Evidence of a contact zone between two *Rhabdomys dilectus* (Rodentia: Muridae) mitotypes in Gauteng Province, South Africa

Anja le Grange[1], Armanda D.S. Bastos[1], Helene Brettschneider[1, 3], Christian T. Chimimba[1, 2 *]

[1] Mammal Research Institute (MRI), Department of Zoology and Entomology, University of Pretoria, Private Bag X20, Hatfield, 0028 South Africa

[2] DST-NRF Centre of Excellence for Invasion Biology (CIB), Department of Zoology and Entomology, University of Pretoria, Private Bag X20, Hatfield, 0028 South Africa

[3] Molecular Genetics Research Department, National Zoological Gardens of South Africa, Pretoria, 0002 South Africa

Recent studies have described the presence of several mitochondrial lineages within *Rhabdomys* which was previously considered to be a monotypic genus. The exact distributional limits of the species and subspecies and their contact zones are unclear. In this study we demonstrate that two monophyletic *Rhabdomys dilectus* mitochondrial lineages are present at two northern Gauteng Province sampling sites in South Africa. Cytochrome *b* gene sequences, 896 nucleotides in length generated for 36 *Rhabdomys* samples identified 10 unique haplotypes corresponding to eight *R. dilectus dilectus* haplotypes (from 32 individuals) and two *R. d. chakae* haplotypes (from four individuals). The present study provides the first empirical evidence for a contact zone for two *R. dilectus* conspecific mitochondrial lineages and contributes to the refinement of *Rhabdomys* distributional maps in southern Africa.

Keywords: Cytochrome *b, Rhabdomys dilectus, chakae Rhabdomys dilectus dilectus*

*Author for correspondence: Department of Zoology and Entomology, University of Pretoria, Pretoria 0002, South Africa. E-mail: ctchimimba@zoology.up.ac.za

Rhabdomys is a widespread murid genus endemic to Africa that is generally found in grassland (Skinner and Chimimba 2005). This genus is grouped among Arvicanthine rodents, together with the genera *Arvicanthis, Desmomys, Lemniscomys, Mylomys* and *Pelomys* (Ducroz *et al.* 2001; Lecompte *et al.* 2008). *Rhabdomys* occurs throughout southern Africa, including most of Namibia, Botswana, Zimbabwe, Mozambique, Swaziland, Lesotho, and South Africa but has a limited range in eastern Africa where it is restricted to small areas in Zambia, Malawi, Tanzania, the Democratic Republic of Congo and Kenya (Musser and Carleton 2005).

The taxonomy of the genus *Rhabdomys* has been problematic (Castiglia *et al.* 2012). It has historically been considered to be a monotypic genus containing only one species *R. pumilio*, with a varying number of subspecies (Skinner and Chimimba 2005). As early as 1905, four distinct morphological groups were identified within the genus and were referred to as subspecies, a view that was accepted by several authors (Musser and Carleton 1993). Based on pelage colour, body size and morphological characteristics, such as tail length; Roberts (1951) described 20 subspecies from southern Africa. However, some authors disputed the number of subspecies described. De Graaff (1981) suggested the presence of two subspecies, whereas Missone (1974) maintained *Rhabdomys* to be a monotypic genus. In a subsequent review only seven of the subspecies proposed by Roberts (1951) were retained (Meester *et al.* 1986). Karyotypic and allozyme studies revealed the presence of two karyotypes (2*n* = 46 and 2*n* = 48) and restricted gene flow between widely separated populations in southern Africa (Ducroz *et al.* 1999; Mahida 1999; Taylor 2000). Breeding and behavioural studies were also not able to provide conclusive evidence on species and subspecies boundaries (Pillay 2000a; b; Pillay *et al.* 2006).

