



**DEPARTMENT: ENVIRONMENTAL AFFAIRS AND TOURISM
REPUBLIC OF SOUTH AFRICA**

Tel: (012) 310-3911

Fax: (012) 322-2682

Ref: C10/2/1/2/7/2

Enquiries: Ms K Ngxabani

Tel: 021 405 9421 Fax: 021 405 9424 E-mail: kusi@antarc.wcape.gov.za

Mr J Cooper (Avian Demography Unit)
Department of Statistical Sciences
University of Cape Town
RONDEBOSCH
7701

RECEIVED
28 OCT 2003
AMC

2 October 2003

Dear John

**PRINCE EDWARD ISLANDS MANAGEMENT COMMITTEE (PEIMC) MEETING:
29 AUGUST 2003**

1. Emanating from 16th PEIMC meeting held at our offices in Cape Town, please refer to the revised version of document 4.5. (It was discovered that even numbers of the document were missing, hence the revised one).
2. Please confirm receipt thereof to kusi@antarc.wcape.gov.za
3. Should you have any queries, you are welcome to contact me.

Kind regards

Kusi Ngxabani
for DIRECTOR-GENERAL

SANAP INTERIM PROGRESS REPORT

PROJECT TITLE:

Threats to biodiversity and ecosystem functioning at the Prince Edward Islands: developing a conservation strategy for endemic and keystone insect species

RESPONSIBLE PROJECT LEADER (AND CO-LEADERS)

Dr. A.D. Slager-Bastos

Department of Zoology & Entomology

University of Pretoria

Pretoria 0002

South Africa

Prof. C.T. Chimimba

Department of Zoology & Entomology

University of Pretoria

Pretoria 0002

South Africa

Prof. S.L. Chown

Department of Zoology

University of Stellenbosch

Private Bag X1

Matieland 7602

South Africa

PROJECT RESEARCHERS

Ms. L.J. van Rensburg

Department of Zoology & Entomology

University of Pretoria

Pretoria 0002

South Africa

Mr. G. Grobler

Department of Zoology & Entomology

University of Pretoria

Pretoria 0002

South Africa

DATE OF REPORT

Third Interim Progress Report, June 2002 - June 2003

(a) Objectives

Hypothesis 1. There is only a single species of *Pringleophaga* (Lepidoptera: Tineidae) on the Prince Edward Islands and the populations do not differ genetically to the extent that they should be managed as separate units.

This is a straightforward phylogeographic hypothesis that must be tested. Essentially we will determine the genetic distance between populations of the *Pringleophaga* species on Marion and Prince Edward Island, and between the extreme phenotypes of these species found on each of the islands. In each case, at least 10 adults and 40 larvae (largest to be found) of each population will be sampled. The cytochrome oxidase subunit I mitochondrial DNA (mtDNA) gene sequences will be determined as previously described by Bogdanowicz *et al.* (2000). Neighbor-joining, minimum evolution, parsimony and maximum-likelihood analyses will be carried out with PAUP* and MEGA (Swofford 1999; Kumar *et al.* 2002). Sequence data will also be generated for representatives of *Pringleophaga crozetensis* and *Pringleophaga kerguelensis* from Possession and Kerguelen Islands, respectively. The inclusion of these species will permit assessment of the degree to which the Prince Edward and Marion Island populations are genetically distinct from each other relative to other known species.

Hypothesis 2. Although they differ morphologically, and to some extent in their biology, the two *Ectemnorhinus* species found on Marion Island do not show sufficient genetic distance for them to be considered separate species.

This is also a phylogeographic hypothesis that will be tested. However, because *E. similis* and *E. marioni* vary considerably depending on their habitat of origin, material will have to be collected from all of these habitats. Thus, at least 20 specimens will be collected from *Sanionia uncinata* and *Blepharidophyllum densifolium* mires, *Acaena magellanic* drainage lines, *Cotula plumosa* herbfield *Pringlea antiscorbutica*, *Callitriche antarctica*, *Ditrichum strictum* and *Andreaea acuminata* moss cushions, and *Poa cookii* tussock grassland. In addition, because of the complex situation on *Azorella selago* cushions, 40 specimens of each morphological type (recognized as *E. similis* and *E. marioni*) will be collected. For each specimen partial sequences of the cytochrome oxidase I (COI) gene will be generated. The usefulness of genetic characterization of this region of the mitochondrial genome in determining patterns of colonization of the Canary Islands by darkling beetles has previously been demonstrated. In addition to the Marion island specimens, representatives of *Ectemnorhinus* species, *E. richtersi* material from the Crozet archipelago and *E.*

viridis from Heard Island will be genetically characterized so as to obtain reasonable indications of the evolutionary differences between populations. Material for the afore-mentioned species has already been made available. Phylogenetic analyses will be performed as described in hypothesis 1, the results of which will assist in future conservation management strategies.

