CHAPTER 2

Molecular and morphometric assessment of the taxonomic status of *Ectemnorhinus* weevil species (Coleoptera: Curculionidae, Brachycerinae) from the sub-Antarctic Prince Edward Islands

G.C. Grobler[1], L. Janse van Rensburg[1], A.D.S. Bastos[1], C.T. Chimimba[1] and S.L. Chown[2]

[1]*Department of Zoology and Entomology, University of Pretoria, 0002 South Africa;*

[2]*DST-NRF Centre of Excelolence for Invasion Biology (Biology), Department of Botany and Zoology, Stellenbosch University, 7602 South Africa*

(All morphometric data reported in this chapter was generated by L. Janse van Rensburg)

Abstract: There are long-standing controversies on the taxonomic status of *Ectemnorhinus* weevil species occurring on the sub-Antarctic Prince Edward Islands. Since the two islands that constitute the Prince Edward Islands archipelago (PEIA), Marion Island (MI) and Prince Edward Island (PEI) differ in terms of alien invasive species, such as the introduced house mouse *Mus musculus*, and in conservation management strategies, it is important to consider inter-island dynamics when investigating inter-specific relationships. Using a combined molecular phylogenetic and morphometric approach, we attempted to resolve the taxonomic status of the PEIA *Ectemnorhinus* weevil species. A COI gene phylogeny was inferred following the genetic characterization of 52 *Ectemnorhinus* weevils from both islands, and morphometric assessment using a set of 15 linear, external measurements was used to differentiate between the two currently recognized species, *E. similis* and *E. marioni*. Analyses revealed the presence of two genetically and morphometrically distinct species on PEI, whilst evidence for a single species, comprising diverse genetically discrete populations was found on MI. Based on these results, the species unique to PEI has been designated *E. kuscheli* n. sp. whilst we confirm the synonymy between *E. similis* and *E. marioni*, the two species originally described from MI. *E. kucheli* appears to be restricted to PEI, whereas *E. similis* occurs on both MI and PEI.

Key words: Weevils, *Ectemnorhinus*, Prince Edward Islands, COI gene, phylogenetics, morphometrics, conservation

Introduction

The weevils of the South Indian Ocean province of the Southern Ocean belong to a single, monophyletic unit centred around the genus *Ectemnorhinus* G. R. Waterhouse, 1853 (Kuschel and Chown 1995). There are approximately 36 species in the group, and they have proven to be taxonomically difficult (Brown 1964; Kuschel 1970; Dreux & Voisin 1989; Chown 1991). In particular, the taxonomic status of *Ectemnorhinus marioni* and *E. similis* from the sub-Antarctic Prince Edward Islands has long been controversial. *Ectemnorhinus similis* C. O. Waterhouse, 1885 was the first *Ectemnorhinus* species described from Marion Island. Subsequently, Jeannel (1940) described *E. marioni* Jeannel, 1940, which was distinguished from *E. similis* based on of the form of the humeri, and interstrial and strial morphology. However, Kuschel (1971) synonymized the two species due to the lack of consistent differences in either internal or external morphology. Subsequently, Dreux & Voisin (1986) continued to recognise the two species, noting that they differed in the form of their elytral striae and interstriae, and the elytral punctuation.

Crafford et al. (1986) recognized three distinct ecotypes within *E. similis* based on body size and colour. Following a detailed investigation of habitat use, feeding biology, life history, morphology, and mating preferences, Chown (1990), noted that the use of vesture colour and body length to distinguish between ecotypes was not justified. Rather, he argued that the species complex should be separated into two morphologically similar, but ecologically distinct species. Small-sized (3.77 mm – 7.79 mm; median: 5.53 mm) bryophyte-feeding individuals associated with vegetation types dominated by the plants *Azorella selago* Hook. f. and *Agrostis magellanica* Lam, and including bryophytes such as *Campylopus spp., Ptychomnion ringianum* Broth. & Kaal., *Ditrichum strictum* (Hook. f. & Wils) Hampe and others, were referred to as *E. marioni* (Chown 1990). The larger (4.51 mm − 8.69 mm, median: 6.44 mm) angiosperm-feeding individuals associated with *Acaena magellanica* (Lam.) Vahl. herbfields, *Callitriche antarctica* Engelm. ex Hegelm., *Pringlea antiscorbutica* R.Br. ex Hook. f., *Poa cookii* Hook. f. and *A. selago* were designated *E. similis*. Although *E. similis* feeds mainly on angiosperms, bryophytes, other cryptogams are incorporated into their diet at the end of the growing season, when vascular plant foliage deteriorates (Chown 1989; Chown and Scholtz 1989; Chown 1990). Both *E. marioni* and *E. similis* can be found on *A. selago*, but the former species feeds only on epiphytic bryophytes growing on this plant species (Chown and Scholtz 1989), whereas the latter species feeds

both on the *A. selago* and on epiphytic species including the grass, *A. magellanica*, and bryophytes.

Apart from variation in body size and diet, *E. marioni* and *E. similis* also differ in the length of their life cycles and times of emergence (Chown 1990). *Ectemnorhinus marioni* exhibits a shorter life cycle with fewer instars, and adults are present throughout the year, while adults of *E. similis* only emerge during summer months, and their emergence appears to be synchronized with the first flushes of angiosperm growth and flowering. Apart from body size, there are neither consistent differences in the male genitalia (Chown 1990), nor consistent differences in either the ovipositor or the spermatheca in females of the two species. Chown (1990) suggested that *E. marioni* and *E. similis* evolved sympatrically in a manner similar to that proposed by Rice (1984), with reproductive isolation being induced by size-based assortative mating associated with differences in food preference (Chown 1990; Crafford and Chown 1991). It is important to note that both *E. similis* and *E. marioni* were described from Marion Island, while no species was formally based on weevils from Prince Edward Island. However, because the weevils from Prince Edward Island appeared to be morphologically and ecologically similar to MI individuals, it was concluded that both species occur on Prince Edward Island too (Dreux, 1971). Many studies have subsequently followed these taxonomic decisions (reviewed by Chown et al. 2002; Klok and Chown 2003), and they have also been applied in conservation management of the Prince Edward Islands (Anonymous 1996).

Factors influencing population size and density of *Ectemnorhinus* species on the Prince Edward islands have been documented. While the house mouse (*Mus musculus* Linnaeus, 1758, *sensu lato*) was introduced by sealers on Marion Island (hereafter referred to as MI), the larger of the two Prince Edward Islands, more than 180 years ago (Watkins and Cooper 1986), the smaller Prince Edward Island (hereafter referred to as PEI) has remained mouse-free. Mice feed on a variety of invertebrates and plants on the islands, and especially weevils (Gleeson and van Rensburg 1982; Smith et al. 2002). In addition, mean temperature in the sub-Antarctic has increased by approximately 1°C in the last 50 years (Smith and Steenkamp 1990), and is believed to have led to an increase in the survival rate of mice during winter months, resulting in an overall population increase (Smith and Steenkamp 1990; Smith 2002). The mean volume contribution of weevil adults found in the guts of mice increased from 7 % in 1979/1980 (Gleeson and Van Rensburg 1982) to 11 % in 1992/1993 (Smith et al. 2002). House mice are thus considered to be responsible for the significant change in the populations of *Ectemnorhinus* species on MI, amounting to almost an order of

magnitude decline in biomass between 1976 and 1996 (Chown et al. 2002), and a pronounced difference between population densities on MI and PEI (Crafford and Scholtz 1987).

Mice are also thought to have caused a reduction in body size of weevil species on MI relative to PEI as frequency distributions of the size of the *Ectemnorhinus* species differ considerably between the islands (Chown and Smith 1993). The authors of this study noted that the situation across the two islands seemed to reflect predation by mice, but also concluded that further investigations were necessary, due particularly to taxonomic difficulties with the genus (see also Chown 1991).

Given the severity of weevil predation on MI and uncertainties on the taxonomic status of the *Ectemnorhinus* species on both islands (Chown 1990, 1991), it is not clear whether the PEI populations alone are sufficient to ensure conservation of the two *Ectemnorhinus* weevil species, especially if predictions that mice predation is likely to continue escalating (Chown et al. 2002) are realized. If the same species occur on both islands and if the populations are not so dissimilar that they should be considered different management units, then the current management regime (Anonymous 1996) will suffice. An essential assumption of this regime is that Prince Edward Island serves largely as a mouse-free haven for species occurring on both islands (but see contrary views in Gremmen and Smith 1999; Chown et al. 2002). However, if the species or populations differ, then management practices would have to change, and serious consideration would have to be given to eradication of mice on Marion Island. Thus, it is clear that resolving the status of the two currently recognized *Ectemnorhinus* species is important from a conservation perspective.

The aim of the present study was, therefore, to evaluate the current taxonomic status of the *Ectemnorhinus* weevil species occurring on the Prince Edward Islands using both molecular and morphometric techniques. The COI gene, that has been successfully used to differentiate between island-bound coleopteran species (Caccone and Sbordoni 2001; Emerson et al. 1999; Sequeira et al. 2000; Trewick 2000), was selected for genetic characterization, whilst a set of 15 linear external measurements (Janse van Rensburg et al. 2003) was used for the morphometric assessment.

