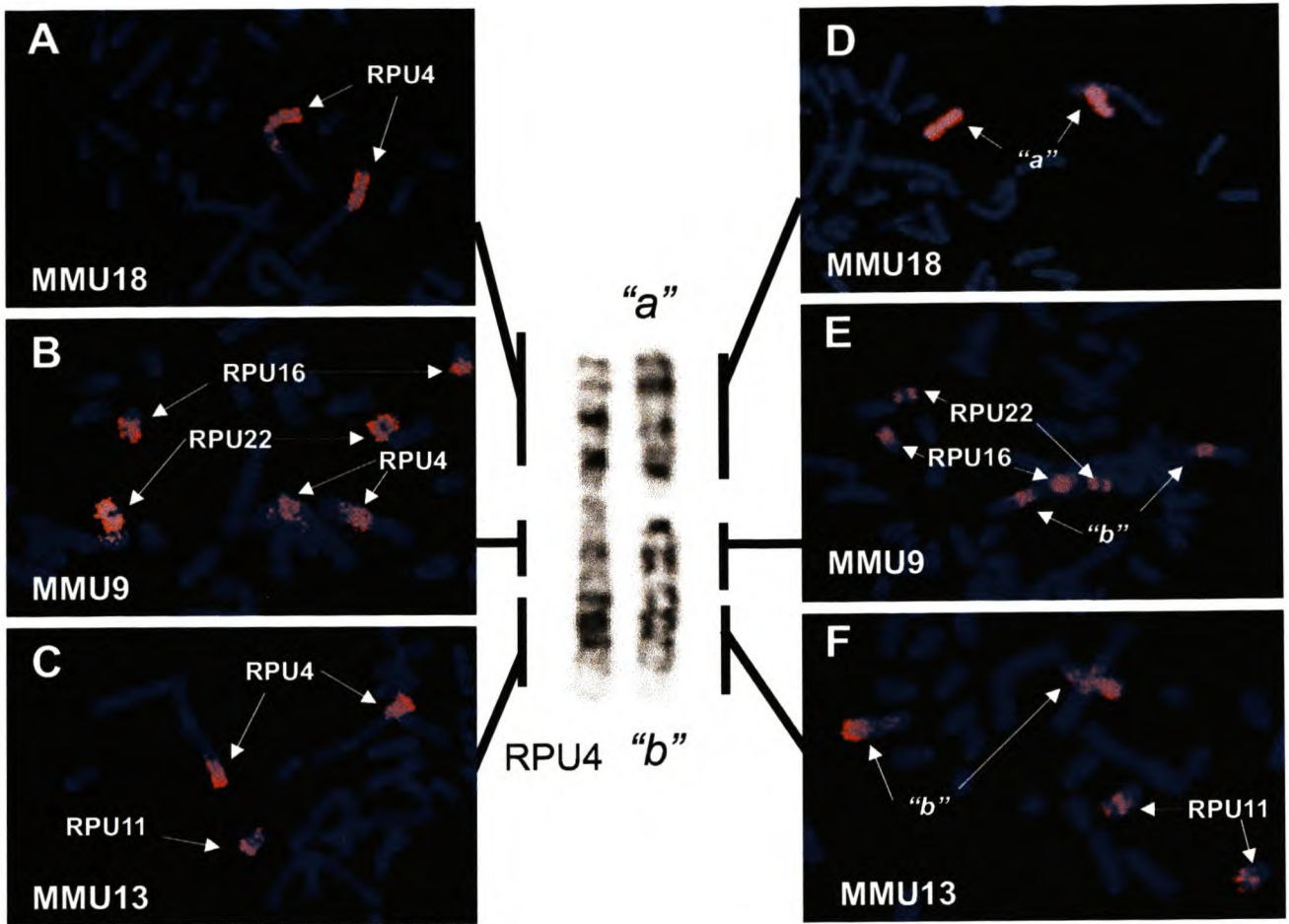


Figure 5: FISH of MMU9, 13 and 18 on the metaphases from $2n = 46$ (A-C) and $2n = 48$ (D-F). The central panel shows the side-by-side G band comparisons of the metacentric Robertsonian fusion chromosome (RPU4) characteristic of $2n = 46$, and the unfused acrocentric chromosomes "a" and "b" of the $2n = 48$ cytotype. (A) Hybridization of MMU18 to RPU4p in $2n = 46$ cytotype. (B) Hybridization of MMU9 to the proximal regions of RPU4q and RPU16, and entire RPU22 in the $2n = 46$ cytotype. (C) Hybridization of MMU13 to the distal RPU4q and entire RPU11 in the $2n = 46$ cytotype (this is a partial spread containing only one homologue of RPU11). (D) Hybridization of mouse chromosome 18 to the acrocentric homologues of pair "a" in $2n = 48$. (E) Mouse chromosome 9 painting probe showing hybridization to the proximal regions of autosomal pair "b", the greater part of pair RPU16, and the entire RPU22 in the $2n = 48$ cytotype. (F) Hybridization of mouse chromosome 13 to distal third of "b" and the whole of RPU11 in the $2n = 48$ cytotype.

Sequence data

The full *cyt b* gene sequence (1140 bp) was obtained for each of the 35 specimens included in our study. The nucleotide composition is typical of the mammalian *cyt b* gene (Irwin *et al.* 1991): Guanine = 13%, Cytosine = 27%, Adenine = 29% and Thymine = 31%. Of the 1140 bp, 364 sites were variable of which 269 were parsimony informative (excluding outgroup taxa). Transitional changes outweighed transversions by 5:1.

Structural analysis of the three functional domains of the *cyt b* data-set (including the two outgroup taxa) did not reveal significant deviation from that of other mammals (Irwin *et al.* 1991). The transmembrane had the highest percentage of variable amino acids ($Q = 50\%$) followed by the inner membrane ($Q = 38\%$). The outer membrane had the least number of variable sites ($Q_o = 24\%$, Table 2). No stop codons, or shifts in the reading frame, were evident in the data.

Table 3: Distribution of variable and invariable amino acid residues in the three functional domains of the 1140 bp of *cyt b* gene

Domains	Number of sites		
	Variable	Invariable	Sum
Transmembrane (Q)	49	48	97
Inner surface (Qi)	65	107	172
Outer surface (Qo)	27	84	111
Total	141	239	380

Saturation analysis revealed a positive correlation between substitutional changes and sequence divergence values, and between transitions and transversions i.e. that there is no saturation (Figure 6). However, detailed analysis of codon changes indicate that third position transitional mutations were saturated.

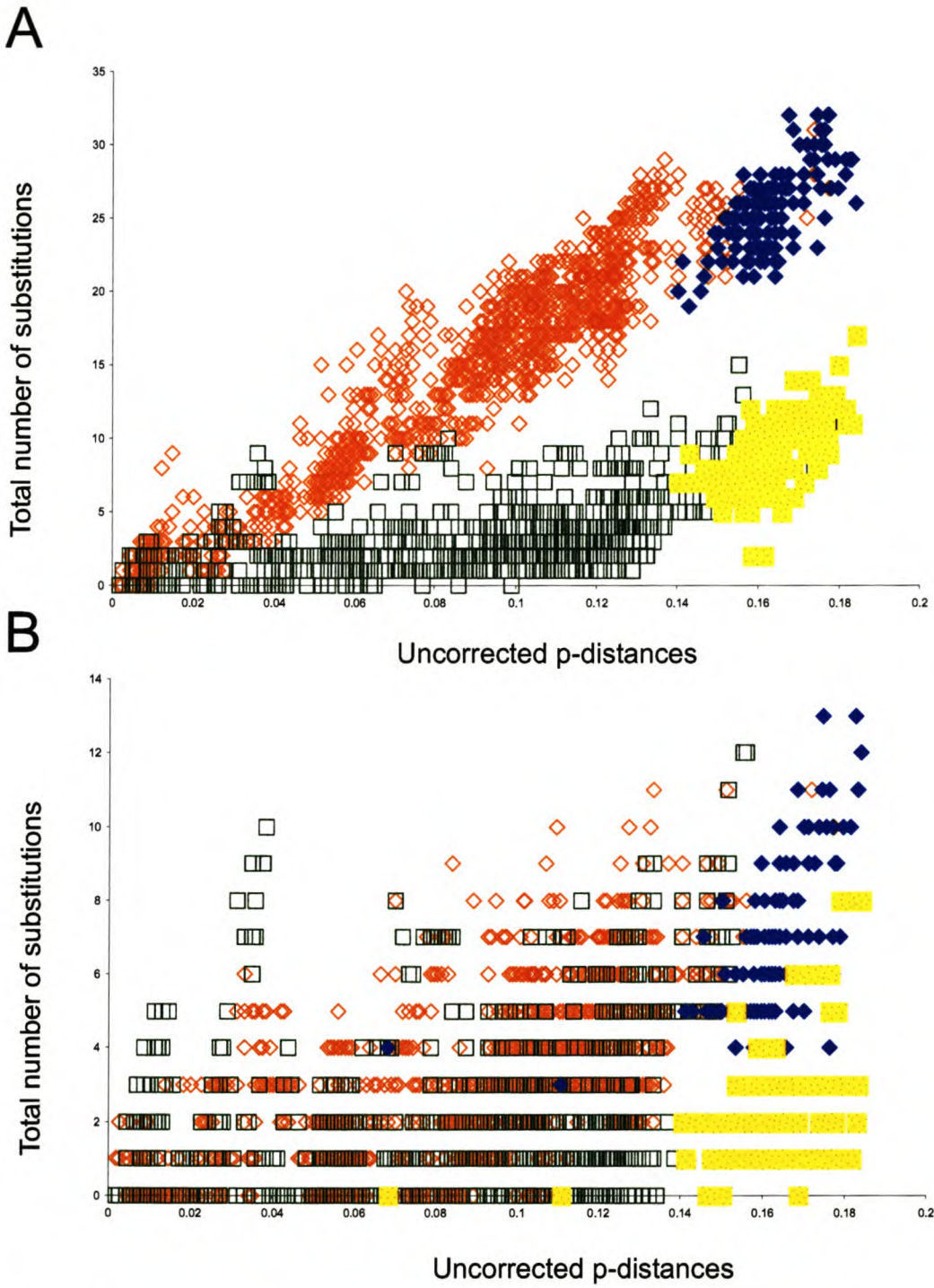
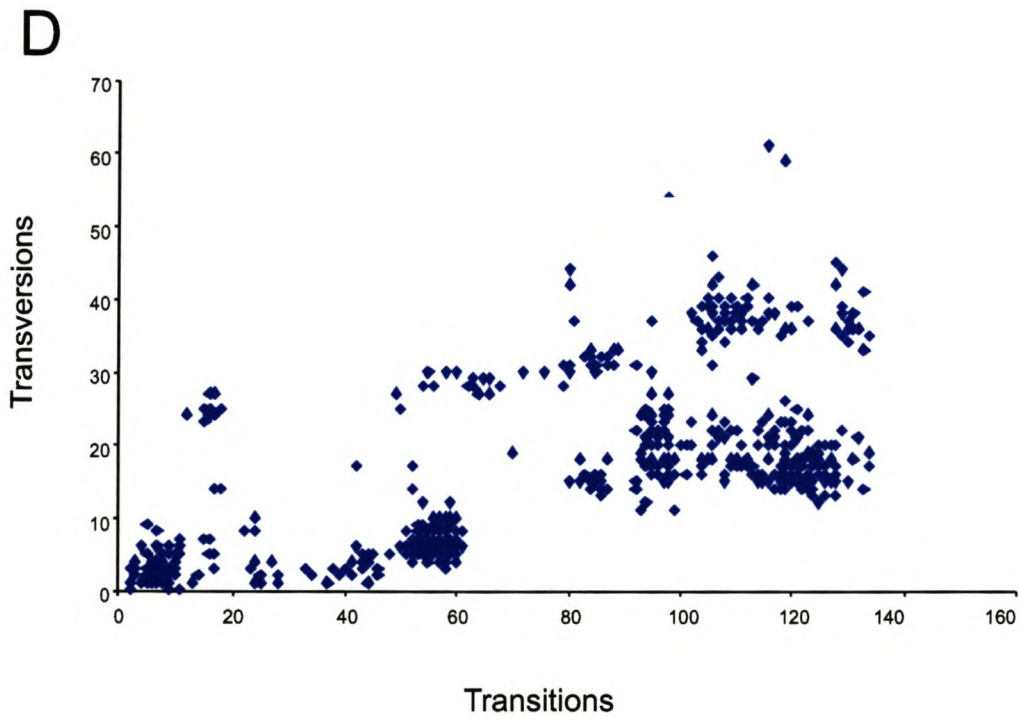
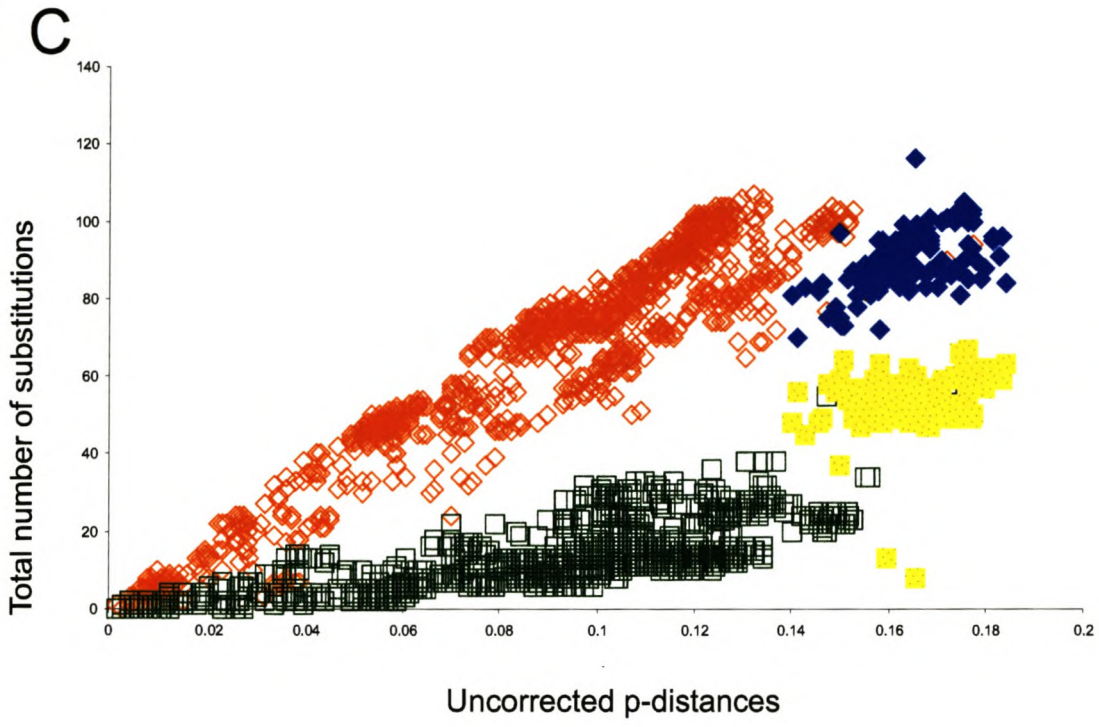


Figure 6: Saturation plots for the first (A), second (B), and third (C) codon positions. (D) Shows the transition versus transversion plots. The Y-axis is the number of substitutions while the X-axis is the uncorrected p-distances: \diamond = transitions for ingroup; \blacklozenge = transversions for outgroup; \square = transversion for ingroup; and \blacksquare = transversions for outgroup.



Phylogenetic analysis

Under equal weighting, the parsimony analysis resulted in 36 equally parsimonious trees of 913 steps (Consistency index = 0.595, Retention index = 0.812) from which a strict consensus was computed. Apart from terminal branch swapping, the derived strict consensus tree was the same as that obtained with empirical weighting and neighbour joining analysis employing the TrN+G model selected by Modeltest (base frequency A = 0.3038, C = 0.2607, G = 0.1263, T = 0.3092 with gamma distribution shape parameter = 0.345). For illustrative purposes the NJ tree topology is presented and discussed. The monophyly of *R. pumilio* is well supported and two main clades were retrieved (Clade 1 and 2, Figure 7). These clades are separated by mean sequence divergence of 12.0% (8.3 – 15.6%, Table 4). Clade 1 is further subdivided into two sister-groups, A (2n = 46) and B (2n = 48). The mean sequence divergence between these two subgroups groups is 6% (range 5.0% - 8.0%). Subgroup A (2n = 46) has NJ bootstrap support of 97% and is comprised of specimens collected at Mt Elgon (Uganda), Nyika Plateau (Malawi), and Inyanga (Zimbabwe), and in northern parts of South Africa (Potchefstroom, Irene and Willem Prinsloo Nature Reserve). Mean sequence divergence within this group is 2.7% (range = 0.3% - 4%). Subgroup B (2n = 48) comprises specimens from the South African localities of Alice and King William's Town (both in Eastern Cape Province), Cathedral Peak (Kwazulu Natal Province), Lydenburg and Pilgrim's Rest (both in Mpumalanga), Willem Pretorius Nature Reserve (Free State Province) and Suikerbosrand Nature Reserve (Gauteng Province). Although sub-group B enjoys 100% bootstrap support, the terminal nodes are unresolved reflecting the low sequence divergences among haplotypes (mean = 1.3%; 0.4% - 3.8%), and a relatively recent radiation.

Clade 2 has modest NJ bootstrap support (79%) and comprises 13 samples from 7 localities, all of which share a diploid number of 2n = 48. Specimens included in this clade were collected in Swakopmund and Keetmanshoop (both in Namibia), and the South African localities of Gariiep Dam (Free State Province) and Grotto Bay, Bottelary, Swartberg (all from Western Cape). The Springbok (Northern Cape) sample appears basal to this lineage. Mean sequence divergence within this clade is 11% (range = 1.5% - 14%).

Figure 7: Neighbour joining tree showing the two major mitochondrial lineages which are separated by mean sequence divergence of 12%. Clade 1 contains specimens with both $2n = 46$ and 48 while Clade 2 comprises specimens with $2n = 48$. Representatives of Clade 1 are suggested to represent a single species, *R. dilectus*, which can be subdivided into two subspecies, *R. d. dilectus* (subgroup A) and *R. d. chakae* (subgroup B) while Clade 2, represents *R. pumilio* (see text). Numbers at the nodes show bootstrap values for NJ (above nodes) and equal weighting parsimony (below nodes). The numbers in parenthesis indicate locality numbers (corresponding to those in Fig 2 and Table 2) while a and b refer to multiple specimens from the same locality. Single and double arrowheads distinguish the two competing evolutionary scenarios that can be invoked to explain the patterns suggested by the combination of cytogenetic and MtDNA data (see text for details). Single arrowhead on the branch leading to subgroup A indicates the Robertsonian fusion that was fixed in the ancestor of this lineage (first hypothesis). Double arrowheads on the branch leading to Clade 1 indicates the fixation of the $2n = 46$ characterizing the 2.9MYA divergence from Clade 2. Double arrowheads on the branch leading to subgroup B indicate the reversal (fission) of the fusion that changed the diploid number back to the $2n = 48$ found in representatives of this lineage.

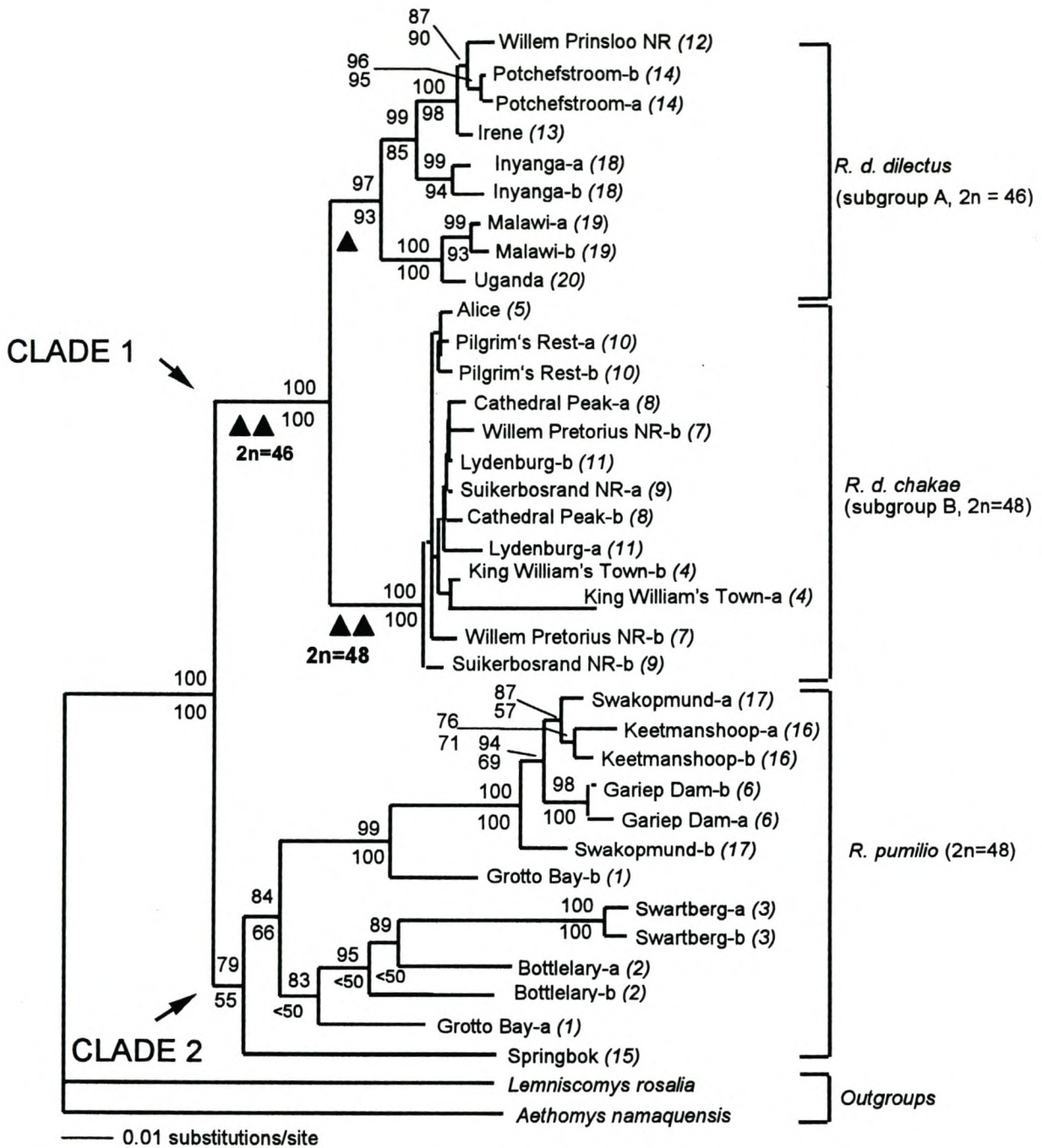


Figure 7

Table 4: Uncorrected (p-) distance matrix (%) for *cyt b* (1140 bp) for all *R. pumilio* in relation to the outgroups (*L. rosalia* and *A. namaquensis*)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	
1 Willem Prinsloo NR	-																							
2 Swakopmund-a (Namibia)	11.14	-																						
3 Swakopmund-b (Namibia)	10.88	1.75	-																					
4 Keetmanshoop-a (Namibia)	11.93	1.58	2.98	-																				
5 Keetmanshoop-b (Namibia)	11.4	1.05	2.28	1.23	-																			
6 Alice	5.53	11.84	11.23	12.28	11.84	-																		
7 Swartberg-a	13.6	14.65	14.65	15.18	14.83	12.37	-																	
8 Swartberg-b	13.68	14.39	14.39	14.91	14.56	12.46	0.88	-																
9 Cathedral Peak-a	6.05	12.37	11.75	12.9	12.46	1.05	12.72	12.81	-															
10 Cathedral Peak-b	5.88	12.02	11.4	12.54	12.11	0.88	12.72	12.81	0.7	-														
11 King William's Town-b	5.7	12.02	11.4	12.54	12.11	0.88	12.9	12.98	0.88	0.7	-													
12 King William's Town-a	8.33	14.65	14.04	15.18	14.74	3.6	15.53	15.61	3.51	3.6	3.16	-												
13 Willem Pretorius NR-a	5.7	11.49	10.88	11.75	11.75	0.88	12.72	12.81	1.23	1.05	1.05	3.77	-											
14 Willem Pretorius NR-b	6.14	12.19	11.49	12.63	12.37	1.05	12.9	12.98	0.88	0.88	1.23	3.77	1.23	-										
15 Grotto Bay-a	8.68	10.61	10.7	11.4	10.79	8.68	7.46	7.37	8.86	8.68	8.86	11.58	8.86	9.21	-									
16 Grotto Bay-b	9.91	5.18	5.26	6.23	5.35	9.74	10.35	10.09	10.09	9.91	9.91	12.63	9.74	10.26	5.79	-								
17 Springbok	9.65	12.03	12.12	12.29	12.03	10.09	10.89	10.71	10.62	10.44	10.44	13.34	10.27	10.71	8.96	9.4	-							
18 Suikerbosrand NR-a	5.61	11.93	11.32	12.46	12.02	0.61	12.63	12.72	0.44	0.44	0.79	3.33	0.79	0.61	8.6	9.83	10.36	-						
19 Suikerbosrand NR2-b	5.44	11.93	11.32	12.46	12.02	0.79	12.28	12.37	1.14	0.97	0.79	3.51	0.97	1.32	8.33	9.83	10.18	0.7	-					
20 Pilgrim's Rest-a	5.61	11.84	11.23	12.37	11.93	0.44	12.63	12.72	0.97	0.79	0.79	3.51	0.79	1.14	8.77	9.74	10.36	0.53	0.7	-				
21 Pilgrim's Rest-b	5.53	11.93	11.32	12.46	12.02	0.53	12.54	12.63	1.05	0.88	0.88	3.6	0.88	1.23	8.68	9.83	10.09	0.61	0.79	0.44	-			
22 Lydenburg-a	5.97	12.11	11.49	12.72	12.19	1.32	12.98	13.07	1.14	1.14	1.32	3.86	1.49	1.32	8.77	10	10.71	0.88	1.4	1.23	1.32	-		
23 Lydenburg-b	5.79	12.11	11.49	12.63	12.19	0.79	12.81	12.9	0.44	0.44	0.79	3.33	0.97	0.61	8.77	10	10.44	0.18	0.88	0.7	0.79	0.88	-	
24 Malawi-a	4.21	12.46	12.19	13.25	12.72	5	13.6	13.68	5.7	5.53	5.53	8.25	5.35	5.79	9.74	11.14	10.79	5.26	5.44	5.26	5.35	5.79	5.44	
25 Malawi-b	4.21	12.46	12.19	13.42	12.9	5.35	13.95	14.04	5.88	5.7	5.7	8.42	5.53	5.97	9.91	11.32	10.97	5.44	5.61	5.44	5.53	5.97	5.61	
26 Uganda	4.39	12.63	12.19	13.42	12.9	4.65	13.33	13.42	5.35	5.18	5.18	7.9	5.18	5.44	9.39	11.05	10.97	4.91	5	4.91	5	5.44	5.09	
27 Inyanga-a (Zimbabwe)	2.46	11.93	11.67	12.72	12.19	4.91	13.07	13.16	5.61	5.44	5.26	7.98	5.26	5.7	8.86	10.53	10.09	5.18	5	5.18	5	5.53	5.35	
28 Inyanga-b (Zimbabwe)	2.72	11.93	11.67	12.9	12.37	5.18	13.51	13.6	5.88	5.7	5.53	8.16	5.53	6.05	9.3	10.53	10.18	5.44	5.26	5.44	5.26	5.7	5.61	
29 Potchefstroom-b	0.88	11.32	11.05	12.11	11.58	5.18	13.25	13.33	5.88	5.7	5.35	7.98	5.53	5.97	8.86	10.09	9.65	5.44	5.26	5.44	5.35	5.79	5.61	
30 Potchefstroom-a	0.97	11.4	11.14	12.19	11.67	5.26	13.33	13.6	5.97	5.79	5.44	8.07	5.61	6.05	8.95	10.18	9.74	5.53	5.35	5.53	5.44	5.88	5.7	
31 Irene	0.97	11.4	11.14	12.02	11.49	4.91	13.07	13.16	5.61	5.44	5.26	7.9	5.26	5.7	8.51	9.74	9.3	5.18	5	5.18	5.09	5.53	5.35	
32 Gariep Dam-b	11.42	1.93	2.64	2.81	1.93	11.77	14.67	14.4	12.47	12.12	12.12	14.75	11.77	12.47	10.8	5.71	12.31	12.03	12.03	11.94	12.03	12.21	12.21	
33 Gariep Dam-a	11.93	2.02	2.81	2.72	1.84	12.28	14.83	14.56	12.98	12.63	12.63	15.26	12.28	12.81	11.05	5.79	12.56	12.54	12.54	12.46	12.54	12.72	12.72	
34 Bottlelary-a	10.7	11.58	12.11	12.46	12.02	10.44	6.67	6.58	10.44	10.44	10.61	13.33	10.44	10.97	6.05	7.81	10.36	10.35	10	10.35	10.26	10.7	10.53	
35 Bottlelary-b	10.26	9.12	9.3	9.91	9.65	10.26	7.72	7.19	10.44	10.44	10.44	13.16	10.09	10.61	5.79	5.18	7.9	10.35	9.83	10.26	10.09	10.7	10.53	
36 <i>Lemniscomys rosalia</i>	16.32	17.37	17.37	17.63	17.46	16.05	16.84	16.75	16.05	16.05	15.7	17.98	16.05	16.23	14.56	16.4	17.12	15.97	15.26	15.79	15.97	16.32	16.14	
37 <i>Aethomys namaquensis</i>	16.32	17.37	17.46	17.81	17.72	15.79	17.28	17.02	16.32	16.14	16.14	18.42	15.79	16.4	15.97	17.37	17.47	15.88	15.61	15.61	15.7	16.23	16.05	

Table 4 (continued)

	24	25	26	27	28	29	30	31	32	33	34	35	36	37
24 Malawi-a	-													
25 Malawi-b	0.53	-												
26 Uganda	1.23	1.4	-											
27 Inyanga-a (Zimbabwe)	3.16	3.16	3.33	-										
28 Inyanga-b (Zimbabwe)	3.77	3.77	3.95	0.97	-									
29 Potchefstroom-b	4.04	4.21	4.21	2.28	2.54	-								
30 Potchefstroom-a	4.12	4.3	4.3	2.37	2.63	0.26	-							
31 Irene	3.6	3.95	3.6	2.19	2.28	0.79	0.88	-						
32 Gariep Dam-b	12.38	12.73	12.56	11.68	11.68	11.42	11.5	11.33	-					
33 Gariep Dam-a	13.07	13.42	13.25	12.19	12.37	11.93	12.02	11.84	0.62	-				
34 Bottlelary-a	11.49	11.49	11.23	10.53	10.97	10.7	10.79	10.44	12.56	12.81	-			
35 Bottlelary-b	11.32	11.32	11.23	10.18	10.7	10.26	10.35	10.09	10.1	10.26	5.18	-		
36 <i>Lemniscomys rosalia</i>	16.75	17.11	16.58	16.05	16.4	16.14	16.23	16.23	17.56	17.54	14.3	15.44	-	
37 <i>Aethomys namaquensis</i>	16.23	16.58	16.4	16.32	16.4	16.4	16.49	16.05	17.74	18.16	15.88	16.75	16.84	-

Minimum spanning tree

The minimum spanning tree showing the minimum number of mutational steps between haplotypes is given in Figure 8. No shared haplotypes were detected. Clade 1 and 2, as evidenced from the phylogenetic analysis (Figure 7), were similarly retrieved and separated by 95 mutational steps. Additionally, the subdivision within Clade 1 is also reflected in the minimum spanning tree; haplotypes characterizing the $2n = 46$ populations (Subgroup A) are separated by 53 substitutions from those belonging to subgroup B ($2n = 48$). Haplotypes comprising subgroup B are separated by relatively low numbers of steps (the lowest being two steps between haplotypes 18 and 23 occurring in Suikerbosrand Nature Reserve and Lydenburg respectively, while the King William's Town haplotypes, numbers 11 and 12, are the most divergent, separated by 36 mutational steps. In addition, subgroup B is characterized by a star-like phylogeny with most haplotypes connecting to either mtDNA lineage 18 or 20. The low number of mutational steps, together with the star-like pattern, is indicative of a recent expansion event. In sharp contrast, relatively higher numbers of mutational steps join haplotypes in the second major mtDNA grouping (Clade 2). The lowest number of mutational changes is seven between two Gariiep Dam haplotypes (numbers 32 and 33), and the highest is 90 between Springbok and Bottletary haplotypes (numbers 17 and 35). This indicates a more ancient divergence for Clade 2.

