Chapter I

General Introduction:

Rationale for investigating the phylogeny and phylogeography of *Scarabaeus (Pachysoma)* MacLeay (Scarabaeidae: Scarabaeinae).

Conservation Genetics, Pattern and Process

"The overarching aim of conservation biology is to protect biological diversity and the processes that sustain it in the face of perturbations caused by human activity," (Moritz, 2002). Challenges we face are therefore threefold, 1) our knowledge of pattern and process is incomplete, 2) natural and anthropogenic change are bound to occur within a system and 3) conflict between human societies and biological needs is inevitable and reconciliation will only be achieved through trade-offs and priority setting (Moritz, 2002).

Conservation biology is therefore aptly described as a "crisis discipline." The magnitude of this crisis is evident by the large number of species being endangered or facing extinction. Presently 713 species are categorised as extinct/extinct in the wild, 5483 species are classified as critically endangered, endangered or vulnerable and 12,716 species as lower risk/conservation dependent, near threatened, data deficient and least concern (according to IUCN redlist of Threatened Status Category (2005): Summary for all Classes and Orders: www.redlist.org). In an attempt to prevent crisis management we need to understand the patterns and processes that conservation biology aims to describe by including detailed and comprehensive studies of organisms to date (DeSalle & Amato, 2004). The idea, therefore, is that conservation genetics aims at creating an accurate picture of pattern and process in the endangered species.

Conservation biology thus far is expanding to incorporate many disciplines, which allow for conservation biologists to more effectively address critical problems regarding the management of endangered species and critical areas. Genetic information not only allows for many conservation decisions to be placed in context but also adds unprecedented precision and understanding to decision making (DeSalle & Amato, 2004).

The integration of demographic factors (biology of population growth and life history) and genetic approaches often allow for strong inferences to be made regarding conservation biology. Conservation genetics allows for the quantification of processes, such as inbreeding depression, effective population size, minimum viable population size, levels of genetic variation and gene flow, that may all affect endangered populations. Conservation decisions often rely on the designation of species boundaries, which in itself is a contentious issue in both systematic and evolutionary biology. The delineation of conservation units - environmentally significant units (ESU's) and management units (MU's) (Moritz, 1994a & b) may help designate conservation priorities and are thought to be of paramount importance while other units such as semi-species, incipient species and subspecies are thought to be of lesser concern due to high levels of confusion surrounding their definitions. The challenge in conservation genetics is to firstly integrate the genetic data with both biological and non-biological data and secondly to use the results obtained from these studies in the implementation of a successful conservation decision in the context of social, cultural and political issues.

Phylogeography, Molecules and Morphology

Phylogeography is the study of genes and geography. By overlaying molecules and geographic data over time and space, historical inferences about evolutionary processes at the population level can be inferred (Avise, 2000). Inferences include the restriction of gene flow by geographical and historical barriers, colonisation success of some lineages and the effects of population bottlenecks (Diniz-Filho *et al*., 1999).

Phylogeography, by revealing divergent evolutionary lineages often overlooked by traditional taxonomy and by identifying biotic processes, can help direct conservation biology (DeSalle & Amato, 2004). A crisis discipline often sees periods of expansion for tools used to solve problems that the crises pose. Proliferation of the technologies for genomics, systematics and population biology over the past decade has been a key factor for the integration of genetics into conservation biology (DeSalle & Amato, 2004).

DNA sequence data from the mitochondrial genome are being increasingly used to estimate phylogenetic relationships between taxa. The use of DNA sequence data provides an empirical means of understanding the processes governing the evolution and inheritance of DNA. Mitochondrial genes are chosen for study as they are easy to manipulate, clonally inherited, single copy, non recombining and abundant (Simon et al., 1994). Accurate estimates of species limits are imperative for biodiversity assessments especially in areas of endemism. Species are the basic units of biodiversity on which evolutionary biology focuses (Puorto *et al*., 2001). Given the fact that morphology and molecules evolve at different rates, these characters within the same taxa will have been exposed to similar vicariant biogeography as well as climatic changes and will therefore exhibit similar histories.