Materials and methods

Study area and samples

   Ectemnorhinus weevil specimens were collected over three consecutive years (April 2001 – April 2003) from 28 localities (Fig. 1) on MI. Samples from PEI which has restricted access, were collected along an altitudinal gradient (0 – 675 m a.s.l.) at 200 m intervals and from an additional locality with vegetation consisting largely of *Ditrichum strictum*, in April 2003. Coordinates for all the sampling localities are summarized in Table 1. All specimens were collected by hand and preserved in absolute ethanol.
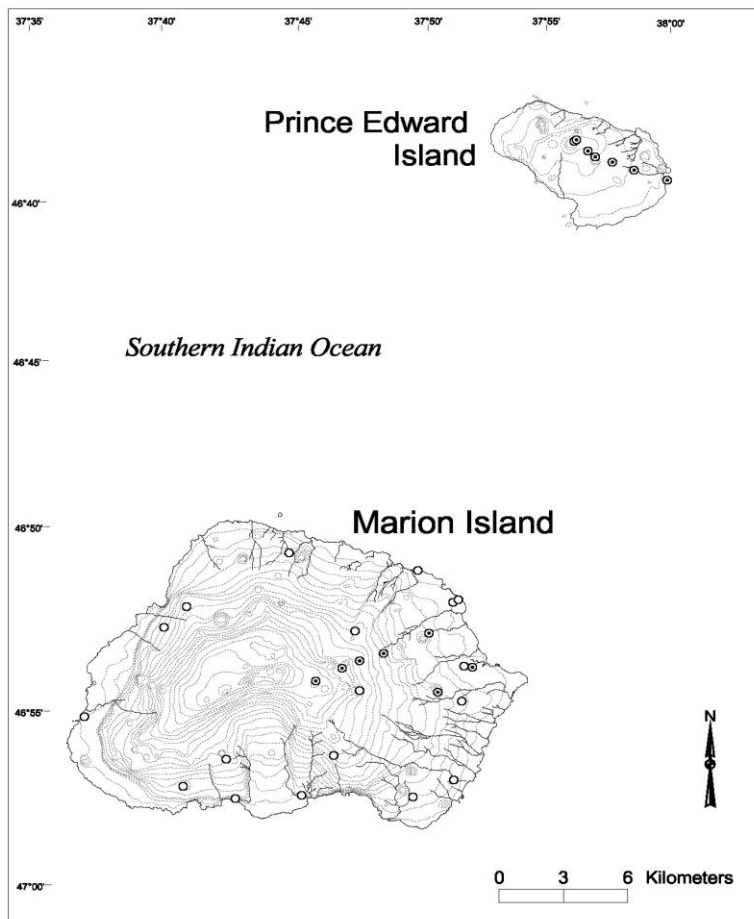

Fig. 1. Map indicating *Ectemnorhinus* weevil sampling localities on Marion Island and Prince Edward Island that correspond to the coordinates summarised in Table 1. Samples collected from all localities were included in the morphometric analyses while only those samples collected from the dotted localities were included in the genetic analyses.

TABLE 1. Summary of sampling locality coordinates

Sampling Locality	Coordinates
MI 200 m Junior's Kop	S 46º52.794' E 37º50.083'
MI 400 m First Red Hill	S 46º53.412' E 37º48.21'
MI 600 m First Red Hill	S 46º53.647' E 37º47.208'
MI 800 m Katedraalkrans	S 46º53.896' E 37º46.482'
MI 1000 m	S 46º54.29' E 37º45.375'
MI Tate's Hill *Pringlea*	S 46º54.6' E 37º50.478'
MI Albatros Lakes	S 46º53.82' E 37º51.916'
PE Cave Bay	S 46º38.752' E 37º59.780'
PE 200 m	S 46º38.457' E 37º58.396'
PE 400 m	S 46º38.211' E 37º57.482'
PE 600 m	S 46º37.533' E 37º55.985'
PE TvZB 672 m	S 46º37.590' E 37º55.891'
PE Ditrichum	S 46º38.057' E 37º56.771'

TvZB indicates samples collected at the top of Van Zinderen Bakker Peak.

For the morphometric component of the study, between five and 30 *Ectemnorhinus* specimens per locality were measured. Due to the uncertainty regarding the taxonomic status of *Ectemnorhinus* species on both MI and PEI, individuals were only identified as belonging to the genus, based on the generic descriptions provided by Kuschel and Chown (1995), with *a priori* rather than *a posteriori* multivariate morphometric analyses (Sneath and Sokal 1973) being used to define phenetic groupings.

Fifty-two *Ectemnorhinus* individuals from seven localities on both MI and PEI (Fig. 1) were analysed for the molecular component of the study. In an attempt to ensure adequate representation of species-associated feeding preferences as indicated by Chown (1990), *Ectemnorhinus* individuals were collected from *D. strictum*, *A. selago*, and *P. antiscorbutica*. Individuals collected from *D. strictum* on MI were collected from an *A. selago*-free polar desert site near Albatross Lakes while those collected from *D. strictum* on PEI were collected from a site comprising mainly of *D. strictum* (coordinates given in Table 1). As gut contents were not evaluated, we were, however, mindful that sampling from a particular plant species did not necessarily imply feeding preference for that plant species. Body size variation, another criterion used by Chown (1990) to distinguish between *E. marioni* and *E. similis*, was accommodated by including the extreme size classes (largest two and the smallest two

individuals) per locality. *A priori* assignment into different species was not taken into account in subsequent molecular analyses. *Ectemnorhinus viridis* (G. R. Waterhouse 1853) from Heard Island was selected as an outgroup, since it is a congener of *E. marioni* (Kuschel and Chown 1995).

Molecular characterization

Following rehydration of ethanol-preserved weevils with water, one to two weevil legs per specimen were frozen in liquid nitrogen before being ground and mixed with phosphate-buffered saline (PBS). DNA was extracted using a modified guanidinium thiocyanate (GuSCN)/silica-based method (Boom et al. 1990).

Published primers C1-J-1718 and TL2-N-3014 (Simon et al. 1994) were initially used to generate partial sequence data for representatives of all six weevil species currently considered to occur on MI namely, *Bothrometopus elongatus* (Jeannel 1953), *Bothrometopus parvulus* (Waterhouse 1885), *Bothrometopus randi* (Jeannel 1953), *E. marioni* (Jeannel 1940), *E. similis* (Waterhouse 1885) and *Palirhoeus eatoni* (Waterhouse 1879). As these primers generally resulted in poor quality sequences, two MI weevil-specific COI primers were designed from the aligned partial sequences, following the guidelines of Rychlik (1993). These MI weevil-specific COI primers termed GF and GR1 (Table 2) amplified a 1059 bp PCR product under the following conditions: 1×Buffer, 0.2 mM dNTP, 0.4 µM of each primer and 1 U *Taq* polymerase in a final volume of 50 µl containing 200 ng of template DNA. A typical temperature profile consisted of an initial denaturation step at 94ºC for 90 s, followed by 40 cycles at 94ºC for 22 s, 46ºC for 30 s and 72ºC for 1 min. PCR products were purified and DNA sequences were determined by automated cycle sequencing on an ABI PRISM™ 3100 Analyser using the ABI PRISM Big Dye™ Terminator version 3.0 sequencing standard.

Internal primers termed GF3, GF4, GF5 and GR5 (Table 2) were designed from the sequences initially generated with the MI weevil-specific primers. The latter two primers were used in all subsequent cycle-sequencing reactions to generate a homologous 885 bp region of sequence data. The sequences were viewed and edited in Chromas version 1.43 (McCarthy 1996-1997) and aligned with DAPSA version 4.9 (Harley 2000).

TABLE 2 List of oligonucleotide primers used in this study.

Name	Orientation	Sequence	Tm
C1-J-1718	Forward	5'GGAGGATTTGGAAATTGATTAGTTCC 3'	60ºC
TL2-N-3014	Reverse	5'ATTATACCGTCTAATCACGTAACCT 3'	58ºC
GF-1858	Forward	5' GGGACAGGTTGAACAGTTTATC 3'	58ºC
GR1-2938	Reverse	5' ATGTTGTTATTCTTGAAGATGAAAG 3'	54ºC
GF3-2206	Forward	5'GGTCACCCAGAAGTATATAT3'	53ºC
GF4-2662	Forward	5'GCTGGAATAGTACAATGATT3'	53ºC
GF5-1940	Forward	5' TACATATAGCAGGTGTATCATC 3'	54ºC
GR5-2935	Reverse	5' GTTATTCTTGAAGATGAAAGATT 3'	51ºC

Tm: Melting temperature, calculated using the formula:

Tm = [69.3+(0.41*%GC)]-650/primer length

Phylogenetic analyses

Three sequence datasets were compiled, a MI dataset, a PEI dataset, and a combined MI and PEI dataset. Neighbor-Joining (NJ; Saitou and Nei 1987) and Minimum Evolution (ME; Rzhetsky and Nei 1992) algorithms in MEGA version 2 (Kumar et al. 2001) were used to construct phylogenies for the combined dataset with nodal support being assessed by 100 000 bootstrap replications.

Model Test version 3.06 (Posada and Crandall 1998) was used to identify the model of evolution that best fits the data with parameters identified under the Akaike Information Criterion (Akaike 1974) being used for subsequent Maximum Likelihood analyses (ML; Felsenstein 1981). In each case, the TrN + I model with equal rates for all sites that correspond to the General time–reversible model, GTR + I (Rodriguez et al. 1990) was selected. The proportion of invariable sites (I) and three different substitution types estimated for the dataset was as follows: I = 0.7935, rate [A-G] = 48.19, rate [C-T] = 11.65, and other rates = 1.00.

Maximum Likelihood analyses were performed in PhyML 3.0 (Guindon and Gascuel 2003) under the TN93 model (with all parameters estimated and optimised over tree, length and rate) prior to 5000 bootstrap re-sampling replications. Bayesian phylogenetic analyses (BPA) using MrBayes version 3.0B4 (Huelsenbeck and Ronquist 2001) were performed with the same models and parameters recovered for each of the respective datasets. Analyses were

initiated with random starting trees and run for 10 000 000 generations with Markov chains sampled every 1000 generations. Of the 10 000 trees obtained, 2500 were discarded as "burn-in".

Parsimony analyses performed with PAUP* version 4.0b10 (Swofford 1999) included equal weighting and differential weighting schemes such as character weighting where third base positions were given a weight of 1, and first base positions were up-weighted to 9.76923; successive weighting (Farris 1969); 6 parameter parsimony on its own and combined with both character and successive weighting (Williams and Fitch 1990).

The equality of evolutionary rates between lineages was tested using the relative rate test (Li and Bousquet 1992) in PHYLTEST version 2.0 (Kumar 1996). In addition, the likelihood ratio test (Felsenstein 1981, 1988) was performed, and log likelihood scores obtained with and without the molecular clock enforced, were compared. Divergence times were calculated from uncorrected pairwise values and calibrated using 2.3% nucleotide sequence divergence per million years based on the arthropod mtDNA survey of Brower (1994). BEAST 1.5.3 (Drummond & Rambaut 2007) was used to obtain an ultrametric tree using Bayesian MCMC analysis orientated towards rooted, time-measured phylogenetics. Well supported nodes identified following NJ, ME, ML and BI analyses were constrained to be monophyletic and the GTR+I model identified in Model Test version 3.06 (Posada and Crandall 1998) under the AIC was enforced using a strict molecular clock model. The results of two independent runs of 20,000,000 generations, with Markov chains sampled every 1,000 generations, were merged and analyzed with Tracer v1.4 and TreeAnnotator v1.4.7 (Drummond & Rambaut 2007). Of the 40,000 trees obtained 5,000 were discarded as 'burn-in'. Haplotype (h) and nucleotide diversities (π) were estimated for each island individually in DNASP 3.51 (Rozas and Rozas 1999). Differences in total body lengths of individuals between clades were assessed using analysis of variance (ANOVA; Zar 1996) for PEI.