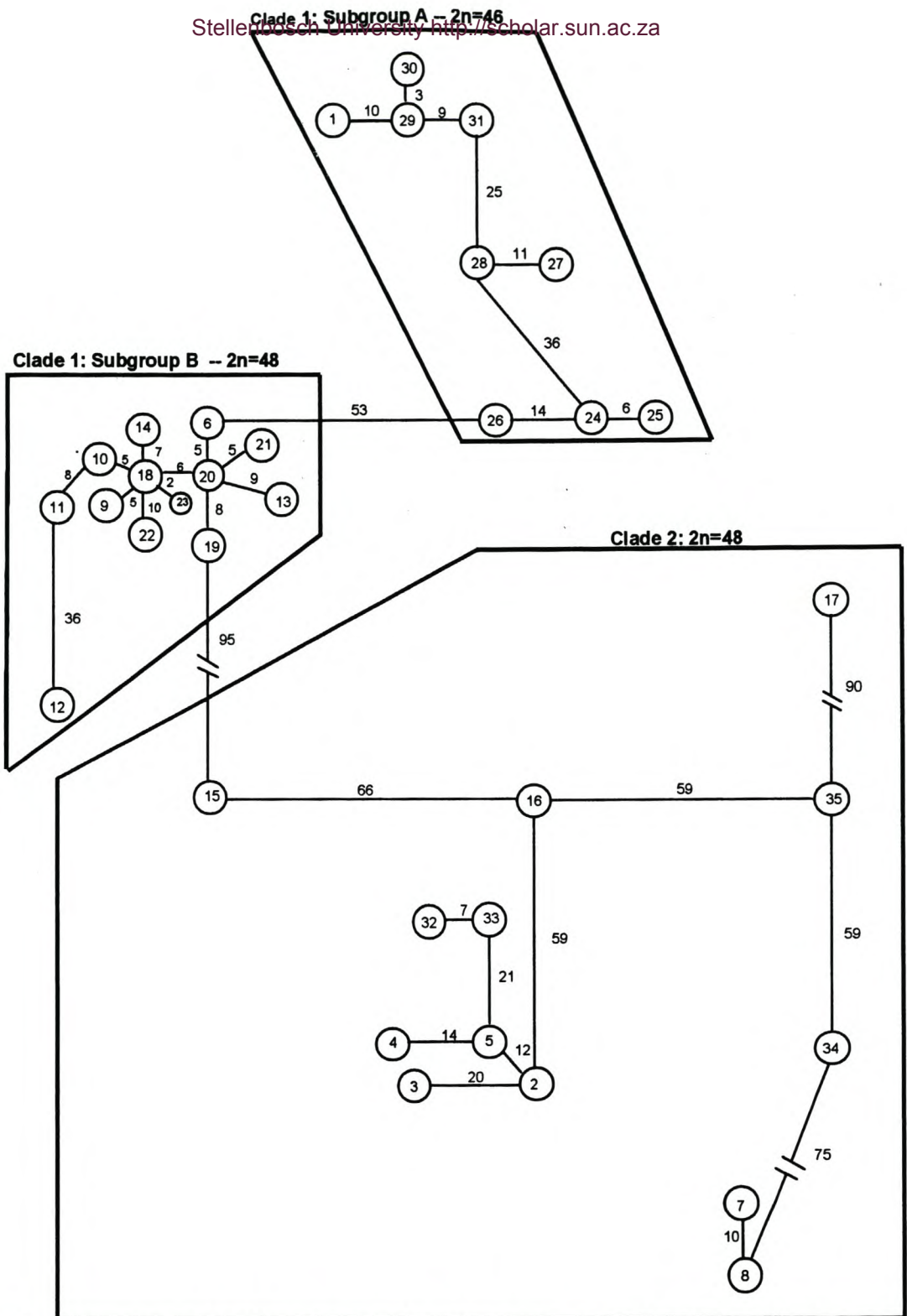


Figure 8: An unrooted minimum spanning tree constructed for the 35 haplotypes (numbered 1 – 35) detected in this investigation. Numbers of mutational steps joining the haplotypes are indicated along the connecting branches. Clades 1 and 2 also retrieved by NJ and MP are separated by 95 mutational steps, while the two cytotypic subgroups (A and B) of Clade 1 are separated by 53 mutational steps. Correspondence between haplotype numbers and their geographic origins are given in Table 2.

Discussion

One of the main findings of our investigation is the presence of two distinct and highly divergent mtDNA clades within *R. pumilio*. These comprise a mesic group in which animals are characterised by a dark grey/ dark brown pelage, and a xeric group in which specimens have a lighter coloration thus following Gloger's rule (Feldhamer *et al.* 1999). The mesic clade has an east-central distribution that is further sub-divided into two sister groups, each with a characteristic karyotype ($2n = 46$ and $2n = 48$). The second mtDNA clade comprises specimens that are all $2n = 48$ which are distributed predominantly in the western regions of South Africa and Namibia (Figure 2). Interestingly, these two clades were not retrieved in a previous study using allozymes and specimens from Malawi, Zimbabwe and Potchefstroom (all possessing $2n = 46$ and forming part of subgroup A in the present investigation) failed to cluster together (Mahida *et al.* 1999). A similar xeric and mesic dichotomy is evident in another African rodent species for which comprehensive data are available (*Aethomys namaquensis*), although in this case it is underpinned by morphological data (Chimimba, 2001) and not mtDNA.

Divergence times

In spite of the debate on the accuracy of molecular clocks (see Chapter 1; also Gillespie 1986; Martin & Palumbi 1993; Avise 1994; Rambaut & Bromham 1998) they have nonetheless proved useful for dating cladogenic events in rodents (e.g. Smith & Patton 1999; Jolley *et al.* 2000; Ducroz *et al.* 2001). Further, since the relative rate test (Robinson *et al.* 1998; Robinson-Rechavi & Huchon 2000) suggests that there is no significant difference in the substitution rates among sequences ($P > 0.05$) in this study, the utility of this approach was explored. Although *R. pumilio* fossils have been recorded from Holocene (over the last 10,000 years) and Pleistocene (over the last 2 MYA) deposits, the accurate dating of these fossils is lacking. I therefore follow the calibration used by Ducroz *et al.* (1998, 2001) involving the so-called "Arvicanthis Division" of murids (which includes *R. pumilio*, Musser 1987) which is based on the third position transversions and the 12 million years divergence time estimated between *Mus* and *Rattus* (Jacobs & Pilbeam 1980). Applying Ducroz *et al.*'s calibration (1.53% per million years), the average number of third codon transversions distinguishing *R. pumilio* mtDNA Clades 1 and 2 (16.7) converges on a divergence estimate of 2.9 MYA, and that between subgroup A ($2n = 46$) and B ($2n = 48$) at roughly 600,000 years. My estimate of 2.9 MYA only slightly predates the oldest *R. pumilio* fossils thus far documented which

have early Pleistocene origins (Kingdon 1974; Pocock 1987; De Graaff 1961; Avery 1991).

Ancestral karyotype

The subject of whether chromosomal fissions (Todd 1970) or fusions (Wurster & Bernishke, 1968) are driving karyotypic evolution is contentious (see Chapter 1; Shi *et al.* 1980; Lin *et al.* 1991; Graphodatsky *et al.* 2001). The presence of interstitial telomeres in low diploid number species, for example in muntjacs, has been used as evidence that the high diploid number is ancestral (Shi *et al.* 1980; Lin *et al.* 1991; Yang *et al.* 1995, 1997). On the other hand, the most recent ancestor of all canid species had a low diploid number (Graphodatsky *et al.* 2001). However, my mtDNA data places the $2n = 48$ basal in the phylogenetic tree suggesting that it is ancestral, and that the $2n = 46$ is the derived state.

Biogeographic interpretation

Fossil remains in East Africa indicate that some of the Murinae emerged approximately 5 MYA (Denys & Jaeger 1986). During this period, rifting and uplifting of the East African landscape was at its maximum (Patridge *et al.* 1995). Changes in topography were accompanied by a major temperature drop resulting from formation of the ice cap on Antarctica (Van Zinderen Bakker 1986; Bonnefille 1985; Lindesay 1998). Palynological evidence indicates that the change in temperature caused the vegetation to change from predominantly sub-tropical, to a mosaic comprised of a variety of vegetation types including grasslands (Bonnefille 1985). The prevailing cooler and drier climatic conditions (around 3.7 - 1.7 MYA) in East Africa led to grassland extensions which are correlated with an increase in the arid faunal component of both bovids and rodents (Bonnefille 1985). During the same period, southern Africa was experiencing cold temperatures resulting from the drop in oceanic temperatures in the southern hemisphere leading to a gradient of decreasing aridity extending from west to east (Lindesay 1998). This resulted in a change from subtropical vegetation to fynbos in the western Cape, and to grasslands in the rest of southern Africa. Because *R. pumilio* relies on grass for nest building, and for supplementing its herbivorous diet (Chapter 1; Stiemie & Nel 1973), it is likely that demographic fluctuations would be closely linked to range contraction and expansion of grasslands, as would other small mammal fauna with similar habitat requirements (Thackeray 1987). The interglacial grassland extensions of east Africa (Bonnefille 1985) would have connected with those in the south

creating favourable conditions for dispersal of *R. pumilio* between east and southern Africa.

Against this background it is suggested that *R. pumilio* survived the climatic oscillations that characterised the Pliocene in two refugia – one in the mesic regions, the other in the xeric zone of southern Africa. Molecular clock calibrations indicate the establishment of the two refugia at roughly 2.9 MYA. At this point two competing hypotheses can be invoked to explain the patterns suggested by the combination of cytogenetic and mtDNA data. First, that superimposed on this initial cladogenic event followed a second more recent (600,000 years ago) divergence involving the descendants of the mesic refugium that gave rise to subgroups (A and B). A single fusion, with the concomitant reduction in diploid number to $2n = 46$, was fixed in the ancestor to subgroup A (B remained unaffected with $2n = 48$). Fixation probably involved small effective population size, inbreeding and drift, all factors that are conventionally associated with population fragmentation (Wilson *et al.*, 1975; Bush *et al.*, 1977; Lande, 1985; Walsh, 1982; King, 1993).

However, while the two refugia hypothesis corresponds well with the topology in Figure 7, and with the ecological and geographic distribution of the two mtDNA clades, it does not adequately explain the differences in haplotype diversity between subgroups (Figures 7 and 8) where the relatively long terminal branches in A contrast with the short branches and star-like phylogeny depicted for subgroup B. To do so requires the establishment of the $2n = 46$ karyotype during the initial cladogenic event at 2.9 MYA (i.e. in the ancestor to mesic group). Subsequently a reversal (fission) of this fusion, fixed in an ancestral population in the eastern regions of South or southern Africa during the more recent retreat to refugia (600,000 years before present), caused a return to the $2n = 48$ state. This was followed by the subsequent rapid expansion evident in subgroup B (second hypothesis). Although the second evolutionary scenario more adequately explains the mtDNA patterns, the most parsimonious interpretation of the cytogenetic data is that a single fusion punctuated the evolution of a lineage leading to subgroup A (first hypothesis). The converse (i.e. a reversal of the fusion in subgroup B) would require two independent rearrangements of the same chromosome. These results clearly show the value of using multiple independent data sets in retrieving information on the historical evolutionary events that give rise to species complexes. Either approach in isolation (mtDNA sequencing or comparative cytogenetics) would have led to incomplete or partly erroneous interpretations. Moreover, our data also highlight the dangers inherent in arranging taxa into monophyletic clades based on diploid number and the number of autosomal chromosome arms (Nfa) in chromosomal

phylogenies (e.g. Smith, 1990; Britton-Davidian *et al.*, 1995; Vassart *et al.*, 1995). Had this course been followed (and in the absence of the mtDNA data), the 2n = 48 populations would have been grouped together to the exclusion of those with 2n = 46 on the grounds that they had identical karyotypes.

Taxonomic considerations

Genetic distance, ecological divergence and evidence of differences in mating behavior among representatives of the two mtDNA clades suggest that there may be grounds for the recognition of two species within *R. pumilio*. The high genetic distance delimiting the two mtDNA clades (mean 12%) is comparable to among-species variation within other murid genera. For example, the interspecific cytochrome *b* sequence variation detected in *Peromyscus* (Bradley *et al.*, 2000; Harris *et al.*, 2000), *Sigmodon* (Peppers and Bradley, 2000) and *Akodon* (Smith and Patton, 1993) rodents approaches 10%. However, using mtDNA genetic distances to assign taxonomic rank (Johns and Avise, 1998; Bradley and Baker, 2001) is problematic, and is not without its detractors (e.g. Ferguson, 2002). Nonetheless, we believe it may serve as a guide in *R. pumilio* particularly in view of the behavioural data where laboratory based experiments involving mice from Alice and Irene (both Clade 1) and Springbok (Clade 2) showed that oestrous females generally preferred homotype stimuli (Pillay, 2000b).

Following the rules of nomenclature ("rule of priority", Simpson 1945; Roberts 1951), *R. dilectus* De Winton, 1897 would have priority for populations falling within the geographic range encompassed by mtDNA Clade 1. Within this species there appear to be two discrete subgroups delimited by 2n = 46 and 2n = 48 which are referred to as *R. d. dilectus* (subsuming *R. p. nyasae*, *R. p. dilectus* and *R. p. vaalensis*) and *R. d. chakae* (Wroughton, 1905; subsuming *R. p. chakae* and *R. p. mosheshi*). The second mtDNA clade comprises the apparently monotypic *R. pumilio*. Although there is clearly evidence of structure, I chose to err on the side of caution given the presence of shared lineages (Grotto Bay) and limited sampling which, at this juncture, preclude a firmer statement on the variation detected within *R. pumilio*.

In summary, this study suggests the presence of two major mtDNA lineages within what has conventionally been referred to as a single species, *R. pumilio* which are divided along ecological lines. The large genetic distances between them strongly suggest a historical separation promoted, in part, by climatic factors in the Pliocene. Although clearly not definitive, the results suggest that a taxonomic revision of *Rhabdomys* is

warranted and that the four-striped mouse is probably comprised of two species, *R. pumilio*, and the newly erected *R. dilectus*. Moreover, that within the latter there is good evidence for the recognition of two subspecies: *dilectus* and *chakae*. Further breeding experiments, behavioural comparisons, and more comprehensive sampling (particularly for the xeric areas occupied by *R. pumilio*) will allow for a more rigorous assessment of their status.

CHAPTER 3¹PHYLOGENOMIC ANALYSIS OF *RHABDOMYS PUMILIO*

Introduction

Fluorescence *in situ* hybridization (FISH) involving chromosome specific probes (thereafter named chromosome painting) was developed independently in 1988 by Pinkel *et al.* and Cremer *et al.*. Since then it has proved to be an indispensable tool in comparative chromosome analysis and study of chromosomal rearrangements in a wide range of mammals (for reviews see Ried *et al.* 1998; Murphy *et al.* 2001). Chromosome painting has been instrumental in transferring linkage information from "map-rich" taxa such as human and mouse to "map-poor" taxa (e.g. Yang *et al.* 2000; Cavagna *et al.* 2002). Treatment of phylogenomic markers in a cladistic manner both at the intraordinal and intrageneric level has resulted in a better understanding of mode and tempo of chromosome evolution in mammals (see Chapter 1). For instance, the rate of evolutionary change is very slow (one rearrangement per 10 million years) in the genomes of cats, the mink, ferrets, dolphin and some primates and is relatively faster in the dog, bear, cow, lesser apes and several New World primates. At the one extreme are murids, represented by rat and mouse, with higher rates of genome evolution (in the order of one rearrangement per million year; Murphy *et al.* 2001). This 10 fold disparity emphasizes the relevance for assessing lineage-specific rates, particularly for murids which are thought to have an elevated evolutionary rate at molecular sequence level (Catzeflis *et al.* 1987).

Somewhat surprisingly, even though the order Rodentia is the most speciose of all mammals with more than 1800 recognised species (Musser & Carleton 1993; Nowak 1999), and the commercial availability of mouse chromosome painting probes (Rabbits *et al.* 1995), relatively few ZOO-FISH comparisons among rodent taxa have been undertaken. In fact, comparisons are limited to assessing variation within the Black rat, *Rattus rattus* ($2n = 38-42$; Cavagna *et al.* 2002), between mouse, *Mus musculus* ($2n =$

1. The data presented in this chapter form the substance of a paper that has been accepted for publication: Rambau R. V. & Robinson T. J. Chromosome painting in the African four-striped mouse *Rhabdomys pumilio*: detection of murid specific contiguous segment combinations. *Chromosome Research*

40) and the Norway rat, *Rattus norvegicus* ($2n = 42$; Scalzi & Hozier 1998; Guilly *et al.* 1999; Grutzner *et al.* 1999; Stanyon *et al.* 1999; Helou *et al.* 2001), and between mouse and the Chinese hamster, *Cricetulus griseus* ($2n = 22$; Yang *et al.* 2000). Additionally, four mouse chromosome specific probes (MMU 2, 3, 6 and 11) have been used to search for regions of homology within the laminate-toothed rats of the subfamily Otomyinae, and more specifically the Vlei rat *Otomys irroratus* ($2n = 23-31$), the Bush Karoo rat *O. unisulcatus* ($2n = 28$), and the Whistling rat *Parotomys brantsii* ($2n = 42$; Rambau *et al.* 1997; Rambau 1998). While most of these studies have been driven by their importance as models for biomedical research (Gill *et al.* 1989; Copeland *et al.* 1993; Szpirer *et al.* 1996; Botting & Morrison 1997; Overbeek *et al.* 2001), the data have provided insights into rodent chromosomal evolution that were previously intractable using conventional cytogenetics.

These investigations have demonstrated that although mouse and rat last shared an ancestor some 8-14 million years ago (Jacobs & Pilbeam 1980), they have accumulated equivalent numbers of chromosomal rearrangements as is evident between human and cats which diverged 65-90 million years ago (Stanyon *et al.* 1999). Moreover, these investigations have shown that there may be selection against the disruption of gene synteny in some mouse chromosomes (e.g. 3, 4, 9, 18 and 19) since they have been retained as complete chromosomes or chromosome blocks. Others (MMU 1, 10 and 17) tend to be more prone to fragmentation during the course of evolution appearing as multiple hybridization signals in the genomes of the rat and the Chinese hamster (Stanyon *et al.* 1999; Yang *et al.* 2000; Cavagna *et al.* 2002).

By extending the comparative approach to the other murids it will be increasingly possible to more accurately reconstruct the patterns and processes that have shaped chromosomal evolution in rodents. Critical to this endeavor, however, is that detailed chromosome maps must include reference to an index species such as the laboratory mouse for which there are extensive gene mapping data (Davisson *et al.* 1998; Copeland *et al.* 1993; Nadeau & Taylor 1984). The mouse particularly lends itself to investigating chromosomal homologies in a ZOO-FISH context since it has a highly rearranged genome making mouse chromosome specific painting probes useful for dissecting chromosome rearrangements in rodents.

The data presented herein follow directly from the investigations conducted in chapter 2 and include the results of the karyotypic comparisons between *M. musculus* and *R. pumilio* (both murine species) that involved a combination of conventional banding

protocols and FISH. Second, the conserved contiguous chromosome segment combinations detected in the homology map of *M. musculus* and *R. pumilio* are compared to those obtained in two other murids, *R. norvegicus* (Stanyon *et al.* 1999; Guilly *et al.* 1999; Helou *et al.* 2001) and *R. rattus* (Cavagna *et al.* 2002). These data are discussed in an evolutionary context (cladistic analysis) through comparisons with the more distantly related cricetid, the Chinese hamster, *C. griseus* (Yang *et al.* 2000).

Methods

Samples and spleen cultures

R. pumilio: Chromosome painting data obtained from the analysis of the *R. pumilio* $2n = 46$ cytotype (from Irene, South Africa) were used for comparison with *M. musculus*.

M. musculus: Mouse splenic lymphocyte cultures were propagated following the protocol of Davisson & Akesson (1987) and Rabbits *et al.* (1995). Briefly, the spleen was aseptically removed and minced in a sterile petridish. Blood was rinsed off the spleen using RPMI 1640 medium and the spleen was finely minced. Small amount of RPMI 1640 medium was added to resuspend the cells which were then transferred to tissue culture flask. Alternatively, the spleen was minced and then digested twice, 20 min in each case, in 15 ml of medium containing 10 mg/ml collagenase (in RPMI 1640). After each digest the suspended cells in the supernatant were pelleted by centrifugation, and then transferred to tissue culture flasks. Cells were grown for 44 h in closed culture flasks using RPMI 1640 medium enriched with 10% fetal calf serum, 10 mg/ml Lipopolysaccharide (LPS), 5 $\mu\text{g/ml}$ Concanavalin A (ConA), 20 $\mu\text{l/ml}$ heparin and 1x L-Glutamine. Harvesting of cells and slide preparations were done as previously described in Chapter 2.

Chromosome nomenclature

Chromosome numbering in *R. pumilio* and *M. musculus* is in accordance with the standards published, respectively, by Ducroz *et al.* (1999) and Evans (1996); the abbreviated species nomenclature follows international norms (ISCN 1978).

Results and discussion

Chromosome painting between *M. musculus* and *R. pumilio*

All the mouse chromosomes, excluding the Y, annealed to complementary sites in the *R. pumilio* genome with hybridization limited to the euchromatic regions of chromosomes. The 19 autosomal painting probes and the X defined 40 regions of homology between mouse and four-striped mouse (Figure 9).

Three patterns of hybridization were observed: (1) *Single hybridizations*: Ten mouse chromosomes 2-4, 7, 14-16, 18, 19 and X were retained either as a single chromosome, as chromosomal arms, or as a conserved block in *R. pumilio* chromosomes; (2) *Double hybridizations*: Six mouse chromosomes (5, 6, 8, 11, 12 and 13) produced two signals (i.e. on different chromosomes) in the *R. pumilio* karyotype; and (3) *Multiple hybridizations*: Grouped in this category are four mouse chromosome paints (MMU 1, 9, 10 and 17) that hybridize to three or more chromosomes in the *R. pumilio* genome (Figure 10).

Of the ten mouse chromosomes that hybridized to discrete *R. pumilio* chromosomes, six (MMU 3, 4, 7, 14, 18, 19) are also conserved *in toto* (without disruption) in the Norway rat (Stanyon *et al.* 1999; Helou *et al.* 2001), the Black rat (Cavagna *et al.* 2002) and the more distantly related Chinese hamster (Yang *et al.* 2000). This suggests selection against disruption, and their possible retention as symplesiomorphic chromosomal states in most rodents. MMU 15 and 16 hybridized to one chromosome in *R. pumilio* but to two segments in both rat species, as well as the Chinese hamster, indicating its derived state in *R. pumilio*. The remaining mouse chromosome paint that produced a single hybridization in *R. pumilio* is MMU 2 which paints to single chromosomes in the Norway and the Black rat (also representatives of the Murinae, Family Muridae; Nowak 1999) but to two segments in the cricetids, represented by the Chinese hamster. The fact that MMU 2 hybridizes to single chromosomes in *R. pumilio*, *R. rattus* and *R. norvegicus*, but to two chromosomes in the Chinese hamster (a cricetid) suggests that it is a shared, derived murid character. The X chromosome, as with other mammals, is conserved across all taxa although differences in banding homology (see below) suggests that some intrachromosomal repatterning has occurred.

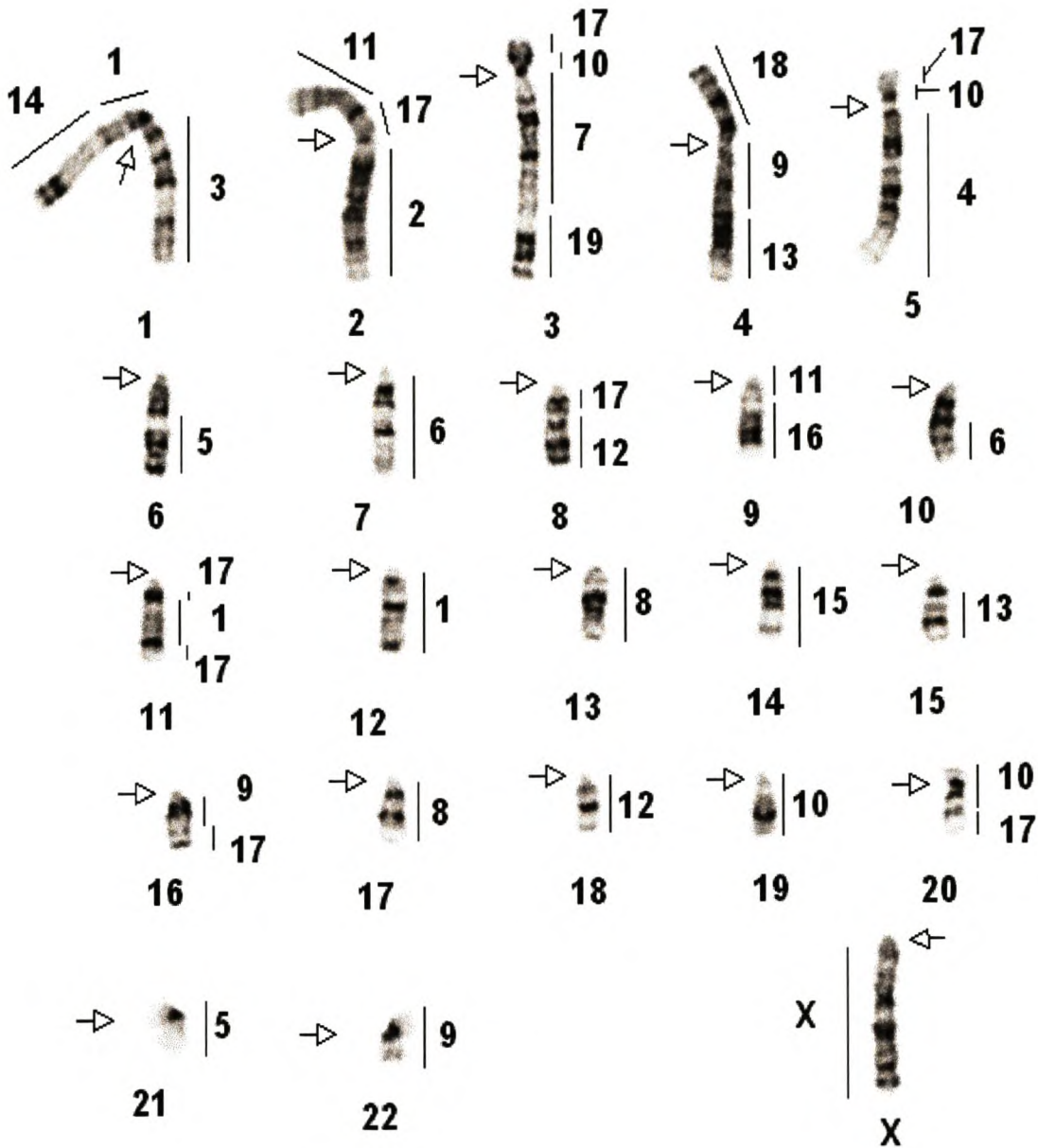


Figure 9: Homology map summarising hybridization patterns produced by FISH using mouse specific painting probes onto *R. pumilio* metaphase chromosomes. Chromosome numbering (below the chromosomes) of the *R. pumilio* half-karyotype follows Ducroz *et al.* (1999; see Figure 3 in Chapter 2). Lines to the right of each chromosome show the extent of banding homology between *R. pumilio* and *M. musculus*. Numbers beside the lines correspond to *M. musculus* chromosomes. Arrows indicate the position of the centromeres.