The overall availability of the number and diversity of characters is increasing at a remarkable rate in phylogenetic studies. How, therefore, to successfully integrate molecular and morphological data is one of the challenges of phylogenetics today. Different data sets often exhibit similar topologies with differences restricted to the positions of a few taxa, so may tell us different stories (Baum, 1992; Marshall, 1992). Three approaches have been suggested when combining datasets: (1) separate analysis, where trees are estimated separately from each partition, and the different estimates compared using taxonomic congruence (Miyamoto & Fitch, 1995); (2) the total evidence approach, whereby all available data are combined in a simultaneous analysis (Kluge, 1989); and (3) conditional data combination, whereby only homogenous data partitions (estimated by a statistical test of homogeneity) are combined in a simultaneous analysis (Bull *et al.*, 1993; de Queiroz *et al.*, 1995). It is desirable to know, when combining data sets, how each data partition contributes to the final tree topology. This can be achieved by comparing the overall tree topology with the individual trees of each data partition (Creer *et al.*, 2003).

Inferences in evolutionary history are often based on the determination of genetic relatedness among individuals and the extent of the differences between them. The patterns of relatedness are often a result of processes occurring over two time scales: evolutionary time that encompasses broad-scale changes in prevailing environmental conditions, and ecological time over which population processes (e.g. migration, local extinction and colonisation) occur (Martin & Simon, 1990). Evolutionary biology, therefore, aims to unravel these interactions and assess the importance of short- and long- term processes. Understanding of evolutionary processes can be brought about by the study of closely related taxa representing a spectrum of divergence levels (Martin & Simon, 1990).

Genetic structure of a population is generally a result of both biogeographical factors and ongoing ecological and demographic processes (Carisio *et al.*, 2004). Our understanding of species formation from an evolutionary paradigm is based on the foundation of population level comparisons. By examining the variation among populations, their historical associations and the processes of genetic restructuring, what may have lead to speciation can often be revealed (Wright, 1931).

Scarabaeus (Pachysoma) MacLeay (1821)

Dung beetles are probably the first insects to be considered divine. In ancient Egypt the beetles were worshiped in the form of the solar deity, Khepera who controlled the sun's daily path across the sky, where the beetle represents the sun god 'Ra' and the ball the sun moving across the sky (Forgie, 2003). The rolling of the dung ball is one of the methods used by dung beetles to move and process dung. Dung represents a patchy, ephemeral and limited food source. These characteristics would have been the most probable factors allowing for the diversity in morphology, behaviour and ecology presently seen. Although many species form balls and roll them backwards with their hind limbs exceptions to this exist in that some species drag preformed dung pellets/detritus forward (Scholtz, 1989; Philips *et al*., 2002) while others may carry dung pellets with their front legs and sometimes heads (Halffter & Matthews, 1966; Zunino *et al*., 1989; Philips *et al*., 2002).

Scarabaeus (Pachysoma) is a subgenus of the Scarabaeini (Scarabaeidae: Scarabaeinae), a tribe whose members are found in moist savanna through to drier regions including very hot dry deserts (Scholtz, 1989) of the Afrotropics and southern latitudes of the Palaearctic. Scarabaeines predominantly feed on dung, but have also been known to feed on humus, carrion and fungi (Scholtz & Chown, 1995). Scarabaeini are one of 12 tribes in the Scarabaeinae that are differentiated in part by behavioural trichotomy between those that breed inside the dung pad (endocoprids), those that bury the dung in preformed burrows at the food source (paracoprids), and those that remove the dung and bury it some distance from the food source (telecoprids) (Balthasar, 1963; Halffter & Edmonds, 1982; Scholtz & Holm, 1985; Hanski & Cambefort, 1991).