Morphometric analyses

Fifteen morphometric measurements were recorded by a single observer (L.J.v.R.) using a stereomicroscope fitted with a calibrated eyepiece micrometer. Measurements, defined and selected based on a morphometric character selection procedure followed by Janse van Rensburg et al. (2003) included: Total body length (TL), pronotum breadth (PB), femur length (FL), interocular distance (O), metacoxal distance (MT), maximum breadth of elytra (EW), length of first three tarsal segments (T3), meso/metacoxal distance (MM),

interantennal distance (A), mesocoxal distance (MS), femur breadth (FB), funicle segments 1, 2, and 3 (F1, F2 and F3), and rest of funicle (FR). Measurements were recorded to the nearest 0.05 mm (TL and EW), 0.03 mm (PB and FL), and 0.01 mm (O, A, F1, F2, F3, FR, T3, MS, MT, MM and FB).

For multivariate morphometric analyses, the absence of multivariate sexual dimorphism (Janse van Rensburg et al. 2003) permitted pooling of sexes for subsequent analyses. For MI, a total of 807 individuals from 28 localities, which provided adequate geographical coverage of *Ectemnorhinus* species, were used for morphometric analysis. A total of 240 *Ectemnorhinus* specimens from six localities on PEI were analyzed. Data screening revealed five outlier specimens, not considered representative of the populations. A re-examination of these specimens revealed outlier values arising from damaged parts, and to avoid the introduction of bias in the sample, they were excluded from subsequent analyses. After determining the absence of multivariate sexual dimorphism using principal components analysis, these datasets were subjected to a randomization procedure (Manly 1991), where a new dataset with an equal number of individuals as the original dataset were randomly sampled with replacement for each island to assess whether the absence of multivariate sexual dimorphism in the original dataset was significantly different from the randomly selected dataset.

Following Chimimba et al. (1999), sampled localities on MI and PEI were grouped into a number of computationally manageable geographical subsets to accommodate for the unweighted pair-group arithmetic average (UPGMA) cluster analyses for the MI (*n* = 807) and the combined island (*n* = 1047) data matrices, since the data matrices were too large for simultaneous specimen-level analyses. The results of the individual-level analyses of the geographical subsets facilitated the grouping of locality mean values in subsequent analyses that accommodated entire island data that were similar to the results of the individual-level analyses. The 28 and 24 genetically identified *Ectemnorhinus* individuals from MI and PEI, respectively, were included in all morphometric analyses as references in defining phenetically-derived groupings.

Multivariate analyses included principal components analysis (PCA) and unweighted pair-group arithmetic average (UPGMA) cluster analysis to assess whether species could be identified based on morphometric characters (Sneath and Sokal 1973). Canonical variates analysis (CVA) of genetically defined groupings was also undertaken (Pimentel and Smith 1986) to define phenetic groups *a posteriori*. The CVA was followed by a multivariate analysis of variance (MANOVA: Zar 1996) to test for statistically significant differences

between pre-defined groups. UPGMA cluster analysis was based on both Euclidean distances and product-moment correlation coefficients among Operational Taxonomic Units (OTUs; Sneath and Sokal 1973), while the PCA was computed from product-moment coefficients among variables (Sneath and Sokal 1973). All statistical procedures were performed using Statistica version 5.5 (Statsoft 1995).
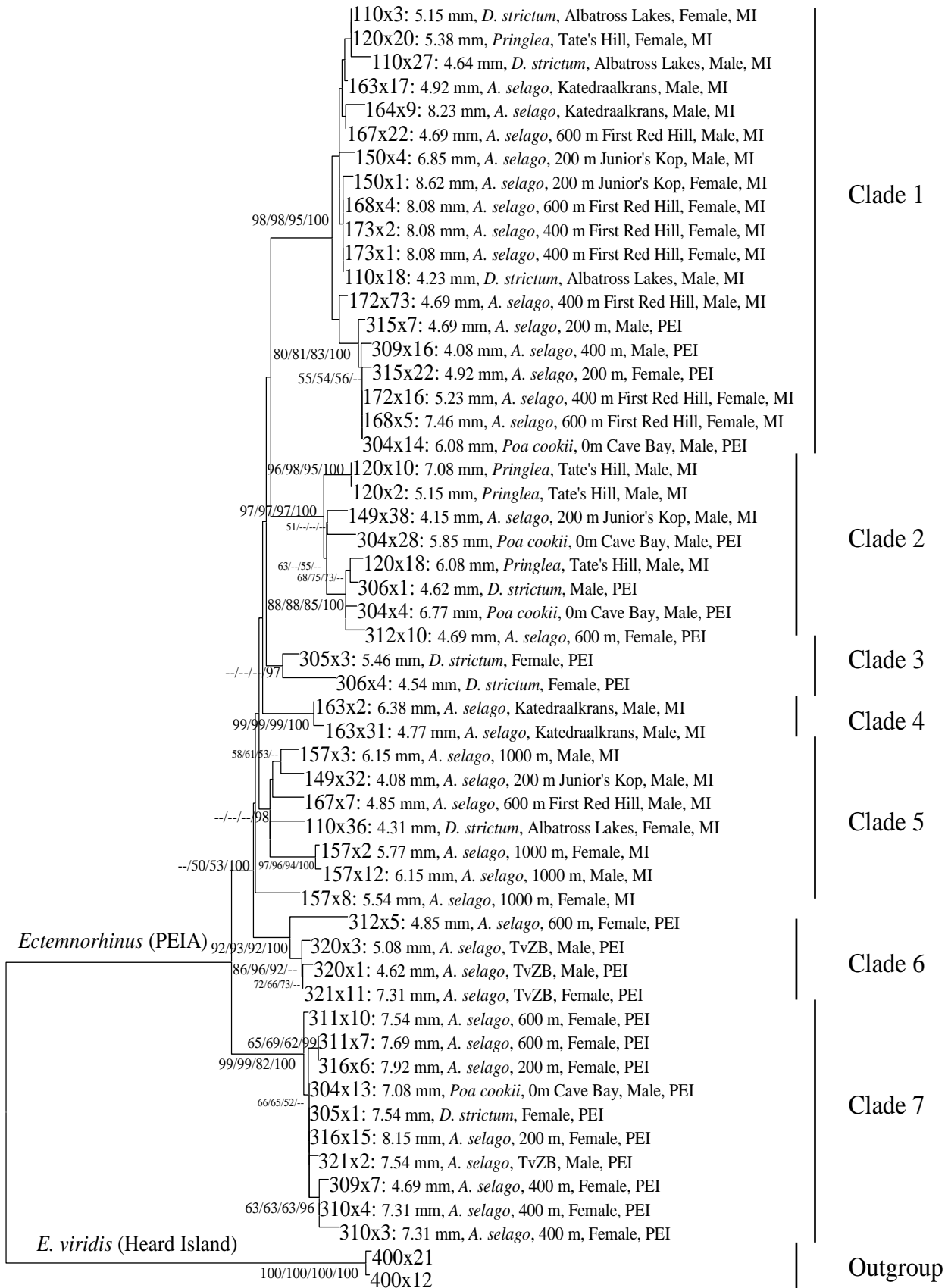
Results

Molecular analyses

An homologous region of 885 bp corresponding to nucleotide positions 514 to 1399 of the COI gene was generated for 52 PEIA *Ectemnorhinus* individuals and two *E. viridis* outgroup specimens. All sequences have been deposited in the Genbank database under accession numbers AY762267- AY762320. For the combined dataset, 775 of the 885 sites were conserved across all 54 sequences and 98 of the 110 variable sites were parsimony informative. The % A + T was 68.6 % and the transition (ti)/transversion (tv) ratio was 6. Third base position substitutions accounted for 88.2 % of the variation and the remaining 11.8 % was due to the first base substitutions. Mutations at nucleotide level gave rise to five non-synonymous amino acid substitutions at codons 19, 85, 241, 279, and 289. Of the 52 PEIA *Ectemnorhinus* individuals sequenced, 42 had unique haplotypes. When the individuals collected from MI and PEI were pooled, a nucleotide diversity (π) and haplotype diversity (h) of 0.02032 and 0.990 respectively, was obtained. From the 28 MI sequences, 22 unique haplotypes were obtained with a π of 0.01217 and an h of 0.976. The π and h for the PEI was estimated to be 0.01687 and 0.986, respectively, with 21 unique haplotypes being identified from the 24 PEI sequences generated. Although all major clades (numbered 1-7; Fig. 2) did not have high levels of support across all methods of phylogenetic analysis utilised, clade topology was consistent across all methods. Clade 5 which has low levels of bootstrap support in ML and ME, had high support (0.9) with Bayesian analysis. Clades 6 and 7 consisted solely of individuals collected on PEI, while clades 1 and 2 incorporated individuals from both islands. In clade 1, MI individuals ranging in size from 4.23 mm to 8.08 mm, and collected on *A. selago*, *P. antiscorbutica*, and *D. strictum*, grouped together. Similarly in clade 7, individuals collected on both *A. selago* and *D. strictum* from PEI grouped together, indicating that assignment of species according to host plant preference

does not appear to hold. Clade 7 was consistently basal to clades 1 to 6 with the grouping of the latter clades having ML and 6-parameter parsimony bootstrap values of 80 % and 92 %, respectively. When the individuals in clade 7 were compared to the PEI individuals in each of the other clades, it was found that they were significantly larger (ANOVA: $F(8, 34) = 9.38$, $P < 0.05$). Sequence divergence values of 2.2 % were observed between clade 7 and clades 1 to 6 when all the individuals from both MI and PEI were pooled, while sequence divergence values of 1.5 % were observed between clade 7 and clades 1 to 6 when only the individuals collected on PEI were used.