Some of this material is already available and was collected by previous studies as part of the general collection procedures. The material is stored in the collection of S.L. Chown or at the National Collection of Insects, Pretoria.

Hypothesis 3. The populations of the two *Ectemnorhinus* species found on Marion Island and Prince Edward Island are not sufficiently distinct genetically for them to be managed independently.

This hypothesis is similar to the previous one. To complete the work here, the collections will have to be made on Prince Edward Island. Some of this material was collected as part of the qualitative invertebrate survey undertaken by A.G.A. Gabriel and J. Barendse in 2000 for the project run at that time by the co-project leader. Given the usefulness of CO I and CO II gene sequences for evolutionary studies from intraspecific phylogenies up to higher evolutionary levels, the molecular characterization and analyses will be performed in an identical manner to that outlined above. In this phase of the study, all of the information will be combined.

Hypothesis 4. There has been no consistent change in the population densities of the *Ectemnorhinus* species and the *Pringleophaga* species on Marion Island.

In 1975/6, 1983-1985, 1986-1988, 1991-1993, and from 1996-2000, numerous density estimates of these species were made in a variety of contexts. To date there has been no attempt to compile these data and analyze them in such a way as to determine just how significant the impact of mice has been on populations of these species. We intend to compile these data and to investigate whether there are significant differences in the densities of each species between years and whether there is any trend detectable. As part of a pilot study we have already secured the original invertebrate density data collected by Alan Burger in the mid-1970s. We intend to secure the original data of all of the other authors mentioned here. These data will then be subject to standard statistical analyses (mostly Generalized Linear Modelling) taking year, habitat and collector into account.

Hypothesis 5. There has been no change in the sizes or body shapes of the populations of the *Ectemnorhinus* and *Pringleophaga* species on each of the two Prince Edward Islands, relative to each other and to the populations when sampled fourteen years ago.

Since the mid 1980s, and as part of a study of the weevil diversity of the Prince Edward Islands, material of all six weevil species has been collected and analyzed both for body size and other morphometric characters. The collections made in 1986/7 were repeated in 1992 and formed the basis of the first work demonstrating the effects of mice on these species. Similar material was collected in 1995 and again in 1999/2000 either as part of physiological or ecological research being undertaken at the time. Our approach here will be to compare the body sizes and shapes of the individuals of each of the six weevil species from Marion and Prince Edward Island for each of the years. Specifically we will determine whether there is any temporal body size trend in the adults of the species that form the most common prey types of house mice (*E. similis*, *E. marioni* and *Bothrometopus randi*) for both Marion and Prince Edward Island. Using the less-preferred or rarely taken species (*Palirhoeus eatoni*, *Bothrometopus elongatus*, *B. parvulus*) we will determine the extent of the background, inter-annual variability that might be a consequence simply of stochastic variation, or perhaps a change in climates at the islands. We will also determine whether there has been a change in the morphological space (i.e. size and shape) occupied by these species since the first analysis undertaken in 1986/7. This will be done using standard and geometric ecomorphological techniques.

Pringleophaga sp. larvae have also been collected routinely for either ecological or physiological research work. Most of this material has been preserved and either resides in national museums (e.g. Transvaal Museum) or in collections of current or past principal investigators. Because determining larval instars for *Pringleophaga* species is extraordinarily difficult, our approach will be to construct body size frequency distributions for each collection that has been made on a different date and on a different island, and then to determine whether the upper tail of this distribution has moved significantly to the left in any consistent way.

Because there is a strong relationship between body size and virtually every component of insect life history (e.g. fecundity, feeding rate, metabolic rate, growth rate), alterations in body size of keystone species such as *P. marioni* will alter ecosystem functioning on Marion Island. If we detect trends in the body sizes of our focal species, we will use previously established estimates of the relationships between body size and physiological

rates to provide a first approximation of the likely effects of such changes on ecosystem functioning.