Members of *Scarabaeus* (*Pachysoma)* are flightless and have feeding and foraging adaptations that are unique within the Scarabaeinae (Scholtz, 1989). *Scarabaeus* (*Pachysoma)* is an exception to the conventional backward dung ball rolling of the Scarabaeini. The beetles randomly move in search of dry dung pellets or plant matter (detritus) which, when found, is gathered up and held in the long comb-like setae on the hind limbs and dragged forwards to be buried in a preconstructed holding chamber (Scholtz, 1989; Harrison, 1999). This is repeated to provision the holding chamber. The nest is then expanded to below the moisture line (Scholtz, 1989). Moisture from the surrounding soil re-hydrates the stored food supply making it suitable for consumption.

The Namaqualand and the Namib Desert

Scarabaeus (Pachysoma) distribution extends from just north of Cape Town, in South Africa, to Walvis Bay, in Namibia and encompasses three distinct biomes. The southern tip comprises the western extreme of the fynbos biome, the area up to the Orange River is geographically considered to be Namaqualand and the section north of the Orange River to Mossamedes in Angola is considered Namib Desert (van Zinderen Bakker, 1975; Rutherford

& Westfall, 1994; Pickford & Senut, 1999). Widespread aridity on the west coast of Africa is related to the up welling of cold surface water, the Benguela Current, and the continental rain shadow – rain originates from moist air blown in from the Indian Ocean, east coast. Aridity becomes more intense as one moves northward, culminating in the Namib Desert (Tankard & Rogers, 1978). Rainfall is minimal but constant moisture is available to the fauna and flora through the formation of coastal fog banks, which are wind blown up to 50km inland (Logon, 1960; Seely & Louw, 1980). Presently the Namib Desert is one of the driest parts of the African continent and from a taxonomic point of view one of the richest deserts in the world (van Zinderen Bakker, 1975). The evolutionary processes resulting in the great number of endemic taxa points to a great age of the Namib with an undisturbed climatic history (van Zinderen Bakker, 1975). However some physical, chemical and biological attributes suggest that the aridity is youthful, developing progressively since the Miocene (Tankard & Rogers, 1978), indicating that relatively rapid radiation has occurred in most taxa found in this area.

Adaptations to the desert and flightlessness

The evolution of flight is thought to have contributed to the diversity and evolutionary success of insects. Flight allows for certain benefits including dispersal, the successful searching for mates, food and habitats (Roff, 1990, Scholtz, 2000). Contrary to these benefits certain species have secondarily become flightless (Scholtz, 2000). Some of the factors said to influence flightlessness are habitat persistence or environmental heterogeneity, geographic variables, alternative modes of migration and taxonomic variation (Roff, 1990).

Deserts are thought to pose considerable constraints on organisms occurring there. Many morphological, behavioural and physiological adaptations exist within desert animals permitting them to survive under harsh conditions. For all desert arthropods living in arid environments life is complicated by being small and having a relatively large surface area, which in turn leads to rapid exchange of heat and water with the surrounding area (Nicolson, 1990).

A possible physiological advantage of wing loss is that it allows an insect to divert energy associated with the wing and wing muscle development to some other use such as increased fecundity. Wing muscles are relatively massive structures within insects comprising 10 – 20% of the body mass of most insects (Roff, 1990). It has been shown that many insects histolyse their wing muscles during egg production, leading us to believe that this is a means to increase egg/sperm/offspring production, thereby increasing their overall fecundity (Roff, 1990).

Morphologically, flightlessness is associated with a secure joining of the elytra along the midline. The fusion of the elytra creates a hermetically sealed chamber called the subelytral cavity (Byrne & Duncan, 2003). This fusion of the elytra is thought to be a modification to prevent water loss through evaporation (Chown *et al*., 1998; Scholtz, 2000). Many desert beetles have a subelytral cavity with representatives being found in tenebrionids, carabids and scarabs (Byrne & Duncan, 2003). Flightless beetles have been shown to exhibit unidirectional tidal airflow (forward airflow, i.e. airflow from the posterior to the anterior body) as opposed to the previously thought convention of respiratory airflow moving from the anterior to posterior of the body (Duncan, 2003). The combination of tidal airflow and a subelytral cavity has allowed for arid-dwelling beetles to reduce water loss by releasing respiratory CO_2 via a single mesothoracic spiracle into the atmosphere (Byrne & Duncan, 2003). In this way water loss is, therefore, confined to a small area of the total respiratory system, with beetles losing up to 4% total water as opposed to 74% if all the spiracles were exposed to the atmosphere (Duncan, 2002; Duncan, 2003).