No significant rate heterogeneity was found among the substitution rates at $P < 0.05$ according to both relative rate and likelihood ratio tests. Therefore, it was concluded that *Ectemnorhinus* individuals from the Prince Edward Islands do not evolve at markedly different rates and a molecular clock based on that calibrated for arthropods (Brower 1994) could, therefore, be imposed. BEAST estimates and uncorrected pairwise estimates differed considerably with BEAST estimates generally being much older than uncorrected pairwise estimates (Fig. 3). Uncorrected pairwise estimates showed that the *Ectemnorhinus* weevil lineages coalesced at approximately 0.49 million years ago (MYA) while individuals from clade 7 coalesced at approximately 0.049 MYA and individuals from clades 1 to 6 coalesced at approximately 0.33 MYA. BEAST estimates for the same nodes recovered estimates of approximately 0.70 million years ago (MYA) for all individuals characterized, while individuals from clade 7 coalesced at approximately 0.18 MYA and individuals from clades 1 to 6 coalesced at approximately 0.47 MYA.

Fig. 2 Minimum Evolution (ME) tree based on 885 base pairs of the mitochondrial COI gene and inferred using the Tamura-Nei distance correction algorithm for the combined data set. For each specimen, the sample number is indicated followed by the body length measurement, plant species it was collected from, locality, sex and the island of origin (where 'MI' denotes Marion Island and 'PEI' indicates Prince Edward Island). Nodal support was assessed by 100 000 bootstrap replications. Support values indicated in brackets were obtained from Neighbour Joining (NJ) analysis (based on 100 000 bootstrap replications), Maximum Likelihood (ML) analysis (based on 5000 bootstrap replications) and Bayesian phylogenetic analyses (BPA) from 10 000 000 generations with Markov chains sampled every 1000 generations and 2500 of the 10 000 trees obtained discarded as "burn-in". Nodal support values ≥ 50 obtained from ME, NJ and ML and ≥ 90 from BPA, expressed as a percentage, are indicated. '--' denotes bootstrap support values below 50 (NJ and ML) and below 90 (BI). TvZB indicates samples collected at the top of Van Zinderen Bakker Peak at an elevation of 672 m above sea level.

Phylogenetic tree showing:

Clade 1
- 110x3: 5.15 mm, *D. strictum*, Albatross Lakes, Female, MI
- 120x20: 5.38 mm, *Pringlea*, Tate's Hill, Female, MI
- 110x27: 4.64 mm, *D. strictum*, Albatross Lakes, Male, MI
- 163x17: 4.92 mm, *A. selago*, Katedraalkrans, Male, MI
- 164x9: 8.23 mm, *A. selago*, Katedraalkrans, Male, MI
- 167x22: 4.69 mm, *A. selago*, 600 m First Red Hill, Male, MI
- 150x4: 6.85 mm, *A. selago*, 200 m Junior's Kop, Male, MI
- 150x1: 8.62 mm, *A. selago*, 200 m Junior's Kop, Female, MI
- 168x4: 8.08 mm, *A. selago*, 600 m First Red Hill, Female, MI
- 173x2: 8.08 mm, *A. selago*, 400 m First Red Hill, Female, MI
- 173x1: 8.08 mm, *A. selago*, 400 m First Red Hill, Female, MI
- 110x18: 4.23 mm, *D. strictum*, Albatross Lakes, Male, MI
- 172x73: 4.69 mm, *A. selago*, 400 m First Red Hill, Male, MI
- 315x7: 4.69 mm, *A. selago*, 200 m, Male, PEI
- 309x16: 4.08 mm, *A. selago*, 400 m, Male, PEI
- 315x22: 4.92 mm, *A. selago*, 200 m, Female, PEI
- 172x16: 5.23 mm, *A. selago*, 400 m First Red Hill, Female, MI
- 168x5: 7.46 mm, *A. selago*, 600 m First Red Hill, Female, MI
- 304x14: 6.08 mm, *Poa cookii*, 0m Cave Bay, Male, PEI

Clade 2
- 120x10: 7.08 mm, *Pringlea*, Tate's Hill, Male, MI
- 120x2: 5.15 mm, *Pringlea*, Tate's Hill, Male, MI
- 149x38: 4.15 mm, *A. selago*, 200 m Junior's Kop, Male, MI
- 304x28: 5.85 mm, *Poa cookii*, 0m Cave Bay, Male, PEI
- 120x18: 6.08 mm, *Pringlea*, Tate's Hill, Male, MI
- 306x1: 4.62 mm, *D. strictum*, Male, PEI
- 304x4: 6.77 mm, *Poa cookii*, 0m Cave Bay, Male, PEI
- 312x10: 4.69 mm, *A. selago*, 600 m, Female, PEI

Clade 3
- 305x3: 5.46 mm, *D. strictum*, Female, PEI
- 306x4: 4.54 mm, *D. strictum*, Female, PEI

Clade 4
- 163x2: 6.38 mm, *A. selago*, Katedraalkrans, Male, MI
- 163x31: 4.77 mm, *A. selago*, Katedraalkrans, Male, MI

Clade 5
- 157x3: 6.15 mm, *A. selago*, 1000 m, Male, MI
- 149x32: 4.08 mm, *A. selago*, 200 m Junior's Kop, Male, MI
- 167x7: 4.85 mm, *A. selago*, 600 m First Red Hill, Male, MI
- 110x36: 4.31 mm, *D. strictum*, Albatross Lakes, Female, MI
- 157x2: 5.77 mm, *A. selago*, 1000 m, Female, MI
- 157x12: 6.15 mm, *A. selago*, 1000 m, Male, MI
- 157x8: 5.54 mm, *A. selago*, 1000 m, Female, MI

Clade 6
- 312x5: 4.85 mm, *A. selago*, 600 m, Female, PEI
- 320x3: 5.08 mm, *A. selago*, TvZB, Male, PEI
- 320x1: 4.62 mm, *A. selago*, TvZB, Male, PEI
- 321x11: 7.31 mm, *A. selago*, TvZB, Female, PEI

Clade 7
- 311x10: 7.54 mm, *A. selago*, 600 m, Female, PEI
- 311x7: 7.69 mm, *A. selago*, 600 m, Female, PEI
- 316x6: 7.92 mm, *A. selago*, 200 m, Female, PEI
- 304x13: 7.08 mm, *Poa cookii*, 0m Cave Bay, Male, PEI
- 305x1: 7.54 mm, *D. strictum*, Female, PEI
- 316x15: 8.15 mm, *A. selago*, 200 m, Female, PEI
- 321x2: 7.54 mm, *A. selago*, TvZB, Male, PEI
- 309x7: 4.69 mm, *A. selago*, 400 m, Female, PEI
- 310x4: 7.31 mm, *A. selago*, 400 m, Female, PEI
- 310x3: 7.31 mm, *A. selago*, 400 m, Female, PEI

Outgroup
- 400x21
- 400x12

Ectemnorhinus (PEIA)

E. viridis (Heard Island)

Support values: 98/98/95/100, 80/81/83/100, 55/54/56/--, 96/98/95/100, 97/97/97/100, 51/--/--/--, 63/--/55/--, 68/75/73/--, 88/88/85/100, --/--/--/97, 99/99/99/100, 58/61/53/--, --/--/--/98, --/50/53/100, 97/96/94/100, 92/93/92/100, 86/96/92/--, 72/66/73/--, 65/69/62/99, 99/99/82/100, 66/65/52/--, 63/63/63/96, 100/100/100/100
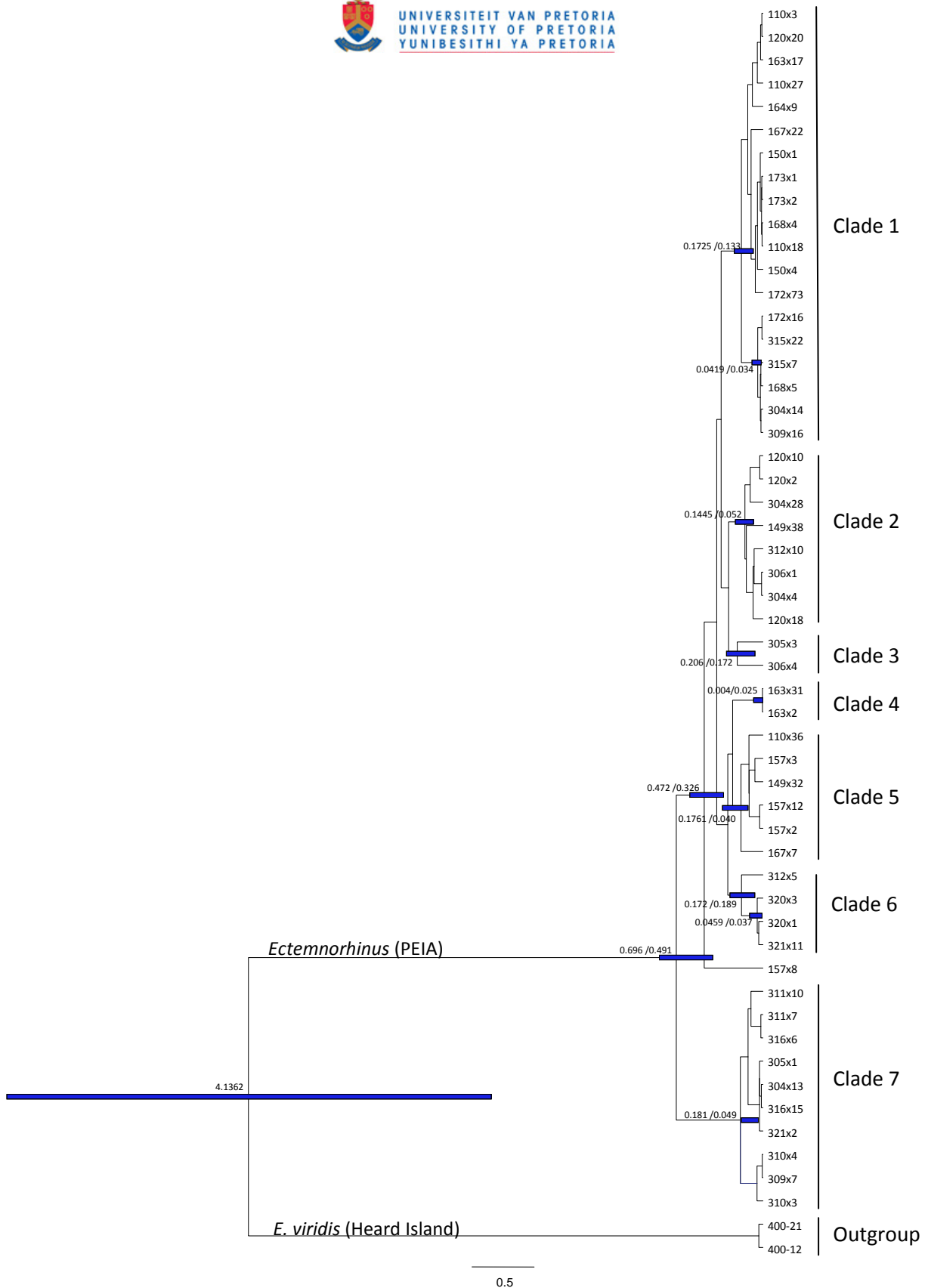
Scale: 0.01

Fig. 3 Ultrametric tree obtained with BEAST. The numbers in the nodes (Beast estimates / uncorrected pairwise estimates) correspond to the estimated age in MY. Blue bars correspond to the 95 % confidence interval. A clock rate of 2.3 % sequence divergence per million years (MY) was used.