Subsequent phylogenetic analysis of cytochrome *b* DNA sequences revealed the presence of two distinct mitochondrial lineages within *Rhabdomys* suggesting that in southern Africa this genus comprises at least two species, namely *R. pumilio* and the newly erected *R. dilectus* (Rambau *et al.* 2003). Lighter coloured animals with a diploid number of 2*n* = 48 from the drier western and southern central regions of South Africa, Namibia and Botswana to south-western Angola were assigned to *R. pumilio*; whereas darker individuals with diploid numbers of either 2*n* = 48 or 2*n* = 46 from the more mesic regions of southern and eastern Africa were assigned to *R. dilectus* (Rambau *et al.* 2003; Musser and Carleton 2005). Based on the two chromosomal forms and mtDNA subclades within *R. dilectus*, this

taxon was provisionally sub-divided into two subspecies. *Rhabdomys dilectus chakae* (2*n* = 48) that is restricted to South Africa and *R. d. dilectus* (2*n* = 46) that occurs from southern to eastern Africa (Rambau *et al.* 2003). Furthermore a new *R. d. dilectus* karyotype with 2*n* = 38 was recently discovered in high-altitude populations in northern Tanzania (Castiglia *et al*. 2012). Recent studies confirming the monophyly of these lineages with a nuclear DNA marker have revealed both *R. pumilio* and *R. dilectus* to be even more diverse than previously thought (du Toit *et al.* 2012). Although the above-mentioned studies included samples from a wide geographic range in southern and eastern Africa, the exact species and subspecies distributional limits and possible contact zones are still not well-understood (Skinner and Chimimba 2005). This taxonomic uncertainty not only has implications for population studies but also impacts epidemiological studies as host identification is crucial for understanding disease dynamics (Bastos *et al.* 2005).

Comparative studies have shown two sibling species of multimammate mice, *Mastomys coucha* and *M. natalensis* to differ in their susceptibility to experimental infection with the well-known plague bacterium, *Yersinia pestis* (Arntzen *et al.* 1991). Experimental evidence suggests that different *Rhabdomys* lineages may also display differences in susceptibility to plague infection as *Rhabdomys* sampled from geographically distant populations in South Africa exhibited different levels of susceptibility to experimental plague infection (Shepherd *et al.* 1986). These studies highlight possible problems with previous epidemiological research involving the genus *Rhabdomys*, as without refined distributional ranges, it is not clear which of the species or subspecies play a definitive role in zoonotic disease transmission (Davis *et al.* 1968; Hallet *et al.* 1970).

The present study used phylogenetic analysis of cytochrome *b* sequences to genetically identify the *Rhabdomys* mitochondrial lineages present in northern Gauteng Province, South Africa and contributes to refinement of distributional data for the proposed species and subspecies within the genus in southern Africa.

Rodent samples were collected at 4-6 week intervals between August 2010 and July 2011 in Hammanskraal (1111 metres above sea level (m.a.s.l.)) and the University of Pretoria (UP) Experimental farm (1375 m.a.s.l.; Fig. 1), Gauteng Province, South Africa (Table 1). Animals were live-trapped using Sherman traps (H.B. Sherman Traps Inc. Florida, U.S.A.) and housed as per the guidelines of the American Society of Mammalogists (ASM, www.mammalogy.org/committees/index.asp; Animal Care and Use Committee 1998).

