Chapter VI

Concluding comments on the phylogeny and phylogeographic patterns of *Scarabaeus (Pachysoma)* MacLeay (Scarabaeidae: Scarabaeinae).

Throughout the preceding chapters I have attempted to identify species relationships at both a morphological and molecular level and key patterns and processes in phylogeographic history that may have shaped the population structure within *Scarabaeus (Pachysoma)* seen today. I have also attempted to highlight conservation concerns based on some of the analyses done and questions posed. In this chapter I therefore attempt to summarise the key findings of each chapter and bring together the ideas and theories to identify the most important processes affecting or having affected *Scarabaeus (Pachysoma).*

Phylogenetic history

Much contention has surrounded the taxonomy of *Scarabaeus (Pachysoma)* and related taxa over the last 50 years. One of the primary aims of this study was therefore to produce an estimate of *Scarabaeus (Pachysoma)* phylogeny using both molecular and morphological data as individual datasets and then combined, in order to address whether the group was monophyletic, how *Pachysoma* was related to *Scarabaeus*, whether *Neopachysoma* was a valid genus and whether there were 13 good species within *Pachysoma*. The phylogenetic analysis was conducted using 64 morphological characters (obtained from Harrison and Philips (2003) and 1197 bp of the Cytochrome Oxidase I (COI) gene (Sole *et al*., 2005)).

Scarabaeus (Pachysoma) was found to be a monophyletic clade within *Scarabaeus* and was therefore classified as a derived subgenus thereof (Harrison *et al*., 2003; Forgie *et al*., 2005; Sole, 2005, Chapter 3). The synonymy of *Neopachysoma* with *Pachysoma* is supported even though it is clearly a distinct lineage within *Scarabaeus (Pachysoma)* (Sole *et al*., 2005; Forgie *et al*., 2005). Morphologically there were 13 good species within *Scarabaeus (Pachysoma)*. At a molecular level strong resolution was found for 11 of the 13 species with *S. (P.) hippocrates* and *S. (P.) glentoni* forming a species complex called the hippocrates/glentoni complex. The phylogenetic tree produced from the combined dataset showed strong support for all 13 species. The morphological and molecular data partition phylogenies showed congruence with the combined phylogeny, lending strong support for combining datasets using total evidence. Phylogenetic trees based on combined data

partitions were relatively more resolved than those based on the individual data analyses. Both the data partitions contributed to the overall combined phylogeny without the morphological data being overshadowed by the large molecular dataset, indicating that both the gene chosen as well as the characters had good resolving ability and were adequate for the level of phylogenetic information required.

Phylogeographic history

Biogeographic inferences could be made due to a recent comprehensive history of the geology and palaeo-climate of the Namib Desert being available (Pickford & Senut, 1999). Speciation events and divergence times were estimated by applying a molecular clock, which was based on Brower's (1994) 2.3 % divergence per million years, to the molecular data. The use of molecular data allowed for the relation of species age to past geological and climatic events rendering a base from which to infer phylogeographic history of the species of *Scarabaeus (Pachysoma)*.

Scarabaeus (Pachysoma) is estimated to have arisen about 2.9 million years ago, which appears to be young when compared with the age of the Namib Desert - dating back to the Miocene (*ca* 15 Ma). A consistent and reliable source of water in the form of advective fog (Nicolson, 1990), which is blown up to 50 km inland, can be associated with the radiation of *Scarabaeus (Pachysoma)* into inhospitable areas along the west coast of southern Africa (Logon, 1960; Seely & Louw, 1980; Nicolson, 1990; Pickford & Senut, 1999). Clear south-north evolutionary gradients can be seen within the species of *Scarabaeus (Pachysoma)*, that are consistent with the unidirectional wind regime, indicating that the psammophilous taxa disperse with their substratum and habitat the barchan dune (Penrith, 1979; Endrödy-Younga, 1982; Prendini, 2001). Major ancient rivers such as the Orange, Buffels and Holgat appear to be gene barriers to certain species of '*Pachysoma*' as well as areas of origin of speciation events (Irish, 1990).

Strong geographic association can be seen within the phylogenies where species that group together within the clades share similar distributions along the total *Scarabaeus (Pachysoma)* distribution. Species with a suite of mostly plesiomorphic characters have a southerly distribution while their derived psammophilous relatives have central to northern Namib distributions.

Population demographics

Three species of *Scarabaeus (Pachysoma)* were selected for detailed population studies, based on the fact that they exhibited distinct south-north morphological clinal variation as seen in the study by Harrison and Philips (2003). Using distance methods, basic population analyses methods and coalescent theories (Schneider *et al*., 2000; Beerli & Felsenstein, 1999; 2001; Kuhner *et al*., 2004) an attempt was made to answer questions aimed at assessing factors that could have contributed to the population structure exhibited by these species of *Scarabaeus (Pachysoma)*.