28

Morphometric analyses

Principal component analyses based on both original and randomly selected data for both MI and PEI, showed no grouping of the sexes indicating the absence of multivariate morphometric sexual dimorphism within datasets.

All multivariate morphometric analyses of both individual and combined island datasets as well as individual-level analyses and those based on mean values were similar, and are best illustrated by PCA results. The PCA of the combined MI and PEI dataset (encompassing all size classes) showed neither a distinct separation with reference to the two currently recognized *Ectemnorhinus* species nor with regard to island of origin (Fig. 4). Lack of separation, however, seems to be largely confounded by a large degree of body size variation among individuals from the two islands. This is reflected by the high positive loadings of the measurements on the first PCA axis that accounted for 86.08% of the total variance (Table 3).
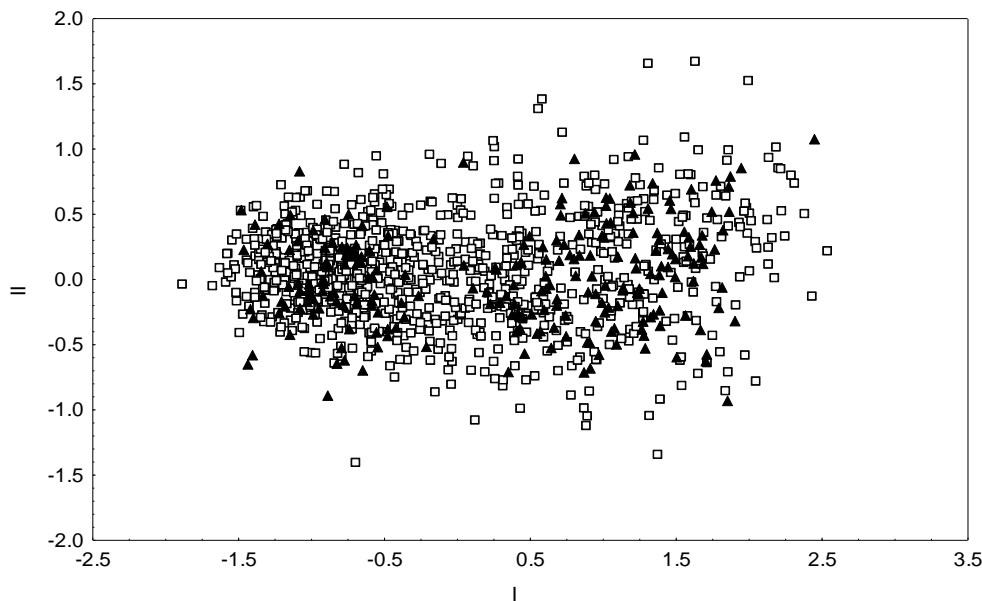
Fig. 4 Components I and II from a principal components analysis of *Ectemnorhinus* species collected from both Marion Island (open squares) and Prince Edward Island (closed triangles), indicating no distinct separation with reference to the two currently recognized *Ectemnorhinus* species nor with regard to island of origin.

TABLE 3 Loadings of variables on components I and II from principal components analyses of pooled samples of *Ectemnorhinus marioni* and *E. similis* from a) Marion Island b) Prince Edward Island and c) Marion and Prince Edward Islands combined.

*Variable	a) Marion Island		b) Prince Edward Island		c) Marion and Prince Edward Island	
	Principal components		Principal components		Principal components	
	I	II	I	II	I	II
TL	0.974	-0.029	0.989	0.057	0.976	-0.012
EW	0.956	-0.036	0.976	0.041	0.960	-0.038
PB	0.946	-0.042	0.986	0.080	0.952	-0.066
O	0.952	-0.009	0.977	-0.006	0.952	-0.058
A	0.897	-0.012	0.957	0.090	0.906	-0.065
F1	0.940	-0.047	0.959	-0.020	0.944	-0.041
F2	0.938	-0.059	0.961	-0.051	0.941	-0.062
F3	0.884	-0.052	0.921	-0.115	0.890	-0.093
FR	0.931	-0.054	0.938	-0.064	0.930	-0.013
T3	0.956	-0.043	0.807	-0.517	0.949	-0.010
MS	0.555	0.830	0.833	0.258	0.712	0.698
MT	0.936	0.044	0.945	0.071	0.937	0.082
MM	0.875	-0.071	0.950	0.089	0.889	-0.045
FL	0.986	-0.061	0.988	0.020	0.986	-0.054
FB	0.958	-0.023	0.973	0.011	0.960	-0.055
% trace	**84.23**	**4.78**	**89.37**	**2.58**	**86.08**	**3.54**

* Total body length (TL), pronotum breadth (PB), femur length (FL), interocular distance (O), metacoxal distance (MT), maximum breadth of elytra (EW), length of first three tarsal segments (T3), meso/metacoxal distance (MM), interantennal distance (A), mesocoxal distance (MS), femur breadth (FB), funicle segments (F1, F2 and F3) and rest of funicle (FR).

A PCA restricted to the 52 genetically identified specimens from both MI and PEI indicated no separation based on either body size or shape in the MI sample. However, two groups are observed for PEI *Ectemnorhinus* weevils, with only a single *Ectemnorhinus* individual from group A (encompassing individuals of clades 1, 2, 3 and 6, Fig. 2), falling within *Ectemnorhinus* group B, comprising individuals from clade 7 (Fig. 5a). The 52 genetically identified specimens showed a more pronounced separation between *Ectemnorhinus* group A, B and the MI *Ectemnorhinus* specimens on the first CVA axis. A shape related separation

was observed on the second CVA axis between the MI *Ectemnorhinus* species and *Ectemnorhinus* group A from PEI (Fig. 5b). A MANOVA showed a statistically significant phenetic difference between these pre-defined groupings ($F_{(30,62)} = 4.34$, $P < 0.0001$).
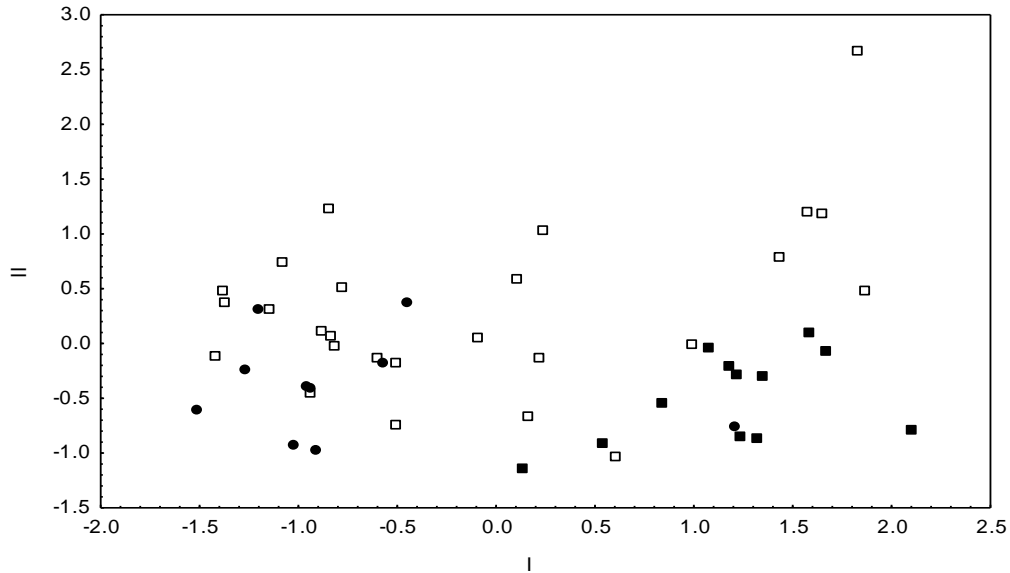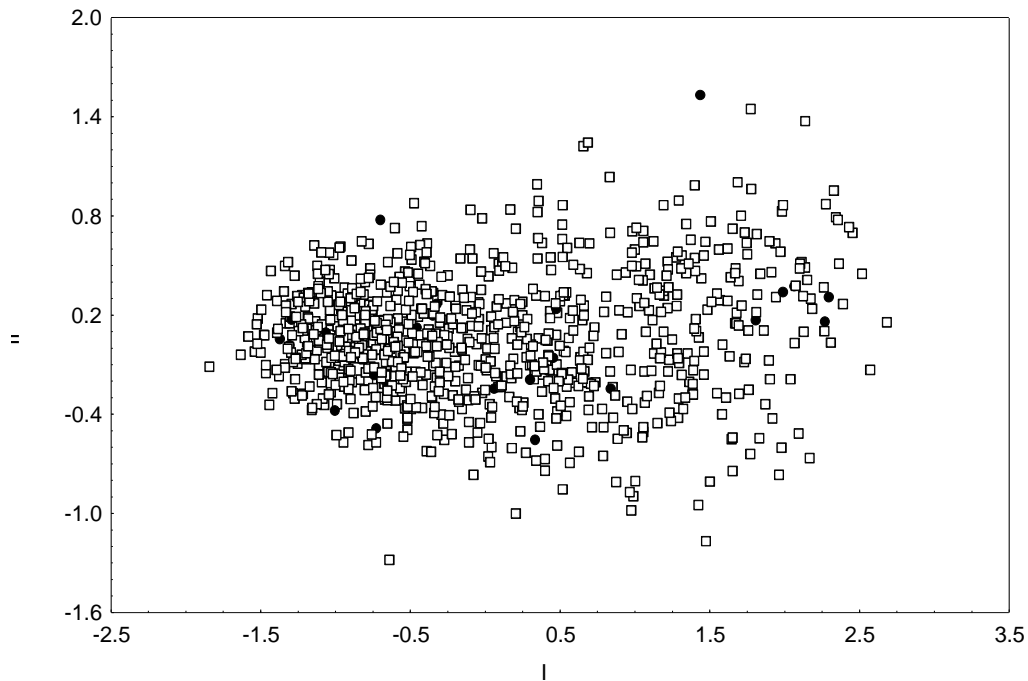