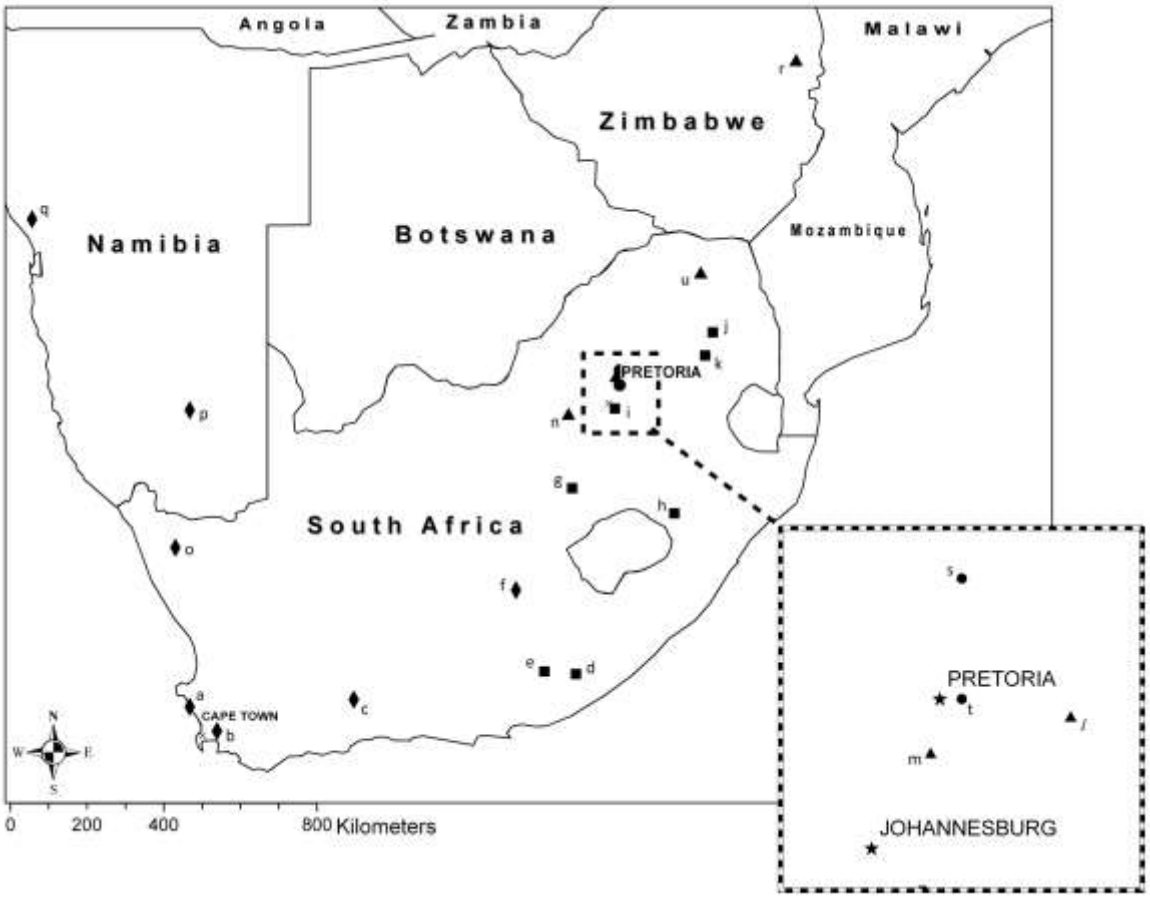

Fig. 1. Sampling localities of *Rhabdomys* sampled in the current study (s and t; ●). Additional *Rhabdomys* samples collected in previous studies are also indicated (a-r and u; Rambau *et al.* 2003; Castiglia *et al.* 2012). *Rhabdomys* species and subspecies are represented by the following symbols: *R. pumilio* (♦), *R. d. chakae* (■), *R. d. dilectus* (▲).

Animals were euthanized as approved by the Animal Ethics Committee of the University of Pretoria (Ethics Clearance number: EC026-10). Kidney tissue was removed and stored at minus 20°C for further analyses.

Total genomic DNA was extracted from kidney tissue using the High Pure PCR template preparation kit (Roche) according to the prescribed manufacturer's protocol. Primers L14724 and H15915 were used to amplify the cytochrome *b* gene region of the mitochondrial genome as previously described (Bastos *et al.* 2005). The resulting amplicon was purified directly from the tube using the High Pure PCR product purification kit (Roche) according to manufacturer's specifications. Nucleotide sequences were determined with each of the external PCR primers by cycle sequencing with Big Dye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems) v3.1, at an annealing temperature of

Table 1. Summary of the 36 *Rhabdomys* specimens sampled from four localities in Gauteng Province, South Africa.

Locality	GPS	N	N (per species)	Haplotypes	Samples used in Figure 2	GenBank Accession no.
University of Pretoria Experimental farm	25°75'S 28°26'E	4	*R. d. dilectus* (2)	Rdd03	EF4-8	KJ939542
				Rdd05	EF8-14	KJ939544
			R. d. chakae (2)	Rdc02	EF4-9	KJ939550
Hammanskraal Informal settlement	25°36'S 28°27'E	5	*R. d. dilectus* (5)	Rdd01	-	-
				Rdd08	-	-
Hammanskraal Prestige College Campus	25°42'S 28°27'E	8	*R. d. dilectus* (8)	Rdd01	-	-
				Rdd02	HPB3-2	KJ939541
				Rdd06	HPB2-3	KJ939546
				Rdd07	HPB4-12	KJ939547
				Rdd08	HPB4-6	KJ939548
University of Pretoria Hammanskraal Campus	25°40'S 28°26'E	19	*R. d. dilectus* (17)	Rdd01	HUPII9-11	KJ939540
				Rdd04	HUP10-18	KJ939543
				Rdd05	HUPI9-28	KJ939545
			R. d. chakae (2)	Rdc01	HUP5-1	KJ939549