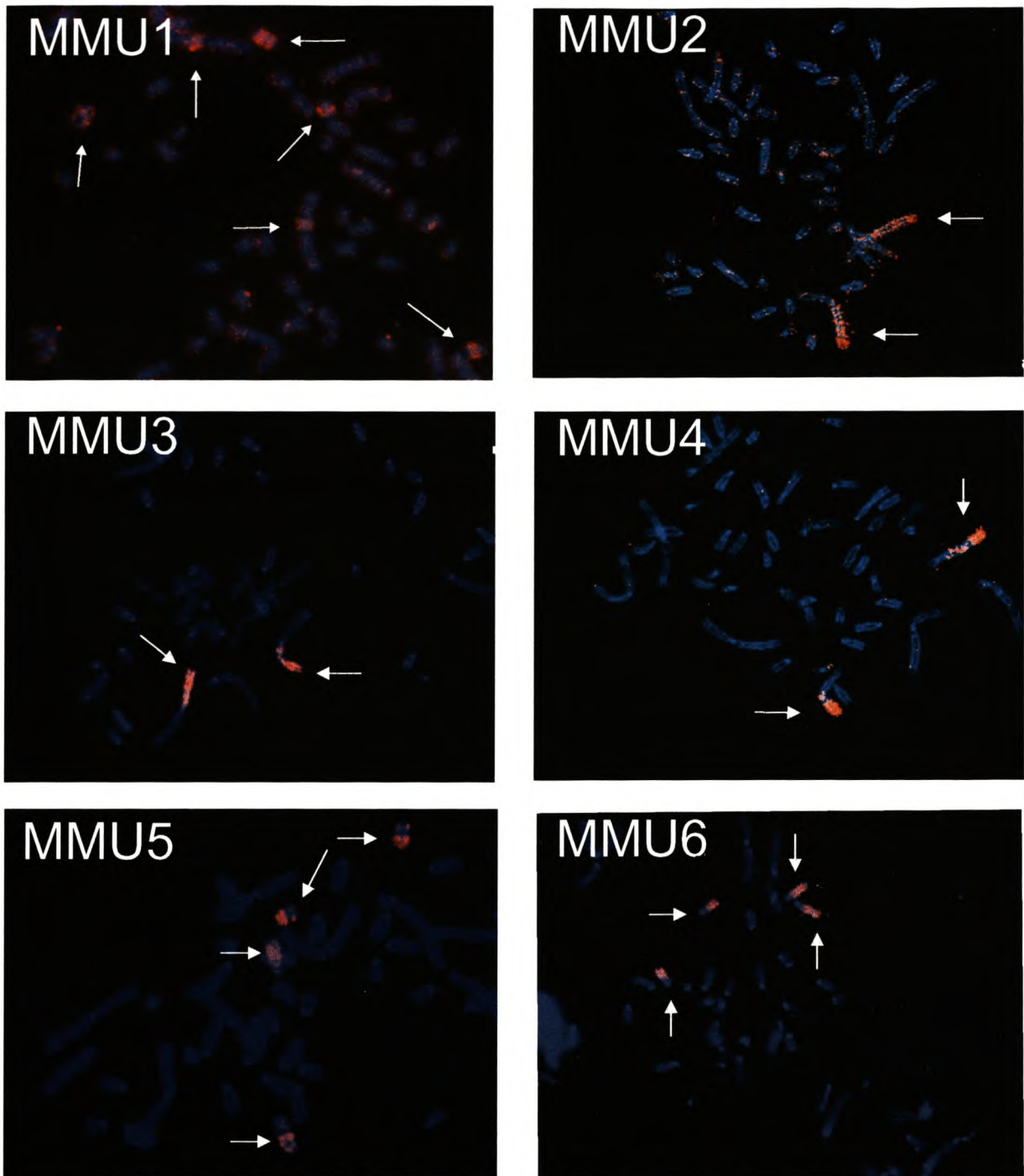


Figure 10: FISH results with all *M. musculus* chromosome paints on *R. pumilio* metaphase spreads. The paints used are indicated in the left corner of each plate while the hybridized chromosomes are indicated by arrows. Green color indicates detection using FITC-avidin (1 mg/ml; Amersham) for biotinylated probes and FITC-antidigoxigenin (200mg/ml; Roche) for digoxigenin labelled probes and red staining indicates detection with CY3-avidin conjugate. Metaphase spreads were counterstained with DAPI.

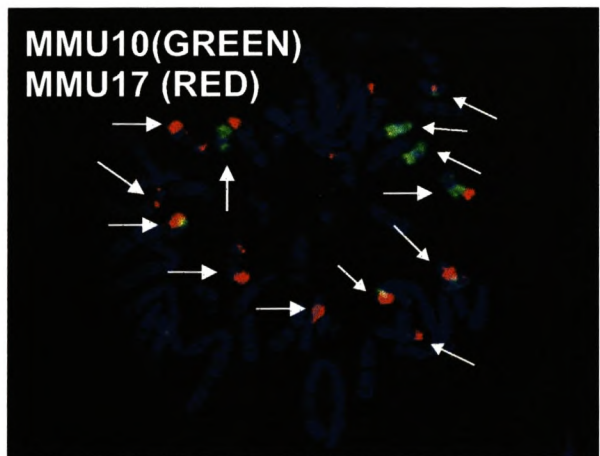
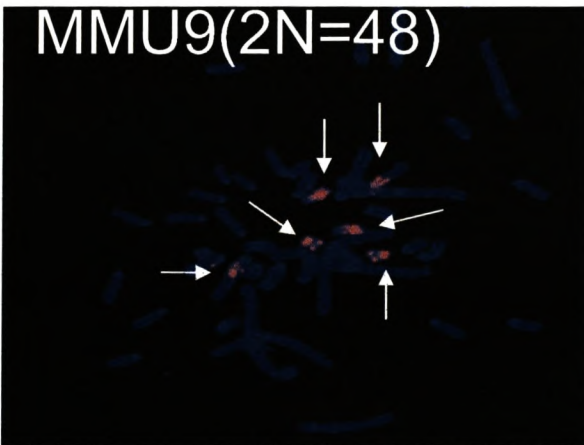
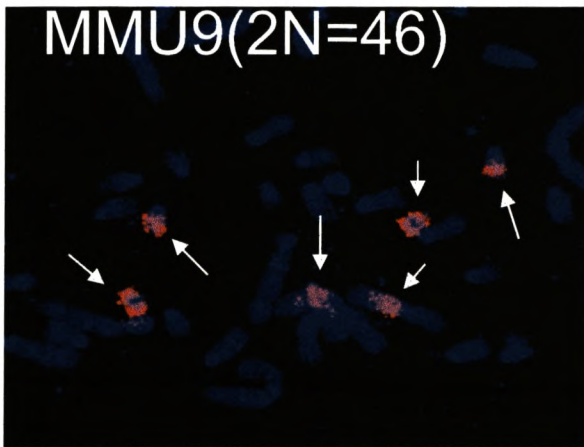
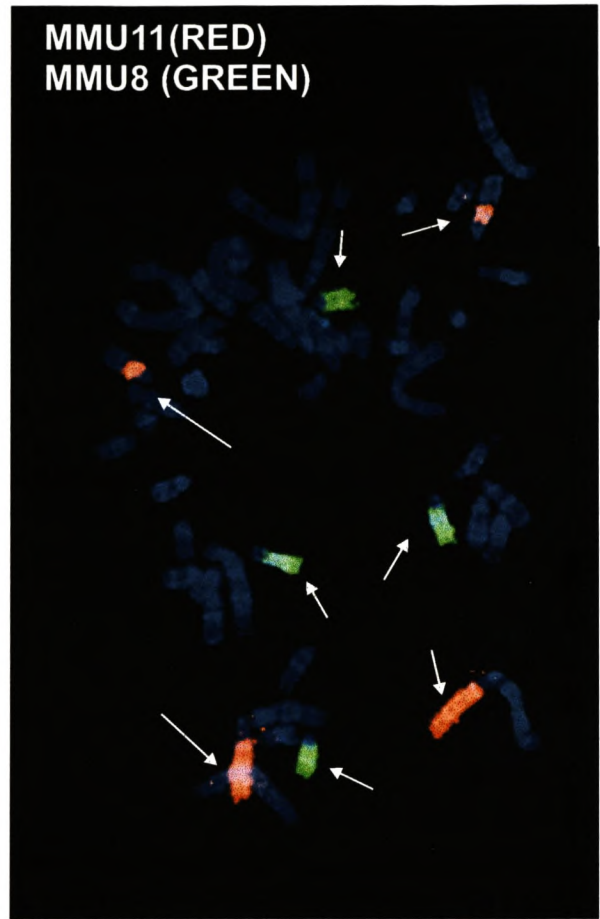
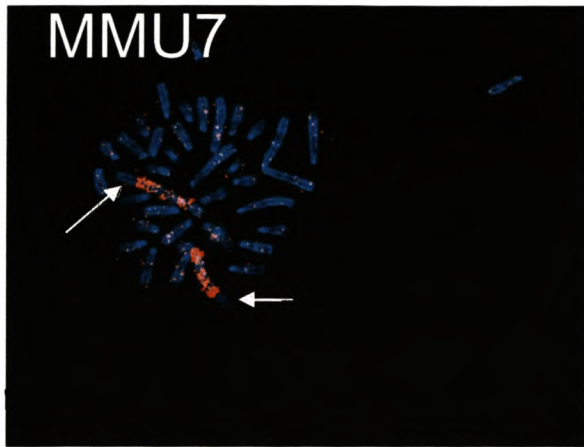


Figure 10 (continued)

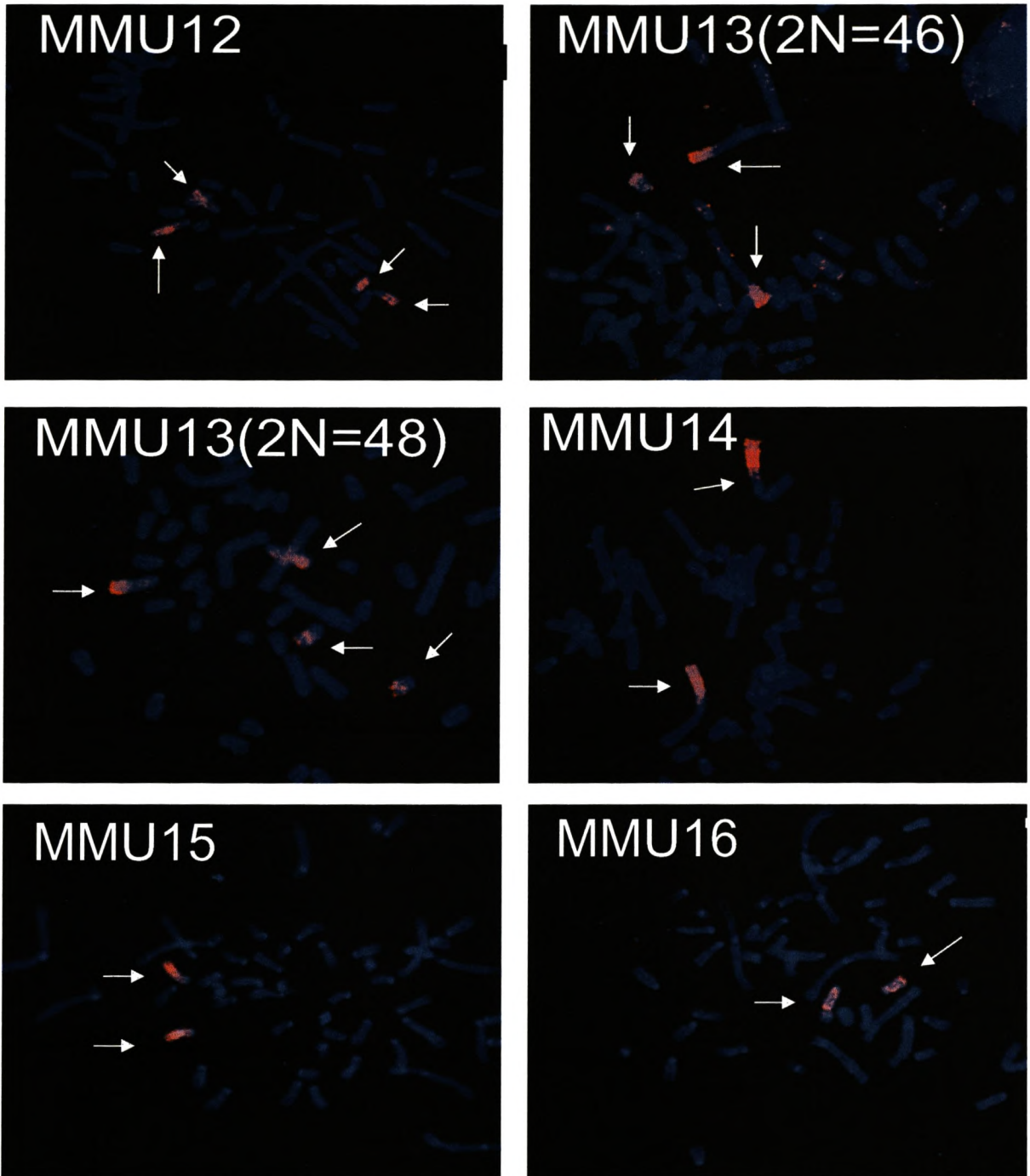


Figure 10 (continued)

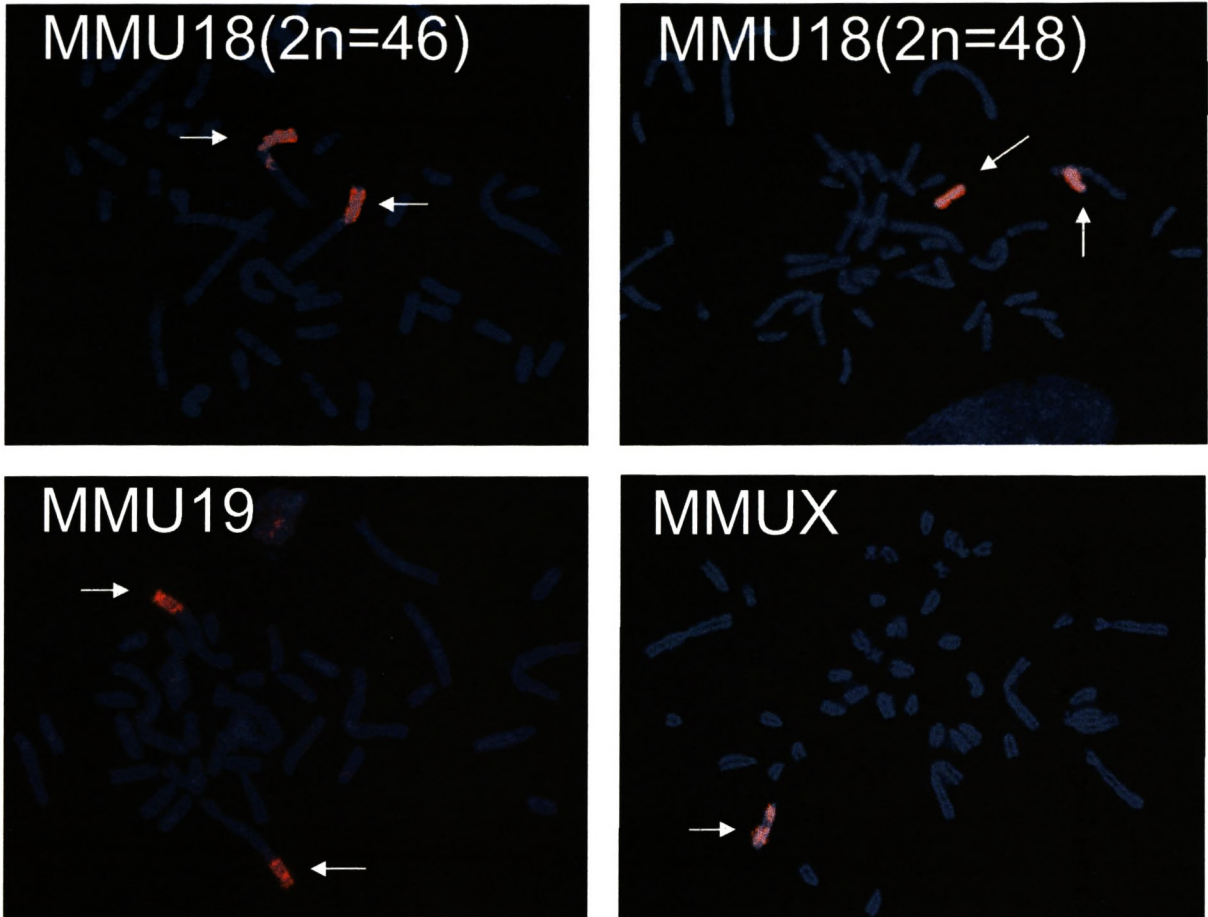


Figure 10 (continued)

G-band comparison between mouse and *R. pumilio*

Since whole chromosome painting probes do not allow for the detection of intrachromosomal changes, side by side G band comparisons were done of the chromosomes and chromosomal regions that were established by FISH (Figure 11). All mouse painting probes that hybridize to only one region on *R. pumilio* chromosomes (MMU 2, 3, 4, 7, 14, 15, 16, 18, 19 and the X) have reasonable banding homology between the species with little or no evidence of intrachromosomal rearrangements at least at this level of resolution. Exceptions to this are MMU 7, MMU 18 and the X which show relatively poor correspondence, presumably resulting from some internal rearrangement.

The mouse chromosome paints that produced split signals (e.g. mouse chromosome 1, 5, 6, 8, 11, 12 and 13) show, in some instances, some banding homology with corresponding portions of the *R. pumilio* chromosomes (see Figure 11). However, in general, the G-band data indicate that intrachromosomal rearrangements underpin a substantial part of the chromosomal difference between these two rodent species, and that reciprocal painting and the use of subchromosomal probes would be required to accurately define these rearrangements.

Contiguous segment combinations

The *R. pumilio* chromosomes painted by multiple mouse paints collectively comprise 10 contiguous associations: MMU 14/1/3 (on RPU 1), MMU 11/17/2 (RPU 2), MMU 17/10/7/19 (RPU 3), MMU 18/9/13 (RPU 4), MMU 17/10/4 (RPU 5), MMU 17/12 (RPU 8), MMU 11/16 (RPU 9), MMU 17/1/17 (RPU 11), MMU 9/17 (RPU 16), and MMU 10/17 (RPU 20). Comparison of these syntenic associations sheds light on primitive and shared chromosomal segment combinations among the murid species (*R. pumilio*, *R. norvegicus*, *R. rattus*) and a cricetid (*C. griseus*). The syntenic segment combination of mouse chromosome 17/10/7/19 in *R. pumilio* is almost identical to that obtained in the two rat taxa except for a pericentric inversion, and a minute insertion of a portion of MMU 13 in the two rat taxa (Helou *et al.* 2001; Cavagna *et al.* 2002). Together, segments of mouse 17 and 10 and whole chromosomes 7 and 19 constitute a large syntenic association that probably predates the divergence of these taxa (see below). Further, the insertion of a segment of chromosome 13 which was absent in *R. pumilio* may be shown to be a synapomorphy for the Black and the Norwegian rats.

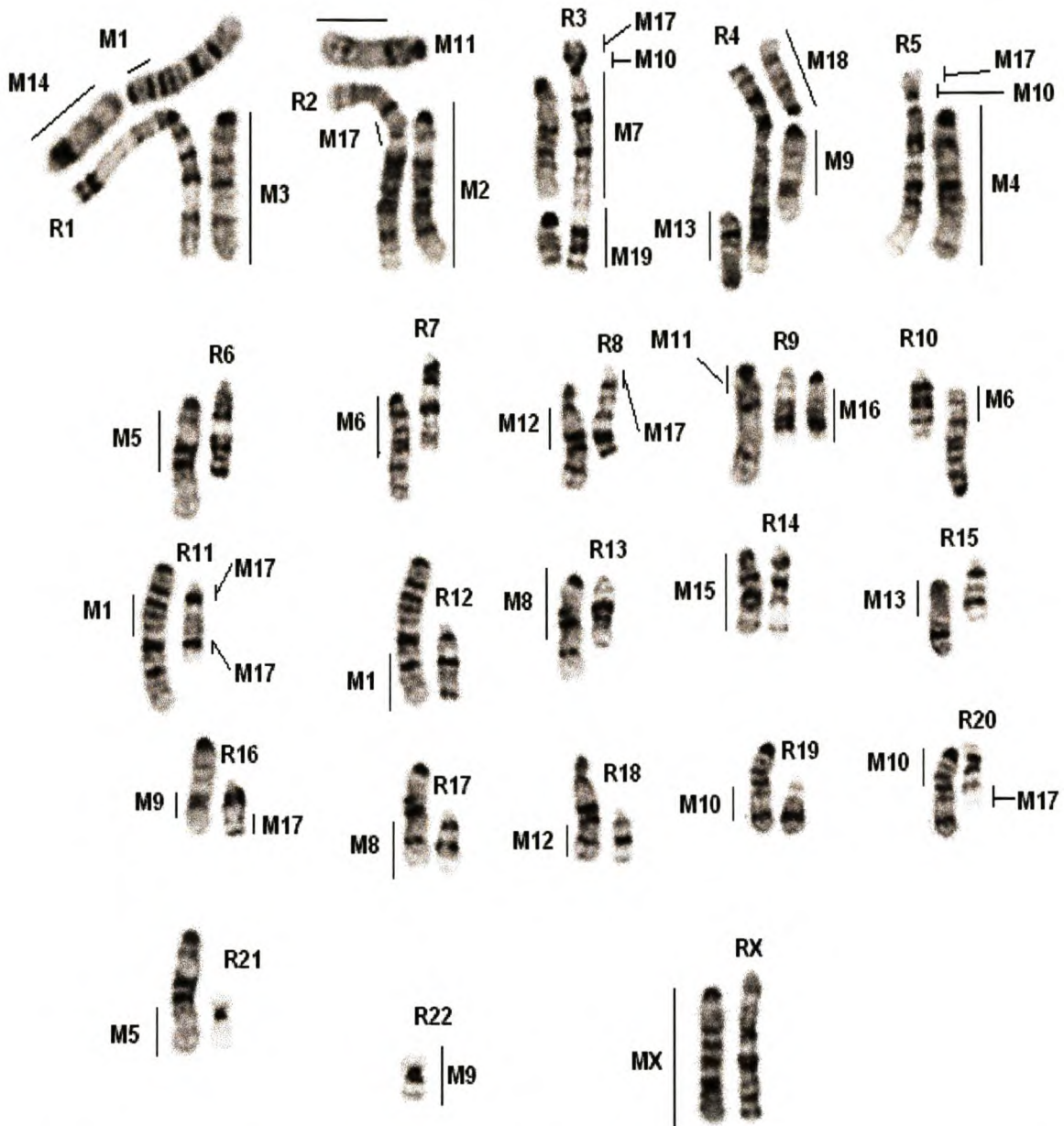


Figure 11: G-banding comparisons between *M. musculus* and *R. pumilio* chromosomes that were identified as homologous by FISH. *Rhabdomys pumilio* chromosome numbers are preceded by R while *M. musculus* chromosome numbers are preceded by M. The centromere positions of all acrocentric chromosomes are at the tip with the exception of MMU 18 (corresponding to RPU4p), 6 (corresponding to RPU 10) and 13 (corresponding to *R. pumilio* 15) which have been inverted to emphasize banding homology. Lines indicate the extent of banding homology between chromosomes of *R. pumilio* and *M. musculus*.

Additionally, two segment combinations (17/1/17 and 17/12) unite the two rat species and *R. pumilio* to the exclusion of the single cricetid for which data are available. However, it is noteworthy that a centromeric shift and addition of a segment of mouse chromosome 1 to the synteny defined by mouse chromosomes 17/12 (in the rats and *R. pumilio*) allows for the derivation of Chinese hamster chromosome 5 suggesting that it may possibly be a recent event (see Figure 2; Yang *et al.* 2000). Gene mapping data indicate that the syntenic associations identified by fragments of mouse chromosome 1, 10 and 17 may be ancestral for most taxa (Nadeau & Taylor 1984; Lyons *et al.* 1997; Copeland *et al.* 1993). For instance, the combination of segments of mouse chromosomes 10 and 17 (which were evident in our homology map) were also detected in man, *Homo sapiens* (HSA6), the domestic cat, *Felis catus* (FCA B2), and cattle, *Bos taurus* (BTA1; Lyons *et al.* 1997). Additionally, segments of mouse chromosomes 1 and 17 and mouse chromosomes 11 and 16 were detected in the human chromosomes 21 and 22 respectively, clearly indicating that they have ancient origins (Nadeau & Taylor 1984; Scherthan *et al.* 1994; Lyons *et al.* 1997). It is remarkable that mouse chromosome 1, 10, and 17 consistently appear as fragments in these varied mammalian taxa indicating that they are derived in the mouse.

In summary, the *R. pumilio*/*M. musculus* chromosome homology map allows detection of syntenies which may be specific for the Murinae although this is not definitive due to limited taxon sampling. However, the data will contribute to the accurate reconstruction and revision of the ancestral murid/cricetid karyotype suggested by Koop *et al.* (1984) based solely on G-banding. This should be possible as more representatives of murids and cricetids are analysed by ZOO-FISH. Clearly, mouse chromosomes 1, 10 and 17 appear to be more prone to chromosome breakage than many of the other chromosomes which show a high degree of conservation (MMU2, 3, 4, 7, 14, 15, 16, 18, 19). To this end my homology map is useful and provides a step towards a better understanding of genome evolution in murids for which both molecular sequence data and basic cytogenetic data (diploid number) are abundant, but for which chromosome painting data is generally lacking.

CHAPTER 4

STRUCTURAL ANALYSIS OF THE CONTROL REGION OF THE MTDNA OF
R. PUMILIO

Introduction

The high turn over rate in the control region of mammals makes it a useful marker for investigating intraspecific variation (Chapter 1; Taberlet 1996). Since a preliminary investigation based on the sequences of the 5' region indicated that there is paucity of parsimony informative characters for phylogenetic analysis in *R. pumilio*, it was hypothesised that this could be due to some structural rearrangement of the three main domains (left, central and right) in this species. As a result, the complete control regions of selected *R. pumilio* specimens were sequenced to establish the structural organization of this commonly used molecular marker. The selected specimens were representative of the two main groupings evident in the *cyt b* phylogeny (Chapter 2): Clade 1 which is divided into subgroup A ($2n = 46$) and subgroup B ($2n = 48$), and Clade 2 ($2n = 48$). I first compared the complete sequence of the *R. pumilio* control region to the mouse (*M. musculus*), platypus (*Ornithorhynchus anatinus*), chimpanzee (*Pan troglodytes*), human (*H. sapiens*), rat (*R. norvegicus*), rabbit (*Oryctolagus cuniculus*), dolphin (*Cephalorhynchus commersonii*), cattle (*Bos taurus*), seal (*Phoca vitulina*), and opossum (*Didelphis virginiana*) used by Gemmel *et al.* (1996). Secondly, the control region sequences were analysed using PAUP*version 4.0b6 (Swofford 2001) to determine their utility for retrieving phylogenetic information on this species, and to compare the resulting topologies with those obtained from the larger *cyt b* data set (Chapter 2).

Materials and methods

Samples

A total of 10 samples representing the two main *cyt b* groupings of *R. pumilio* (Chapter 2) were used in this investigation. The first group comprises specimens that form a monophyletic clade in Figure 7 (Chapter 2) which have a diploid number of $2n = 46$, from Uganda, Malawi, Inyanga, and Potchefstroom. The second group is a sister-clade to the Uganda-Malawi-Inyanga-Potchefstroom assemblage but has a diploid number of $2n = 48$. Representatives of this group have a north-eastern distribution and were collected at Pilgrim's Rest, Lydenburg, Cathedral Peak and King William's Town. For the third group ($2n = 48$) representatives were drawn from Grotto Bay and Gariiep Dam. Geographic location and collection sites are illustrated in Figure 2 and Table 2.

DNA extraction, amplification and sequencing

DNA extracts were obtained from muscle tissue following the phenol/chloroform protocol suggested by Maniatis *et al.* (1982). Polymerase chain reaction was used to amplify the control region sequences. The primers selected were: universal primers N777 and DLH1 (Paabo & Wilson 1988; Kocher *et al.* 1989; Irwin *et al.* 1991) and a custom made primer (5'AATT AAT TAA AGG CCA GGA CCA AAC3') which is located in the tRNA bordering the 12S rRNA gene. PCR conditions were 94°C for 3 min and 94°C for 30 s (denaturation), 48°C for 45 s (annealing) and 72°C for 45 s (elongation). PCR products were checked on 1% agarose gel and then excised and purified with the QIAquick PCR purification kit (Qiagen Ltd.). Cycle sequencing was done using the three primers under the following conditions: 96°C for 10 s (denaturation); 50°C for 5 s (annealing); 60°C for 4 min (elongation) after which the products were purified and sequenced using the ABI 3100 automated sequencer.