(b) History of the Project

This project commenced in April 2001. The first 3 months, reported on in the first interim report detailed the insect sampling carried out during the 2001 relief period and the approaches that were to be followed with respect to the genetic and morphological analyses. The second progress report dealt with the development and application of molecular methods for characterising the six weevil species on Marion Island. Emphasis was placed on discerning the genetic relationships of the two *Ectemnorhinus* species previously distinguished on the basis of size and feeding preferences. Phylogenetic analysis of the COI gene sequences generated for weevils representative of the size and feeding diversity within this genus on the island indicated that there was no genetic support for the two species presently recognised. Instead a genetically diverse species-complex exists. The morphometric component of the study was aimed at reducing the number of measurements, without compromising on informational content. By economising on measurements, the sample size can be increased. A summary of the progress made in the 12 months since this 2nd report, is detailed below.

Project Funding to Date

Category	2001/2	2002/3	2003/4
Human resources costs	R80 000	R80 000	R80 000
Running expenses	R93 590	R100 900	R110 955
Capital equipment	-	-	-
TOTAL	173 590	R180 900	R190 955

(c) Scientific progress

[1] MARION ISLAND SAMPLING - 2003

Field sampling was conducted at Prince Edward Island in April 2003 by G. Grobler under the supervision of R. Mercer. The same six species previously collected on Marion Island, namely, *Ectemnorhinus similis*, *E. marioni*, *Bothrometopus randi*, *B. parvulus*, *B. elongatus* and *Palirhoeus eatoni* were sampled on Prince Edward. Sampling on Marion Island in 2003 was directed at obtaining representative specimens from localities that were previously not sampled. The localities sampled from in 2003 include Kildalkey, Johnny's Hill, Watertunnel, Greyheaded, Pyroxene kop, Good hope bay and Sidney Hill. An altitudinal sampling method ranging from 0m to 675m a.s.l (Van Zinderen Baker Peak) was followed on Prince Edward Island. In addition to securing weevil specimens, approximately 80 *Pringleophaga marioni* larvae were also collected at Prince Edward Island.

As before, all 2003 specimens have been sorted, individually labelled and stored. Each specimen is also being photographed at the Electron Microscopy Department on the UP main campus and these visual recordings are being archived. These electronically captured dorsal, ventral and lateral images will be used for identification purposes as well as for geometric morphometric analyses.

[2] MORPHOMETRIC ANALYSES

Measurement recording for all the specimens collected from PEI between 2001 and 2003 has been completed. In addition, measurement recording for approximately 2000 weevils was performed on samples obtained from the National Insect Collection in Pretoria, the Transvaal Museum and from the private collection of Prof. S.L. Chown. The latter samples were collected between 1952 and 1992 and will permit assessment of morphometric changes in the weevil species over time.

Previous morphometric studies of the six weevil species occurring on Marion Island were based on 23 linear measurements defined and illustrated by Chown (1989).

Initially, the same 23 measurements were considered for the present study. However, it has been shown that after the assessment of linear dependence (redundancy) and colinearity, sets of many quantitative measurements can be reduced and still contain equivalent information (Chimimba & Dippenaar 1995). Consequently, the 23 linear measurements were subjected to a series of cluster analyses and ordination procedures in order to partition redundant and colinear measurements. Various criteria developed by Chimimba & Dippenaar (1995) were used as guidelines for the subsequent removal of redundancy and the selection of a final set of 13 measurements. While reducing the number of measurements for subsequent recording, these analyses permitted an adequate representation of the phenotype, consistent with the concept of morphological integration. In addition, these analyses allowed the assessment of sexual dimorphism.

[3] GENETIC ANALYSIS

This study represents the first attempt at genetic characterisation of PEI weevil and moth species. The taxonomy of the *Ectemnorhinus* genus within and between islands of the PEI archipelago is unclear but molecular markers are powerful tools, not only for clarifying taxonomic status but also for determining patterns of colonization of islands by beetles and for determining the relative ages of weevils and the islands they inhabit (Juan *et al.* 1995; Sequeira *et al.* 2000). The cytochrome oxidase I gene of the mitochondrial DNA (mtDNA genome) revealed the presence of a single *Ectemnorhinus* species on Marion Island, but did not address the effects of predation (2nd interim report). Sampling from mice-free localities on Marion Island and altitudinal sampling from Prince Edward in 2003, makes it possible to address hypotheses 2 and 3.

3.1 Materials & Methods

3.1.1 Genetic characterisation of moths

Attempts to amplify the COI gene of *Pringleophaga* specimens with universal insect primers (Simon *et al.* 1994) proved unsuccessful. This is presumably due to primer-binding failure as the quality and integrity of the DNA extracted has been confirmed. The complete failure to amplify COI indicates that *Pringleophaga* probably differs markedly at its COI gene compared to other

Lepidoptera genera, as various reports from the literature attest to the success of the available 'universal' insect primers with these genera.