Fig. 5a Components I and II from a principal components analysis based on genetically identified individuals. No separation is observed in the Marion Island sample (open squares), while two groups are observed for samples collected on Prince Edward Island, *Ectemnorhinus* group A (closed circles; individuals of clades 1, 2, 3 and 6, Fig. 2c) and *Ectemnorhinus* group B (closed squares; individuals from clade 7)
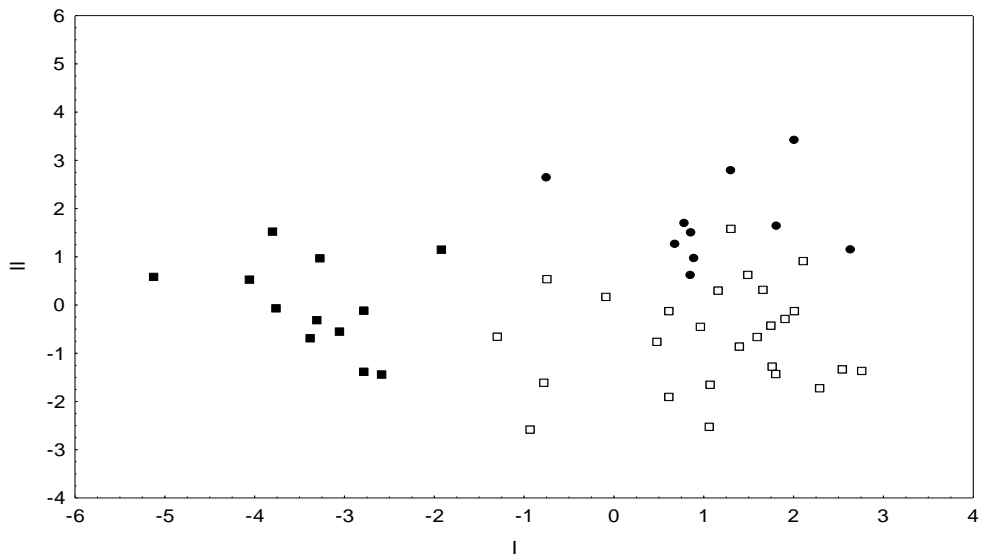
Fig. 5b Components I and II from a canonical variates analysis (CVA) showing a more pronounced separation of genetically identified individuals from Prince Edward Island, *Ectemnorhinus* group A (closed circles) and *Ectemnorhinus* group B (closed squares) with no separation in the Marion Island samples (open squares).

A PCA of MI samples (represented by all size classes) showed a considerable degree of phenetic variation, but no separation based on either size or shape variables (Fig. 6a). Principal component I (84.23% of the total variance) had high positive loadings on all measurements (Table 3), highlighting the importance of size variation. No separation was observed on the second (Table 3) and subsequent PCA axes.

Fig. 6a Components I and II from a principal components analysis of *Ectemnorhinus* species collected from Marion Island, indicate no separation based on either size or shape variables. Genetically identified individuals are indicated by black circles.

A PCA, comprising all size classes and including genetically identified PEI samples as references (Fig 6b), however, showed two phenetic groupings for the *Ectemnorhinus* species. Only a single genetically-identified individual from the *Ectemnorhinus* group A clustered with individuals of *Ectemnorhinus* group B. Separation between the two groups is based on a size- rather than a shape-related variation, as is shown by PCA axis I (89.37 %), which generally has high positive loadings on measurements analysed (Table 3).
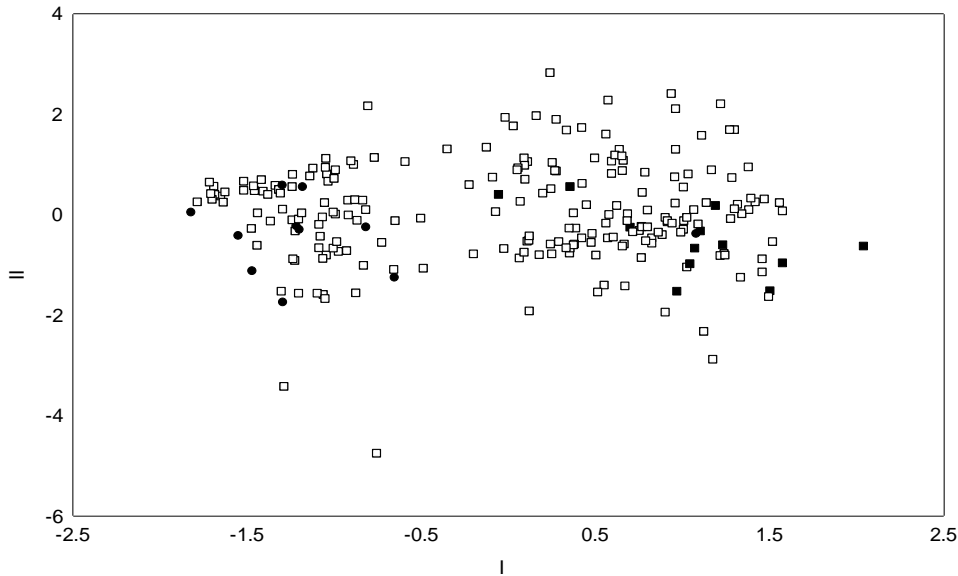
Fig. 6b Components I and II from a principal components analysis of *Ectemnorhinus* species collected from Prince Edward Island indicate separation between two group based on size variation. Genetically identified *Ectemnorhinus* group A (closed circles) and *Ectemnorhinus* group B (closed squares) are indicated.

Discussion

Assessment of the taxonomic status of weevil species originally described from MI was undertaken using a combined molecular and morphometric approach. The COI gene phylogeny identified seven recently diverged, well-supported clades on the PEIA, but gave no support for the presence of the two species, designated *E. marioni* and *E. similis* by Chown (1990) from MI. When investigating individuals collected from MI, it was found that none of the clades in the molecular phylogeny containing MI individuals displayed clustering on the basis of body size or according to plant species from which they were collected. In addition, multivariate analyses of the MI sample showed no separation of *Ectemnorhinus* individuals according to either body size or body shape variation. The molecular analyses, therefore, suggest that previous morphologically- and ecologically-defined distinguishing characteristics for MI *Ectemnorhinus* weevils (Crafford et al. 1986; Chown and Scholtz 1989; Chown 1990) do not consistently correspond to the clades identified using genetic markers. Moreover, the current morphometric analysis confirms that size variation is a major source of difficulty when attempting to establish species limits within the genus (see Brown 1964; Kuschel 1970, 1971; Crafford et al. 1986; Chown and Scholtz 1989; Chown 1990, 1991).

In contrast to the results obtained for the individuals collected on MI, two major, size-distinct clades were discernible for the individuals collected on PEI from both the genetic

(clade 7 as opposed to the PEI individuals collectively grouped within clades 1 to 6) and the morphometric analyses. A sequence divergence value of 1.5 % was observed between the two major PEI clades that corresponds well with the intra-generic Kimura-2-parameter genetic distances of 1.5 % and 2.1 % reported for arthropods from other island systems (Trewick 2000). This, together with the high levels of bootstrap support for the two size- and genetically-distinct clades, suggests that the present recognition of two species distinguishable on the basis of size is supported on PEI. The presence of two size-discrete groupings of *Ectemnorhinus* individuals on PEI was also confirmed by the morphometric analyses, where all multivariate analyses indicated the presence of two size-related phenetic groupings. The single *Ectemnorhinus* individual from group A, that clustered within the *Ectemnorhinus* group B assemblage is indicative of the extent of body size variation within *Ectemnorhinus* species.

In the light of these results a re-evaluation of the species status of the PEIA *Ectemnorhinus* weevils is necessary. Because individuals from Prince Edward Island have not been used in formal taxonomic assessments (i.e. they have not been physically identified and labelled, with a corresponding description or specimen listing in the literature), the larger PEI-restricted weevils that constitute clade 7 have been designated *Ectemnorinus kuscheli* (see Appendix A to this chapter for a full species description). Likewise, because neither the phylogenetic nor the morphometric data support the existence of two species on MI, *E. similis* and *E. marioni* from MI, together with those weevils from PEI that group within clades 1 to 6, have been synonymized, under the name *Ectemnorhinus similis*, which has priority.

The different methods used to determine the time of coalescence of weevil lineages produced different results. Uncorrected pairwise estimates showed that the *Ectemnorhinus* weevil lineages coalesced at approximately 0.49 million years ago (MYA), that *E. kuscheli* coalesced at approximately 0.049 MYA and *E. similis* coalesced at approximately 0.33 MYA. BEAST recovered much older estimates with the estimate for the *Ectemnorhinus* weevil lineage coalescence occurring approximately 0.70 million years ago (MYA), *E. kuscheli* coalescence occurring approximately 0.18 MYA and *E. similis* coalescence around 0.47 MYA. While all the dates estimated by uncorrected pairwise estimates fall within the parameters of the oldest rocks dated on the PEIA (Boelhouwers *et al.* 2008) the dates estimated by BEAST exceed these dates, but remain within the estimated emergence time of the islands of less than 1 MYA (Boelhouwers *et al.* 2008).

While our data suggest the presence of two species on PEI and only one on MI, it is

important to remember that two species, *E. marioni* and *E. similis*, were originally described from MI. The critical question that may now be posed relates to the apparent disappearance of one species of *Ectemnorhinus* on MI. Was there only one *Ectemnorhinus* species to begin with on Marion Island or is it possible there were indeed two species 65 years ago when Jeannel (1940) first described *E. marioni* as a second species distinct from *E. similis*?