Sequence alignment and analysis

Sequences were checked and edited using Sequence Navigator (v1.0.1). Alignment was done using both Clustal W version 1.6 (Thompson *et al.* 1994) and then manually. Regions of overlap between sequences were identified and ambiguous sequences were excluded. Orthologous sequences of the mouse (Bibb *et al.* 1981) were used as a reference. For the comparative analysis, and for the identification of conserved

nucleotide sequences, the *R. pumilio* sequences were aligned to those of human (Anderson *et al.* 1981), cattle (Anderson *et al.* 1982), chimpanzee (Foran *et al.* 1988), platypus (Gemmell *et al.* 1996), dolphin (Southern *et al.* 1988), rat (Brown *et al.* 1986), seal (Amason & Johnson 1992), rabbit (Mignotte *et al.* 1990) and opossum (Janke *et al.* 1994).

Putative secondary structures in the conserved regions were examined using the program MFOLD (Zuker 1989; Jaeger *et al.* 1989; Jaeger 1989). In order to determine the utility of the control region sequences for phylogenetic analysis neighbour joining and parsimony analysis were performed in PAUP*version 4.0b6. Phylogenetic reconstructions of *R pumilio* were performed using the mouse (*M. musculus*) as the outgroup.

Results and discussion

The *R. pumilio* control region is comprised of roughly 994 bp including the flanking tRNAs. Repeat motifs and length variation among *R. pumilio* specimens were not detected. For comparison, positional assignment of the 994 bp nucleotide bases was obtained by aligning them to sequences of the mouse (Bibb *et al.* 1981) which is the closest available evolutionary relative to *R. pumilio*. This revealed that the structural organization of *R. pumilio* control region is comparable to that of other mammals in that the three main domains (left, central and right domain) were clearly distinguishable (Saccone *et al.* 1987). The mouse regions homologous to the 5'end (on the L-strand) extend from position 15490 - 15600, the central region from 15601 - 15990 and the 3'end from 15991-16296.

Base composition and nucleotide heterogeneity

The three domains of *R. pumilio* control region displayed base compositional bias. In the 5'end (left domain), the frequency of adenine was highest (41.8%) followed by thymine (34.8%), cytosine (16.7%) and guanine (7%). The central domain contained a higher percentage of cytosine (31.3%) followed by thymine (29.6%), adenine (23.5%) and guanine (15.5%). Lastly, the 3'end (right domain) has a base composition similar to that of the 5' end in that adenine predominated (33.1%), followed by thymine (29.6%), cytosine (25%), and guanine (11.7%). Base composition in these three regions is comparable (but not identical) to that found in the 26 mammal species used by Sbisa *et al.* (1997).

For instance, the G content contained in the left domain of *R. pumilio* is comparable with the lowest observed values across divergent mammals (7-15%; Saccone *et al.* 1987; Sbisa *et al.* 1997). In the case of the *R. pumilio* central domain, the G content was similarly low (15.5%) compared with sequences from the suite of species used in the comparison (17-24%, Sbisa *et al.* 1997). However, in the right domain, the G content (11.7%) is within the range obtained in other mammals (7-14%, Saccone *et al.* 1987; Sbisa *et al.* 1997). Overall the low G content in the three domains of the *R. pumilio* control region is consistent with what has been found in other mammals where A+T > C+G (Saccone *et al.* 1987; Sbisa *et al.* 1997; Matson & Baker 2001).

Further comparison of the three domains among the ten *R. pumilio* samples revealed, as expected, an unequal distribution of variable sites. Of the 177 characters comprising the 5' end only 59 sites were variable (66% were conserved). The 358 bp comprising the 3' end contained 102 variable characters indicating that 71% of nucleotides were conserved, and only 25 bp out of 311 bp of the central domain were variable; this equates to 91% conservation among sequences. The distribution of variable sites is also similar to that obtained in the European house mouse, *M. musculus*, where the hypervariable region is located in the 5' end region, while the moderately variable, and highly conserved sequences were on the 3' end, and in the central domain respectively (Nachman *et al.* 1994). This pattern is different to the general pattern recently observed in the red-backed vole where 40 % variation is in the right domain, 22% in the left domain and 12% in the central domain (Matson & Baker 2001). In the case of cervids, however, 39% of variation occurs in the left domain, 33% variation occurs in the right domain, and only 15% in the central conserved block (excluding indels; Randi *et al.* 2001).

Conserved Sequence Blocks

In an attempt to detect any peculiarities in the *R. pumilio* control region sequences a consensus sequence was made and aligned to sequences drawn from GenBank following Gemmel *et al.* (1996). These included human, chimpanzee, dolphin, cattle, seal, opossum, platypus and the closely related mouse and rat. The comparative synthesis of Gemmel *et al.* (1996) revealed that these divergent taxa have four highly conserved sequence motifs namely TAS, CSB1, CSB2 and CSB3 (Figure 12). Homologous regions to these sequences in *R. pumilio* were identified in the mouse/*R. pumilio* alignment, and then extrapolated to the homologous regions identified by Gemmel *et al.* (1996). First, the 15 bp comprising the TAS sequences show remarkable similarity to homologous regions in mouse, rat, rabbit, human and chimpanzee, dolphin, cattle, seal, opossum and platypus (Gemmel *et al.* 1996), but not in *R. pumilio*. The high degree of conservation of TAS is thought to be indicative of an evolutionary function, specifically for signaling the termination of d-loop synthesis (Saccone *et al.* 1987). It is, however, noteworthy that while the primary sequence has not been conserved in *R. pumilio*, the flanking regions show potential to form secondary structures (Figure 13). Nevertheless, the apparent lack of conservation in primary sequence of TAS in my *R. pumilio* raises questions about their purported function.

The CSB1-3, which lies in the hypervariable 3'end of the control region, has been highly conserved in all the taxa that Gemmel *et al.* (1996) used in his investigation; it is similarly conserved in *R. pumilio*. Contrary to what was obtained for TAS however, the high degree of similarity in these sequences among Gemmel *et al.*'s taxa was similarly mirrored in *R. pumilio*. Simple pairwise comparisons between corresponding CSB sequences of *R. pumilio* and the Gemmel *et al.* (1996) taxa confirmed the conservativeness of these regions. First, CSB1 sequences of *R. pumilio*, and each of the taxa analysed by Gemmel *et al.*, revealed similarity values ranging from 47% between *R. pumilio* and the more distantly related opossum, to 86% between *R. pumilio* and the closely related mouse. Second, CSB2 showed similarity values > 50% and lastly, CSB3 was remarkably conserved with values ranging from 68% to 95% in all taxa except cattle, dolphin and platypus where homologous sequences were not found.

Apparently the retention of these conserved sequence blocks is due to their ability to form stable secondary structures. Therefore, the primary sequences (L-strand) of *R. pumilio*, which were homologous to these conserved regions, were checked to determine whether they can form secondary structures. The TAS, CSB1 and CSB3 sequences formed part of the secondary structures (Figure 13), while the CSB2 had a formation immediately after the primary sequence. The nature of the conservation, and the capacity for the formation of stable secondary structures of these sequences is accepted as evidence that they have some functional role in the mtDNA (Brown *et al.* 1986). It has been shown that absence of these regions, or structural mutations in these regions, leads to replication failure in *E. coli* (Masukata & Tomizawa 1984). Brown *et al.* (1986) adds that these conserved sequences play a key role in the replication, initiation and in the termination of replication, and that they also "facilitate formation of primer-precursor/template complex, a necessary step for primer generation by RNase H". Therefore, the ability of the flanking regions of these conserved sequence blocks to form secondary structures probably underscores the viability of their functional role (transcription and regulation of replication) in the control region (Saccone *et al.* 1987).

(a) CSB1

Human:	TTAATGCTTGTAGGACA-TAAT
Chimpanzee:	TTAATGCTTGCAGGACA-TAAC
Mouse:	TTCATGCTTGTTAGACA-TAAA
Rhodomys:	TTAATGCTTGATAGACA-T-AT
Rat:	TCCATGTTTGTAAGACA-TAAA
Rabbit:	TTAATGCTTGTCGGACA-TAAA
Dolphin:	T-AATGGTTACAGGACA-TATT
Cattle:	TCAATGGTCACAGGACA-TAAA
Seal:	TCAATGGTAGCGGGACA-TAGT
Opossum:	TTAATATACGAAGGACAATAAA
Platypus:	TTAATGCTTGACGGACA-TAAA

(b) CSB2

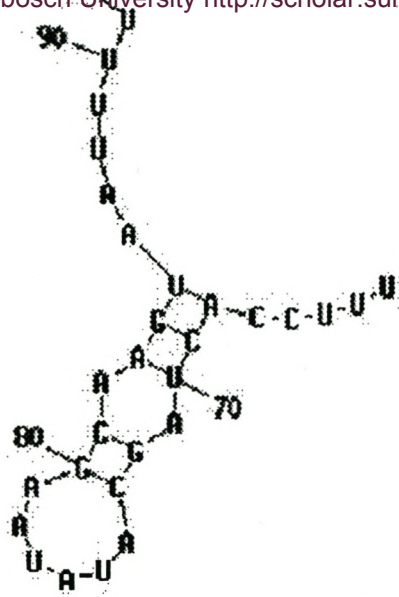
Human:	AAACCCCCCCTCCCCCGG
Chimpanzee:	AAACCCCCCTTCCCCCGG
Mouse:	AAACCCCC---ACCCCTC
Rhodomys:	AAACCCCTTTCCCC----
Rat:	AAACCCCCC--ACCCCTA
Rabbit:	AAACCCCCC-TACCCCTC
Dolphin:	AAA-CCCCCTTCCCCCTTA
Cattle:	---CCCCCTTC-----
Seal:	AAACCCCC-TTACCCCGG
Opossum:	AAA--CCCCCTTACCCCTA
Platypus:	--CCCCCCTTCCCCCGG

(c) CSB3

Human:	TCTGCCAAACCCCAAAAAC
Chimpanzee:	TCTGCCAAACCCCAAAAAC
Mouse:	--TGCCAAACCCCAAAAAC
Rhodomys:	-ATGCCAAACCCCAAAAAC
Rat:	--TGCCAAACCCCAAAAAC
Rabbit:	CCTGCCAAACCCCAAAAAC
Seal:	TCTGCCAAACCCCAAAAAC
Opossum:	TCCGTCAAACCCCAAAAAC

Figure 12: Alignment of the *R. pumilio* consensus sequence to the conserved homologues in the CSB1-3 (a-c) of human, chimpanzee, mouse, rat, rabbit, dolphin, cattle, seal, opossum. Gaps were introduced to emphasize regions of alignment.

(a)



(b)

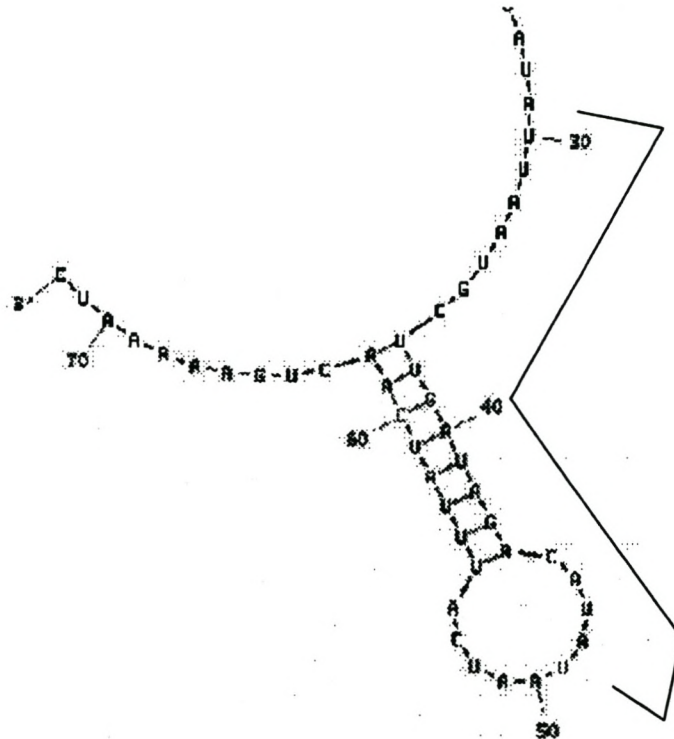
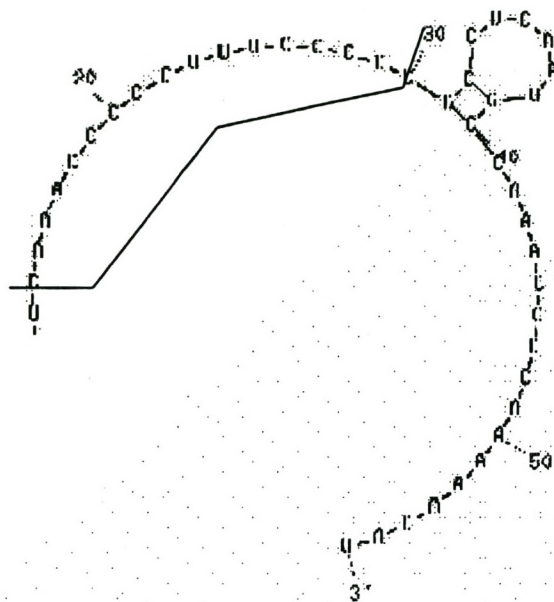


Figure 13: Putative secondary structures in *R. pumilio* obtained with (a) TAS and three conserved sequences (b) CSB1, (c) CSB2 and (d) CSB3. Lines show length and position of the primary sequences in relation to the secondary structures.

(c)



(d)

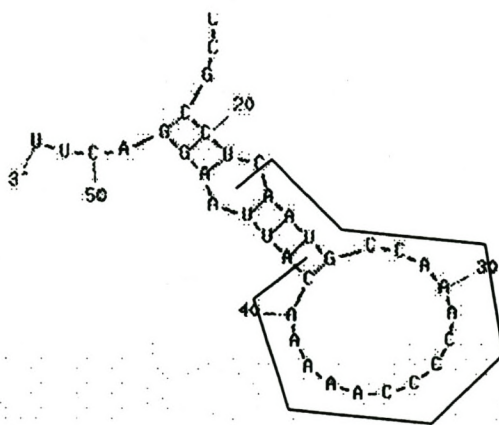


Figure 13 (continued)

Phylogenetic analysis and implications

In the analysis incorporating complete sequences of the control region, all the gaps that were introduced to obtain alignment with the mouse were excluded. This was also done for areas where homology was equivocal. Of the 871 bp of the remaining sequences, 218 bp were variable of which 110 bp were parsimony informative. Comparisons of these control region sequences to the larger *cyt b* data set revealed that the *cyt b* data contains a high proportion of parsimony informative sites: out of the 1140 bp, 350 bp were variable and 209 bp were parsimony informative. As expected, uncorrected sequence divergences for the *cyt b* were generally higher. For instance, comparison of representatives of the most geographically distant localities of subgroup B (King William's Town) and A (Uganda) have genetic distances of 11.3% for control region (Table 6) and 13% for the *cyt b* gene (Table 7). Further comparisons of representatives of subgroup C (Grotto Bay) and subgroup A (Malawi) confirms this discrepancy with the control region showing only 6.7% divergence compared to 11.1% of the *cyt b* gene. However, the tree topologies of each data set were the same (see below).

In the case of the control region data, three equally parsimonious trees of 489 steps were retrieved (Consistency index = 0.824, Retention index = 0.563, Homoplasy index = 0.176). Apart from terminal branch swapping, the derived strict consensus was identical to the NJ tree (Figure 14). The *cyt b* data resulted in two equally parsimonious trees (length = 533; CI = 0.76; RI = 0.69; HI = 0.23) which were used to construct the strict consensus which, incidentally, was also identical to the NJ (except for terminal branch swapping). The groupings retrieved by the larger *cyt b* data (Figure 7, Chapter 2) were retained in the smaller data sets, the control region (Figure 14) and *cyt b* (Figure 15): (1) Subgroup A (characterised by $2n = 46$) comprised of samples from Malawi, Uganda, Inyanga and Potchefstroom; (2) Subgroup B, ($2n = 48$) includes samples from King William's Town, Cathedral Peak, Lydenburg and Pilgrim's Rest; and (3) Subgroup C ($2n = 48$) is composed of samples from Grotto Bay and Gariep Dam. The *cyt b* data similarly supports these three groupings with bootstrap values generally >80% (Figure 15) while the control region bootstrap values are lower (Figure 14).

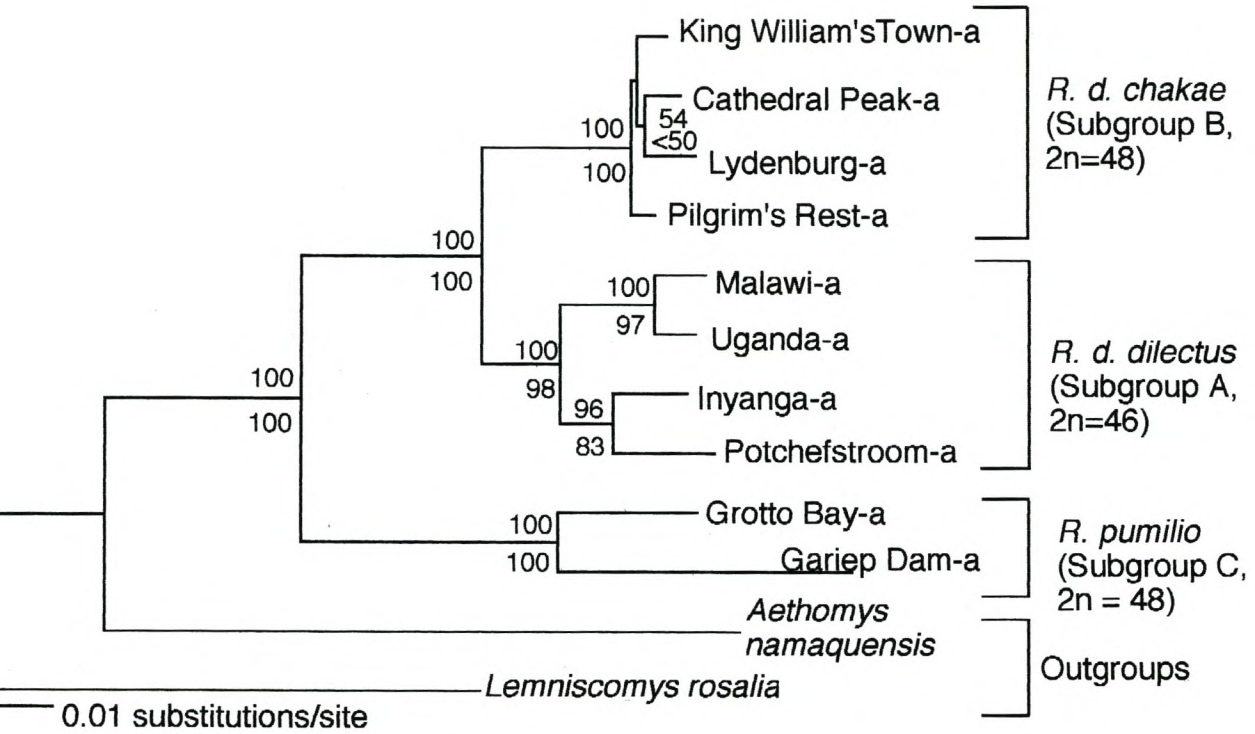


Figure 15: Neighbour joining phylogram topology obtained from the complete *cyt b* (1140 bp) showing the three subgroups. Two equally parsimonious trees were obtained (length = 533; CI =0.76; RI =0.69; HI = 0.23).

Table 5: Uncorrected (p-) distance matrix for the control region (871 bp)

	1	2	3	4	5	6	7	8	9	10	11
1 KingWilliam'sTown-											
2 Grotto Bay	0.074	-									
3 Cathedral Peak	0.013	0.073	-								
4 Pilgrim's Rest	0.018	0.075	0.012	-							
5 Lydenburg	0.018	0.079	0.009	0.015	-						
6 Malawi	0.037	0.067	0.035	0.038	0.038	-					
7 Uganda	0.114	0.140	0.107	0.108	0.109	0.094	-				
8 Inyanga	0.061	0.091	0.057	0.057	0.058	0.038	0.099	-			
9 Potchefstroom	0.039	0.068	0.035	0.036	0.034	0.016	0.091	0.041	-		
10 Gariep Dam	0.112	0.094	0.116	0.112	0.117	0.116	0.186	0.135	0.117	-	
11 Mouse	0.215	0.229	0.215	0.214	0.216	0.225	0.246	0.233	0.216	0.242	-

Table 6: Uncorrected (p-) distance matrix for cyt b (1140 bp) for the reduced data

	1	2	3	4	5	6	7	8	9	10	11	12
1 KingWilliam'sTown	-											
2 Grotto Bay	0.099	-										
3 Cathedral Peak	0.008	0.101	-									
4 Pilgrim's Rest	0.007	0.097	0.009	-								
5 Lydenburg	0.013	0.100	0.011	0.012	-							
6 Malawi	0.055	0.111	0.057	0.053	0.058	-						
7 Uganda	0.052	0.110	0.053	0.049	0.054	0.012	-					
8 Inyanga	0.053	0.105	0.056	0.052	0.055	0.031	0.033	-				
9 Potchefstroom	0.054	0.101	0.059	0.055	0.059	0.041	0.043	0.024	-			
10 Gariep Dam	0.126	0.058	0.129	0.125	0.127	0.130	0.132	0.122	0.120	-		
11 Lemniscomyspp	0.157	0.164	0.160	0.158	0.163	0.168	0.165	0.160	0.162	0.175	-	
12 Aethomyssp	0.161	0.174	0.163	0.156	0.162	0.162	0.164	0.163	0.165	0.182	0.168	-

The congruence between topologies of these two mtDNA markers suggest that both may be suitable for phylogenetic investigation. However, the higher proportion of parsimony informative characters contained in *R. pumilio* *cyt b* suggest that it is more appropriate for resolving species complexes – hence its utility in rodents (e.g. *Akodon*, Smith & Patton 1993; *Sigmodon*, Peppers & Bradley 2000; *Peromyscus*, Bradley *et al.* 2000; Harris *et al.* 2000). As shown in Chapter 2, the levels of sequence variation detected within *R. pumilio* *cyt b* sometimes exceeds the levels of genetic divergences which demarcate species in other murids (Johns & Avise 1998; Baker & Bradley 2001). In contrast, the lower divergences obtained from the *R. pumilio* control regions indicate a slower turn over rate raising questions as to its usefulness in detecting fine-scale geographic structure within *R. pumilio*.

In summary, and contrary to the norm (see Taberlet 1996), the *R. pumilio* control region appears to have a slower mutational rate than the *cyt b*. However, the results of this study support recent finding in the garter snakes (genus *Thamnophis*) where the protein coding gene appears to be evolving more rapidly than the noncoding region (De Queiroz *et al.* 2002). Despite the congruence between the *cyt b* and control region tree topologies, it was considered appropriate to use the former to investigate the phylogeography of *R. pumilio* in view of its elevated substitution rate. Two observations suggest that the control region of *R. pumilio* appears to be evolving in a relatively unique way. First, the G content specifically in the left domain is well below that contained in other mammals (Saccone *et al.* 1987; Sbisa *et al.* 1997). Second, the high similarity of the TAS in other taxa (Gemmell *et al.* 1996), and its lack of sequence conservation in *R. pumilio*, suggests that its function may have been lost, or altered, in *R. pumilio*. Nevertheless, the structural arrangement of the control region of *R. pumilio* is similar to that of other mammalian taxa in that it has retained the three functional domains. Further, the base compositional bias in these regions detected in other mammal species is similarly retained in *R. pumilio*. With the exception of minor sequence differences, the three functional conserved sequence blocks, CSB1, 2, and 3 have also been conserved. As expected, selection against disruption of primary sequences of the conserved sequence block (and their functional role) is indicated by formation of stable secondary structures.

CHAPTER 5

SUMMARY AND CONCLUDING REMARKS

Rodent species generally occupy a wide range of habitats. Because of their low dispersal capabilities it is expected that they will exhibit some degree of genetic differentiation among geographic localities (Avice 1994). This trend has been observed in several rodent population studies using maternally inherited mtDNA profiles to tease evolutionary associations apart (Smith & Patton 1993; peppers & Bradley 2000; Bradley 2000; Harris 2000). Despite rodent species richness in Africa, there are relatively few investigations that have investigated phylogeographic patterns. This is particularly important since rodents often show uniform phenotypic characters (cryptic species). In this study, an attempt is made to address this by investigating one of the most widely distributed endemic African rodents, *R. pumilio*, using mtDNA and molecular and conventional cytogenetic approaches. The aims of this study were threefold: First, I set out to examine the phylogeography of *R. pumilio* in southern Africa by analysing complete sequences of mtDNA *cyt b* and the control region from specimens drawn from localities throughout the species' range in South Africa. This was supplemented with limited extralimital sampling. Second, I attempted to more clearly delimit the extent of the distribution of the two cytotypes detected in southern African *R. pumilio* specimens by karyotyping representatives drawn from localities across the species' range, and from areas where cytogenetic analyses had not previously been undertaken. Additionally, fluorescence *in situ* hybridization (FISH) using flow-sorted mouse chromosome specific painting probes and high resolution G-banding were implemented in the hope of detecting subtle interchromosomal rearrangements in the genomes of the two cytotypes. Third, a comparative phylogenomics approach was followed that utilized the *M. musculus* / *R. pumilio* FISH experiments to identify shared derived chromosomal states in other murids for which comparable data are available. These were then placed in a broader phylogenetic context.

Analysis of the South African *R. pumilio* showed the populations to be fixed for either $2n = 46$ or $2n = 48$; no intra-population karyotypic variation was detected. Specimens collected at Irene (Gauteng Province) had $2n = 46$, while those sampled at the Suikerbosrand Nature Reserve (Gauteng Province) with approximately 70km separating the two localities had $2n = 48$. A diploid number of $2n = 48$ was obtained for specimens collected in the remainder of South Africa, the Eastern Cape (Alice), Free State (Gariiep Dam, Willem Pretorius Nature Reserve), Mpumalanga (Lydenburg and Pilgrim's Rest), Western Cape (Bottlelary, Grotto Bay) and Northern Cape (Springbok). Samples from

two Namibian localities (Swakopmund and Keetmanshoop) also had a diploid number of $2n = 48$.

Fluorescence *in situ* hybridization using mouse chromosome specific painting probes produced similar patterns for the two cytotypes revealing that no interchromosomal rearrangements (other than the centric fusion difference) distinguish the apparently parapatrically distributed cytotypes. The chromosomes involved in the fusion/ fission were hybridized by MMU18, MMU9 and MMU13. FISH signals on the $2n = 46$ cytotype were on the short arm (p arm) of the fusion chromosome (RPU 4) corresponding to MMU18, while the long arm was hybridized by segments of MMU9 and MMU13. FISH signals on the $2n = 48$ cytotype were restricted entirely to acrocentric autosomes.

The mtDNA data retrieved two major lineages that roughly correspond to the xeric and mesic biotic zones of southern Africa. One mtDNA clade comprises specimens with $2n = 48$ and the other representatives of two cytotypes ($2n = 48$ and $2n = 46$). The mtDNA phylogeny suggests that the $2n = 48$ is ancestral, and $2n = 46$ was recently derived. Further, the mean sequence divergence (12%, range 8.3 -15.6%) separating the two mtDNA clades is comparable to among-species variation within murid genera suggesting their recognition as distinct species, the prior names for which would be *R. dilectus* and *R. pumilio*. Low sequence divergences, and the diploid number dichotomy within the mesic lineage, support the recognition of two subspecies corresponding to *R. d. dilectus* ($2n = 46$) and *R. d. chakae* ($2n = 48$). MtDNA data do not support subspecific delimitation within the nominate, *R. pumilio*.

In the absence of any rate heterogeneity in the *cyt b* data the molecular clock was applied using a calibration derived for the Arvianthine rodents. This calibration is based on third position transversions since they accumulate linearly (i.e. they are not saturated) and the 12 million years divergence time estimated between *Mus* and *Rattus*. This clock places the divergence between *R. pumilio* and *R. dilectus* (mtDNA Clades 1 and 2) at 2.9 MYA, and that between *R. d. dilectus* and *R. d. chakae* (mtDNA subgroup A, $2n = 46$ and B, $2n = 48$) at roughly 600,000 years. Although the first of these estimates falls within the hypothesised Miocene-Pliocene (5 MYA) emergence of the African Murinae it predates the oldest *R. pumilio* fossils thus far documented which have early Pleistocene origins. Correspondence between the molecular dating and past climate data allows for the development of hypotheses that explain the evolutionary history of the two species. It is suggested that the ancestral *R. pumilio* survived the climatic oscillations that characterised the Pliocene in two refugia – one in the mesic regions, the other in the xeric zone of southern Africa. Molecular clock calibrations indicate the establishment of

the two refugia at roughly 2.9 MYA. Superimposed on this initial cladogenic event followed a second, more recent (600,000 years ago), divergence involving the descendants of the mesic refugium that gave rise to two DNA subgroups (A and B) that are underpinned by differences in $2n$. Fixation of the derived chromosomal state probably involved small effective population size, inbreeding and drift, all factors conventionally associated with population fragmentation. Should this hypothesis hold, it is not unlikely that the present day distribution of what is conventionally known as *R. pumilio* reflects the relatively recent contact between these refugial populations that followed the climatic changes at the end of the Pliocene.