The species of *Scarabaeus* (*Pachysoma)* feed on dry rodent or herbivore pellets and/or detritus. Due to the dryness in the desert, rates of decay are slowed down considerably so insects feeding on detritus, carcasses or the persistent parts of desert plants have their food sources persist for long periods of time (Roff, 1990; Scholtz, 2000). *Scarabaeus* (*Pachysoma)* beetles drag the dry dung or detritus to below the moisture line allowing for re-hydration (Scholtz, 1989). Most beetles do not take advantage of the hygroscopic water absorption by detritus as they feed only during the day, in which the detritus has only 2% water content. If the beetles were to feed on the detritus when the fog was present they could be consuming detritus containing 60% water (Nicolson, 1990). This could be one of two reasons for *Scarabaeus* (*Pachysoma)* beetles dragging the dry dung or detritus to below the moisture line prior to feeding on it. Another reason for feeding below the moisture line could be that they are dependent on micro-organisms such as fungi and bacteria in the dry dung or detritus for food but these need moisture for development (Scholtz pers. comm.).

Systematic concerns

The diversity we see today and the uniqueness of its components is one of the more remarkable aspects of life. No two individuals in a sexually reproducing population are the same, nor are any two populations, species or higher taxa. According to Mayr & Ashlock (1991), '*Taxonomy is the theory and practise of classifying organisms*' and much, if not all, biological research is based on a sound phylogeny. Taxonomy *s.l.* serves not only to identify and classify organisms but also allows for the comparative study of organisms as well as the role of lower and higher taxa in nature and evolutionary history (Mayr & Ashlock, 1991). Delimiting a species is important for understanding many evolutionary mechanisms and processes. Species are also used as the fundamental units of analysis in biogeography, ecology, macroevolution and conservation biology (Sites & Marshall, 2003). Two goals for systematic studies are to: 1) discover monophyletic groups at higher levels and 2) discover lineages (i.e. species) at lower levels (Sites & Marshall, 2003). A good phylogeny is therefore of paramount importance if good phylogeographic and population studies are to follow.

The genus *Pachysoma* was first described by MacLeay (1821). *Pachysoma* was defined by aptery, absence of humeral calli, semi-contiguous mesocoxae and short mesosterna (Ferreira, 1953). An evaluation by Holm & Scholtz (1979) concluded that these characteristics were either due to convergence or were too variable and inconsistent to use as the justification for a genus. In spite of this its generic status was maintained. The genus was later synonomised with *Scarabaeus* Linnaeus, 1758 by Mostert & Holm (1982). Endrödy-Younga (1989) and Scholtz (1989) questioned the synonymy of *Pachysoma* with *Scarabaeus* as the former have a unique set of morphological and behavioural apomorphies including unique feeding and foraging biology, a rounded body shape due to flightlessness and are restricted to the south-west coast of Africa. In a recent phylogenetic analysis of *Scarabaeus* (*Pachysoma)* by Harrison & Philips (2003) *Pachysoma s.l* forms a distinct clade within *Scarabaeus* and is therefore considered a subgenus thereof.