The first hypothesis for the observed difference in weevil assemblage between the two islands is the loss of one of the originally described *Ectemnorhinus* species from MI. One possible cause for the loss could be that the reduction in body size of *E. similis* through size-selective predation by mice (Chown and Smith 1993; Smith et al. 2002) which would have removed the size-induced reproductive barrier that was proposed on grounds of the significant relationship between female and male body size in *in-copula* pairs observed by Chown (1990) on MI. This scenario would allow the two previously recognized species to interbreed. In addition to mice predation, it is also possible that climate change may play a role as temperature on the Prince Edward Islands has increased by an average of 0.04°C per year since the late 1960s (Smith 1991). It is well-known that in arthropods, and indeed most invertebrates, increasing developmental temperatures often lead to a decline in body size (Atkinson 1994). Moreover, if temperatures increase to such an extent that generation time is much shorter than season length, then additional declines in body size with increasing temperature can be expected (Kozłowski et al. 2004). Investigations of weevil species on MI have shown that long-term warming (at least since the 1960s) may well have led to on-going declines in body size, accompanied by a secondary, significant influence of mouse predation (L. Janse van Rensburg, 2005). These changes in the environment may select for smaller individuals within the populations, leading eventually to introgression.

The second hypothesis is that there was originally only one *Ectemnorhinus* species on MI that was erroneously described twice. The original descriptions of *E. similis* by Waterhouse (1885) and *E. marioni* by Jeannel (1940) indicate that there are distinct differences between the two species. The controversial taxonomic status of these two species is, however, a clear indication of the difficulty taxonomists were faced with in the past when using morphological characteristics alone. One possible reason for the different status of *Ectemnorhinus* species between the two islands may also be due to differences in glaciation histories, as MI was extensively glaciated whilst PEI was not (Verwoerd 1971). As a result, weevils on PEI would have had longer exposure to vascular plants as an additional, more nutritious food source to bryophytes, than those on MI. This may have given rise to two

species, a smaller one with a preference for bryophytes and a larger one with a preference for angiosperms, as suggested by Chown (1990). Another possible explanation for the dissimilar status of these island species may be due to differences in coalescence times.

Whatever the underlying cause(s), there is no doubt that a marked difference in the species exists between the islands with PEI harbouring both the newly described *E. kuscheli* and *E. similis* and MI only *E. similis*. The two islands also differ considerably with respect to the genetic composition of their respective populations of *E. similis*, with each island having numerous island-unique haplotypes and only one haplotype of *E. similis* being shared between the islands. PEI may thus not be considered as a safe haven for *E similis* populations that are on MI. The *Ectemnorhinus* weevils from the two islands are distinct and should therefore be considered as different management units. Even if the PEI populations were not drastically different from those on MI, and the MI populations were completely removed by mice predation, the restocking of an island with specimens from another island may not be appropriate. Management regimes at the Prince Edward islands should instead focus on preserving the genetic variation unique to MI. There is an urgent need to explore possibilities of either eradicating or drastically controlling mice on MI as a long-term goal. In the interim, the current policy of restricting human visits to PEI should be strictly maintained.

References

Akaike, H., 1974: A new look at the statistical model identification. IEEE Trans. Autom. Contr. 19, 716-723.

Anonymous, 1996: Prince Edward Islands Management Plan. Department of Environmental Affairs and Tourism, Pretoria.

Atkinson, D., 1994: Temperature and organism size-A biological law for ectotherms? Advances in Ecological Research 25, 1-58.

Boelhouwers, J.; Meiklejohn, I.; Holness, S.; Hedding, D., 2008: Geology, geomorphology and climate change. *In* Chown, S.L.; Froneman, P.W., eds. The Prince Edward Islands. Land-Sea Interactions in a Changing Ecosystem. African Sun Media, Stellenbosch, 65-96.

Boom, R.; Sol, C. J. A.; Salimans, M. M. M.; Jansen, C.L.; Wertheim-van Dillen, P. M. E.; van der Noordaa, J., 1990: Rapid and Simple Method for Purification of nucleic Acids. J. Clinical Microbiol. 28, 495-503.

Brower, A. V. Z., 1994: Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. Proc. Nat. Acad. Sci. USA 91, 6491-6495.

Brown, K. G., 1964: The insects of Heard Island. A. N. A. R. E. Report 1, 1-39.

Caccone, A.; Sbordoni, V., 2001: Molecular biogeography of cave live: A study using mitochondrial DNA from Bathysciine beetles. Evolution 55, 122-130.

Chimimba, C. T.; Dippenaar, N. J.; Robinson, T. J., 1999: Morphometric and morphological delineation of southern African species of *Aethomys* (Rodentia:Muridae) Biol. J. Linn. Soc. 67, 501-527.

Chown, S. L., 1989: Habitat use and diet as biogeographic indicators for sub-Antarctic Ectemnorhinini (Coleoptera: Curculionidae). Antarct. Sci. 1, 23-30.

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

Chown, S. L. 1991: Species problems in the Ectemnorhinini (Coleoptera: Curculionidae) of sub-Antarctic Marion Island. In: Zunino, M.; Bellés, X.; Blas, M. (eds.) Advances in Coleopterology. European Association of Coleopterology, Barcelona, pp. 201-210.

Chown, S. L.; McGeoch, M. A.; Marshall, D. J., 2002: Diversity and conservation of invertebrates on the sub-Antarctic Prince Edward Islands. African Entomology 10, 67-82.

Chown, S. L.; Scholtz, C. H., 1989: Biology and ecology of the *Dusmoecetes* Jeannel (Col. Curculionidae) species complex on Marion Island. Oecologia 80, 93-99.

Chown, S. L.; Smith, V. R., 1993: Climate change and the short-term impact of feral house mice at the sub-Antarctic Prince Edward Islands. Oecologia 96, 508-516.

Crafford, J. E.; Chown, S. L., 1991: Comparative nutritional ecology of bryophyte and angiosperm feeders in a sub-Antarctic weevil species complex (Coleoptera: Curculionidae). Ecol. Entomol. 16, 323-329.

Crafford, J. E.; Scholtz, C. H., 1987: Quantitative differences between the insect faunas of sub-Antarctic Marion and Prince Edward Islands: a result of human intervention? Biological Conservation 40, 255-262.

Crafford, J. E.; Scholtz, C. H.; Chown, S. L., 1986: The insects of sub-Antarctic Marion and Prince Edward Islands; with a bibliography of entomology of the Kerguelen biogeographical province. S. Afr. J. Antarc. Res. 16, 42-84.

Dreux, P. H., 1971: Insecta. In: Van Zinderen Bakker, E. M.; Winterbottom, J. M.; Dyer, R. A., (eds) Marion and Prince Edward Islands: Report on the South African Biological and Geological Expedition 1965-1966. A.A. Balkeman, Cape Town, pp. 335-343.

Dreux, P.; Voisin, J. F., 1986: Révsion du genre *Dusmoecetes* Jeannel 1940, et description de deux espèces et d'une sous-espèce nouvelles (Col. Curculionidae Ectemnorhininae). *Bulletin de la Société entomologique de France* 91, 28-40.

Dreux, P.; Voisin, J. F., 1989: Sur le systématique des genres de la sous-famille des Ectemnorrhininae (Coleoptera, Curculionidae). Nouvelle Revue d'Entomologie (N.S.) **6**, 111–118.

Drummond, A.J.; Rambaut, A., 2007: BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evolutionary Biology **7**, 214.

Emerson, B. C.; Oromi, P.; Hewitt, G. M., 1999: MtDNA phylogeography and recent intra-island diversification among Canary Island *Calathus* beetles. Mol. Phylogen. Evol. 13, 149-158.

Farris, J. S., 1969: A successive approximations approach to character weighting. Syst. Zool. 18, 374-385.

Felsenstein, J., 1981: Evolutionary trees from DNA sequences: a maximum likelihood approach. J. Mol. Evol. 17, 368-376.

Felsenstein, J., 1988: Phylogenies from molecular sequences: inference and reliability. Ann. Rev. Gen. 22, 521-565.

Gleeson, J. P.; Van Rensburg, P. J. J., 1982: Feeding ecology of the house mouse *Mus musculus* on Marion Island. S. Afr. J. Antarct. Res. 12, 34-39.

Gremmen, N. J. M.; Smith, V. R., 1999: New records of alien vascular plants from Marion and Prince Edward Islands, sub-Antarctic. Polar Biol. 21, 401-409.

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

Harley, E. H., 2000: Dapsa 4.9. Department of Chemical Pathology, University of Cape Town.

Huelsenbeck, J. P.; Ronquist, F., 2001: MR-BAYES: Bayesian inference of phylogeny. Bioinformatics 17, 754-755.

Janse van Rensburg, L.; Chimimba, C. T.; Bastos, A. D.; Chown, S. L., 2003: Morphometric measurement selection: an invertebrate case study based on weevils from sub-Antarctic Marion Island. Polar Biol. 27, 38-49.

Janse van Rensburg, L., 2005: A morphological analysis of weevils from sub-Antarctic Prince Edward Islands: an assessment of ecological influences. Doctoral Thesis, University of the Pretoria, South Africa

Jeannel, R., 1940: Coléoptères. Croisière du Bougainville aux îles australes francaises. Mém. Mus. Nation. Hist. Nat. (N.S.) 14, 63-201.

Jeannel, R., 1953: Sur la faune entomologique de l'ile Marion. Revue Francaise d'Entomologie 31, 319-417.

Klok, C. J.; Chown, S. L., 2003: Resistance to temperature extremes in sub-Antarctic weevils: interspecific variation, population differentiation and acclimation. Biological Journal of the Linnean Society 78, 401-414.

Kozłowski, J.; Czarnołęski, M.; Dańko, M., 2004: Can optimal resource allocation models explain why ectotherms grow larger in cold? Integrative and Comparative Biology 44, 480-493.

Kumar, S., 1996: PHYLTEST: A Program for Testing Phylogenetic Hypothesis, Version 2.0. Institute of Molecular Evolutionary Genetics and Department of Biology, The Pennsylvania State University, University Park, Pennsylvania, USA.

Kumar, S.; Tamura, K.; Jakobsen, I. B.; Nei, M., 2001: MEGA2: Molecular Evolutionary Genetics Analysis software, Bioinformatics.17, 1244-1245.