Finally, phylogenomic analysis of the mouse/ *R. pumilio* chromosome map produced by FISH and G-banding indicates a high degree of homology has been retained between the species. Ten mouse chromosomes (2-4, 7, 14-16, 18, 19 and the X) were retained as chromosomal arms, or intact chromosome blocks. Five mouse chromosome painting probes that correspond to mouse autosomes 5, 6, 11, 12 and 13 produced double signals; the remaining four painting probes (1, 9, 10 and 17) hybridized to three or more *R. pumilio* chromosomes respectively. In total, the 20 mouse chromosome paints revealed 40 segments of conserved synteny in the *R. pumilio* genome. Most of the mouse chromosomes that produced single signals in *R. pumilio* have previously been shown to be conserved in the Black and Norwegian rats and the Chinese hamster. Eight contiguous segment associations appear to be *R. pumilio* specific, two were shared by *R. pumilio* and the Black and Norwegian rats, but to the exclusion of the Chinese hamster. The data suggest that mouse chromosomes 1, 10, and 17 have undergone extensive rearrangements during genome evolution in the murids and may be useful markers for enhancing our understanding of the mode and tempo of chromosome evolution in rodents.

In conclusion, a combination of the mtDNA (showing elevated divergences) and cytogenetics using molecular probes (confirming the centric fusion) suggest that the $2n = 48$ is ancestral and that $2n = 46$ is derived. Further, the data indicate that a taxonomic revision of *Rhabdomys* is warranted, and that the four-striped mouse is probably comprised of two species, *R. pumilio*, and the newly erected *R. dilectus*. Moreover, that within the latter there is good evidence from my data for the recognition of two subspecies: *dilectus* and *chakae*. Laboratory based breeding and mate-choice experiments between specimens collected in areas representing the newly erected *R. dilectus* and the nominate species (Pillay 2000a, b) confirm and extend the molecular conclusions suggesting the presence of pre-mating isolating mechanisms which could act to provide an effective barrier to gene flow. These corroborative data satisfy criteria

for species recognition under both the biological species concept (reproductive isolation) and phylogenetic species concept (distinct phylogenetic patterns).

REFERENCES

- ALLARD, M. W. & MIYAMOTO, M. M. 1991. Tests for rodent polyphyly. *Nature* 353: 610-611
- ANDERSON, S., BANKIER, A. T., BARREL, B. G., DE BRUIN, M. H. L., COULSON, A. R., DROUIN, J., EPERON, I. C., NIERLICH, D. P., ROE, B. A., SANGER, F., SCHREIER, P. H., SMITH, A. J. H., STADEN, R. & YOUNG, I. G. 1981. Sequence and organization of the human mitochondrial genome. *Nature* 290: 457-465.
- ANDERSON, S., DEBRUIJN, M. H. L., COULSON, A. R., EPERON, I. C., SANGER, F. & YOUNG, I. G. 1982. Complete sequence of bovine mitochondrial DNA. *J. Mol. Biol.* 156: 683-717.
- APPLIED BIOSYSTEMS. Inc. 1994. Sequence Navigator DNA and Protein sequence comparison Software, Perkin-Elmer Corp., Forster City, CA.
- ARNASON, U. & JOHANSSON, E. 1992. The complete mitochondrial DNA sequence of the Harbour Seal, *Phoca vitulina*. *J. Mol. Evol.* 34: 493-505.
- ARCTANDER, P. 1995. Comparison of a mitochondrial gene and a corresponding nuclear pseudogene. *Proc. R. Soc. Lond. B* 262: 13-19.
- AVERY, D. M. 1991. Late quaternary incidence of some micromammalian species in Natal. *Durban Mus. Novit.* 16: 1-11.
- AVISE, J. C. & AQUADRO, C. F. 1982. A comparative summary of genetic distances in the vertebrates. *Evol. Biol.* 15: 151-184.
- AVISE, J. C. 1991. Ten unorthodox perspectives on evolution prompted by comparative population genetic findings on mitochondrial DNA. *Annu. Rev. Genet.* 25: 45-69.
- AVISE, J. C. 1994. *Molecular Markers, Natural History and Evolution*. Chapman Hall, New York.

- AVISE, J. C. & WOLLENBERG, K. 1997. Phylogenetics and the origin of species. *Proc. Natl. Acad. Sci. USA* 94: 7748-7755.
- AVISE, J. C. 2000. *Phylogeography: The history and formation of species*. Harvard University Press, London.
- AYALA, F. J. 1997. Vagaries of the molecular clock. *Proc. Natl. Acad. Sci. USA* 94: 7776-7783.
- BAKER, R. J. & BICKAM, J. W. 1986. Speciation by monobrachial centric fusions. *Proc. Natl. Acad. Sci. USA*. 83: 8245-8248.
- BANAROV, V. S. 1980. Mice with Robertsonian translocations in experimental biology and medicine. *Genetica* 52/53: 23-32.
- BARBER, P. H., PALUMBI, S. R., ERDMANN, M. V. & MOOSA, M. K. 2002. Sharp genetic breaks among populations of *Haptosquilla pulchella* (Stomatopoda) indicate limits to larval transport: patterns, causes, and consequences. *Mol. Ecol.* 11: 659-674.
- BAROME, P-O., LYMBERAKIS, P., MONNEROT, M., & GAUTUN, J-C. 2001. Cytochrome *b* sequences reveal *Acomys minous* (Rodentia, Muridae) paraphyly and answer the question about the ancestral karyotype of *Acomys dimidiatus*. *Mol. Phylogenet. Evol.* 18: 37-46.
- BIBB, M. J., VAN ETEN, C. T., WRIGHT, M. W., WALBERG, M. W. & CLAYTON, R. A. 1981. Sequence and gene organization of mouse mitochondrial DNA. *Cell* 26: 167-180.
- BIELEC, P. E., GALLAGHER, D. S., WOMACK, J. E. & BUSBEE, D. L. 1998. Homologies between human and dolphin chromosomes detected by heterologous chromosome painting. *Cytogenet. Cell Genet.* 81: 18-25.
- BOND, W., FERGUSON, M. & FORSYTH, G. 1980. Small mammals and habitat structure along altitudinal gradients in the southern Cape mountains. *S. Afr. J. Zool.* 15: 34-43.
- BONNEFILLE, R. 1985. Evolution of the continental vegetation: the palaeobotanical record from East Africa. *S. Afr. J. Sci.* 81: 267-270.

BOTTING, J.H. & MORRISON, A. R. 1997. Animal Research is vital to medicine. *Sci. Am.* 276: 83-85.

BRADLEY, R. D., TIEMANN-BOEGE, I., KILPATRICK, C. W. & SCHMIDLY, D. J. 2000. Taxonomic status of *Peromyscus boylii sacarensis*: inferences from DNA sequences of the mitochondrial cytochrome *b* gene. *J. Mammal.* 81: 875-884.

BRADLEY, R. D. & BAKER, R. J. 2001. A test of the genetic species concept: cytochrome *b* sequences and mammals. *J. Mammal.* 82: 960-973.

BRANT, J. F. 1855. Beiträge zur nähern Kenntniss der Säügethiere Russlands. *Mem. Acad. Imp. St. Petersbourg* 6-9: 1-375.

BRITTON-DAVIDIAN, J., CATALAN, J., GRANJON, L. & DUPLANTIER, J-M. 1995. Chromosomal phylogeny and evolution in the genus *Mastomys* (Mammalia, Rodentia). *J. Mammal.* 76: 248-262.

BRITTON-DAVIDIAN, J., CATALAN, J., DA GRACA RAMALHINHO, M., GANEM, G., AUFFRAY, J.-C., CAPELA, R., BISCOITO, M., SEARLE, J. B. & DA LUZ MATHIAS, M. 2000. Rapid chromosomal evolution in island mice. *Nature* 403: 158.

BROOKS, P. M. 1974. The ecology of the four-striped field mouse, *Rhabdomys pumilio* (Sparrman 1784), with particular reference to a population on the van Riebeeck Nature Reserve, Pretoria. D.Sc. Thesis. University of Pretoria, Pretoria.

BROWN, G. G., PEPE, G., SACCONI, C. & SBISA, E. 1986. Structural conservation and variation in the D-loop-containing region of vertebrate mitochondrial DNA. *J. Mol. Biol.* 192: 503-511.

BULTE, E. & VAN KOOTEN, G. C. 1999. Economic Science, endangered species, and biodiversity loss. *Conserv. Biol.* 14: 113-119.

BUONGIORNO-NARDELLI, M. & AMALDI, F. 1969. Autoradiographic detection of molecular hybrids between rRNA and DNA in tissue sections. *Nature* 225: 946-948.

- BURDA, H. 2001. Determinants of the distribution and radiation of African mole-rats (Bathyergidae, Rodentia): Ecology or geography?. In: *African Small Mammals*. (eds) C. Denys, L. Granjon, A. Poulet, pp. 263-277, IRD Editions, Paris.
- BUSH, G. L., CASE, S. M., WILSON, A. C. & PATTON, J. L. 1977. Rapid speciation and chromosomal evolution in mammals. *Proc. Natl. Acad. Sci. USA* 74: 3942-3946.
- CAO, Y., ADACHI, J., YANO, T. & HASEGAWA, M. 1994. Phylogenetic place of guinea pigs: no support of the rodent-polyphyly hypothesis from maximum-likelihood analyses of multiple protein sequences. *Mol. Biol. Evol.* 11: 593-604.
- CAO, Y., OKADA, N. & HASEGAWA, M. 1997. Phylogenetic position of guinea pigs revisited. *Mol. Biol. Evol.* 14: 461-464.
- CAPANNA, E., GROPP, A., WINKING, H., NOACK, G. & CIVITELLI, M.-V. 1976. Robertsonian metacentrics in the mouse. *Chromosoma* 58: 341-353.
- CARLETON, M. D & MARTINEZ, C. 1991. Morphometric differentiation among West African populations of the rodent genus *Dasymys* (Muroidea; Murinae), and its taxonomic implications. *Proc. Biol. Soc. Wash.* 104: 419-435.
- CATZEFLIS, F. M., AGUILAR, J-P. & JAEGER, J-J. 1992. Muroid rodents: Phylogeny and evolution. *Trends Ecol. Evol.* 7:122-126.
- CAVAGNA, P., MENOTTI, A. & STANYON, R. 2002. Genomic homology of the domestic ferret with cats and humans. *Mammal. Genome* 11:866-870.
- CHEVRET, P., CATZEFLIS, F. & MICHAUX J. R. 2001. "Acomyinae" new molecular evidences for a muroid taxon (Rodentia; Muridae). In: *African Small Mammals*. (eds) C. Denys, L. Granjon, A. Poulet., pp. 109-125, IRD Editions, Paris.
- CHIMIMBA, C. T. 2001. Intraspecific morphometric variation in *Aethomys namaquensis* (Rodentia: Muridae) from southern Africa. *J. Zool., Lond.* 253: 191-210.
- CHRISTIAN, D. P. 1977. Diurnal activity of the four-striped mouse, *Rhabdomys pumilio*. *Zoologica Africana*. 12: 238-239.

- CHRISTIAN, D. P. 1979a. Comparative demography of three Namib desert rodents: responses to the provision of supplementary water. *J. Mammal.* 60: 679-690.
- CHRISTIAN, D. P. 1979b. Physiological correlates of demographic patterns in three sympatric Namib desert rodents. *Physiol. Zool.* 52: 329-339.
- CHURCHFIELD, S. 1985. Diets of two syntopic mammals in the Inyanga National Park Zimbabwe. *S. Afr. J. Zool.* 20: 65-67.
- CHOATE, T. S. 1972. Behavioural studies on some Rhodesian rodents. *Zoologica Africana* 7: 103-118.
- CHOWDHARY, B. P., RAUDSEPP, T., FRONICKE, L. & SCHERTHAN, H. 1998. Emerging patterns of comparative genome organization in some mammalian species as revealed by Zoo-FISH. *Genome Res.* 8: 577-589.
- CINCOTTA, R. P., WISNEWSKI, J., & ENGELMAN, R. 2000. Human population in the biodiversity hotspots. *Nature* 404: 990-992.
- COETZEE, C. 1970. The relative tail-length of striped mice *Rhabdomys pumilio* Sparrman 1784 in relation to climate. *Zoologica Africana* 5: 1-6.
- CONROY, C. J. & COOK, J. A. 1999. MtDNA evidence for repeated pulses of speciation within Arvicolinae and murid rodents. *J. Mammal. Evol.* 6: 221-245.
- CONTRAFATTO, G., MEESTER, J. A., BRONNER, G., TAYLOR, P. J. & WILLAN, K. 1992. Genetic variation in the African rodent subfamily Otomyinae (Muridae). IV. Chromosome G-banding analysis of *Otomys irroratus* and *O. angoniensis*. *Israel J. Zool.* 38: 277-291.
- COPELAND, N. G., JENKINS, N. A., GILBERT, D. J., EPPIG, J. T., MALTAIS, L. J., MILLER, J. C., DIETRICH, W. F., WEAVER, W. F., LINCOLN, S. E., STEEN, R. G., NAGEAU, J. H. & LANDER, E. S. 1993. A genetic linkage map of the mouse current applications and future prospects. *Science* 262: 57-66.
- CORBERT, G. B. 1997. The species in mammals. In: *Species: The units of biodiversity*, (eds) M. F. Claridge, H. A. Dawah, M. R. Wilson, pp. 341-356, Chapman & Hall, London.

- CRACRAFT, J. 1983. Species concepts and speciation analysis. In: *Current ornithology*, (ed) R. H. Johnson, pp. 159-187, Plenum Press, New York.
- CRACRAFT, J. 1997. Species concepts in systematics and conservation biology - an ornithological viewpoint. In: *Species: The units of biodiversity*, (eds) M. F. Claridge, H. A. Dawah, M. R. Wilson, pp. 325-380, Chapman & Hall, London.
- CRANDAL, K. A., BININDA-EDMONDS, O. R. P., MACE, G. M. & WAYNE, R. K. 2000. Considering evolutionary processes in conservation biology. *Trends. Ecol. Evol.* 15: 290-295.
- CREMER, T., LICHTER, P., BORDEN, J., WARD, D. C., & MANUELDIS, L. 1988. Detection of chromosome aberrations in metaphase and interphase tumor cells by *in situ* hybridization using chromosome specific library probes. *Hum. Genet.* 80: 235-246.
- DALLAS, J. F., DOD, B., BOURSOT, P., PRAGER, E. M. & BONHOMME, F. 1995. Population subdivision and gene flow in Danish house mice. *Mol. Ecol.* 4: 311-320.
- DAVID, J. 1979. Fieldmice are more interesting than you think. *African Wildlife* 33: 44-45.
- DAVISSON, M. T. & AKESON, E. C. 1987. An improved method for preparing G-banded chromosomes from mouse peripheral blood. *Cytogenet. Cell Genet.* 45:70-74.
- DAVISSON, M. T., BRADT, D. W., MERRIAM, J. J., ROCKWOOD, S. F. & EPPING, J. T. 1998. The mouse gene map. *ILAR J.* 39: 96-131.
- DEFILIPPIS, V. R. & MOORE, W. S. 2000. Resolution of phylogenetic relationships among recently evolved species as a function of amount of DNA sequence: an empirical study based on woodpeckers (Aves: Picidae). *Mol. Phylogenet. Evol.* 16: 143-160.
- DE GRAAFF, G. 1961. A short survey of investigations of fossil rodents in African deposits. *S. Afr. J. Sci.* 57: 191-196.
- DE GRAAFF, G. 1981. *The rodents of southern Africa*. Butterworths, Durban.

- DENYS, C. & JAEGER, J.-J. 1986. A biostratigraphic problem: The case of the East African Plio-Pleistocene rodent faunas. *Modern Geol.* 10: 215-233.
- D'ERCHIA, A. M., GISSI, C., PESOLE, G., SACCONI, C. & URNASON, U. 1996. The guinea-pig is not a rodent. *Nature* 381: 597-599
- DE QUEIROZ, A., LAWSON, R. & LEMOS-ESPINAL, J. A. 2002. Phylogenetic relationships of North American garter snakes (*Thamnophis*) based on four mitochondrial genes: how much DNA sequence is enough? *Mol. Phylogenet. Evol.* 22: 315-329.
- DIXKENS, C., KLETT, C., BRUCH, J., KOLLAK, A., SEROV, O. L., ZHDANOVA, N., VOGEL, W. & HAMEISTER, H. 1998. Zoo-Fish analysis in insectivores: "Evolution extols the virtue of the status quo". *Cytogenet. Cell Genet.* 80: 61-67.
- DOBIGNY, G., ANISKIN, V. & VOLOBOUEV, V. 2002. Explosive chromosome evolution and speciation in the gerbil genus *Taterillus* (Rodentia, Gerbillinae): a case of two new cryptic species. *Cytogenet. Genome Res.* 96: 117-124.
- DODA, J. N., WRIGHT, C. T. & CLAYTON, D. A. 1981. Elongation of displacement-loop strands in human and mouse mitochondrial DNA is arrested near specific template sequences. *Proc. Natl. Acad. Sci. USA* 78: 6116-6120.
- DUCROZ, J.-F., GRANJON, L., CHEVRET, P., DUPLANTER, J.-M., LOMBARD, M. & VOLOBOUEV, V. 1997. Characterisation of two distinct species of *Arvicanthis* (Rodentia: Muridae) in West Africa: cytogenetic, molecular and reproductive evidence. *J. Zool., Lond.* 241: 709-723.
- DUCROZ, J.-F., VOLOBOUEV, V. & GRANJON, L. 1998. A molecular perspective on the systematics and evolution of the genus *Arvicanthis* (Rodentia, Muridae): inferences from complete cytochrome *b* gene sequences. *Mol. Phylogenet. Evol.* 10: 104-117.
- DUCROZ, J.-F., GRANJON, L., LOMBARD, M. & VOLOBOUEV, V. 1999. Comparative chromosome analysis (R- and C-bands) of two South African murid species, *Lemniscomys rosalia* and *Rhabdomys pumilio* (Rodentia, Murinae). *Cytogenet. Cell Genet.* 87: 69-74.

- DUCROZ, J-F., VOLOBOUEV, V. & GRANJON, L. 2001. An assessment of the systematics of Arvicanthine rodents using mitochondrial DNA sequences: evolutionary and biographical implications. *J. Mammal. Evol.* 8: 173-206.
- DYER, K., & MEYNE, J. 1991. Molecular Cytogenetics: use of DNA probes as an adjunct to classical cytogenetics, In: *The association of Cytogenetics Technologists, Cytogenetics Laboratory manual*, (ed) M. J. Barch pp. 525-532, 2nd Edition. Raven Press Ltd, New York.
- EIZIRIK, MURPHY, W. J., & O'BRIEN, S. J. 2001. Molecular dating and biogeography of the early placental mammal radiation. *J. Hered.* 92: 212-219.
- EHRICH, D., FEDOROV, V. B., STENSETH, N. C., KREBS, C. J. & KENNEY, A. 2000. Phylogeography and mitochondrial DNA (mtDNA) diversity in North American collared lemmings (*Dicrostonyx groenlandicus*). *Mol. Ecol.* 9: 329-337.
- ELLERMAN, J. R. 1941. *The families and genera of living rodents*. British Museum. London.
- EVANS, E. P. 1996. Standard G-banded karyotype. In: *Genetic Variants and Strain of the Laboratory Mouse*, (eds) M. F. Lyon, A. G. Searle, pp. 1446-1449, Oxford University Press, Oxford.
- FAGUNDES, V., VIANNA-MORGANTE, A. M. & YONENAGA-YASSUDA, Y. 1997. Telomeric sequences localization and G-banding patterns in the identification of a polymorphic chromosomal rearrangement in the rodent *Akodon cursor* (2n = 14, 15 and 16). *Chromosome Res.* 5: 228-232.
- FEDOROV, V. B. & STENSETH, N. C. 2001. Glacial survival of the Norwegian lemming (*Lemmus lemmus*) in Scandinavia: inference from mitochondrial DNA variation. *Proc. R. Soc. Lond.* 268: 809-814.
- FELDHAMER, G. A., DRICKAMER, L. C., VESSEY, S. H. & MERRITT, J. F. 1999. *Mammalogy: adaptation, diversity, and ecology*. McGraw-Hill, Boston.
- FELSENSTEIN, J. 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. *J. Mol. Evol.* 17: 368-376.

- FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783-791.
- FERGUSON, J. W. H. 2002. On the use of genetic divergence for identifying species. *Biol. J. Linn. Soc.* 75: 509-516.
- FERGUSON-SMITH, M. A., YANG, F. & O'BRIEN, P. C. M. 1998. Comparative mapping using chromosome sorting and painting. *ILAR J.* 39: 68-76.
- FERNANDO, P., PFRENDER, M. E., ENCALADA, S. E. & LANDE R. 2000. Mitochondrial DNA variation, phylogeography and population structure of the Asian elephant. *Heredity* 84: 362-372.
- FLAGSTAD, O., SYVERTSEN, P. O., STENSETH, N. C., STACY, J. E., OLSAKER, I., ROED, K. H. & JAKOBSEN, K. S. 2000. Genetic variability in Swayne's hartebeest, an endangered antelope of Ethiopia. *Conservation Biol.* 14: 254-264.
- FORAN, D. R., HIXSON, J. E. & BROWN, W. M. 1988. Comparison of ape and human sequences that regulate mitochondrial DNA transcription and D-loop DNA synthesis. *Nucleic Acids Res.* 16: 5841-5861.
- FORSTER, L., FORSTER, P., LUTZ-BONENGEN, S., WILLKOMM, H. & BRINKMANN, B. 2002. Natural radioactivity and human mitochondrial DNA mutations. *Proc. Natl. Acad. Sci. USA.* 99: 13950-13954.
- FRONICKE, L., CHOWDHARY, B. P., SCHERTHAN, H. & GUSTAVSSON, I. 1996. A comparative map of the porcine and human genome demonstrates ZOO-FISH and gene mapping-based chromosomal homologies. *Mammal. Genome* 7: 285-290.
- FRONICKE, L., MULLER-NAVIA, J., ROMANAKIS, K. & SCHERTHAN, H. 1997. Chromosomal homeologies between human, harbor seal (*Phoca vitulina*) and the putative ancestral carnivore karyotype revealed by Zoo-Fish. *Chromosoma* 106: 108-113.
- GALL, J. G. & PARDUE, M. L. 1969. Formation and detection of RNA-DNA hybrid molecules in cytological preparations. *Genetics* 63: 378-383.

GARAGNA, S., BROCCOLI, D., REDI, C. A., SEARLE, J. B., COOKE, H. J. & CAPANNA, E. 1995. Robertsonian metacentrics of the house mouse lose telomeric sequences but retain some minor satellite DNA in the pericentromeric area. *Chromosoma* 103: 685-692.

GARAGNA, S., MARZILIANO, N., ZUCCOTTI, M., SEARLE, J. B., CAPANNA, E., & REDI, C. A. 2001. Pericentromeric organization at the fusion point of mouse Robertsonian translocation chromosomes. *Proc. Natl. Acad. Sci. USA* 98: 171-175.

GAVA, A. & DE FREITAS, T. R. O. 2002. Characterization of a hybrid zone between chromosomally divergent populations of *Ctenomys minutus* (Rodentia: Ctenomyidae). *J. Mammal.* 83: 843-851.

GEISE, L., SMITH, M. F. & PATTON, J. L. 2001. Diversification in the genus *Akodon* (Rodentia: Sigmodontinae) in southeastern South America: mitochondrial DNA sequence analysis. *J. Mammal.* 82: 92-101.

GEMMEL, N. J., WESTERN, P. S., WATSON, J. M. & MARSHALL GRAVES J. A. 1996. Evolution of the mammalian mitochondrial control region - comparisons of control region sequences between monotreme and therian mammals. *Mol. Biol. Evol.* 13: 798-808.

GILES, R. E., BLANC, H., CANN, H. M. & WALLACE, D. C. 1980. Maternal inheritance of human mitochondrial DNA. *Proc. Natl. Acad. Sci. USA* 77: 6715-6719.

GILLESPIE, J. H. 1986. Variability of evolutionary rates of DNA. *Genetics* 113: 1077-1091.

GILL, T., SMITH, G., WISSLER, R. & KUNZ, H. 1989. The rat as an experimental animal. *Science* 245: 269-276.

GOUREAU, A., YERLE, M., SCHMITZ, A., RIQUET, J., MILAN, D., PINTON, P. & GELLIN, J. 1996. Human and porcine correspondence of chromosome segments using bidirectional chromosome painting. *Genomics* 36: 252-262.

GULLISON, R. E., RICE, R. E., & BLUNDELL, A. G. 2000. Marketing species conservation. *Nature* 404: 923-924.

GRAPHODATSKY, A. S., YANG, F., O'BRIEN, P. C. M., PERELMAN, P., MILNE, B. S., SERDUKOVA, N., KAWADA, S. I. & FERGUSON-SMITH, M. A. 2001. Phylogenetic implications of the 38 putative ancestral chromosome segments for four canid species. *Cytogenet. Cell Genet.* 92: 243-247.

GRAUR, D., WINSTON, A. H. & LI, W.-H. 1991. Is the guinea-pig a rodent. *Nature* 351: 649-652.

GRAY, J. W., CARRANO, A. V., STEINMETZ, L. L., VAN DILLA, M. A., MOORE, D. H., MAYALL, B. H., & MENDELSON, M. L. 1975. Chromosome measurement and sorting by flow systems. *Proc. Natl. Acad. Sci. USA* 72: 1231-1234

GRIFFITHS, C. S. 1997. Correlation of functional domains and rates of nucleotide substitution in cytochrome b. *Mol. Phylogenet. Evol.* 7: 352-365.

GRUTZNER, F., HIMMELBAUER, H., PAULSEN, M., ROPERS, H.-H. & HAAF, T. 1999. Comparative mapping of mouse and rat chromosomes by fluorescence *in situ* hybridization. *Genomics* 55: 306-313.

GUILLY, M.-N., FOUCHET, P., DE CHAMISSO, P., SCHMITZ, A. & DUTRILLAUX, B. 1999. Comparative karyotype of rat and mouse using bidirectional chromosome painting. *Chromosome Res.* 7: 213-221.

GUNDUZ, I., COSKUN, T., MALIKOV, V., VAZIRI, A., POLYAKOV, A. V. & SEARLE, J. B. 2000. Mitochondrial DNA and chromosomal studies of wild mice (*Mus*) from Turkey and Iran. *Heredity* 84: 458-467.

GUNDUZ, I., AUFRAY, J.-C., BRITTON-DAVIDIAN, J., CATALAN, J., GANEM, G., RAMALHINHO, M. G., MATHIAS, M. L. & SEARLE, J. B. 2001. Molecular studies on the colonization of the Madeira archipelago by house mice. *Mol. Ecol.* 10: 2023-2029.

GUSTAVSSON, I. 1988. Reciprocal translocation in four boars producing decreased litter size. *Hereditas* 109: 159-168.

HALANYCH, K. M., DEMBOSKI, J. R., JANSEN VAN VUUREN, B., KLEIN, D. R. & COOK, J. 1999. Cytochrome b phylogeny of North American hares and jackrabbits (*Lepus*, Lagomorpha) and the effects of saturation in outgroup taxa. *Mol. Phylogenet. Evol.* 11: 213-221.

- HAPPOLD, D. C. D. 2001. Ecology of African small mammals: recent research and perspectives. In: *African Small Mammals*, (eds) C. Denys, L. Granjon, A. Poulet, pp. 377-414, IRD Editions, Paris.
- HARRIS, M. J., WALLACE, M. E. & EVANS, E. P. 1986. Aneuploidy in the embryonic progeny of females heterozygous for the Robertsonian chromosome (9.12) in genetically wild Peru-Coppock mice (*Mus musculus*). *J. Reprod. Fert.* 76: 193-203.
- HARRIS, D., ROGERS, D. S., & SULLIVAN, J. 2000. Phylogeography of *Peromyscus furvus* (Rodentia: Muridae) based on cytochrome *b* sequence data. *Mol. Ecol.* 9: 2129-2135.
- HASEGAWA, M., CAO, Y. & ADACH, J. 1992. Rodent polyphyly? *Nature* 355: 595.
- HAYES, H. 1995. Chromosome painting with human chromosome-specific DNA libraries reveals the extent and distribution of conserved segments in bovine chromosomes. *Cytogenet. Cell Genet.* 71: 168-174.
- HELOU, K., VALENTINSSON, A., LEVAN, G. & STAHL, F. 2001. Between rat and mouse Zoo-FISH reveals 49 chromosome segments that have been conserved in evolution. *Mammal. Genome* 12: 765-771.
- HENEGARIU, O., HEEREMA, N. A., WRIGHT, L. L., BRAY-WARD, P., WARD, D.C. & VANCE, G. H. 2001. Improvements in cytogenetic slide preparation: controlled chromosome spreading, chemical aging and gradual denaturing. *Cytometry* 43: 101-109.
- HEY, J. 2000. Human mitochondrial DNA recombination: can it be true? *Trends Ecol. Evol.* 15: 181-182.
- HILLIS, D. M., MABLE, B. K. & MORITZ, C. 1996. Applications of molecular systematics. In: *Molecular systematics* (eds) D. M. Hillis, B. K. Mable, and C. Moritz, pp. 515-544. Sinauer Associates, Sunderland, Massachusetts.
- HOELZEL, A. R., HANCOCK, J. M. & DOVER, G. A. 1991. Evolution of the cetacean mitochondrial D-loop region. *Mol. Biol. Evol.* 8: 475-493.