Relevance of this study

Habitat destruction and or deterioration are arguably the greatest threats to insect diversity (Samways, 1994). *Scarabaeus (Pachysoma)* occurs in the Succulent Karoo, Fynbos and Desert biomes (Rutherford & Westfall, 1994). Within this large range the species exhibit discontinuous distribution owing to their low vagility. Their distribution therefore consists of pockets of isolated populations some of which are threatened by the removal of the natural vegetation for large scale wheat farming in the south-western Cape, commercial development on the West Coast for holiday and recreational purposes e.g. Lambert's Bay and Strandfontein, mining for diamonds and other minerals and by exotic plant invaders e.g. Port Jackson (*Acacia saligna*) and Rooikrans (*Acacia cyclops*), modifying dune systems. Furthermore, some of the species are potentially threatened through their collection and sale to collectors (Harrison, 1999). Therefore, knowledge of their habitat requirements, taxonomy, behaviour and distribution is of vital importance for the initiation of conservation strategies to ensure their survival.

Key Questions of this thesis:

Given the background above the objectives and key questions of the present study were:

Chapter 2 **- Phylogeography of the Namib Desert dung beetles *Scarabaeus (Pachysoma)* MacLeay (Coleoptera: Scarabaeidae).**

Key Questions

Q1. To resolve the relationships of the 13 species of *Scarabaeus (Pachysoma)* based on mitochondrial cytochrome oxidase I.

Q2. To estimate the divergence times and ages of the species within *Scarabaeus (Pachysoma)* and to relate these to past geological and climatic events

Chapter 3 - **Testing for congruence between morphological and molecular characters of *Scarabaeus (Pachysoma)* MacLeay (Coleoptera: Scarabaeidae).**

Key questions:

Q1. To resolve the phylogenetic relationships between the 13 species of *S. (Pachysoma)* using Parsimony and other methods based on both morphological and molecular data partitions.

Q2. To test for monophyly of *Scarabaeus (Pachysoma)* within *Scarabaeus*

Q3. To test whether there is congruence between the morphological and molecular datasets using the total evidence approach.

Chapter 4 - **Phylogeographic patterns of *Scarabaeus (Pachysoma)* (Coleoptera: Scarabaeidae) inferred from gene genealogies and coalescent theory.**

Key Questions

Q1. To what degree has geographic isolation led to the genetic restructuring between populations of the same species.

Q2. What is the extent of gene flow between populations of the same species and does it correlate with patterns of geographic proximity?

Q3. Where geographically did *Scarabaeus (Pachysoma)* originate and how are the populations of each species related to one another?

Q4 What are the effective/actual population sizes of the species in question?

***Chapter 5* – Isolation of microsatellite markers from *Scarabaeus (Pachysoma)* MacLeay (Scarabaeidae: Scarabaeinae).**

Key Questions

Q1. To successfully optimise the FIASCO enrichment protocol for the genus *Scarabaeus*.

Q2. To design at least five polymorphic microsatellite loci for the genus *Scarabaeus*.

***Chapter 6* – Concluding comments**

Based on the key questions above the essence of this project was three-fold. It was: firstly, to resolve the phylogenetic relationships between the 13 species of *Scarabaeus (Pachysoma)*; secondly, to elucidate phylogeographic patterns of the species through inferences from historical population dynamics; and lastly to identify and delineate genetically meaningful conservation units, environmentally significant units (ESU's) and management units (MU's) (Moritz, 1994a & b) within the different species. This information would be useful for developing sound conservation management recommendations, as they would be based on a good phylogeny with both strong molecular and morphological inferences as well as ecological data.

Thesis outline

Each of the chapters of this thesis has been compiled as a separate paper for publication purposes. Chapter 2 has been published in the Journal of Biogeography and is formatted for the journal. Chapter 3 has been submitted to Molecular Ecology. Chapter 3 and all the other chapters were formatted for Molecular Ecology. Chapter 4 comprises three sub-chapters based on the three species identified for population analysis. At the start of chapter 4 there is a general introduction and methods used for each species, each sub-chapter has a short introduction, results and discussion. Each chapter contains its own set of references and all appendices can be found at the end of the thesis. Both the general introduction and conclusion are tailored from the respective chapters, which give an overview of what to expect within the thesis and what conclusions were drawn.

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