3.1.2 Genetic characterisation of weevils

Individuals representative of the six weevil species occurring on both Marion Island and Prince Edward Island were selected for the study. All weevils were photographed and body length measurements were recorded. In order to address the diversity of habitats, differences in size and variation across altitudinal ranges, weevils from different *Ectemnorhinus* vegetation types, of varying size and from different altitudes (0 m - 1000 m on Marion Island and 0m – 600m on Prince Edward Island) were selected for this study.

Between two and three weevil legs were ground with liquid nitrogen and total genomic DNA was extracted using a guanidine thiocyanate / silica-based method (Boom *et al.* 1990). Due to technical difficulties encountered with the older samples collected from other non-PEI islands, genomic DNA had to be extracted using a commercial kit, following which a region of approximately 1.1 kbp corresponding to the CO I gene was targeted for amplification with PEI-specific weevil primers (Grobler & Bastos unpublished). Amplicon sizes were estimated following agarose gel electrophoresis and the products of the appropriate size were purified directly from the tube. DNA sequences were generated for each of the weevils by an automated cycle sequencing approach.

3.1.3 Phylogenetic analysis

Nucleotide sequences were aligned in DAPSA (Harley 2001) and a homologous region of 850 bp was identified for phylogenetic analysis. Gene trees were constructed using neighbor-joining (NJ), maximum parsimony (MP), minimum evolution (ME) and maximum likelihood (ML) methods included in PAUP* (Swofford 1999). Trees constructed using NJ and ME algorithms assumed a variety of substitution models in order to assess the sensitivity of results to the reconstruction methods, whilst those constructed using ML methods were based on the HKY 85 base substitution model. 1000 bootstrap replications were performed for each analysis in order to determine support for the clades / clusters identified by the different methods of reconstruction.