48°C. Ethanol precipitated reactions were run on an ABI PRISM™ 3100 Analyser (Applied Biosystems) and sequence chromatograms were viewed, edited and aligned in Mega5 (Tamura *et al.* 2011). Sequences were submitted to GenBank (http://www.ncbi.nlm.nih.gov) under accession numbers KJ939540-KJ939550.

For phylogenetic analyses, the 36 sequences generated in the current study were complemented and aligned with all available *Rhabdomys* cytochrome *b* sequences in GenBank (Accession Numbers AF141214, AF533083-AF533116, FR837633-FR837651) (Ducroz *et al.* 2001; Rambau *et al.* 2003; Castiglia *et al.* 2012). Sequences of *Rattus norvegicus* (NC001665) and *Mus musculus* (EF108340) were included for outgroup purposes.

Neighbour Joining (NJ) analyses were performed in MEGA5 (Tamura *et al.* 2011), Maximum Parsimony (MP) analyses were performed in PAUP4.0b10 (Swofford 2000), Maximum Likelihood (ML) in PhyML (Guidon and Gascuel 2003) and Bayesian Inference (BI) with MrBayes (Huelsenbeck and Ronquist 2001). NJ and ML analyses were performed using the best-fit model and parameters identified under the Akaike Information Criterion (AIC), chosen with jModelTest (Posada 2008), which guided selection of priors for BI. For the latter, four Markov chain Monte Carlo simulations were run for 10 000 000 generations using default heating and swap settings, and were sampled every 100 generations. Trace files were viewed using Tracer version 1.5 (http://beast.bio.ed.ac.uk/) after which 20% of

the runs were discarded as burn-in. For the MP analysis heuristic searches with random addition of sequences (10 replicates) and tree-bisection-reconnection were performed. Successive (*a posteriori*) weighting with the rescaled consistency (RC) to reduce the contribution of homoplasious characters in the parsimony analysis (Farris 1989) was also performed. Nodal support was assessed from 10 000 bootstrap replicates for NJ, 1000 replicates each for ML and MP and from Bayesian posterior probabilities (BPP). Pairwise uncorrected p-distances were determined in MEGA5 (Tamura *et al.* 2011).

The 95-taxon dataset (inclusive of outgroup sequences) contained 338 variable and 249 parsimony-informative sites across the 869 nucleotide gene region characterised. The proportion of base position mutations was $3^{rd} > 1^{st} > 2^{nd}$, with 232 (69%) of the mutations occurring in the 3^{rd} base position, 74 (22%) in the 1^{st} base position and the remaining 32 (9%) being attributed to 2^{nd} base position mutations. The HKY+I+G (I = 0.513, G = 2.296) model of sequence evolution was selected as the best-fit model under the AIC in jModelTest. The unweighted parsimony analysis recovered 108 equally parsimonious trees (Tree Length (TL) = 642 steps, Consistency Index (CI) = 0.52, Retention Index (RI) = 0.86, RC = 0.45). Reweighting with the RC resulted in 18 equally parsimonious trees (TL = 261 steps) and CI = 0.69, RI = 0.93 and RC = 0.62. As MP, ML, BI and NJ analyses recovered similar tree topologies, support indices are summarised on relevant nodes of the NJ tree (Fig. 2).

Analyses recovered two distinct monophyletic groups corresponding to *R. pumilio* and *R. dilectus* (Fig. 2). The *R. pumilio* group is well-supported (NJ: 90%; MP: 99%; BPP: 0.90), however, the *R. dilectus* group is only supported by NJ and MP analyses. The average genetic distance between these two species, is relatively high (11.6%). The *R. pumilio* group includes samples from south-western South Africa and Namibia only (2*n* = 48; Rambau *et al.* 2003) and showed high (8.5%) within species variation. None of the samples from the current study fell within this group.