- HOOGSTRAAL, H. 1979. The epidemiology of tick-borne Crimean-Congo hemorrhagic fever in Asia, Europe, and Africa. *J. Med. Entomol.* 15: 307-417;
- HOLLOCHER, H., TING, C. T., WU, M. L. & WU, C-L. 1997a. Incipient speciation by sexual isolation in *Drosophila melanogaster*: extensive genetic divergence without reinforcement. *Genetics* 147: 1191-1201.
- HOLLOCHER, H., TING, C. T. & WU C-I. 1997b. Incipient speciation by sexual isolation in *Drosophila melanogaster*: variation in mating preference and correlation between sexes. *Evolution* 51: 1175-1181.
- HOUSEAL, T., COOK, J. A., MODI, W. S. & HALE, D. W. 1995. Identification of highly conserved loci by genome painting. *Chromosome Res.* 3: 175-181.
- HUCHON, D., CATZEFLIS, F. M. & DOUZERY, E. J. P. 1999. Molecular evolution of the nuclear von Willebrand factor gene in mammals and the phylogeny of rodents. *Mol. Biol. Evol.* 16: 577 - 589.
- HUCHON, D., MADSEN, O., SIBBALD, M. J. J. B., AMENT, K., STANHOPE, M. J., CATZEFLIS, F., DE JONG, W. W. & DOUZERY, E. J. P. 2002. Rodent phylogeny and a time scale for the evolution of Glires: evidence from an extensive taxon sampling using three nuclear genes. *Mol. Biol. Evol.* 19: 1053-1065.
- IANNUZZI, L., DI MEO, G. P., PERUCATTI, A. & BARDARO, T. 1998. ZOO-FISH and R-banding reveal extensive conservation of human chromosome regions in euchromatic regions of river buffalo chromosomes. *Cytogenet. Cell Genet.* 82: 210-214.
- IRWIN, D. M., KOCHER, T. D. & WILSON, A. C. 1991. Evolution of cytochrome *b* gene of mammals. *J. Mol. Evol.* 32: 128-144.
- ISCN. 1978. An international system for human cytogenetic nomenclature. Report of the Standing Committee on Human Cytogenetic nomenclature. *Cytogenet. Cell Genet.* 21: 309-404.
- JACOBS, L. L. & PILBEAM, D. 1980. Of mice and men: fossil based divergence dates and molecular "clocks". *J. Hum. Evol.* 9: 551-555.

- JAEGER, J. A., TURNER, D. & ZUKER, M. 1989. Improved predictions of secondary structures for RNA. *Proc. Natl. Acad. Sci. USA* 86: 7706-7710.
- JAEGER, J. A.. 1989. Predicting optimal and suboptimal secondary structures for RNA. *Methods Enzymol.* 183: 281-306.
- JANKE, A., GEMMEL, G., FELDMAIER-FUCHS, G., VON HAESLER, A. & PAABO, S. 1996. The mitochondrial genome of a monotreme - the platypus (*Ornithorhynchus anatinus*). *J. Mol. Evol.* 42: 153-159.
- JANSEN VAN VUUREN, B. & ROBINSON, T. J. 2001. Retrieval of four adaptive lineages in duiker antelope: evidence from mitochondrial DNA sequences and fluorescence *in situ* hybridization. *Mol. Phylogenet. Evol.* 20: 409-425.
- JOHNS, G. C. & AVISE, J. C. 1998. A comparative summary of genetic distances in the vertebrate from the mitochondrial cytochrome *b* gene. *Mol. Biol. Evol.* 15: 1481-1490.
- JOLLEY, T. W., HONEYCUTT, R. L. & BRADLEY, R. D. 2000. Phylogenetic relationships of pocket gophers (genus *Geomys*) based on the mitochondrial 12S rRNA gene. *J. Mammal.* 81: 1025-1034.
- KIMURA, M. 1968. Evolution rate at the molecular level. *Nature* 217: 624-626.
- KIMURA, M & OHTA, T. 1971. Protein polymorphism as a phase of molecular evolution. *Nature* 229: 467-469.
- KIMURA, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* 16: 111-120.
- KING, M. 1993. *Species evolution: The role of chromosome change*. University Press. Cambridge.
- KINGDON, J. 1974. *East African mammals: An Atlas of Evolution in Africa*. Academic Press. London.

KINGSWOOD, S. C., KUMAMOTO, A. T., CHARTER, S. J., AMAN, R. A. & RYDER, O. A. 1998. Centric fusion polymorphisms in waterbuck (*Kobus ellipsiprymnus*). *J. Heredity* 89: 96-99.

KOCHER, T. D., THOMAS, W. K., MEYER, A., EDWARDS, S. V., PAABO, S., VILLABLANCA, F. X. & WILSON, A. C. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proc. Natl. Acad. Sci.* 86: 6196-6200.

KOH, H. S., LEE, W-J. & KOCHER, T. D. 2000. The genetic relationships of two subspecies of striped field mice, *Apodemus agrarius coreae* and *Apodemus agrarius chejuensis*. *Heredity* 85: 30-36.

KOLNICKI, R. 2000. Kinetochore reproduction in animal evolution: Cell biological explanation of karyotypic fission theory. *Proc. Natl. Acad. Sci. USA.* 97: 9493-94-97.

KORSTANJE, R., O'BRIEN, P. C. M., YANG, F., RENS, W., BOSM, A. A., LITH, H. A., VAN ZUTPHEN, L. F. M. & FERGUSON-SMITH, M. A. 1999. Complete homology maps of the rabbit (*Oryctolagus cuniculus*) and human by reciprocal chromosome painting. *Cytogenet. Cell Genet.* 86: 317-322.

KOOP, B. F., BAKER, R. J., HAIDUK, M. W. & ENGSTROM, M. D. 1984. Cladistic analysis of primitive G-band sequence for the karyotype of the ancestor of the Cricetidae complex of rodents. *Genetica* 64: 199-208.

KURAMOTO, M. 1984. Systematic implication of hybridization experiments with some Eurasian tree frogs (genus *Hyla*). *Copeia* 1984: 609-616.

LANDE, R. 1985. The fixation of chromosomal rearrangements in a subdivided population with local extinction and colonization. *Heredity* 54: 323-332.

LECOMPTE, A., DENYS, C. & GRANJON, L. 2001. An identification key for species within the genus *Praomys* (Rodentia: Muridae). In: *African Small Mammals*. (eds) C. Denys, L. Granjon, A. Poulet, pp. 127-139, IRD Editions, Paris.

LEE, M. R. & ELDER, F. F. B. 1980. Yeast stimulation of bone marrow mitosis for cytogenetic investigations. *Cytogenet. Cell Genet.* 26: 36-40.

- LEE, C., SASI, R. & LIN, C. C. 1993. Interstitial localization of telomeric DNA sequences in the Indian muntjac chromosomes: further evidence for tandem chromosome fusions in the karyotypic evolution of the Asian muntjacs. *Cytogenet. Cell Genet.* 63: 156-159.
- LI, W., GOUY, M., SHARP, P. M., O'HUIGIN, C. & YANG, Y. 1990. Molecular phylogeny of Rodentia, Lagomorpha, Primates, Artiodactyla, and Carnivora and molecular clocks. *Proc. Natl. Acad. Sci. USA.* 87: 6703-6707.
- LI, W.-H., HIDE, W. A. & GRAUR, D. 1992. Origin of rodents and guinea pigs *Nature* 359: 277-278.
- LICHTER, P. & WARD, D. C. 1990. Is nonisotopic hybridization finally coming of age? *Nature* 345: 93-94.
- LIMING, L., AND YE, Y. & DUAN, X. 1980. Comparative cytogenetic studies on the red muntjac, Chinese muntjac and their F1 hybrids. *Cytogenet. Cell Genet.* 26: 22-27.
- LIMING, S. & PATHAK, S. 1981. Gametogenesis in a male Indian muntjac x Chinese muntjac hybrid. *Cytogenet. Cell Genet.* 30: 152-156.
- LIN, C., C., SASI, R., FAN, Y., -S., & CHEN, Z -Q. 1991. New evidence for tandem chromosome fusions in the karyotypic evolution of Asian muntjacs. *Chromosoma* 101: 19-24
- LINDESAY, J. 1998. Past Climates of Southern Africa. In: *Climates of the southern Continent: Present, Past and Future*, (eds) J. E. Hobbs, J. A. Lindesay, H. A. Bridgman, pp. 161-206, John Wiley & Sons Ltd. New York.
- LUCKET, W. P. & HARTENBERGER, J.-L. 1993. Monophyly or polyphyly of the order Rodentia: possible conflict between morphological and molecular interpretations. *J. Mamm. Evol.* 1: 127-147.
- LYONS, L. A., LAUGHLIN, T. F., COPELAND, N. G., JENKINS, N. A., WOMACK, J.E. & O'BRIEN, S. J. 1997. Comparative anchor tagged sequences (CATS) for integrative mapping of mammalian genome. *Nat. Genet.* 15:47-56.

- MADDISON, W. P., & MADDISON, D. R. 1992. *McClade: Analysis of phylogeny and character evolution*, version v3.04 Sinauer, Sutherland, MA.
- MADSEN, O., SCALLY, M., DOUDY, C. J., KAO, D. T., DEBRY, R. W., ADKINS, R., AMRINE, H., STAMHOPE, M. J., DE JONG, W. W. & SPRINGER, M. S. 2001. Parallel adaptive radiation in two major clades of placental mammals. *Nature* 409: 610-614.
- MAGANO, S. R., ELS, D. A. & CHOWN, S. L. 2000. Feeding patterns of immature stages of *Hyalomma truncatum* and *Hyalomma marginatum rufipes* on different hosts. *Exp. Appl. Acarol.* 24: 301-313.
- MAHIDA, H., CAMPBELL, G. K. & TAYLOR, P. J. 1999. Genetic variation in *Rhabdomys pumilio* (Sparman 1784) - an allozyme study. *S. Afr. J. Zool.* 34: 91-101.
- MANIATIS, T., FRITSCH, E. F. & SAMBROOK, J. 1982. *Molecular cloning: A laboratory manual*. Cold Spring Harbor Laboratory, USA.
- MARSHAL GRAVES, J. A. M. 1998. Background and overview of comparative genomics. *ILAR J.* 39: 48-65.
- MARSHALL, H. D. & RITLAND, K. 2002. Genetic diversity and differentiation of Kermode bear populations. *Mol. Ecol.* 11: 685-697.
- MARGOLIASH, E. 1963. Primary structure and evolution of cytochrome *c*. *Proc. Natl. Acad. Sci. USA.* 50: 672-679.
- MARTIGNETTI, J. A. & BROSIUS, J. 1993. Neural BC1 RNA as an evolutionary marker; guinea pig remains a rodent. *Proc. Natl. Acad. Sci. USA.* 90: 9698-9702.
- MARTIN, A. P. & PALUMBI, S. R. 1993. Body size, metabolic rate, generation time, and the molecular clock. *Proc. Natl. Acad. Sci. USA* 90: 4087-4091.
- MASCARELLO, J. T., STOCK, A. D. & PATHAK, S. 1974. Conservatism in the arrangement of genetic material in rodents. *J. Mammal.* 55: 695-704.
- MASUKATA, H. & TOMIZAWA, J. 1984. Effects of point mutations on formation and structure of the RNA primer for ColE1 DNA replication. *Cell* 36: 513-522.

- MATSON, C. W & BAKER, R. K. 2001. DNA sequence variation in the mitochondrial control region of red-backed voles (*Clethrionomys*). *Mol. Biol. Evol.* 18: 1494-1501.
- MAYR, E. 1963. *Animal species and evolution*. McGraw-Hill Press, New York.
- MAYR, E. & ASHLOCK, P. B. 1991. *Principles of systematic Biology*. New York: McGraw-Hill Inc.
- MEESTER, J., RAUTENBACH, I. L., DIPPENAAR, N. J. & BAKER, C. M. 1986. Classification of southern African mammals. *Transvaal Mus. Monogr.* 5:1-359.
- MEYER, A. 1994. Shortcomings of the cytochrome *b* gene as a molecular marker. *Trends Ecol. Evol.* 9: 278-280.
- MICHAUX, J. & CATZEFLIS, F. 2000. The bushlike radiation of muroid rodents is exemplified by the molecular phylogeny of the LCAT nuclear gene. *Mol. Phylogenet. Evol.* 17: 280-293.
- MIGNOTTE, F., GUERIDE, M., CHAMPAGNE, A. M. & MOUNOLOU, J.-C. 1990. Direct repeats in the non-coding region of rabbit mitochondrial DNA: involvement in the generation of intra- and inter-individual heterogeneity. *Eur. J. Biochem.* 194: 561-571.
- MISSONE, X. 1969. African and Indo-Australian Muridae. Evolutionary trends. *Mus. Roy. l'Afrique Cent., Tervuren, Zool.*, 172: 1-219.
- MORITZ, C., DOWLING, T. E. & BROWN, W. M. 1987. Evolution of animal mitochondrial DNA: relevance for population biology and systematics. *Ann. Rev. Ecol. Syst.* 18: 269-292.
- MORITZ, C. 1994. Defining 'evolutionary significant units' for conservation. *Trends Ecol. Evol.* 10: 373-375.
- MULLER, S., STANYON, R., O'BRIEN, P. C., FERGUSON-SMITH, M. A., PLESKER, R. & WIENBERG, J. 1999. Defining the ancestral karyotype of all primates by multidirectional chromosome painting between tree shrew, lemurs and humans. *Chromosoma* 108: 393-400.

- MULLIS, K. B. & FALOONA, F. A. 1987. Specific synthesis of DNA *in vitro* via a polymerase catalyzed chain reaction. *Meth. Enzymol.* 155: 335-350.
- MURPHY, W. J., EIZIRIK, E., O'BRIEN, S. J., MADSEN, O., SCALLY, M., DOUADY, C. J., TEELING, E., RYDER, O. A., STANHOPE, M. J., DE JONG, W. W. & SPRINGER, M. S. 2001a. Resolution of the early placental mammal radiation using Bayesian phylogenetics. *Science* 294: 2348-2351.
- MURPHY, W. J., EIZIRIK, E., JOHNSON, W. E., ZHANG, Y. P., RYDER, O. & O'BRIEN, S. J. 2001b. Molecular phylogenetics and the origins of placental mammals. *Nature* 409: 614-618.
- MURPHY, W. J., STANYON, R., & O'BRIEN, S. J. 2001c. Evolution of mammalian genome organization inferred from comparative gene mapping. *Genome Bio.* 2: 1-8.
- MUSSER, G. G. 1987. The occurrence of *Hydromys* (Rodentia: Muridae) in early Pleistocene Siwalik strata in northern Pakistan and its bearing on biogeographic affinities between Indian and Northeastern African murine faunas. *Am. Mus. Nov.* 10024: 1-36.
- MUSSER, G. D & CARLETON, M. D. 1993. Family Muridae. In: *Mammal Species of the world: A Taxonomic and Geographic Reference*, (eds) D. E. Wilson, D. M. Reeder, pp.501-755, Smithsonian Institution Press, Washington, DC.
- MYERS, N., MITTERMEIER, R. A, MITTERMEIER, C. G., DA FONSECA, G. A. B. & KENT, J. 2000. Biodiversity hotspots for conservation priorities. *Nature* 403: 853-858.
- NACHMAN, M., BOYER, S. N., SEARLE, J. B. & AQUADRO, C. F. 1994. Mitochondrial DNA variation and the evolution of Robertsonian chromosomal races of house mice, *Mus domesticus*. *Genetics* 136: 1105-1120.
- NADEAU J. H. & TAYLOR, B. A. 1984. Lengths of chromosomal segments conserved since divergence of man and mouse. *Proc. Natl. Acad. Sci. USA* 81: 814-818.
- NASH, W. G., WIENBERG, J., FERGUSON-SMITH, M. A., MENNINGER, J. C. & O'BRIEN, S. J. 1998. Comparative genomics: tracking chromosome evolution in the family Ursidae using reciprocal chromosome painting. *Cytogenet. Cell Genet.* 83: 182-192.

- NEVO, E. 1978. Genetic variation in natural populations: patterns and theory. *Theoret. Pop. Biol.* 13: 121-177.
- NIE, W., WANG, J., O'BRIEN, P. C. M., FU, B., YING, T., FERGUSON-SMITH, M. A. & YANG, F. 2002. The genome phylogeny of domestic cat, red panda and five mustelid species revealed by comparative chromosome painting and G-banding. *Chromosome Res.* 10: 209-222.
- NOWAK, R. M. 1999. Order Rodentia. In: *Walker's mammals of the world*. Vol II, Sixth Edition, pp. 1243-1712, The Johns Hopkins University Press. Baltimore and London.
- NOOR, M. A., GRAMS, K. L., BERTUCCI, L. A. & REILAND, J. 2001. Chromosomal inversions and the reproductive isolation of species. *Proc. Natl. Acad. Sci. USA.* 98: 12084-12088.
- O'BRIEN, S. J. & STANYON, R. 1999. Ancestral primate viewed. *Nature* 402: 365-366.
- OVERBEEK, P. A., GORLOV, I. P., SUTHERLAND, R. W., HOUSTON, J. B., HARRISON, W. R., BOETTGER-TONG, H. L., BISHOP, C. E. & AGOULNIK, A. L. 2001. A transgenic insertion causing cryptorchidism in mice. *Genesis* 30: 26-35.
- PAABO, S. & WILSON, A. C. 1988. Polymerase chain reaction reveals cloning artifacts. *Nature* 334: 387-388.
- PAGE, S. L., SHIN, J., HAN, J., ANDY CHOO, K. H. & SHAFFER, L. G. 1996. Breakpoint diversity illustrates distinct mechanisms for Robertsonian translocation formation. *Human Mol. Genet.* 5: 1279-1288.
- PASCALE, E., VALLE, E. & FURANO, A. 1990. Amplification of an ancestral mammalian L1 family of long interspersed repeated DNA occurred just before the murine radiation. *Proc. Natl. Acad. Sci. USA.* 87: 9481-9485.
- PATRIDGE, T. C., WOOD, B. A. & DE MENCAL, P. B. 1995. The influence of global climatic change and regional uplift on large-mammalian evolution in east and southern Africa. In: *Paleoclimate and Evolution with Emphasis on Human Origins*, (eds) E. S. Vrba, Denton, G. H., Partridge T. C. and Burckle, L. H., pp. 385-424. Yale University Press, London.

- PEPPERS, L. L. & BRADLEY, R. 2000. Cryptic species in *Sigmodon hispidus*: evidence from DNA sequences. *J. Mammal.* 81: 332-343.
- PERRIN, M. R. 1980. The feeding habits of two co-existing rodents, *Rhabdomys pumilio* (Sparman, 1784) and *Otomys irroratus* (Brants, 1827). *Acta Oecol. Gener.* 1: 71-89.
- PHILIPPE, H. 1997. Rodent monophyly: pitfalls of molecular phylogenies. *J. Mol. Evol.* 45: 712-715.
- PIALEK, J., HAUFFE, H. C., RODRIGUEZ-CLARK, K. M. & SEARLE, J. B. 2001. Racialization and speciation in house mice from the Alps: the role of chromosomes. *Mol. Ecol.* 10: 613-625.
- PILLAY, N. 2000a. Reproductive isolation in three populations of the striped mouse *Rhabdomys pumilio*: interpopulation breeding studies. *Mammalia* 64: 461-470.
- PILLAY, N. 2000b. Female mate preference and reproductive isolation in populations of the striped mouse *Rhabdomys pumilio*. *Behaviour* 137: 1431-1441.
- PINKEL, D., LANDEGENT, J., COLLINS, C., FUSCOE, J., SEGRAVES, R., LUCAS, J., & GRAY, J. W. 1988. Fluorescence *in situ* hybridization with human chromosome specific libraries: detection of trisomy 21 and translocation of chromosome 4. *Proc. Natl. Acad. Sci. USA* 85: 9138-9142.
- POCOCK, T. N. 1987. Plio-pleistocene fossil mammalian microfauna of southern Africa - A preliminary report including description of two new fossil muroid genera (Mammalia: Rodentia). *Palaeont. afr.* 26: 69-91.
- POSADA, D. & CRANDALL, K. A. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817-818.
- QUMSIYEH, M. B., COATE, J. L., PEPPERS, J. A., KENNEDY, P. K. & KENNEDY, M. L. 1997. Robertsonian chromosomal rearrangements in the short-tailed shrew, *Blarina carolinensis*, in western Tennessee. *Cytogenet. Cell Genet.* 76: 153-158.

RABBITS, P., IMPLY, H., HEPPELL-PARTON, A., LANGFORD, C., TEASE, C., LOWE, N., BAILEY, D., FERGUSON-SMITH, M. & CARTER, N. 1995. Chromosome specific paints from a high resolution flow karyotype of the mouse. *Nat. Genet.* 9: 369-375.

RAMBAU, R. V., HARRISON, W. R., ELDER, F. F. B. & ROBINSON, T. J. 1997. Chromosomes of Brants' Whistling rat and genome conservation in the Otomyinae revealed by G-banding and fluorescence *in situ* hybridization. *Cytogenet. Cell Genet.* 78: 216-220.

RAMBAU, R. V. 1998. Genome conservation in the Otomyinae revealed by chromosome banding and fluorescence *in situ* hybridization (FISH). MSc. Dissertation. University of Pretoria, Pretoria.

RAMBAU, R. V., ELDER, F. F. B. & ROBINSON, T. J. 2001. Chromosome evolution in the vlei rat, *Otomys irroratus* (Muridae: Otomyinae): a compound chromosomal rearrangement separates two major cytogenetic groups. *Cytogenet. Cell Genet.* 93: 253-257.

RAMBAU, R. V. & ROBINSON, T. J. (In press). Molecular genetics of *Rhabdomys pumilio* subspecies boundaries: MtDNA phylogeography and karyotypic analysis by fluorescence *in situ* hybridization (FISH). *Mol. Phylogenet. Evol.*

RAMBAU, R. V. & ROBINSON, T. J. (In press). Chromosome painting in the African four-striped mouse *Rhabdomys pumilio*: detection of possible murid specific contiguous segment combinations. *Chromosome Res.*

RAMBAUT, A. & BROMHAM, L. 1998. Estimating divergence dates from molecular sequences. *Mol. Biol. Evol.* 15: 442-448.

RANDI, E., MUCCI, N., CLARO-HERGUETA, F., BONNET, A. & DOUZERY, E. 2001. A mitochondrial DNA control region phylogeny of the Cervinae: speciation in *Cervus* and implications for conservation. *Animal Conserv.* 4: 1-11.

RAUDSEPP, T., FRONICKE, L., SCHERTHAN, H., GUSTAVSSON, I. & CHOWDHARY, B. P. 1996. Zoo-Fish delineates conserved chromosomal segments between horse and man. *Chromosome Res.* 4: 218-225.

RAUDSEPP, T., KIJAS, J., GODARD, S., GUERIN, G., ANDERSSON, L. & CHOWDHARY, B. P. 1999. Comparison of horse chromosome 3 with donkey and human chromosomes by cross-species painting and heterologous FISH mapping. *Mamm. Genome* 10: 277-282.

RAUDSEPP, T. & CHOWDHARY, B. P. 2001. Correspondence of human chromosomes 9, 12, 15, 16, 19 and 20 with donkey chromosomes refines homology between horse and donkey karyotypes. *Chromosome Res.* 9: 623-629.

REICHMAN, J. 1998. Handbook of Optical filters for fluorescence microscopy. Chroma Technology Corp.

RENS, W., O'BRIEN, P.C.M., YANG F., SOLANSKY, N., PERELMAN, P., GRAPHODATSKY, M. W., FERGUSON, M. W. J., SVARTMAN, M., DE LEO, A. A., MARSHAL GRAVES, J. A. M. & FERGUSON-SMITH, M. A. 2001. Karyotypic relationships between distantly related marsupials from South America and Australia. *Chromosome Res.* 9: 301-308.

REYES, A., GISSI, C., PESOLE, G., CATZEFLIS, F. M. & SACCONI, C. 2000. Where do rodents fit? Evidence from the complete mitochondrial genome of *Sciurus vulgaris*. *Mol. Biol. Evol.* 17: 979-983.

RIDDLE, B. R., HAFNER, D. J. & ALEXANDER, L. F. 2000. Phylogeography and systematics of the *Peromyscus eremicus* species group and the historical biogeography of North American warm regional deserts. *Mol. Phylogenet. Evol.* 17: 145-160.

RIED, T., SCHROCK, E., NING, Y. & WIENBERG, J. 1998. Chromosome painting: a useful art. *Human Mol. Genet.* 79: 1619-1626.

ROBERTS, A. 1951. *The mammals of South Africa*. The mammals of South Africa Book Fund, Johannesburg.

ROBINSON, M., GOUY, M., GAUTIER, C. & MOUCHIROUD, D. 1998. Sensitivity of the relative-rate test to taxonomic sampling. *Mol. Biol. Evol.* 15: 1091-1098.

ROBINSON-RECHAVI, M. & HUCHON, D. 2000. RRTree: Relative-Rate Tests between groups of sequences on a phylogenetic tree. *Bioinformatics* 16: 296-297.

ROBINSON, T. J., WILSON, V., GALLAGHER, JR, D. S., TAYLOR, J. F., DAVIS, S. K., HARRISON, W. R. & ELDER, F. F. B. 1996. Chromosomal evolution in duiker antelope (Cephalophinae: Bovidae): karyotype comparisons, fluorescence *in situ* hybridization, and rampant X chromosome variation. *Cytogenet. Cell. Genet.* 73: 116-122.

ROBINSON, T. J. & MATTHEE, C. A. 1999. Molecular genetic relationships of the extinct ostrich, *Struthio camelus syriacus*: consequences for ostrich introductions into Saudi Arabia. *Animal Conserv.* 2: 165-171.

ROBINSON, T. J. 2001. The comparative cytogenetics of African small mammals in perspective. In: *African Small Mammals*, (eds) C. Denys, L. Granjon, A. Poulet, pp. 185-214, IRD Editions, Paris.

ROBINSON, T. J., YANG, F. & HARRISON, W. R. 2002. Chromosome painting refines the history of genome evolution in hares and rabbits (order Lagomorpha). *Cytogenet. Genet. Genome Res.* 96: 223-227.

ROKAS, A., & HOLLAND, P. W. H. 2000. Rare genomic changes as a tool for phylogenetics. *Trends Ecol. Evol.* 15: 454-459.

ROURKE, J. & WIENS, D. 1977. Convergent floral evolution in South Africa and Australian Proteaceae and its possible bearing on pollination by non-flying mammals. *Ann. Missouri. Bot. Gard.* 64: 1-17.

ROWE-ROWE, D. T & MEESTER, J. 1982a. Population dynamics of small mammals in the Drakensberg of Natal, South Africa. *Z. Saugetierkunde* 47: 347-356.

ROWE-ROWE, D. T. & MEESTER, J. 1982b. Habitat preferences and abundance relations of small mammals in the Natal Drakensberg. *S. Afr. J. Zool.* 17: 202-209.

ROWE-ROWE, D. T. 1986. Stomach contents of small mammals from the Drakensberg, South Africa. *S. Afr. J. Wildl. Res.* 16: 32-35.

RYDER, O. A. 1986. Species conservation and systematics: the dilemma of subspecies. *Trends Ecol. Evol.* 1: 9-10.

RYDER, O. A., SHAW, J. H. & WEMMER, C. M. 1988. Species, subspecies and *ex situ* conservation. *Int. Zoo. Yb.* 27: 134-140.

SACCONE, C., ATTIMONELLI, M. & SBISA, E. 1987. Structural elements highly preserved during the evolution of the D-loop containing region in vertebrate mitochondrial DNA. *J. Mol. Evol.* 26: 205-211.

SACCONNE, C., PESOLE, G. & SBISA, E. 1991. The main regulatory region of mammalian mitochondrial DNA: structure-function model and evolutionary pattern. *J. Mol. Evol.* 33: 83-91.

SAITOU, N. & NEI, M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4: 406-425.

SARICH, V. M. & WILSON, A. C. 1973. Generation time and genomic evolution in primates. *Science* 179: 1144-1147.

SBISA, E., TANZARIELLO, F., REYES, A., PESOLE, G. & SACCONE, C. 1997. Mammalian mitochondrial D-loop region structural analysis: identification of new conserved sequences and their functional and evolutionary implications. *Gene* 205: 125-140

SCALENENGHE, R., TURCO, E., EDSTROM, J. E., PIRROTTA, V. & MELLI, M. 1981. Microdissection and cloning of DNA from a specific region of *Drosophila melanogaster* polytene chromosomes. *Chromosoma* 84: 205-216.

SCALZI, J. M. & HOZIER, J. C. 1998. Comparative genome mapping: mouse and rat homologies revealed by fluorescence *in situ* hybridization. *Genomics* 47: 44-51.

SCHERTHAN, H., CREMER T, ARNASON U., WEIER, H., LIMA-DE-FARIA, A. & FRONICKE, L. 1994. Comparative chromosome painting discloses homologous segments in distantly related mammals. *Nat. Genet.* 6: 342-347.

SCHLOTTERER, C., AMOS, B. & TAUTZ, D. 1991. Conservation of polymorphic simple sequence loci in cetacean species. *Nature* 354: 63-65.

SCHNEIDER, S., ROESSLI, D. & EXCOFFIER, L. 2000. Arlequin: A software for population genetics data analysis. version 2.0. Genetics and Biometry Laboratory, Department of Anthropology, University of Geneva.