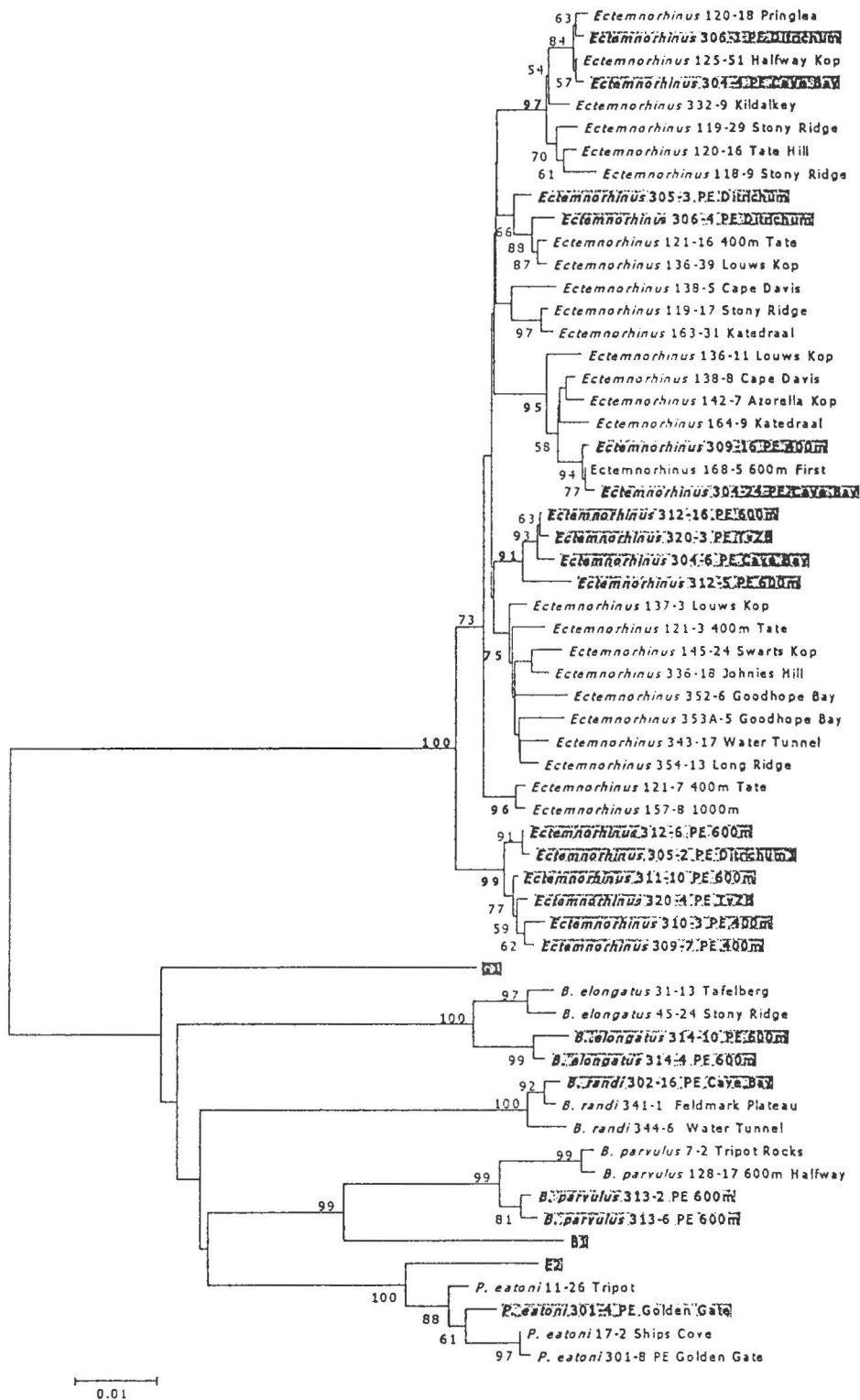


Fig. 1 Minimum evolution tree depicting the CO I gene relationships of six weevil species from Marion Island (indicated in yellow) and from Prince Edward Island (indicated in green). For each taxon, the species name is followed by the individual identity number and the sampling locality (vegetation type / altitude / name) is indicated last. Bootstrap values are based on 1000 pseudo-replications with only those values greater than 50 % being indicated.

3.3 Results

3.3.1 COI gene trees

The within and between genus and island relationships recovered on inferring a COI gene phylogeny are presented in Fig. 1. For *Ectemnorhinus*, two types of clades were recovered, namely those that appear to be island-specific and those containing related haplotypes common to both islands. These clades can be identified by their embolded bootstrap support values. The uppermost two clades (having embolded bootstrap values of 97 and 95) are illustrative of the clades containing weevils from both Marion and Prince Edward Islands, whilst the remaining clades having bootstrap values of 99 and 75 (indicated in bold) correspond to clades that are Prince Edward Island and Marion Island specific, respectively. These results indicate that under excessive predation by mice, the haplotypes unique to Marion Island are under threat. A similar preliminary trend is observed for some species of *Bothrometopus*. In particular *B. elongatus* and *B. parvilus* appear to have island-specific clades, the presence of which needs to be confirmed by more extensive genetic analysis. Although *P. eatoni* and *B. randi* do not display island-specific structuring, no identical haplotypes have thus far been identified.

CONCLUSIONS AND FUTURE PROSPECTS

The presence of a species-complex, rather than two distinct *Ectemnorhinus* species on both Marion Island and Prince Edward Island is indicated by the molecular phylogeny. The possible effects of predation in obscuring the phylogeny have been addressed by the inclusion of specimens from mice-free altitudes and localities. On the basis of these genetic results it is suggested that the taxonomic status of the *Ectemnorhinus* genus be revised for PEI.

The suitability of COI for discerning within-genus relationships is also clearly demonstrated for both sympatric (within-island) and allopatric (between-island) populations of *Bothrometopus*. The existence of island-specific clades should be investigated further by sequencing of additional specimens as this island-specificity indicates that predation may reduce the genetic diversity, should it go unchecked on Marion Island and that Prince Edward Island might not be able to adequately fulfil

a 'back-up' role. It is therefore recommended that genetic characterisation of weevil species on Marion Island be intensified in order to more accurately determine the potential loss in biodiversity.

Our efforts will be directed at concluding the following:

- **Hypothesis 2 & 3:** The molecular data generated for *Ectemnorhinus* component of the study will be subjected to further statistical analyses and two papers will be prepared for publication. The first will be a revision of the taxonomic status of this genus at PEI and will incorporate both morphometric and molecular evidence for the presence of a single species. The second will be a population level study addressing origin and spread of these weevils and suggesting management strategies.
- **Hypothesis 1:** Primers targeting more conserved genomic regions such as 16S have been ordered and alternative COI primers have been designed on the basis of sequences available in the Genbank database. Amplification and sequencing of representative samples will be conducted once suitable primers have been identified.
- **Hypothesis 4 & 5:** All weevil and moth measurements have been taken from specimens collected between 2001 and 2003 from PEI. In addition, 200 previously collected individuals in the National Collection of Insects and 300 individuals previously collected by S.L. Chown were also measured. These data will be complimented by that of approximately 5000 specimens collected primarily from Marion Island, by Prof. S.L. Chown over approximately 14 years. These additional specimens are mainly *Ectemnorhinus* species from Marion Island. Measurements will be recorded for all samples in order to address the question of population changes through time.