Within the *R. dilectus* group two monophyletic groups corresponding to the proposed subspecies *R. d. chakae* and *R. d. dilectus* (Fig. 2) were recovered. The genetic distance between the two proposed subspecies is 5.2%. The *R. d. chakae* grouping was well-supported by all analyses (NJ: 100%; ML: 97%; MP: 100%; BPP: 0.99). This group contained samples from south-eastern South Africa, as well as four samples from the current study. Two distinct *R. d. chakae* haplotypes were identified; one from Hammanskraal (Rdc01) and

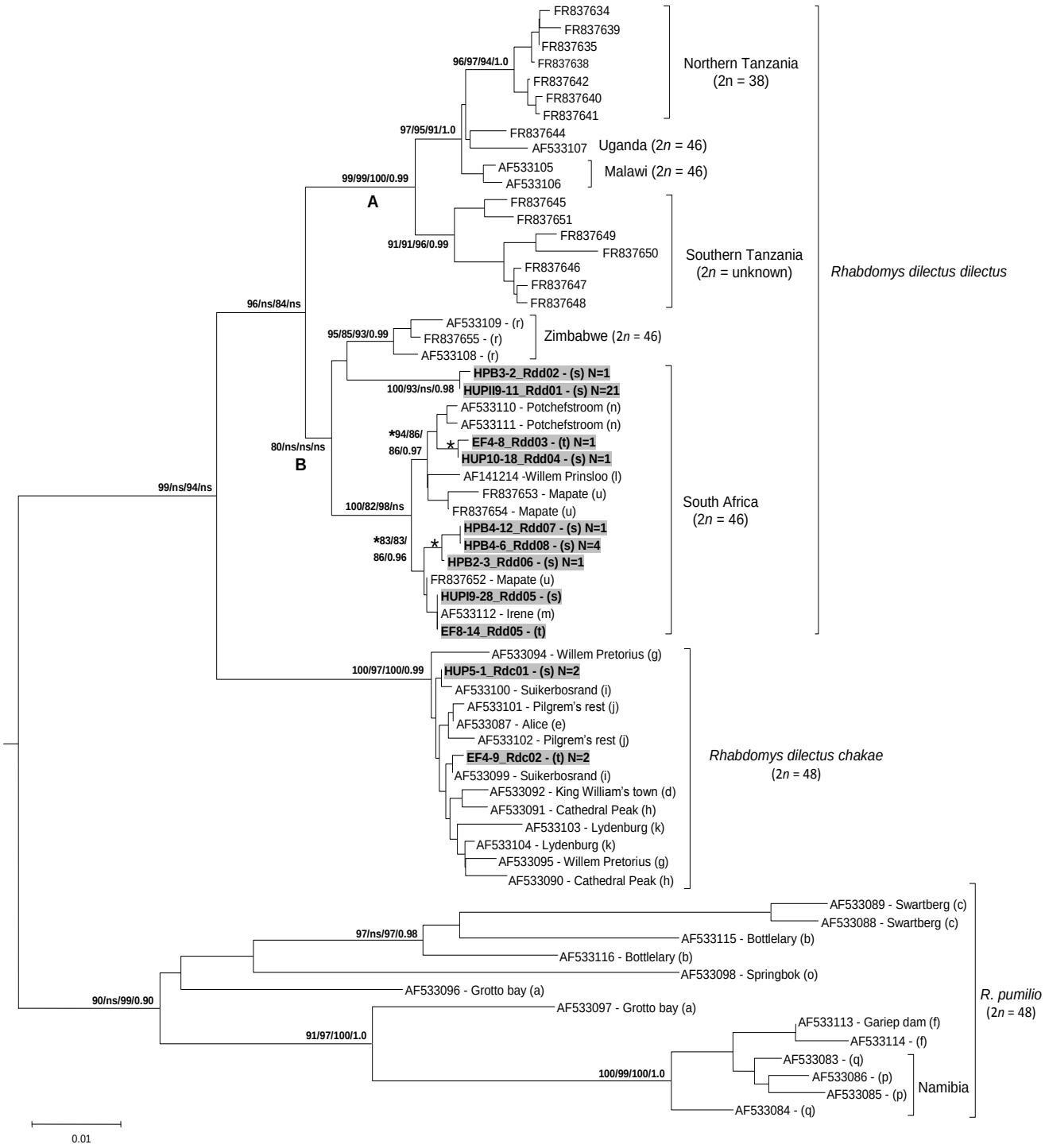

Fig. 2. Neighbour Joining (NJ) tree inferred from partial cytochrome *b* gene sequence data (869 nt) of *Rhabdomys* sampled from Pretoria, Gauteng Province, South Africa during 2010-2011. Bootstrap support (> 70) from NJ, ML and MP analyses and Bayesian posterior probabilities (BPP) (> 0.90) are indicated at relevant nodes (NJ/ML/MP/BPP). Samples highlighted in grey and indicated in bold represent samples from the current study and with accession numbers of sequences from previous studies being provided. Letters in parentheses correspond with sampling locations on map (Fig. 1).

one from the UP Experimental farm (Rdc02; Table 1). Karyotyped *R. d. chakae* samples from a previous study had a diploid number of 2*n* = 48 (Rambau *et al.* 2003).

The remaining samples from this study fell within the more weakly supported *R. d. dilectus* group which can on the basis of geographical distribution be further sub-divided into two lineages designated A and B (Fig. 2). Lineage A was well-supported (NJ: 99%; ML: 99%; MP: 100%; BPP: 1.0) and included specimens from northern and southern Tanzania, Uganda and Malawi. Lineage B contained samples from South Africa and Zimbabwe; however, this lineage was not well supported. Within Lineage B, the Zimbabwean specimens formed a separate well-supported group (NJ: 95%; ML: 85%; MP: 93%; BPP: 0.99). Eight distinct *R. d. dilectus* haplotypes were identified, with two haplotypes from Hammanskraal (Rdd01 and Rdd02) forming a lineage that was sister to samples from Zimbabwe. Two specimens from the University of Pretoria Experimental farm and eight specimens from Hammanskraal clustered with samples previously collected from South Africa, found to have a diploid number of 2*n* = 46 (Rambau *et al.* 2003). Within species variation was low for *R. d. chakae* (0.68%) but was higher for *R. d. dilectus* (2.67%).

