Supplementary material

Wildlife Research **39**(1), 51–61

Experimental treatment-control studies of ecologically based rodent management in Africa: balancing conservation and pest management

Peter J. Taylor[A,F,H], *Sarah Downs*[F], *Ara Monadjem*[E], *Seth J. Eiseb*[C], *Loth S. Mulungu*[D], *Apia W. Massawe*[D], *Themb'a A. Mahlaba*[E], *Frikkie Kirsten*[G], *Emil Von Maltitz*[G], *Phanuel Malebane*[G], *Rhodes H. Makundi*[D], *Jennifer Lamb*[F] *and Steven R. Belmain*[B]

[A]Department of Ecology & Resource Management, University of Venda, Private Bag X5050, Thohoyandou 0950, South Africa.

[B]Natural Resources Institute, University of Greenwich, Central Avenue, Chatham Maritime, Kent ME4 4TB, UK.

[C]National Museum of Namibia, PO Box 1203, Windhoek, Namibia.

[D]Pest Management Centre, Sokoine University of Agriculture, PO Box 3110, Chuo Kikuu, Morogoro, Tanzania.

[E]Department of Biological Sciences, University of Swaziland, Private Bag 4, Kwaluseni, Swaziland.

[F]School of Biological and Conservation Sciences, Westville Campus, Durban, South Africa.

[G]Agricultural Research Council, Plant Protection Research Institute, Private Bag X134, Queenswood 0121, South Africa.

[H]Corresponding author. Email: Peter.Taylor@univen.ac.za

Appendix A1. DNA extraction and sequencing

Cytochrome-*b* sequences were obtained from 36 individuals from Tanzania, Namibia and Swaziland belonging to ten genera of rodents (*Acomys*, *Aethomys*, *Arvicanthis*, *Gerbilliscus*, *Graphiurus*, *Lemniscomys*, *Mastomys*, *Mus* (*Nannomys*), *Rattus* and *Thallomys*) and one of shrews (*Crocidura*) (S. Downs, unpublished data). We selected samples for analysis so as to attempt to include at least two individuals per putative species per country. Soft tissue samples

were dissected and stored in 90% EtOH in Eppendorf™ tubes which were kept at -4C. Total genomic DNA was isolated from tissue samples using a Qiagen DNAeasy kit™ according to the manufacturers protocol. DNA concentration was measured using a NanoDrop ND-1000 Spectrophotometer. Samples of isolated DNA were electrophoresed in 1% agarose gels (containing 200µl of an ethidium bromide (EtBr) solution (0.05 mg ml-1)(Roche) at 80v for 3-4 hours in a 0.5x running buffer. 5µl of a DNA molecular marker ladder (O'GeneRulerTM DNA ladder (Fermentas) were co-electrophoresed with the samples.

PCR (Polymerase Chain Reaction) amplification (Saiki et al., 1988) of essentially the entire mitochondrial cytochrome-*b* gene (~1140bp) was carried out using primers L14724 (5'-CGA AGC TTG ATA TGA AAA ACC ATC GTT G-3') and H15915 (5'-TCT CCA TTT CTG GTT TAC AAG AC-3') at 94°C for 4 min, with 36 Cycles of 94°C for 1 min, 50°C for 1:30 min, 72°C for 2 min, and then 1 Cycle of 72°C for 10 min (Irwin et al., 1990).

Amplified fragments were separated by electrophoresis of the entire PCR product in 1.5% agarose gels following the same procedure as above. Amplicons present as a single band of ~1140bp for cytochrome-*b* were then excised from the gel; DNA was extracted from the gel slices using a Qiagen QIAquick Gel Extraction kit™. Amplicons were sent to Inqaba Biotec (Hatfield, Pretoria, Gauteng, South Africa) for dye-terminator cycle sequencing in both the forward and reverse directions according to the Sanger method.

Sequencing analysis. Consensus sequences were constructed using BioEdit Sequence Alignment Editor (Version 7.0.0 for Windows 95/98/NT/2000/XP) (Hall, 1999). Each sequence was pasted into the BLAST search section of the NCBI website and the closest matches were downloaded and included in multiple sequence alignments for reference or as outgroups, as appropriate. This facilitated the species-level identification of each sample. Multiple sequence alignments were carried out in BioEdit (Hall, 1999) using the Clustal W option. Alignments were further corrected by eye and then trimmed to a uniform length for analysis as follows: 896bp for *Acomys* spp.; 1118bp for *Arvicanthus* spp.; 678bp for *Crocidura* spp.; 626bp for *Graphiurus* spp.; 535bp for *Lemniscomys* spp.; 365bp for *Mastomys* spp; 603bp for *Rattus* spp.; 587bp for *Gerbilliscus* spp.; and 543bp for *Aethomys* spp. and *Thallomys* spp.

Phylogenetic analysis of sequences. Haplotypes were generated using the program DnaSP (DNA sequence polymorphism) version 4.10.9 (Rozas et al., 2003). Information regarding the number of conserved, variable, parsimony informative and singleton sites were also generated using this program. The program jModeltest (Posada, 2008) was used to select the appropriate model of evolution, using the hierarchical likelihood ration test (hLRT) to select the Akaike information criteria (AIC) and the Bayesian information criteria (BIC). The model selected for cytochrome-*b* was GTR+I+G. These models were used for genetic distance, neighbour-joining and Bayesian analyses. Genetic distances were then calculated for each group for taxa within each dataset using PAUP 4.0b10 (Swofford, 2000) according to the selected model selected for each dataset. Neighbour-joining phylograms were created in PAUP 4.0b10 (Swofford, 2000) with support being calculated through bootstrap resampling (Felsenstein, 1985) analysis with 100 replications for each dataset. Bayesian estimation of phylogeny was done carried out using MrBayes version

3.0B4 (Huelsenbeck and Ronquist, 2001). Markov Chain Monte Carlo (MCMC). Four Markov chains were run for over 5000000 generations each was used to calculate posterior probability of tree distribution, with a burn-in value of 22500. Preliminary runs were carried out to ensure that the burn-in value used was more than sufficient to discard less-likely trees created before the probabilities for each chain had plateaued at the most likely-level.

References

Hall, T. A. (1999.) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**, 95–98.

Huelsenbeck, J. P., and Ronquist, F. (2001). Mrbayes: A Bayesian inference of phylogeny. *Bioinformatics* **17**, 754–755.

Posada, D. (2008). jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution* **25** (7), 1253–125.

Rozas, J., Sanchez-DelBarrio, J. C., Messeguer, X., and Rozas, R. (2003). Dnasp, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics* **19**, 2496–2497.

Saiki, R. K. D., Gelford, H., Stoffel, S., Scharf, S. J., Higuchi, R. G., Horn, G. T., Mullis, K. B., and Henlich, H.A. (1988.) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* **239,** 487–491.

Swofford, D. L. (2002). 'PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4.0b10.' (Sinauer Associates: Sunderland, MA.)