Three distinct species within *Scarabaeus (Pachysoma)* have been studied here, all exhibiting very different population demographics with overlap seen in areas of geographic similarity. *S. (P.) hippocrates* was shown to have four distinct populations in South Africa; *S. (P.) gariepinus* had three populations, two in South Africa and one in Namibia and *S. (P.) denticollis* was identified as a single population along a dune field continuum in Namibia. The phylogeographic partitioning seen in the three species was supported by the AMOVA analysis. All three species exhibit high overall haplotype diversity. Both the Stepwise (Mismatch distributions) and Exponential (LAMARC) Expansion Models indicate strong historical population expansion. Fu's UPBLUE and *F*s statistic values, indicative of recent population parameters were not always significant for all populations throughout the three species which may be an indicator that the present populations may not be undergoing population expansion but instead are in a slight decline or are maintaining population numbers. As recent events are shown to be masked by past trends giving rise to conflicting results; species census data collected over a number of years should be conducted in order to resolve this. Application of nested clade analysis (NCPA) (Templeton *et al*., 1995) indicated allopatric speciation for those populations separated by environmental and anthropogenic barriers – such as rivers and towns – while for the Namibia population of *S. (P.) gariepinus* and the species *S. (P.) denticollis* isolation by distance and continuous range expansion could be inferred as defining population structure.

Coalescence for each species was calculated and it was estimated that all three species underwent population expansion within the late Pleistocene era. Analysis of gene flow revealed a strong degree of south-north movement, consistent with the unidirectional wind regime. Large numbers of individuals were shown to have moved between populations. A high degree of historical gene flow indicates that the species were originally continuous populations within the geographic region but extinction of the intermediate populations most

likely occurred through both natural and human factors. Recent events therefore indicate that human induced, environmental barriers and reduced vagility have had a major influence on the population structure seen within these three species.

Conservation recommendations

Populations that show gradual geographic and individual variation at both a molecular and morphological level make defining species delimitations problematic (Drotz & Saura, 2001). Extensive molecular and morphological variation occurs across all three species. However to delimit added species or sub-species based on molecular data would not be desirable and may pose problems with regard to taxonomic concerns. It is clear that selective changes are occurring within the populations and that sufficient mitochondrial divergence has occurred, affecting overall population structure. If these changes are to continue to be observed and the species conserved, conserving authorities need be made aware of the circumstances and each population should be delineated as a Management Unit (Moritz, 1994a, b). Each population is connected by low levels of gene flow and are functionally independent and therefore should be managed as individual entities. To conserve every living creature is beyond our reach but an effort needs to be made where we are aware of changes and threats occurring within species and populations of species.

Isolation of Microsatellite markers

The aim behind this part of the project was to have a nuclear marker with which to compare the mitochondrial COI sequences because, by combining and comparing the same analyses on different genes a better overall picture could be obtained of the population demographics of *Scarabaeus (Pachysoma).* A second objective behind isolating microsatellites was that as these markers are often genus specific it would be interesting to use these powerful loci on different species of the large and variable genus *Scarabaeus,* to answer additional taxonomic and demographic questions that were posed throughout this thesis.

The FIASCO protocol was chosen over other methods of microsatellite isolation as it is fast, efficient, requires only basic skills in molecular biology and limited laboratory equipment as compared to that for traditional methods of microsatellite screening. The FIASCO protocol is an enrichment protocol based on the ability to recover microsatellite DNA by PCR amplification, after selective hybridisation (Zane *et al.*, 2002). As microsatellites need be isolated *de novo* this turned out to be a daunting and labour intensive process and problems resulting in a low yield of polymorphic loci were two-fold. The first

problem encountered was with cloning the DNA fragments into the vector cells for colony growth. Probes were selected based on previous studies of Coleoptera where microsatellites were isolated but this did not improve the cloning procedure. Different agar media were tried in case the competent cells were sensitive to the agar, which they were not. Different time combinations as suggested by the TOPO cloning manual were used and lastly the competent cells were tested on both Avian and Mammalian DNA to test whether they were of poor quality, which they were not. The second problem was encountered during optimisation of a locus where consistent conflicting PCR results were obtained. In some instances the PCR's contained single bands while under the same conditions using the same reagents double bands where obtained in a separate amplification reaction. These two problems were identified in both the orders Coleoptera and Lepidoptera, indicating that they may be common across unrelated taxa (Meglécz *et al*., 2004). However, as failed attempts at microsatellite isolation are generally not published, the underlying cause of the problems experienced can only be speculated upon. Despite these difficulties the FIASCO protocol was optimised for *Scarabaeus* and four polymorphic microsatellite loci were successfully isolated. However, for the analyses to be statistically powerful this is too few to constructively work with, at least one extra locus is needed for the completion of this part of the study.

Future research

Many possibilities for future research can be suggested from this study. I include only those which will most enhance the research done and may be of particular interest.

The resolution of the hippocrates/glentoni complex has been an issue that needs to be resolved. Morphologically these two species are very similar and can reliably be identified based on male genitalia. By increasing the number of specimens and analysing a different gene better phylogenetic resolution at a molecular level, should be obtained for these two species.

An addition of a nuclear gene or genes such as a ribosomal gene - 18S/16S - or a protein-coding gene - elongation factor-1 α - would be of interest to be sequenced for the population study, as this would support or refute the slightly conflicting results regarding the biogeographic history of the group presented here. An added microsatellite locus needs to be isolated to have at least five polymorphic loci so as to ensure the statistical power of the

analyses is sufficient. The microsatellite data should be analysed and published in conjunction with the mitochondrial COI data, so as to ascertain whether overlying patterns exist between the two types of DNA. Once the microsatellite section of this study has been completed these loci can and will hopefully be successfully used within other species of *Scarabaeus* for similar and more detailed studies to elucidate phylogeographic and demographic patterns.

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