The present study recovered mitochondrial lineages shown in previous studies to correspond to *R. pumilio, R. dilectus chakae* and *R. d. dilectus*; however, some of the deeper nodes were not well-supported by all the analyses. No *R. pumilio* was sampled from the current study sites, in agreement with current proposed distributional limits for this species (Castiglia *et al* 2012; du Toit *et al*. 2012).

Within *R. dilectus* both proposed subspecies, *R. dilectus chakae* and *R. d. dilectus*, were sampled from sites within the Gauteng area, which are approximately 50 km apart, *viz.* Hammanskraal and the University of Pretoria Experimental Farm (South Africa). Both *R. d. chakae* and *R. d. dilectus* samples were sampled from Hammanskraal and from the University of Pretoria Experimental Farm (South Africa). This represents the first record of the proposed subspecies occurring sympatrically. Niche modelling predicted large areas of sympatry for the grassland biome, within Gauteng and Free State Provinces of South Africa (du Toit *et al*. 2012; Meynard *et al*. 2012). Despite intensive sampling in previous studies, trapping yielded either only one or the other subspecies (Pillay *et al*. 2006; Ganem *et al.* 2012; Meynard *et al.* 2012). Meynard *et al*. (2012) proposed that competition could be the main driver of the species' distributional limits within this area which seems suitable for both species. Recent behavioural and ecological studies have suggested that the two

subspecies may have fairly distinct and specific environmental requirements (Mackey 2011; Ganem *et al*. 2012). Although *R. d. dilectus* was more frequently sampled at the Hammanskraal study site, the current study confirms that both mitochondrial lineages occur in Hammanskraal and at the University of Pretoria Experimental farm. Therefore, these results provide tentative evidence of a contact zone between *R. dilectus chakae* and *R. d. dilectus* in the Gauteng Province, as predicted by Meynard *et al*. (2012) and highlight the need for more extensive sampling particularly in this area. The discovery of a potential contact zone in an area predicted by previous niche modelling warrants further sampling, especially in areas where other *R. dilectus* contact zones have been predicted such as the Free State Province. Furthermore the small number of *R. d. chakae* collected corresponds with the patchy distribution predicted for this proposed subspecies in Gauteng (Meynard *et al*. 2012).