Kuschel, G., 1970: Coleoptera: Curculionidae of Heard Island. Pacific Insects Monograph 23, 255-260.

Kuschel, G., 1971: Curculionidae. In: Van Zinderen Bakker, E. M.; Winterbottom, J. M.; Dyer, R. A., (eds) Marion and Prince Edward Islands: Report on the South African Biological and Geological Expedition 1965-1966. A.A. Balkeman, Cape Town, pp. 355-359.

Kuschel, G.; Chown. S. L., 1995: Phylogeny and systematics of the *Ectemnorhinus*-group of genera (Insecta: Coleoptera). Invert. Tax. 9, 841-863.

Li, P.; Bousquet, J., 1992: Relative-Rate test for nucleotide substitutions between two lineages. Mol. Biol. Evol. 9, 1185-1189.

Manly, B. F. J., 1991: Randomization and monte carlo methods in biology. Chapman and Hall, London.

McCarthy, C., 1996-1997: School of Biomolecular and Biomedical Science, Faculty of Science and Technology, Griffith University, Brisbane, Queensland, Australia.

Pimentel, R. A.; Smith, J. D., 1986: BIO∑TAT II. A tutorial manual. 2nd edn. Sigma Soft, Placentia, California.

Posada, D.; Crandall, K. A., 1998: MODELTEST: Testing the model of DNA substitution. Bioinformatics 14, 817-818.

Rice, W. R., 1984: Disruptive selection on habitat preference and the evolution of reproductive isolation: a simulation study. Evolution 38, 1251-1260.

Rodriguez, F.; Oliver, J. F.; Marin, A.; Medina, J. R., 1990: The general stochastic model of nucleotide substitution. J. Theoretical Biol. 142, 485-501.

Rozas, J.; Rozas, R., 1999: DnaSP version 3: An integrated program for molecular population genetics and molecular evolution analysis. Bioinformatics 15, 174-175.

Rychlik, W., 1993: Selection of primers for polymerase chain reaction. Methods in Molecular Biology. Vol. 15: PCR Protocols: In: White, B. A., (ed.) Current Methods and

Applications. Humana Press Inc., Totowa, NJ, pp 31-40.

Rzhetsky, A.; Nei, M., 1992: A simple method for estimating and testing minimum-evolution trees. Mol. Biol. Evol. 9, 945-967.

Saitou, N.; Nei, M., 1987: The neighbour-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4, 406-425.

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

Simon, C.; Frati, F.; Beckenbach, A.; Crespi, B.; Lui, H.; Flook, P., 1994: Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann. Entomol. Soc. Am. 87, 651–701.

Smith, V. R., 1991: Climate change and its ecological consequences at Marion and Prince Edward Islands. SA. J. Ant. Res. 21, 223-224.

Smith, V. R., 2002: Climate change in the sub-Antarctic: An illustration from Marion Island. Clim. Change 52, 345-357.

Smith, V. R.; Steenkamp, M., 1990: Climate change and its ecological implications at a sub-Antarctic island. Oecologia 85, 14- 24.

Smith, V. R.; Avenant, N. L.; Chown, S. L., 2002: The diet and impact of house mice on a sub-Antarctic island. Polar Biol. 25, 703-715.

Sneath, P. H. A.; Sokal, R. R., 1973: Numerical Taxonomy. W.H. Freeman, San Francisco, pp. 573.

StatSoft Inc., 1995: STATISTICA for Windows (Computer program manual) StatSoft, Inc. Tulsa, Oklahoma.

Swofford, D. L., 1999: PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Sinauer Associates, Sunderland, Massachusetts.

Trewick, S. A., 2000: Molecular evidence for dispersal rather than vicariance as the origin of flightless insect species on the Chatham Islands, New Zealand. J. Biogeogr. 27, 1189-1200.

Verwoerd, W. J., 1971: Geology. In: Van Zinderen Bakker, E. M.; Winterbottom, J. M.; Dyer, R. A., (eds) Marion and Prince Edward Islands: Report on the South African

Biological and Geological Expedition 1965-1966. A.A. Balkeman, Cape Town, pp. 40-62.

Waterhouse, G. R., 1853: Descriptions of new genera and species of Curculionides. Trans. Ent. Soc. London 2, 172-207.

Waterhouse, C. O., 1879: Zoology of Kerguelen Island: Coleoptera. Phil. Trans. R. Soc. Lond. 168, 230-234.

Waterhouse, C. O., 1885: Description of two new Curculionidae (*Ectemnorhinus*) from Marion Islands. Ann. Mag. Nat. Hist. Lond. 16, 121-123.

Watkins, B. P.; Cooper, J., 1986: Introduction, present status and control of alien species at the Prince Edward Islands. S. Afr. J. Antarc. Res. 16, 86-94.

Williams, P. L.; Fitch, W. M., 1990: Phylogeny determination using the dynamically weighted parsimony method. Methods Enzymol. 183, 615-626.

Zar, J., 1996: Biostatistical Analysis, 3rd edn. Prentice Hall, New Jersey.

Appendix

A Description of *Ectemnorhinus kuscheli* n. sp.

Description: Length: 4-69 – 8.15 mm. (holotype: 7.69 mm with left front leg missing). A large *Ectemnorhinus* species. Brown to black; antennae, abdomen, and base of femora brown to black. Rostrum medially depressed or sulcate. Scrobes below antennal tubercle narrowly exposed. Epistome distinctly asymmetrical due to right side projecting further forward. Mandibles rather slender, moderately curved on outer edge, lacking cusps or scar. Labial palp 2-segmented. Pronotum medially not sulcate. Each elytron rounded at apex leaving part of tergites exposed. Interstriae 1, 3 and 5 not subcostate or uneven. Elytra with distinct erect setae on dorsum although setae sometimes very short. Hind wings very rudimentary, less than 0.1 x length of elytron. Inner flange of elytra rudimentary. Hind margin of prothorax behind coxae truncate. Metepisternal structure obsolete. Tibiae without mucro. Claw segment shorter than other segments combined. Tarsomeres 1-3 with adhesive pads on sole. Lobes of tarsomere 3 of unequal size. Tergite 8 of female exposed beyond 7. Blade of sternite 8 of female not transversely quadrangular. Internal sac with an elongate appendage on each side of gonopore. Gonopore basal on internal sac. Proximal hemisternites with struts. Setae on distal hemisternites long. Bursa without sclerite. Spermathecal duct insertion on bursa median or terminal. Spermathecal gland very long, much longer than duct. Proventricular blades rudimentary, without bristles.

Diagnosis: Identical to *E. similis* in external morphology and internal structure (See Chown 1990). Statistically larger than *E. similis*. Ecology, habitat and feeding preference identical to *E. similis*. Significant difference between *E. similis* and *E. kuscheli* are to be found in genetic evidence (Fig. 2). Four nucleotide positions can be used as diagnostic characters to distinguish between the two species, namely:

   (i)   Position 568 of the COI gene: *E. kuscheli* has a T while *E. similis* has an A
   (ii)   Position 997 of the COI gene: *E. kuscheli* has a T while *E. similis* has a C
   (iii)   Position 1063 of the COI gene: *E. kuscheli* has a C while *E. similis* has a T
   (iv)   Position 1297 of the COI gene: *E. kuscheli* has either an A or G (R) while *E. similis* has either a C or T (Y).

Distribution: Prince Edward Island.

Etymology: This new species is named after G. (Willy) Kuschel in recognition of his work on sub-Antarctic weevils.

Type material examined:

Holotype:

♀, South Africa, Prince Edward Island, on *Azorella selago*, 600 m above sea level, S 46º37.533' E 37º55.985', Genbank no. AY762310, voucher no. 311-7, date collected April 2003, collector G.C. Grobler. Deposited in the Natural History Museum, London, United Kingdom.

Paratypes:

♂, South Africa, Prince Edward Island, on *Azorella selago*, Top of Van Zinderen Bakker Peak, S 46º37.590' E 37º55.891', Genbank no. AY762315, voucher no. 321-2, date collected April 2003, collector G.C. Grobler. Deposited in the Natural History Museum, London, United Kingdom.

♀, South Africa, Prince Edward Island, on *Azorella selago*, 400 m above sea level, S 46º38.211' E 37º57.482', Genbank no. AY762318, voucher no. 310-3, date collected April 2003, collector G.C. Grobler. Deposited in the Natural History Museum, London, United Kingdom.

♀, South Africa, Prince Edward Island, on *Azorella selago*, 400 m above sea level, S 46º38.211' E 37º57.482', Genbank no. AY762316, voucher no. 309-7, date collected April 2003, collector G.C. Grobler. Deposited in the Natural History Museum, London, United Kingdom.

♀, South Africa, Prince Edward Island, on *Azorella selago*, 600 m above sea level, S 46º37.533' E 37º55.985', Genbank no. AY762309, voucher no. 311-10, date collected April 2003, collector G.C. Grobler. Deposited in the Transvaal Museum, Pretoria, South Africa.

♀, South Africa, Prince Edward Island, on *Azorella selago*, 200 m above sea level, S 46º38.457' E 37º58.396', Genbank no. AY762311, voucher no. 316-6, date collected April 2003, collector G.C. Grobler. Deposited in the Transvaal Museum, Pretoria, South Africa.

♂, South Africa, Prince Edward Island, on *Poa cookii*, Cave Bay, S 46º38.752' E 37º59.780', Genbank no. AY762312, voucher no. 304-13, date collected April 2003, collector G.C. Grobler. Deposited in the Transvaal Museum, Pretoria, South Africa.

♀, South Africa, Prince Edward Island, on *Azorella selago*, 200 m above sea level, S 46º38.457' E 37º58.396', Genbank no. AY762314, voucher no. 316-15, date collected April 2003, collector G.C. Grobler. Deposited in the National Insect Collection (SANC), Pretoria, South Africa.

♀, South Africa, Prince Edward Island, on *Ditrichum strictum*, S 46º38.057' E 37º56.771', Genbank no. AY762313, voucher no. 305-1, date collected April 2003, collector G.C. Grobler. Deposited in the National Insect Collection (SANC), Pretoria, South Africa.

♀, South Africa, Prince Edward Island, on *Azorella selago*, 400 m above sea level, S 46º38.211' E 37º57.482', Genbank no. AY762317, voucher no. 310-4, date collected April 2003, collector G.C. Grobler. Deposited in the National Insect Collection (SANC), Pretoria, South Africa.