- SCHWARZACHER, H. G. & WOLF, U. & PASSARGE, E. 1974. *Methods in human genetics*. Springer-Verlag, Berlin.
- SEABRIGHT, M. 1971. A rapid banding technique for human chromosomes. *Lancet* 2:971-972.
- SEARLE, J. B. 1986. Meiotic studies of Robertsonian heterozygotes from natural populations of the common shrew, *Sorex araneus* L. *Cytogenet. Cell Genet.* 41: 154-162.
- SEARLE, J. B. 1998. Speciation, chromosomes, and genomes. *Genome Res.* 8: 1-3.
- SHENBROT, G. & KRASNOV, B. 2001. Geographic variation in the role of gerbils and jirds (Gerbillinae) in rodent communities across the great Palaearctic desert belt. In: *African Small Mammals*, (eds) C. Denys, L. Granjon, A. Poulet, pp. 512-529, IRD Editions, Paris.
- SHEPHERD, A. J. & LEMA, P. A. 1985. Bacterial surveillance of South African rodents. *S. Afr. J. Sci.* 81: 302-308.
- SIMPSON, G. C. 1945. The principles of classification and a classification of the mammals. *Bull. Am. Mus. Nat. Hist.* 85: 1-350.
- SKINNER, J. D. & SMITHERS, R. H. N. 1990. *The mammals of the southern African sub-region*. University of Pretoria, Pretoria.
- SMITH, S. 1990. Cytosystematic evidence against monophyly of the *Peromyscus boylii* species group (Rodentia: Cricetidae). *J. Mammal.* 71: 654-667.
- SMITH, M. F. & PATTON, J. L. 1993. The diversification of South American murid rodents: evidence from mitochondrial DNA sequence data for the Akodontine tribe. *Biol. J. Linn. Soc.* 50: 149-177.
- SMITH, M. F. & PATTON, J. L. 1999. Phylogenetic relationships and the radiation of Sigmodontine rodents in South America: Evidence from cytochrome *b*. *J. Mammal. Evol.* 6: 89-128.

- SOLINA-TOLIDO, S., LENGAUER, C. & FRIES, R. 1995. Comparative genome map of human and cattle. *Genomics* 27: 489-496.
- SOUTHERN, S. O., SOUTHERN, P. J. & DIZON, A. E. 1988. Molecular characterisation of a cloned dolphin mitochondrial genome. *J. Mol. Evol.* 28: 32-42.
- SPRINGER, M. S., BEBRY, R. W., DOUADY, C., AMRINE, H. M., MADSEN, O., DE JONG, W. W. & STANHOPE, M. J. 2001. Mitochondrial versus nuclear gene sequences in deep-level mammalian phylogeny reconstruction. *Mol. Biol. Evol.* 18: 132-143.
- STANYON, R., ARNOLD, N., KOEHLER, U., BIGONI, F. & WIENBERG, J. 1995. Chromosomal painting shows that "marked chromosomes" in lesser apes and Old World monkeys are not homologous and evolved by convergence. *Cytogenet. Cell Genet.* 68: 74-78.
- STANYON, R., YANG, F., CAVAGNA, P., O'BRIEN, P. C. M., BAGGA, M., FERGUSON-SMITH, M. A. & WIENBERG, J. 1999. Reciprocal chromosome painting shows that genomic rearrangement between rat and mouse proceeds ten times faster than between humans and cats. *Cytogenet. Cell Genet.* 84: 150-155.
- STEWART, D. T. & BAKER, A. J. 1994a. Evolution of mtDNA D-loop sequences and their use in phylogenetic studies of shrews in the subgenus *Otisorex* (*Sorex* : Soricidae: Insectivora). *Mol. Phylogent. Evol.* 3: 38-46.
- STEWART, D. T. & BAKER, A. J. 1994b. Patterns of sequence variation in the mitochondrial D-loop region of shrews. *Mol. Biol. Evol.* 11: 9-21.
- STIEMIE, S. & NEL, J. A. J. 1973. Nest-building behaviour in *Aethomys chrysophilus*, *Praomys (mastomys) natalensis* and *Rhabdomys pumilio*. *Zoologica Africana* 8: 91-100.
- SULLIVAN, J. & SWOFFORD, D. L. 1997. Are guinea pigs rodents? The importance of adequate models in molecular phylogenetics. *J. Mammal. Evol.* 4: 77-86.
- SULLIVAN, J., MARKERT, J. A. & KILPATRICK, C. W. 1997. Phylogeography and molecular systematics of the *Peromyscus aztecus* species group (Rodentia: Muridae) inferred using parsimony and likelihood. *Syst. Biol.* 46: 426-440.

- SUMNER, A. T. 1972. A simple technique for demonstrating centromeric heterochromatin. *Expl. Cell Res.* 75: 304-306.
- SVARTMAN, M. & VIANNA-MORGANTE, A. M. 1998. Karyotypic evolution of marsupials: from higher to lower diploid numbers. *Cytogenet. Cell Genet.* 82: 263-266.
- SWANEPOEL, R., STRUTHERS, J. K., SHERPERD, A. J., MCGILLIVRAY, G. M., NEL, M. J. & JUPP, P. G. 1983. Crimean-Congo Haemorrhagic fever in South Africa. *Am. J. Trop. Med. Hyg.* 32: 1407-1415.
- SWOFFORD, D. L. 2001. *PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods), Version 4b6* Sinauer Associates, Sunderland, MA.
- SZPIRER, C., SZPIRER, J., KLINGA-LEVAN, K., STAHL, F. & LEVAN, G. 1996. The rat: an experimental animal in search of a genetic map. *Folia Biol.* 42: 175-226.
- TABERLET, P. 1996. The use of mitochondrial DNA control region sequencing in conservation genetics. In: *Molecular genetic approaches in conservation*, (eds) T. B. Smith, R. K. Wayne, pp. 125-142, Oxford university Press. Oxford 1996.
- TAYLOR, P. 1998. *The smaller mammals of Kwazulu-Natal*. University of Natal Press. Pietermaritzburg, South Africa.
- TAYLOR, P. J. 2000. Patterns of chromosomal variation in southern African rodents. *J. Mammal* 81: 317-331.
- TAYLOR, S. & PERRIN, M. R. 1996. Identification of the rodent species involved in tree damage in commercial forestry in the Natal midlands, South Africa. *Mammalia* 60: 767-773.
- TELENIUS, H., PELMEAR, A. H., TUNNAcliffe A., CARTER, N. P., BEHMEL, A., FERGUSON-SMITH M. A., NORDENSKJOLD, M., PFRANGER, R. & PONDER, B. 1992. Cytogenetic analysis by chromosome painting using DOP-PCR amplified flow-sorted chromosomes. *Genes Chromosomes Cancer* 4: 257-263.
- THACKERAY, J. F. 1987. Late quaternary environmental changes inferred from small mammalian fauna, southern Africa. *Climatic Change* 10: 285-305.

THOMPSON, J. D., HIGGENS, D. G. & GIBSON, T. J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties, and weight matrix choice. *Nuc. Acids Res.* 22: 4673-4680.

TILLEY, S. G. VERRELL, P. A. & ARNOLD, S. J. 1990. Correspondence between sexual isolation and allozyme differentiation: a test in the salamander *Desmognathus ochriphaeus*. *Proc. Natl. Acad. Sci. USA.* 87: 2715-2719.

TODD, N. B. 1970. Karyotypic fission and canid phylogeny. *J. Theor. Biol.* 26: 380-445.

TOLLEY, K. A., ROSEL, P., WALTON, M., BJORGE, A. & OIEN, N. 1999. Genetic population structure of harbour porpoises (*Phocoena phocoena*) in the North Seas and Norwegian waters. *J. Cetacean Res. Manage.* 1: 265-274.

TOLLEY, K. A., VIKINGSSON, G. A. & ROSEL, P. E. 2001. Mitochondrial DNA sequence variation and phylogeographic patterns in harbour porpoises (*Phocoena phocoena*) from the North Atlantic. *Conserv. Genetics* 2: 349-361.

TULLBERG, T. 1899. Ueber das system der nagetiere: Eine phylonetische studie. *Nov. Acat Reg. Soc. Sci. Uppsala Ser. 3* 18: 1-514.

VAN TETS, I. & NICOLSON, S. W. 2001. The nutritional ecology of rodent pollinators of *Protea* in South Africa. In: *African Small Mammals*, (eds) C. Denys, L. Granjon, A. Poulet, pp. 311-320, IRD Editions, Paris, 2001.

VAN ZINDEREN BAKKER, E. M. 1986. African climates and palaeoenvironments since Messian times. *S. Afr. J. Sci.* 82: 70-71.

VASSART, M., SEQUELA, A. & HAYES, H. 1995. Chromosomal evolution in Gazelles. *J. Heredity.* 86: 216-227.

VERHEYEN, E., COLYN, M. & VERHEYEN, W. 1995. The phylogeny of some African muroids (Rodentia) based upon partial mitochondrial cytochrome b sequences. *Belg. J. Zool.* 125: 403-407.

- VIGILANT, L., STONEKING, M., HARPENDING, H., HAWKES, K. & WILSON, A. C. 1991. African populations and the evolution of human mitochondrial DNA. *Science* 253: 1503-1507.
- VOLOBOUEV, V., SICARD, B., ANISKIN, V. M., GAUTUN, J. C. & GRANJON, L. 2000. Robertsonian polymorphism, B chromosomes variation and sex chromosomes heteromorphism in the African water rat *Dasymys* (Rodentia, Muridae). *Chromosome Res.* 8: 689-697.
- WALBERG, M. W. & CLAYTON, D. A. 1981. Sequence and properties of the human KB cell and mouse L cell D-loop regions of mitochondrial DNA. *Nucleic Acids Res.* 9: 5411-5421.
- WALKER, C. W., VILA, C., LANDA, A., LINDEN, M. & ELLEGREN, H. 2001. Genetic variation and population structure in Scandinavian wolverine (*Gulo gulo*) populations. *Mol. Ecol.* 10: 53-63.
- WALLIS, G. P. 1999. Do animal mitochondrial genomes recombine? *Trends Ecol. Evol.* 14: 209-210.
- WALSH, J. M. 1982. Rate of accumulation of reproductive isolation by chromosome rearrangements. *Am. Nat.* 120: 510-532.
- WERGER, M. J. A. 1978. *Biogeography and ecology of Southern Africa*. Dr. W. Junk Publishers. The Hague.
- WIENBERG, J., & STANYON, R. 1997. Comparative painting of mammalian chromosomes. *Curr. Opin. Genet. Develop.* 7: 784-791.
- WIENBERG, J., STANYON, R., NASH, W. G., O'BRIEN, P. C. M., YANG, F., O'BRIEN, S. J. & FERGUSON-SMITH, M. A. 1997. Conservation of human vs feline genome organization revealed by reciprocal chromosome painting. *Cytogenet. Cell Genet.* 77: 211-217.
- WILKINSON, G. S. & CHAPMAN, A. M. 1991. Length and sequence variation in evening bat D-loop mtDNA. *Genetics* 128: 607-617.

- WILLAN, K. & MEESTER, J. 1987. Food deprivation in two African rodents, *Mastomys natalensis* and *Rhabdomys pumilio*. *S. Afr. J. Zool.* 22: 190-194.
- WILLAN, K. 1992. *Problem rodents and their control: A handbook for southern African farmers, foresters and smallholders*. Pietermaritzburg: The Natal Witness.
- WILSON, A. C., BUSH, G. L., CASE, S. M. & KING, M. 1975. Social structuring of mammalian populations and rate of chromosomal evolution. *Proc. Natl. Acad. Sci. USA* 72: 5061-5065.
- WRIGHT, S. 1943. Isolation by distance. *Genetics* 20: 114-130.
- WU, C. & LI, W.-H. 1985. Evidence for higher rates of nucleotide substitution in rodents than in man. *Proc. Natl. Acad. Sci. USA*. 82: 1741-1745.
- WURSTER, D. H. & BENIRSCHKE, K. 1968. Comparative cytogenetic studies of the order Carnivora. *Chromosoma* 24: 336-382.
- YANG, F., CARTER, N. P., SHI, L. & FERGUSON-SMITH, M. A. 1995. A comparative study of karyotypes of muntjacs by chromosome painting. *Chromosoma* 103: 642-652.
- YANG, F., O'BRIEN, P. C. M., WIENBERG, J., NEITZEL, H., LIN, C. C. & FERGUSON-SMITH, M. A. 1997. Chromosomal evolution of the Chinese muntjac (*Muntiacus reevesi*). *Chromosoma* 106: 37-43.
- YANG, F., O'BRIEN, P. C. M. & FERGUSON-SMITH, M. A. 2000. Comparative chromosome map of the laboratory mouse and Chinese hamster defined by reciprocal chromosome painting. *Chromosome Res.* 8: 219-227.
- YANG, F., ALKALAEVA, E. Z., PERELMAN, P. L., PARDINI, A. T., HARRISON, W. R., O'BRIEN, P. C. M., FU, B., GRAPHODATSKY, A. S., FERGUSON-SMITH, M. A. & ROBINSON, T. J. (In press). Reciprocal chromosome painting between human, armadillo and elephant (Superorder Afrotheria) reveals the likely eutherian ancestral karyotype. *Proc. Natl. Acad. Sci. USA*.
- ZUKER, M. 1989. On finding all suboptimal foldings of an RNA molecule. *Science* 244: 48-52.

APPENDIX I: Aligned sequences of the cytochrome *b* gene showing variable sites and their codon positions. Sequences have been aligned to the first sequenced *Rhabdomys pumilio* sample collected from Willem Prinsloo and they are grouped according to species and subspecific delimitations derived from this study. WPri = Willem Prinsloo Nature Reserve, Potc = Potchefstroom, Iren = Irene, Inya = Inyanga (Zimbabwe), Mala = Malawi, Ugan = Uganda, Alic = Alice, CPea = Cathedral Peak, KWTo = King Williams' Town, Suik = Suikerbosrand Nature Reserve, PRes = Pilgrims' Rest, Lyde = Lydenburg, Swak = Swakopmund (Namibia), Keetmanshoop (Namibia), Swar = Swartbert, GBay = Grotto Bay, Spri = Springbok, GDam = Gariep Dam, and Bott = Bottletary. Dots indicate regions of similarity while a and b refer to multiple samples from the same locality.

Site number	4	5	8	9	10	11	13	15	16	18	21	27	28	39	45	48	60	61	62	63	66	67	69	74	75	78	81	93	96	99	105	106	107	108	117	120	121	123	124	126			
Codons	1	2	2	3	1	2	1	3	1	3	3	3	1	3	3	3	3	1	2	3	3	1	3	2	3	3	3	3	3	3	3	1	2	3	3	3	3	1	3	1	3	1	3
<i>R. d. dilectus</i>																																											
WPri	A	C	A	C	A	T	C	A	A	A	T	T	C	T	T	C	T	C	T	C	T	G	T	C	T	C	T	G	C	C	T	C	T	A	C	C	C	A	A	C			
Potc-b	C
Potc-a	C
Iren	C	C
Inya-a	C	C	C
Inya-b	C	C	C
Mala-a	C	C	G	T	.	G	
Mala-b	G	T	.	G	
Ugan	C	C	T	.	G	T	
<i>R. d. chakae</i>																																											
Alic	C	G	C	C	T	.	.
CPea-a	C	C	A	T	.	.	
CPea-b	C	C	T	.	.	
KWTo-b	C	C	T	.	.	
KWTo-a	C	C	T	.	.	
WPre-a	C	C	T	.	.	
WPre-b	G	C	.	.	T	C	T	.	.		
Suik-a	C	C	T	.	.		
Suik-b	C	C	T	.	.		
PRes-a	C	C	T	.	.		
PRes-b	C	C	T	.	.		
Lyde-a	C	C	T	.	.		
Lyde-b	C	C	T	.	.		
<i>R. pumilio</i>																																											
Swak-a	C	.	.	C	.	.	T	C	.	C	C	A	.	T	C	T	T	G				
Swak-b	C	.	.	T	C	.	C	C	A	.	T	C	T	T	G				
Keet-a	T	G	.	.	.	C	C	.	.	T	C	.	C	C	A	.	T	C	T	T	G				
Keet-b	T	G	.	.	.	C	C	.	.	T	C	.	C	C	A	.	T	C	T	T	G				
Swar-a	C	A	T	A	T	C	G	.	G	T	.	C	T	.	T	C	T	C	.	G	.	C	A	T	.	.	A	T	.	.				
Swar-b	C	A	T	A	T	C	G	.	G	T	.	C	T	.	T	C	T	C	.	G	.	C	A	T	.	.	A	T	.	.				
GBay-b	C	T	.	.	.	C	C	.	C	.	C	.	.	A	T	T	.	.			
GBay-b	C	T	.	.	.	C	C	.	C	.	C	.	.	A	T	T	.	.			
Spri	G	C	A	T	T	.	.	.	C	.	.	T	.	.			
GDam-b	C	C	C	.	.	C	C	.	.	T	.	.	C	.	.	.	A	.	A	.	T	C	T	.	.	T	.	T	G					
GDam-a	C	C	C	.	.	C	T	C	.	.	T	C	A	C	.	.	A	C	A	.	T	C	T	.	.	T	G	T	G					
Bott-a	C	.	C	C	.	C	A	T	T	.	.			
Bott-b	C	.	C	C	.	C	A	T	T	.	.			

Site number	132	138	141	144	145	150	165	166	171	174	188	189	192	198	199	202	207	225	228	234	237	243	244	246	255	258	260	261	264	268	270	273	277	279	280	281	285	288	294	297				
Codons	3	3	3	3	1	3	3	1	3	3	3	3	3	3	1	1	3	3	3	3	3	3	1	3	3	3	2	3	3	1	3	3	1	2	3	3	3	3	3					
<i>R. d. dilectus</i>																																												
WPri	A	T	A	G	C	C	C	A	C	T	C	C	T	C	A	C	T	C	C	A	C	T	A	A	C	A	C	A	A	T	T	C	T	C	T	T	C	C	A	C				
Potc-b	C	T		
Potc-a	C	T		
Iren	.	.	.	A		
Inya-a	G	T	.	.	T		
Inya-b	.	.	.	A	G	T	.	.	T		
Mala-a	.	.	.	A	G	T	.	.	T		
Mala-b	.	.	.	A	G	T	G	.	T		
Ugan	.	.	.	A	G	T	G	.	T		
<i>R. d. chakae</i>																																												
Alic	.	.	.	A	.	T	T	T	.	.	T	.	T	G	T	C	.	T	.	.	.		
CPea-a	.	.	.	A	.	T	T	T	.	.	T	.	T	G	T	C	A	T	.	.	.		
CPea-b	.	.	.	A	.	T	T	T	.	.	T	.	T	.	.	.	G	G	T	.	.	.	C	.	.	C	.	T		
KWTo-b	.	.	.	A	.	T	T	T	.	.	T	.	T	G	T	C	A	T		
KWTo-a	.	.	.	A	.	T	T	T	.	.	T	.	T	G	T	C	.	T		
WPre-a	.	.	.	A	.	T	T	C	T	.	.	T	.	T	G	T	C	.	T		
WPre-b	.	.	.	A	.	T	T	T	.	.	T	.	T	G	T	C	.	T		
Suik-a	.	.	.	A	.	T	T	T	.	.	T	.	T	G	T	C	.	T		
Suik-b	.	.	.	A	.	T	T	T	.	.	T	.	T	G	T	C	.	T		
PRes-a	.	.	.	A	.	T	T	T	.	.	T	.	T	G	T	C	.	T		
PRes-b	.	.	.	A	.	T	T	T	.	.	T	.	T	G	T	C	.	T		
Lyde-a	.	.	.	A	.	T	T	T	.	.	T	.	T	G	T	C	.	T		
Lyde-b	.	.	.	A	.	T	T	T	.	.	T	.	T	G	T	C	.	T		
<i>R. pumilio</i>																																												
Swak-a	.	C	T	C	T	T	C	T	.	.	C	.	T	C	.	C	T	.	.	C	.	.	T	.	.	T	.	T		
Swak-b	T	T	T	C	T	T	C	.	.	.	C	.	T	C	.	T	.	.	C	.	.	T	.	.	T	.	.	T		
Keet-a	.	C	T	C	T	T	C	T	.	.	C	.	T	G	C	.	C	T	.	.	C	.	.	T	.	.	T	.	.	T		
Keet-b	.	C	T	C	T	T	C	T	.	.	C	.	T	G	C	.	C	T	.	.	C	.	.	T	.	.	T	.	.	T		
Swar-a	G	C	G	A	T	T	T	.	.	A	.	.	T	G	.	.	T	C	T	G	G	.		
Swar-b	G	C	.	A	.	T	T	.	.	A	.	.	T	G	.	.	T	T	G	.	
GBay-b	G	C	.	A	.	T	T	.	.	A	.	.	T	G	.	.	T	T	.	C	G	.	
GBay-b	G	C	.	A	.	T	T	.	.	A	.	.	T	G	.	.	T	T	.	C	G	.	
Spri	G	C	.	A	.	T	T	.	.	A	.	.	T	
GDam-b	.	?	T	C	T	T	C	A	.	A	C	.	T	C	.	C	T	.	.	C	.	.	.	T	G	T	.			
GDam-a	.	C	T	C	T	T	C	A	.	A	C	.	T	C	.	C	T	.	.	C	.	.	.	T	G	T	.			
Bott-a	G	C	.	A	.	T	T	.	.	A	.	.	T	G	.	.	T	T	.	C	T	G	.	
Bott-b	G	C	.	A	.	T	T	.	.	A	.	.	T	G	.	.	T	T	.	C	T	G	.

Site number	298	299	300	302	303	304	305	306	307	308	309	310	311	312	314	315	316	317	318	319	320	321	322	323	324	325	333	335	336	337	338	339	341	342	343	345	348	353	355	358				
Codons	1	2	3	2	3	1	2	3	1	2	3	1	2	3	2	3	1	2	3	1	2	3	1	2	3	3	2	3	1	2	3	2	3	1	3	3	2	1	1	3				
<i>R. d. dilectus</i>																																												
WPri	C	G	A	G	C	A	T	A	T	A	T	T	A	T	G	G	T	C	C	T	A	T	A	C	A	G	C	A	T	G	A	A	T	A	T	A	C	C	C	C				
Potc-b		
Potc-a		
Iren		
Inya-a	A	.	.	T	T		
Inya-b	T		
Mala-a	G	T	T		
Mala-b	G	T	T		
Ugan	G	T	T			
<i>R. d. chakae</i>																																												
Alic	T	
CPea-a	T	
CPea-b	T	
KWTo-b	T	
KWTo-a	G	A	G	C	A	T	A	T	A	C	.	A	T	G	A	T	C	T	T	A	C	A	C	A	T	C	T	G	C	A	.	T	A	T	G	T				
WPre-a	T	
WPre-b	T	
Suik-a	T	
Suik-b	T	
PRes-a	T	
PRes-b	T	
Lyde-a	T	
Lyde-b	T	
<i>R. pumilio</i>																																												
Swak-a	T	T	T
Swak-b	T	T	T
Keet-a	T	T	T
Keet-b	T	T	T
Swar-a	T	T
Swar-b	T	T
GBay-b	T	T
GBay-b	T	T
Spri	T	T
GDam-b	T	T	T	
GDam-a	T	T	T	
Bott-a	T	T	
Bott-b	T	T	

Site number	363	366	369	375	378	384	387	390	396	399	406	411	420	426	444	445	450	459	462	465	468	471	472	474	475	477	480	489	491	492	498	501	513	514	523	528	531	543	549	552			
Codons	3	3	3	3	3	3	3	3	3	1	3	3	3	3	1	3	3	3	3	3	3	1	3	1	3	3	3	2	3	3	3	3	1	1	3	3	3	3	3	3	3		
<i>R. d. dilectus</i>																																											
WPri	A	T	T	G	C	G	C	A	T	G	A	C	G	C	T	T	T	A	T	T	T	A	C	A	T	A	A	C	C	C	A	T	A	T	T	A	T	C	C	G			
Potc-b	T	C
Potc-a	T	C
Iren	G	T	C	.	.	.	A
Inya-a	.	.	.	A	.	.	.	C	T	C	A
Inya-b	.	.	.	A	.	.	.	C	.	.	.	A	T	C	A
Mala-a	.	.	.	A	.	.	.	C	.	G	A	C	T	C	.	.	.	T	A	
Mala-b	.	.	.	A	.	.	.	C	.	G	A	C	T	C	.	.	.	T	A	
Ugan	.	.	.	A	.	.	.	C	.	G	A	C	T	C	.	.	.	T	A	
<i>R. d. chakae</i>																																											
Alic	.	.	C	A	.	A	.	C	C	.	.	.	C	T	C	C	.	.	T	A	
CPea-a	.	.	C	A	.	A	.	C	.	.	T	C	.	.	.	C	G	T	C	C	.	.	T	A	
CPea-b	.	.	C	A	.	A	.	C	.	.	T	C	.	.	.	C	T	C	C	.	T	T	A	
KWTo-b	.	.	C	A	.	A	.	C	.	.	T	C	.	.	.	C	T	C	C	.	T	T	A	
KWTo-a	.	.	C	A	.	A	.	C	.	.	T	C	.	.	.	C	G	T	C	C	.	.	T	A	
WPre-a	.	.	C	A	.	A	.	C	C	.	.	.	C	T	C	C	.	.	T	A
WPre-b	.	.	C	A	.	A	.	C	.	.	T	C	.	.	.	C	G	T	C	C	.	.	T	A
Suik-a	.	.	C	A	.	A	.	C	C	.	.	.	C	G	T	C	C	.	.	T	A	
Suik-b	.	.	C	A	.	A	.	C	C	.	.	.	C	T	C	C	.	.	T	A	
PRes-a	.	.	C	A	.	A	.	C	C	.	.	.	C	T	C	C	.	.	T	A	
PRes-b	.	.	C	A	.	A	.	C	C	.	.	.	C	T	C	C	.	.	T	A	
Lyde-a	.	.	C	A	.	A	.	C	.	.	T	C	.	.	.	C	G	T	C	C	.	.	T	A	
Lyde-b	.	.	C	A	.	A	.	C	.	.	T	C	.	.	.	C	G	T	C	C	.	.	T	A	
<i>R. pumilio</i>																																											
Swak-a	.	.	.	A	T	A	T	.	C	A	.	.	A	T	C	.	C	.	C	.	G	.	T	.	C	G	.	T	C	.	.	C	T	C	T	T	.		
Swak-b	G	.	.	A	T	A	T	.	C	.	.	.	A	T	C	.	C	.	C	.	G	.	T	.	C	G	.	T	C	.	.	C	T	.	T	T	.		
Keet-a	G	.	.	A	T	A	T	.	C	.	.	.	T	C	.	C	.	C	.	G	.	T	.	C	G	.	T	C	.	.	C	G	C	T	T	.			
Keet-b	G	.	.	A	T	A	T	.	C	.	.	.	T	C	.	C	G	C	.	G	.	T	G	C	G	.	T	C	.	.	C	T	C	T	T	.			
Swar-a	.	A	.	A	.	A	A	G	A	.	.	.	C	T	C	.	.	C	T	C	T	T	T	
Swar-b	.	A	.	A	.	A	A	G	C	C	A	.	.	.	C	.	.	T	.	.	T	C	.	.	C	T	C	T	T	T	.			
GBay-b	.	A	.	A	.	A	T	G	C	.	A	.	.	.	C	.	.	T	.	.	G	C	.	.	C	T	C	T	T	T	.			
GBay-b	.	A	.	A	.	A	T	G	C	.	A	.	.	.	C	.	.	T	.	.	G	C	.	.	C	T	C	T	T	T	.			
Spri	.	A	.	A	.	A	T	G	C	.	.	C	.	.	.	C	.	.	T	T	A	T	C	G	.	.	C	T	C	T	T	T			
GDam-b	G	.	.	A	T	A	T	.	C	.	.	.	A	T	C	.	C	.	C	.	G	.	T	.	C	G	.	T	T	.	.	C	.	.	C	T	C	T	T	.			
GDam-a	G	.	.	A	T	A	T	.	C	.	.	.	T	C	.	C	.	C	.	G	.	T	.	C	G	.	T	T	.	.	C	.	.	C	T	C	T	T	.				
Bott-a	.	A	.	A	.	A	G	G	C	.	A	.	.	.	C	.	.	T	.	.	G	C	.	C	C	T	C	T	.	A			
Bott-b	.	A	.	A	.	A	G	G	C	.	A	.	.	.	C	.	.	T	.	.	G	C	.	C	C	T	C	T	T	T			