References

Chimimba, C.T. and Dippenaar, N.J. 1995. The selection of taxonomic characters for morphometric analysis: A case study based on Southern African *Aethomys* (Mammalia: Rodentia: Muridae). *Annals of Carnegie Museum* 64 (3): 197-217.

Chown, S.L., 1989. Ecology and systematics of the Ectemnorhini: (Coleoptera: Curculionidae: Entiminae). PhD thesis, University of Pretoria.

Chown, S.L. 1989. Habitat use and diet as biogeographic indicators for subantarctic Ectemnorhini (Coleoptera: Curculionidae). *Antarctic Science*. 1: 23-30.

Chown, S.L. 1990. Speciation in the sub-Antarctica weevil genus *Dusmoecetes* Jeannel (Coleoptera Curculionidae). *Systematic Entomology* 15: 283-296.

Gould, S.J., Woodruff, D.F., and Martin, J.P. 1974. Genetics and morphometrics of *Cerion* at Pongo Carpet: A new systematic approach to this enigmatic land snail. *Systematic Zoology* 23: 518-535.

Harley, E.H. 2001. DAPSA: DNA and protein sequence analysis, version 4.9. Department of Chemical Pathology, University of Cape Town, South Africa.

James, F.C., and McCulloch, C.E. 1990. Multivariate analysis in ecology and systematics: Panacea or Pandora's box? *Annual Review of Ecology and Systematics* 21: 129-166.

Juan, C., Oromi, P. & G.M. Hewitt 1996. Phylogeny of the genus *Hegeter* (Tenebrionidae, Coleoptera) and its colonization of the Canary Islands deduced from Cytochrome Oxidase I mitochondrial DNA sequences. *Heredity* 76: 392-403.

Lunt, D.H., Zhang, D.X., Szymura, J.M. & G.M. Hewitt 1996. The insect COI gene: evolutionary patterns and conserved primers for phylogenetic studies. *Insect Molecular Biology* 5: 153-165.

Sequeira, A.S., Lanteri, A.A., Scataglini, M.A., Confalonieri, V.A. & B.D. Farrell 2000. Are flightless *Galapaganus* weevils older than the Galápagos Islands they inhabit? *Heredity* 85: 20-29.

Simon, C., Frati, F., Beckenbach, A., Crespi, B, Liu, H. & Flook, P. 1994. Evolution, weighting and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America*, 87: 651-701.

Swofford, D.L. 1999. PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods). Version 4.0d65. Sinauer Associates, Sunderland, Massachusetts.

Thorpe, R.S. 1980. A comparative study of ordination techniques in numerical taxonomy in relation to racial variation in the ringed snake *Natrix natrix* (L.). *Biological Journal of the Linnean Society* 13: 7-40.

Ward, J. 1963. Hierarchical grouping to optimize an objective function. *Journal of the American Statistics Association* 58: 236-243.

Zar, J. 1984. *Biostatistical Analysis*, 2nd edn. Prentice Hall, New Jersey.

(d) Data and Sample Storage

Data and samples are currently housed in the Department of Zoology & Entomology, University of Pretoria, and the responsible project leader is A.D. Slager-Bastos.

(e) Acknowledgements

All personnel involved in logistic operations at the Prince Edward Islands are thanked for their assistance and the Department of Microscopy, UP is gratefully acknowledged for their assistance and for use of their facilities

(f) Publications (under review)

Janse van Rensburg, L., Chimimba, C.T., Bastos, A.D. & Chown, S.L. Morphometric measurement selection: An invertebrate case study based on weevils from Marion Island. *Polar Biology* (submitted)

(g) Scientific Presentations

L. Janse van Rensburg: Morphometric measurement selection: An invertebrate case study based on weevils from Marion Island, presented at the Annual General Meeting of the department of Zoology and Entomology at the University of Pretoria, November 2002 – Talk



Dr. A.D. Slager-Bastos

30 June 2003