The genetic divergence between the proposed subspecies *R. d. dilectus* and *R. d. chakae* is relatively high (5.2%). In several mammal species, cytochrome *b* sequence differences of > 5% have been found between morphologically distinct sister species (Baker and Bradley 2006). Subsequently it has been suggested that a 5% value may represent a useful lower limit for delineating monophyletic mitochondrial lineages as putative new species (Baker and Bradley 2006) Although hybridization occurred under laboratory conditions (Pillay 2000b), recent studies have suggested that divergent behaviour may act as a pre-mating barrier in the wild (Pillay *et al*. 2006). Consequently, it has been proposed that *R. d. dilectus* and *R. d. chakae* be given full species ranks. Without additional extensive sampling throughout the possible range of both proposed subspecies, sequencing of additional nuclear gene regions, complete morphological investigations and karyotyping of the two different lineages in sympatry; the appropriate taxonomic rank of these two mitochondrial lineages remains unclear.

We gratefully acknowledge all field assistance obtained with trapping and collection of samples for this study. This work is supported by the South African National Research Foundation (NRF).

References

Animal Care and Use Committee. 1998. Guidelines for the capture, handling and care of mammals as approved by the American Society of Mammalogists. *Journal of Mammalogy* 79: 1416-1431.

Arntzen L, Wadee AA, Isaäcson M. 1991. Immune responses of two *Mastomys* sibling species to *Yersinia pestis*. *Infection and Immunity* 59: 1966-1971.

Bastos ADS, Chimimba CT, Von Maltitz E, Kirsten F, Belmain S. 2005. Identification of rodent species that play a role in disease transmission to humans in South Africa. *Proceedings of the South African Society for Veterinary Epidemiology and Preventive Medicine.* pp 78-83. DOI: 10.13140/2.1.2629.4563

Baker RJ, Bradley RD. 2006. Speciation in mammals and the genetic species concept. *Journal of Mammalogy* 87: 643-662.

Castiglia R, Solano E, Makundi RH, Hulselmans J, Verheyen E, Colangelo P. 2012. Rapid chromosomal evolution in the mesic four-striped grass rat *Rhabdomys dilectus* (Rodentia, Muridae) revealed by mtDNA phylogeographic analysis. *Journal of Zoological Systematics and Evolutionary Research* 50: 165-172.

Davis DHS, Eisch RBH, McNeill D, Meyer KF. 1968. Serological survey of plague in rodents and other small mammals in Kenya. *Transactions of the Royal Society of Tropical and Medical Hygiene* 62: 838-861.

De Graaff GD. 1981. *The Rodents of Southern Africa: Notes on their Identification, Distribution, Ecology and Taxonomy*. Pretoria: Butterworths.

Ducroz J, Granjon L, Lombaard M, Volobouev V. 1999. Comparative chromosome analysis (R- and C-bands) of two South African murid species, *Lemniscomys rosalia* and *Rhabdomys pumilio* (Rodentia, Murinae). *Cytogenetics and Cell Genetics* 87: 69-74.

Ducroz J, Volobouev V, Granjon L. 2001. An assessment of the systematics of Arvicanthine rodents using mitochondrial DNA sequences: evolutionary and biogeographical implications. *Journal of Mammalian Evolution* 8: 173-206.

Du Toit N, Jansen Van Vuuren B, Matthee S, Matthee CA. 2012. Biome specificity of distinct genetic lineages within the four-striped mouse *Rhabdomys pumilio* (Rodentia: Muridae) from southern Africa with implications for taxonomy. *Molecular Phylogenetics and Evolution* 65: 75-86.

Farris JS. 1989. The retention index and the rescaled consistency index. *Cladistics* 5: 417-419.

Ganem G, Meynard CN, Perrigault M, Lancaster J, Edwards S, Caminade P, Watson J, Pillay N. 2012. Environmental correlates and co-occurrence of three mitochondrial lineages of striped mice (*Rhabdomys*) in the Free State Province (South Africa). *Acta Oecologica* 42: 30-40.