Site number	555	556	561	567	568	569	571	573	576	578	579	582	584	585	588	591	592	597	601	603	606	609	612	617	618	620	621	624	631	634	636	638	639	640	642	645	648	654	657	660		
Codons	1	3	3	1	2	1	3	3	2	3	3	2	3	3	3	1	3	1	3	3	3	3	2	3	2	3	3	1	1	3	2	3	1	3	3	3	3	3	3	1		
<i>R. d. dilectus</i>																																										
WPri	C	C	C	G	C	G	C	T	T	A	T	T	T	C	C	T	C	C	T	A	A	G	A	C	A	C	C	T	G	C	C	A	G	T	C	C	C	C	T	C		
Potc-b
Potc-a	.	.	.	T
Iren
Inya-a	C
Inya-b	C	G
Mala-a	A
Mala-b
Ugan	G
<i>R. d. chakae</i>																																										
Alic	C	T	.	.	G	G	.
CPea-a	C	T	.	.	G	G	.
CPea-b	C	T	.	.	G	G	.
KWTo-b	C	T	.	.	G	G	.
KWTo-a	C	T	.	.	G	G	.
WPre-a	G	.	.	G	C	T	.	.	G	.	A	G	.	T
WPre-b	A	G	C	T	.	.	G	G	G
Suik-a	C	T	.	.	G	G	.
Suik-b	C	T	.	.	G	G	.
PRes-a	C	T	.	.	G	G	.
PRes-b	C	T	.	.	G	G	.
Lyde-a	C	T	.	.	G	.	C	.	C	G	A	.	.	.	
Lyde-b	C	T	.	.	G	G	.	.	
<i>R. pumilio</i>																																										
Swak-a	.	T	T	A	.	.	T	.	.	.	C	G	.	T	T	C	T	.	C	.	.	A	C	.	T	.	T	A	.	T	T	T	.	.	.		
Swak-b	G	T	.	A	.	.	T	.	.	.	C	G	.	T	T	C	T	T	C	.	A	C	.	T	C	A	.	T	T	T		
Keet-a	.	T	T	A	.	.	T	.	G	.	C	G	.	T	T	C	T	.	C	.	A	C	.	T	T	T	T	T	T	T	.	T	C	A	.	
Keet-b	.	T	T	A	.	.	T	.	.	.	C	.	.	T	T	C	T	.	C	.	A	C	.	T	T	A	.	T	T	T	
Swar-a	.	.	.	A	T	T	C	.	C	.	A	.	T	T	T	C	.	C	.	T	C
Swar-b	.	.	.	A	T	T	C	.	C	.	A	.	T	T	T	C	.	C	.	T	C	
GBay-b	.	.	.	A	.	.	T	T	T	C	.	C	.	A	.	T	T	T	C	G	
GBay-b	T	T	.	A	.	.	T	.	.	.	C	T	T	C	T	.	C	.	A	.	.	.	C	T	A	.	T	T	T	.	.	
Spri	.	.	.	A	T	T	C	.	C	.	A	.	T	T	T	C	C	.	C	.	T	.	C	
GDam-b	.	T	T	A	.	.	T	.	.	.	C	.	C	T	T	C	T	.	C	.	A	C	T	A	.	T	T	T	.	.		
GDam-a	.	T	T	A	.	.	T	.	.	.	C	.	C	T	T	C	T	.	C	.	A	C	T	A	.	T	T	T	.	.		
Bott-a	.	T	.	A	.	.	T	C	.	T	A	.	T	T	T	.	.	T	T	T	A	C	.	
Bott-b	.	.	.	A	G	.	T	T	C	A	.	T	T	T	C	C	.	C	.	T	.	.	C	

Site number	661	662	668	669	672	674	675	684	685	687	688	693	694	695	696	697	702	704	705	706	708	709	711	712	715	723	724	725	726	729	730	736	738	739	744	745	746	748	756	759			
Codons	2	2	3	3	2	3	3	1	3	1	3	1	2	3	1	3	2	3	1	3	1	2	3	1	1	3	1	2	3	3	1	1	3	1	3	1	2	1	3	3			
<i>R. d. dilectus</i>																																											
WPri	A	A	C	C	C	C	C	C	C	C	A	A	T	C	C	A	T	A	A	C	A	T	T	T	C	C	C	T	T	C	T	T	C	C	C	T	T	C	C	T			
Potc-b	T	.	.	C	
Potc-a	T	
Iren	T	
Inya-a	T	T	C	.	.	T	.	.	C		
Inya-b	T	T	T	C	.	.	T	.	.	C		
Mala-a	T	T	
Mala-b	C	.	.	T	T		
Ugan	G	T	T	T		
<i>R. d. chakae</i>																																											
Alic	.	.	.	T	G	G	T	.	.	.	A	T		
CPea-a	.	.	.	T	G	T	.	.	.	A	T		
CPea-b	.	.	.	T	G	T	.	.	.	A	T		
KWTo-b	.	.	.	T	G	T	.	.	.	A	T		
KWTo-a	.	.	.	T	T	G	T	.	.	.	A	T		
WPre-a	.	.	.	T	T	.	.	.	A	T		
WPre-b	.	.	.	T	G	T	.	.	.	A	T		
Suik-a	.	.	.	T	G	T	.	.	.	A	T		
Suik-b	.	.	.	T	G	A	T	.	.	.	A	T		
PRes-a	.	.	.	T	G	G	T	.	.	.	A	T		
PRes-b	.	.	.	T	.	G	G	G	T	.	.	.	A	T		
Lyde-a	.	C	.	T	.	.	.	T	G	T	.	.	.	A	T		
Lyde-b	.	.	.	T	G	T	.	.	.	A	T		
<i>R. pumilio</i>																																											
Swak-a	T	.	.	G	C	.	T	T	.	.	.	A	T	T	C	T	C		
Swak-b	T	.	.	G	C	.	T	A	T	T	C	T	C	
Keet-a	T	T	.	.	G	C	.	T	T	.	.	.	A	T	T	C	T	C	
Keet-b	T	.	.	G	C	.	T	T	.	.	.	A	T	T	C	T	C
Swar-a	.	.	T	T	G	.	T	A	.	.	C	.	A	T	.	T	A	A	.	.	.	T	T	.		
Swar-b	.	.	T	T	G	.	T	A	.	.	C	.	A	T	.	T	C	A	C	.	.	.	T	C	T	.		
GBay-b	.	.	T	T	A	.	T	T	.	.	G	.	G	T	T	T	C	T	.	
GBay-b	T	.	.	G	C	.	T	T	.	.	.	A	T	T	C	T	.
Spri	.	.	T	T	.	T	G	.	T	T	.	T	C	A	.	
GDam-b	T	.	.	G	C	.	T	T	.	.	.	A	T	T	C	T	C	
GDam-a	T	.	.	G	C	.	T	T	.	.	.	A	T	T	C	T	C	
Bott-a	.	.	.	T	.	T	A	G	.	A	C	T	.	.	.	A	.	.	T	G	T	T	.		
Bott-b	.	.	T	T	.	T	G	A	T	.	.	.	C	C	.	.	T	.

Site number	765	768	770	777	780	783	786	792	795	796	798	801	804	807	810	813	816	819	822	828	831	834	835	837	843	844	846	849	852	853	855	857	858	861	862	864	870	873	879	883			
Codons	3	3	2	3	3	3	3	3	3	1	3	3	3	3	3	3	3	3	3	3	3	3	1	3	3	1	3	3	3	1	3	2	3	3	1	3	3	3	1				
<i>R. d. dilectus</i>																																											
WPri	C	T	T	T	C	T	C	C	T	C	C	T	T	A	A	A	A	T	C	C	C	C	G	C	T	C	C	T	C	C	A	A	T	A	T	A	G	T	A	A			
Potc-b	
Potc-a	
Iren	
Inya-a	T	
Inya-b	T	T	
Mala-a	C	.	.	G	.	.	T	
Mala-b	C	.	.	G	.	.	T	
Ugan	T	C	.	.	G	.	.	T	
<i>R. d. chakae</i>																																											
Alic	T	.	.	T	T	A	C	G	
CPea-a	T	.	.	T	C	.	T	A	C	G
CPea-b	T	.	.	T	T	A	C	G
KWTo-b	T	.	.	T	T	A	C	G
KWTo-a	T	.	.	T	T	A	C	G
WPre-a	T	.	.	T	T	A	C	G
WPre-b	T	.	.	T	T	A	C	G
Suik-a	T	.	.	T	T	A	C	G
Suik-b	T	.	.	T	T	A	C	G
PRes-a	T	.	.	T	T	A	C	G
PRes-b	T	.	.	T	C	T	A	C	G
Lyde-a	T	.	.	T	T	A	A	C	G	
Lyde-b	T	.	.	T	T	A	C	G	
<i>R. pumilio</i>																																											
Swak-a	T	T	C	G	T	.	T	.	T	C	C	G	.	A	C	.	
Swak-b	T	T	C	G	T	.	T	.	T	C	C	G	.	A	C	.	
Keet-a	T	T	C	G	T	.	T	.	T	C	C	G	.	A	C	.	
Keet-b	T	T	C	G	T	.	T	.	T	C	C	G	.	A	C	.	
Swar-a	.	C	.	C	.	A	T	T	.	T	A	C	C	G	.	G	.	C	T	T	.	.	A	.	C	T	.	C	.	A	C	T	C	.	C	.	T	C	C	.	.		
Swar-b	.	C	.	C	.	A	T	T	.	T	A	C	C	G	.	G	.	C	T	T	.	.	A	.	C	T	.	C	.	A	C	T	C	.	C	.	T	C	C	.	.		
GBay-b	G	A	.	.
GBay-b	T	T	G	T	.	T	.	T	C	C	G	.	A	C	.	
Spri	.	.	C	A	.	A	.	A	C	.	T	C	.	.	T	.	G	C	.	T	A	.	.	A	A	.	A	A	T	C	.	.	G	A	C	T	G
GDam-b	T	T	C	G	T	.	T	.	T	C	C	G	.	A	C	.	
GDam-a	T	T	C	G	T	.	T	.	T	C	C	G	.	A	C	.	
Bott-a	A	T	T	.	.	.	C	.	G	.	.	.	C	T	T	.	.	A	.	C	T	.	C	.	A	C	T	C	.	C	.	A	C	.	.	.		
Bott-b	A	.	T	.	.	.	C	.	G	.	.	.	C	T	T	.	.	T	.	C	T	C	G	C	.	A	C	.	

Site number	885	886	888	891	894	895	900	901	903	904	905	906	907	909	910	911	912	914	915	916	917	918	921	929	937	939	945	948	954	957	959	960	967	968	969	970	971	972	975	981		
Codons	3	1	3	3	3	1	3	1	3	1	2	3	1	3	1	2	3	2	3	1	2	3	3	2	1	3	3	3	3	3	2	3	1	2	3	1	2	3	3	3		
<i>R. d. dilectus</i>																																										
WPri	C	C	A	A	C	T	T	C	A	G	C	C	C	A	C	T	C	C	C	T	T	T	C	C	C	C	A	A	C	T	T	T	A	C	T	C	T	T	T	A		
Potc-b	T
Potc-a	T
Iren
Inya-a	T	G
Inya-b	T
Mala-a	T	.	C	G
Mala-b	T	.	C	C	T	C	G	
Ugan	T	.	C	C	C	G	
<i>R. d. chakae</i>																																										
Alic	T	C	G	C	
CPea-a	T	C	G	C	
CPea-b	T	C	G	C	
KWTo-b	T	C	G	T	.	.	C	
KWTo-a	T	C	G	T	.	.	C	
WPre-a	T	C	G	C	
WPre-b	T	C	G	C	
Suik-a	T	C	G	C	
Suik-b	T	C	G	C	
PRes-a	T	C	T	G	C	
PRes-b	T	C	G	C	
Lyde-a	C	G	C	
Lyde-b	T	C	C	G	C		
<i>R. pumilio</i>																																										
Swak-a	.	.	.	T	.	C	C	T	C	C	T	T	
Swak-b	.	.	.	T	.	C	C	T	C	C	T	T	
Keet-a	.	.	.	T	.	C	C	T	C	C	T	T	
Keet-b	.	.	.	T	.	C	C	T	C	C	T	T	
Swar-a	.	T	.	T	T	C	.	.	G	.	.	T	C	.	.	A	.	T	.	.	.	C	.	.	T	T	.	T	.	A	.	C	A	C	G		
Swar-b	.	T	.	T	T	C	C	.	G	.	.	T	C	.	.	A	.	T	.	.	.	C	.	.	T	T	.	T	.	A	.	C	A	C	G		
GBay-b	A	T	.	A	.	C	A	C	G	
GBay-b	.	.	.	T	.	C	C	T	C	T	T	
Spri	T	.	C	.	.	C	.	.	.	A	T	T	.	C	A	G	.	T	T	A	C	C	T	T	.	.	C		
GDam-b	.	.	.	T	.	C	C	T	C	C	T	
GDam-a	.	.	.	T	.	C	C	T	C	C	T	T		
Bott-a	.	.	.	T	T	C	T	C	.	.	A	T	.	.	C	.	.	C	.	.	.	A	C	G		
Bott-b	.	.	.	T	.	C	C	T	A	C	.	.	C	.	C	T	C	T	A	.	.	G		

Site number	987	990	994	999	1005	1008	1014	1020	1023	1026	1032	1035	1036	1038	1041	1042	1044	1047	1050	1053	1057	1065	1068	1071	1077	1080	1083	1086	1089	1091	1092	1095	1098	1104	1106	1107	1108	1109	1110	1117		
Codons	3	3	1	3	3	3	3	3	3	3	3	3	1	3	3	1	3	3	3	3	1	3	3	3	3	3	3	3	2	3	3	3	3	2	3	1	2	3	1			
<i>R. d. dilectus</i>																																										
WPri	A	A	T	C	A	C	T	T	G	C	A	C	C	A	C	A	C	T	C	C	T	T	C	T	T	T	C	C	A	T	C	T	G	C	C	A	G	G	T	C		
Potc-b	G
Potc-a	G
Iren	G
Inya-a	A	C	.	.	.	T	G	
Inya-b	A	T	.	.	C	T	G	
Mala-a	.	.	.	T	.	.	C	.	A	C	.	.	T	T	.	.	.	C	.	.	T	G	.	.	.	C	G		
Mala-b	.	.	.	T	.	.	C	.	A	C	.	.	T	T	.	.	C	.	.	T	G	C	G		
Ugan	.	.	.	T	.	.	.	A	T	.	.	.	C	.	.	T	T	.	.	C	C	G		
<i>R. d. chakae</i>																																										
Alic	.	.	.	T	A	T	T	T	.	.	C	.	C	T	.	G	.	.	C	C	G		
CPea-a	.	.	.	T	G	.	.	.	A	T	G	.	.	.	T	.	.	.	T	T	.	.	C	.	C	T	.	G	.	.	C	C	G		
CPea-b	.	.	.	T	G	.	.	.	A	T	G	.	.	.	T	.	.	.	T	T	.	.	C	.	C	T	.	G	.	.	C	C	G		
KWTo-b	.	.	.	T	A	T	T	.	.	.	T	T	.	.	C	.	C	T	.	G	.	.	C	C	G		
KWTo-a	.	.	.	T	A	T	T	.	.	.	T	T	.	.	C	.	C	T	.	G	.	.	C	C	G		
WPre-a	.	.	.	T	A	T	T	.	.	.	T	T	.	.	C	.	C	T	.	G	.	.	C	C	G		
WPre-b	.	.	.	T	G	.	.	.	A	T	G	.	.	.	T	.	.	.	T	A	.	.	C	.	C	T	.	G	.	.	C	C	G		
Suik-a	.	.	.	T	G	.	.	.	A	T	G	.	.	.	T	.	.	.	T	T	.	.	C	.	C	T	.	G	.	.	C	C	G		
Suik-b	.	.	.	T	A	T	T	.	.	.	T	T	C	.	C	C	C	.	G	.	.	C	C	G			
PRes-a	.	.	.	T	.	A	.	.	A	T	T	.	.	.	T	T	.	.	C	.	C	T	.	G	.	.	C	C	G		
PRes-b	.	.	.	T	A	T	T	.	.	.	T	T	.	.	C	.	C	T	.	G	.	.	C	C	G		
Lyde-a	.	.	.	T	A	T	G	.	.	.	T	.	.	.	T	T	.	.	C	.	C	T	.	G	.	.	C	G		
Lyde-b	.	.	.	T	G	.	.	.	A	T	G	.	.	.	T	.	.	.	T	T	.	.	C	.	C	T	.	G	.	.	C	C	G		
<i>R. pumilio</i>																																										
Swak-a	.	T	C	.	.	T	C	A	.	T	.	.	.	C	.	G	T	C	.	A	.	C	.	.	.	C	T	.	.	T	.	A	G	.	.	A	.	.	G			
Swak-b	G	T	C	.	.	T	C	A	.	T	.	.	.	C	.	G	T	C	T	A	.	C	.	.	.	C	T	.	.	T	.	A	A	.	.	A	.	.	G			
Keet-a	.	T	C	.	.	T	C	G	.	T	.	.	.	C	.	G	T	C	.	A	.	C	.	.	.	C	T	.	T	A	T	.	A	G	.	.	A	.	G			
Keet-b	.	T	C	.	.	T	C	G	.	T	.	.	.	C	.	G	T	C	.	A	.	C	.	.	.	C	T	.	.	T	.	A	.	.	.	A	.	.	G			
Swar-a	.	T	C	T	.	.	.	A	.	T	G	T	T	C	T	T	C	C	T	C	C	C	.	T	.	.	A	T	.	.	.	T	.	G				
Swar-b	.	T	C	T	.	.	.	A	.	T	G	T	T	C	T	T	C	C	T	C	C	C	.	T	.	.	A	T	.	.	.	T	.	G				
GBay-b	.	T	C	T	.	.	.	A	.	T	G	T	C	T	T	C	C	T	C	C	C	.	T	.	.	A	G					
GBay-b	G	T	C	.	.	T	C	A	.	T	.	.	.	C	.	G	T	C	T	A	.	C	.	C	.	C	T	.	.	T	.	A	.	.	.	A	.	G				
Spri	.	?	C	C	C	.	.	.	A	T	G				
GDam-b	.	T	C	.	.	T	C	G	.	T	.	T	.	C	.	G	T	C	.	A	.	C	.	.	.	C	T	.	.	T	.	A	.	.	A	.	G					
GDam-a	.	T	C	.	.	T	C	G	.	T	.	T	.	C	.	G	T	C	.	A	.	C	.	.	.	C	T	.	.	T	.	A	.	.	A	.	G					
Bott-a	.	T	C	T	.	.	.	A	.	T	G	T	T	C	C	T	C	C	C	.	T	.	.	A	T	.	.	A	.	G					
Bott-b	.	T	C	.	.	T	C	A	.	T	T	C	T	A	C	C	.	C	C	C	.	T	.	.	A	.	.	.	A	.	G						

Site number	1120	1122	1126	1128
Codons	1	3	1	3
<i>R. d. dilectus</i>				
WPri	A	T	T	A
Potc-b	.	.	A	.
Potc-a	.	.	A	T
Iren	.	.	A	.
Inya-a	.	.	A	.
Inya-b	.	.	A	.
Mala-a	.	.	A	.
Mala-b	.	.	A	.
Ugan	.	.	A	.
<i>R. d. chakae</i>				
Alic	.	.	A	.
CPea-a	.	.	A	.
CPea-b	.	.	A	.
KWTo-b	.	.	A	.
KWTo-a	.	.	A	.
WPre-a	.	.	A	.
WPre-b	.	.	A	.
Suik-a	.	.	A	.
Suik-b	.	.	A	.
PRes-a	.	.	A	.
PRes-b	.	.	A	.
Lyde-a	.	.	A	.
Lyde-b	.	.	A	.
<i>R. pumilio</i>				
Swak-a	G	.	A	.
Swak-b	G	.	A	.
Keet-a	G	.	A	.
Keet-b	G	.	A	.
Swar-a	G	C	A	.
Swar-b	G	C	A	.
GBay-b	G	.	A	.
GBay-b	G	.	A	.
Spri	.	.	A	.
GDam-b	G	.	A	.
GDam-a	G	.	A	.
Bott-a	G	.	A	.
Bott-b	G	.	A	.

APPENDIX II

The distribution of parsimony informative sites among the six types of substitutions and their respective consistency indices (CI) for the cytochrome *b* data

Codon substitutions	Number of informative sites	CI values
First position		
C-T	37	0.5
A-G	24	0.63
A-C	10	0.86
A-T	9	0.93
C-G	2	1
G-T	2	1
Second position		
C-T	14	0.867
A-G	1	1
A-C	3	1
A-T	6	0.9
C-G	1	1
G-T	4	1
Third position		
C-T	170	0.392
A-G	48	0.6
A-C	41	0.76
A-T	52	0.69
C-G	12	0.96
G-T	14	0.94

APPENDIX III: The complete sequence of the control region aligned to the consensus sequence. Sequences are grouped according to the species and subspecific delimitation derived from this study. Cons = Consensus sequence derived from the ten *Rhabdomys pumilio* sequences, Potc = Potchefstroom, Inya = Inyanga (Zimbabwe), Mala = Malawi, Ugan = Uganda, Alic = Alice, CPea= Cathedral Peak, KWTo = King Williams' Town, PRes = Pilgrims' Rest, Lyde = Lydenburg, GBay = Grotto Bay, and GDam = Gariiep Dam. Dots indicate regions of similarity while a and b refer to multiple samples from the same locality.

```

[
[
10      20      30      40      50      60      70      80      90      100]
[
Cons      GACATCAAGAAGAAGGATTATCTCTCTACTATCAGCACCCAAAGC-TGACGTTCTAATTAATACTACTTCTCGCAGTACATAAATTTATATACCACAATA [99]
R. d. dilectus
Mala-a    .....G.....G..... [100]
Inya-a    .....G.....G..... [100]
Ugan      ..... [99]
Potc-a    .....T..... [99]
R. d. chakae
CPea-a    .....AC.....T..... [98]
Lyde-a    .....AC..... [99]
PRes-a    .....AC..... [99]
KWTo-a    .....AC.....G..... [99]
R. pumilio
GBay-a    .....C?.T.....C.....T.....A..... [99]
GDam-a    .....C?.T.....C.....C.....T.....C.....TT..G.. [99]

```

```

[
[
110      120      130      140      150      160      170      180      190      200]
[
Cons      AACATTTATGTATATCGTACATTAAGCAAGTAATTTTACTCATGATTTTAGACTCAAAACTAAAATTCAACTATAA [199]
R. d. dilectus
Mala-a    .....?..?..T..T...C.....G.....C..... [200]
Inya-a    .....T...C...T.....A.....A...C.....T..... [200]
Ugan      .....T...C.....C.....TC..... [199]
Potc-a    .....T.....A.....C..... [199]
R. d. chakae
CPea-a    .....G.....A.....G..T..... [197]
Lyde-a    .....A.....AG..T..... [199]
PRes-a    .....A.....G..T..... [199]
KWTo-a    .....G.....T.....A..C.....G..... [199]
R. pumilio
GBay-a    .....T.....T...C.....AC.T.....C.....C..... [199]
GDam-a    .T.T...A..AC-...GG.G..TC.....T.....A...T.....TA.....GTGAC.....G.C. [197]

```

[210	220	230	240	250	260	270	280	290	300]	
[.]
Cons	ATTTTCATTCAACACAAATATTCATTACC-CATTTTAATTAATGTGAATAGGACATAACTGTGTTATCATAACATACACCATCTTCGTCATAAACCTTTCTC										[298]
R. d. dilectus											
Mala-a	...A.....C.....										[300]
Inya-a	...C.....C..T.....										[300]
UganT.....C..C.....A.....C.....										[299]
Potc-aT.....C.....C.....										[299]
R. d. chakae											
CPea-aCCT.A.....T.....C.....										[297]
Lyde-aCC.TA.....T.....C.....										[299]
PRes-aCC..A.....C.....										[299]
KWTo-aC.....CC..A.....C.....										[299]
R. pumilio											
GBay-aT.....T..C.....A..C.....T..C.....										[299]
GDam-a	T...C..A.....G...T..C..T.....CT.G..A..T.....C.....G.....T..C.....										[297]

[310	320	330	340	350	360	370	380	390	400]	
[.]
Cons	TTCCATATGTCTATCCCCCTTCCCATTGCGTATCAATTCTACCATCCTCCGTGAAACCAACAACCGGCCACCTATGCCCTCTTCTCGCTCCGGGCC										[398]
R. d. dilectus											
Mala-aC.....										[400]
Inya-aA.....C.....										[400]
UganC.....										[399]
Potc-aC.....										[399]
R. d. chakae											
CPea-aC.....										[397]
Lyde-aC.....										[399]
PRes-aC.....C.....										[399]
KWTo-aC.....C.....										[399]
R. pumilio											
GBay-aC.....C.....										[399]
GDam-aA.....C.....C.....										[397]

	410	420	430	440	450	460	470	480	490	500]	
[
[
Cons	ATCACACTTGGGGGTAGCTAACTTGAAACTTTATCAGACATCTGGTTCCTTACCTCAGGGCCATCAATTGCGTTATCGCCCATACGTTCCCCTTAAATAAG										[498]
R. d. dilectus											
Mala-a										[500]
Inya-aC.....T.....										[500]
UganGA.....C.....T.....										[499]
Potc-aC.....C.....T.....										[499]
R. d. chakae											
CPea-a	..T.....C.....										[497]
Lyde-a	..T.....C.....C...T...A.A.....										[499]
PRes-a	..T.....C.....T.....T.....										[499]
KWTo-a	..T.....C.....										[499]
R. pumilio											
GBay-a	..TC.....C..G.....T.....										[499]
GDam-a	..TC.....C.....T.....										[497]

	510	520	530	540	550	560	570	580	590	600]	
[
[
Cons	ACATCTCGATGGTAACGGGTCTAATCAGCCCATGATCAACATAACTGTGGTGTCAGGCATTTGGTATTTT-AAATTTTCGGATGCTATCACTCAACATAG										[597]
R. d. dilectus											
Mala-a-.....										[599]
Inya-a?.....-.....										[599]
Ugan?.....CCA...-..GCA.TTTCA.TGC.TG..TT.TTAT.										[598]
Potc-a-.....										[598]
R. d. chakae											
CPea-a-.....T.....										[596]
Lyde-aG.....-.....T.....?										[598]
PRes-aG.....A.....T.....										[599]
KWTo-aA.....-.....										[598]
R. pumilio											
GBay-aG.....T.....										[599]
GDam-aG.....T.....										[597]

```

[           610           620           630           640           650           660           670           680           690           700]
[           .           .           .           .           .           .           .           .           .           .]
Cons      CCGTCAAGGCATGAAGGCCAGCCCATCATGTAGCCGAACCTCACGGTGAAGGATCATTAGT-CCTCATAACCAACCCACCCAAAGACTATAGATTAATGCTT [696]
R. d. dilectus
Mala-a   .....T..... [698]
Inya-a   .....G.....T.....T.AAG.TC..T.----GTTTCAT.....C.....T..T..... [694]
Ugan     ..T...C.T.AAC...GT..TT.AT..GG.ATA.GGAATT.A.TG..CG.TGA..A---.G.A.T..GG.....TG..CGA.....A. [695]
Potc-a   .....T..... [697]
R. d. chakae
CPea-a   .....C.....T..... [695]
Lyde-a   .....C.....T..... [697]
PRes-a   .....T.....G..... [698]
KWTo-a   .....C.....T.....G..... [697]
R. pumilio
GBay-a   .....C.....GG..... [697]
GDam-a   .....C.....GG..... [695]

```

```

[           710           720           730           740           750           760           770           780           790           800]
[           .           .           .           .           .           .           .           .           .           .]
Cons      GATAGACATATAATCATTTTTCAACTGAAAAATCACTCATCAAACCCCTT--TCCCCGCCTCAATGCCAAACCCCAAAACATTAAGGACTTACTCAT [794]
R. d. dilectus
Mala-a   .....A..... [796]
Inya-a   .GAT.....G.....T.....--T...T.T...G.....C [791]
Ugan     .T..G..A.A.---.??...T..A?...T.C.GGG.....--.....C.....C [790]
Potc-a   .....A.....C..... [795]
R. d. chakae
CPea-a   .....T.....--.....C.....C [793]
Lyde-a   .....T.....--.....C.....C [795]
PRes-a   .....?.....T.....--.....C.....C [796]
KWTo-a   .....?.....T.....--.....C.....C [795]
R. pumilio
GBay-a   ...G.....C..CT...AATTAATC.....TT.....A..T.....T..C..... [797]
GDam-a   ...G.....C..CT...AATTAATC.....--.....A..... [793]

```

	810	820	830	840	850	860	870	880	890	900]	
[
[
Cons	TTCTTACCCATTATTATT	-CATTCTAGTAGTCCAAAAA	TATAACTTAAATT	-CCAGTATTCGTAACATTTTC	--ATAAGA--	-CCAGATTTCACTTATCA					[888]
R. d. dilectus											
Mala-aC.....										[891]
Inya-aA.....										[886]
Ugan	...?.....?T.C.....T.....G.....T.....GCG.G..GG??										[890]
Potc-aGT.....										[890]
R. d. chakae											
CPea-a	..T.....C..A.....										[888]
Lyde-a	..T.....C..A.....										[890]
PRes-a	..T.....C..A.....										[891]
KWTo-a	..T.....C..A.....										[890]
R. pumilio											
GBay-a	..TCC.TT.G..C.C..GT.....T.....G...-T.....										[892]
GDam-a	..T.CG....CC....A.....A..T....G....G...-TT.C...?C.....										[888]

	910	920	930	940	950	960	970	980	990	1000]	
[
[
Cons	ACGCTCAATCAAATTCCTTATCCCCAATAAATTTACGCCGTTAATGTAGCTTAATAATAAAGCAAAGCACTG-AAAATG-CTTAGATGGATTAAAAATC										[986]
R. d. dilectus											
Mala-a										[989]
Inya-a	.A.....T.....										[984]
UganCT.....GA.....C.....T.....C.....										[990]
Potc-a										[988]
R. d. chakae											
CPea-aA..CC.....T.....										[987]
Lyde-aA..CC.....T.....										[989]
PRes-aC.A..CC.....T.....										[990]
KWTo-aA..CC.....T.....										[989]
R. pumilio											
GBay-aC.....T.....T.....C.....										[990]
GDam-aC..G....CCC.TCCCCT..T..C.....C.....										[986]

[]	
[]	
Cons	CCATA	[991]
R. d. dilectus		
Mala-a	[994]
Inya-a	[989]
Ugan	[995]
Potc-a	[993]
R. d. chakae		
CPea-a	[992]
Lyde-a	[994]
PRes-a	[995]
KWTo-a	[994]
R. pumilio		
GBay-a	[995]
GDam-a	[991]