Guidon S, Gascuel O. 2003. A simple, fast and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* 52: 696-704.

Hallet AF, Mcneill D, Meyer KF. 1970. A serological survey of small mammals for plague in southern Africa. *South African Medical Journal* 44: 831-837.

Huelsenbeck JP, Ronquist F. 2001. MrBayes: Bayesian inference of phylogeny. *Bioinformatics* 17: 754-755.

Lecompte E, Aplin K, Denys C, Catzeflis F, Chades M, Chevret P. 2008. Phylogeny and biogeography of African Murinae based on mitochondrial and nuclear gene sequences, with a new tribal classification of the subfamily. *BMC Evolutionary Biology* 8: 199.

Mackey MK, 2011. The behavior of two sub-species of the striped mouse Rhabdomys: the role of phylogeny and the environment. MSc Thesis, University of Witwatersrand, Johannesburg, South Africa.

Mahida H, Campbell GK, Taylor PJ. 1999. Genetic variation in *Rhabdomys pumilio* (Sparrman, 1784) - an allozyme study. *South African Journal of Zoology* 34: 91-101.

Meester JAJ, Rautenbach IL, Dippenaar NJ, Baker CM. 1986. Classification of southern African mammals. *Transvaal Museum Monograph* 5: 1-359.

Meynard CN, Pillay N, Perrigault M, Caminade P, Ganem G. 2012. Evidence of environmental niche differentiation in the striped mouse (*Rhabdomys* sp.): inference from its current distribution in southern Africa. *Ecology and Evolution* 5: 1008-1023.

Missone X. 1974. Order Rodentia. In: Meester J, Setzer HW (eds), *The mammals of Africa: An identification Manual.* Washington: Smithsonian Institution Press. pp 1-39.

Musser GG, Carleton MD. 1993. Superfamily Muroidea. In: Wilson DE, Reeder DM (eds), *Mammal Species of the World a Taxonomic and Geographic Reference.* Washington DC: Smithsonian Institution Press. pp 501-755.

Musser GG, Carleton MD. 2005. Superfamily Muroidea. In: Wilson DE, Reeder DM (eds), *Mammal Species of the World a Taxonomic and Geographic Reference.* Baltimore: Johns Hopkins University Press. pp 894-1531.

Pillay N. 2000a. Female mate preference and reproductive isolation in populations of the striped mouse, *Rhabdomys pumilio*. *Behaviour* 137: 1431-1441.

Pillay N. 2000b. Reproductive isolation in three populations of the striped mouse *Rhabdomys pumilio*: interpopulation breeding studies. *Mammalia* 64: 461-470.

Pillay N, Eborall J, Ganemba G. 2006. Divergence of mate recognition in the African striped mouse (*Rhabdomys*). *Behavioral Ecology* 17: 757-764.

Posada D. 2008. jModelTest: Phylogenetic model averaging. *Molecular Biology and Evolution* 25: 1253-1256.

Rambau RV, Robinson TJ, Stanyo R. 2003. Molecular genetics of *Rhabdomys pumilio* subspecies boundaries: mtDNA phylogeography and karyotypic analysis by fluorescence in situ hybridization. *Molecular Phylogenetics and Evolution* 28: 564-575.

Roberts A. 1951. Rhabdomys. In: Bigalke R, FitzSimons V, Malan DE (eds), *The Mammals of South Africa.* Johannesburg: Trustees of ‘‘The mammals of South Africa’’ Book Fund. pp 498-505.

Skinner JD, Chimimba CT. 2005. *The Mammals of the Southern African Subregion*. Cambridge: Cambridge University Press.

Shepherd AJ, Leman PA, Hummitzsch DE. 1986. Experimental plague infection in South African wild rodents. *Journal of Hygiene (Cambridge)* 96: 171-183.

Swofford DL. 2000. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods), Version 4.0b10. Sinauer Associates, Sunderland, MA.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution* 24: 1596-1599.

Taylor PJ. 2000. Patterns of chromosomal variation in southern African rodents. *Journal of Mammalogy* 81: 317-331.