UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Molecular phylogeny and evolution of the Ectemnorhinus group of weevils in the Prince Edward Islands

by

Gert C. Grobler

Submitted in partial fulfillment of the requirements of the degree of Doctor of Philosophy (Entomology)

Department of Zoology & Entomology
Faculty of Natural & Agricultural Sciences
University of Pretoria
Pretoria
South Africa

December 2011

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Molecular phylogeny and evolution of the Ectemnorhinus group of weevils in the Prince Edward Islands

Student: Gert C. Grobler[1]

Supervisors: Prof. Armanda D. Bastos[1], Prof. Christian T. Chimimba[1,2] & Prof. Steven L. Chown[2]

Departments: [1]Department of Zoology and Entomology, University of Pretoria, Pretoria 0002 South Africa

[2]DST-NRF Centre of Excellence for Invasion Biology (CIB), Department of Botany and Zoology, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa

Degree: Doctor of Philosophy (Entomology)

Declaration

I, Gert Grobler hereby declare that apart from the morphometric data that were generated by Dr Lindie Janse van Rensburg (included in Chapter 2), this thesis which is submitted for the degree of Doctor of Philosophy (Entomology) at the University of Pretoria, is otherwise my own work and has not previously been submitted by me for a degree at this, or or any other tertiary institution.

Signed:……

Date:……

Disclaimer

Each chapter of this thesis has been compiled as a separate paper for publication purposes. Chapter 2 has been published in the *Journal of Zoological Systematics and Evolutionary Research* and is formatted for the journal. Chapters 4 and 5 have been formatted for and submitted to *Antarctic Science*. Each chapter contains its own set of references. The general introduction and conclusion are tailored from the rest of the chapters and give an idea of what to expect from the thesis and about the conclusions drawn. Consequently, unavoidable overlaps may occur between chapters.

Thesis summary: All previous taxonomic studies on the Ectemnorhinus group of weevils have been based primarily on morphological data. While these studies are invaluable, some questions can only be addressed adequately through molecular studies. This is especially true when studying the genetic relationships and phylogeograpic patterns of taxa endemic to the South Indian Ocean Province (SIP) biotas that have long been controversial. The Ectemnorhinus group of genera is a monophyletic unit of weevils endemic to the region. The present study focused mainly on the Ectemnorhinus group of weevils found on the Prince Edward Islands archipelago (PEIA). The mitochondrial cytochrome oxidase I gene was targeted when investigating relationships among members of this weevil group. On the PEIA, it is important to note that 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. Since emergence, a series of volcanic and glaciation events have occurred on Marion Island, whilst Prince Edward Island has remained largely unaffected by glaciation. Phylogenetic analyses revealed the presence of two genetically and morphometrically distinct species of *Ectemnorhinus* weevils 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 the present study confirmed the synonymy between *E. similis* and *E. marioni*, the two species originally described from MI. *Ectemnorhinus kucheli* appears to be restricted to PEI, whereas *E. similis* occurs on both MI and PEI. When investigating the population dynamics of the *Ectemnorhinus* weevils on the PEIA, the data indicated that PEI was the first of the two islands of the PEIA to be colonized by *Ectemnorhinus* weevils, at an estimated time of coalescence of approximately 0.3116 million years ago (MYA). The PEI population then acted as the source population for the colonization of MI by *Ectemnorhinus* weevils some time before the last glaciation, approximately 10 000 to 35 000 years ago. The separation by distance of the PEI *Ectemnorhinus* weevils from those on MI then gave rise to two species by allopatric speciation on MI. During the last glaciations, MI was extensively glaciated with only the southwestern corner of the island being free of ice. This extensive glaciation of MI would have resulted in the eradication of all *E. similis* on MI except for those occurring on the ice-free southwestern corner of the island. At the end of the last glacial maximum, when the ice started to melt, the coastal areas of MI emerged first from beneath the ice and were available for re-colonization by weevils. The movement of weevils that were isolated in the south-western corner of MI, along the coastal areas of the island, was assisted by strong, frequent south-western winds. Subsequent, post-glacial volcanism during the

Holocene was then responsible for the fragmentation of the new migrants, resulting in small population pockets surrounded by fresh, uninhabitable lava and subsequent divergence of each populations. When the Holocene black lava became re-colonizeable, the weevils from the different isolated populations migrated to the remainder of the island. Currently, members of the different genetically-identified populations occur in sympatry and in some cases even on the same plant, but no noticeable geneflow was detected between them. It is thus suggested that the time of isolation, before the post-glacial black lava during Holocene became hospitable, was sufficiently long and the populations sufficiently small that a number of genetically-discrete populations arose. Consequently, the present study recognises two genetically discrete populations of *E. kucheli* on PEI and seven discrete *E. similis* populations on MI that are morphologically indistinct. When examining the relationships among 13 species from five different islands within the South Indian Ocean Province (SIP) that are representative of 22 populations within the genera *Palirhoeus*, *Bothrometopus* and *Ectemnorhinus*, there was little support for separating the genus *Palirhoeus* from *Bothrometopus*, and no support for the morphologically-delineated species groups currently recognized within *Bothrometopus*. The present study shows that colonization of the Prince Edward Islands is likely to have occured repeatedly from other islands within the SIP and that *Bothrometopus parvulus* on the PEIA comprises two species that are not sister taxa. The second novel con-generic species was therefore designated *Bothrometopus huntleyi* n. sp. and examination of the genetically identified specimens resulted in the indentification of distinguishing morphological characteristics. The analyses indicated that *B. huntleyi* arose approximately 0.5 million years ago from a high-altitude population that is still present on MI. The first major intra- and inter-island dispersal event occurred ~0.338 MYA, coinciding with the glaciation-free second volcanic stage on MI. Apart from this early inter-island colonisation, only one other between-island dispersal event, corresponding with the glaciation-free seventh volcanic stage, was detected. Genetically discrete weevil complexes on each of the islands of the PEIA together with the low levels of inter-island gene flow reaffirm the need to control alien invasive mice, which are restricted to MI, and which prey on these weevil species.

Key words: Ectemnorhinus group of weevils, mtDNA, COI gene, conservation, biogeography, dispersal, speciation, invasion biology, phylogeography, Prince Edward Island, Marion Island, Coleoptera, Curculionidae, evolution, phylogeny, Southern Ocean islands, sub-Antarctic.

Acknowledgements

This project was conducted under the auspices of the Department of Zoology and Entomology of the University of Pretoria, South Africa. The Directorate Antarctica and Islands of the South African Department of Environmental Affairs and Tourism provided logistic support on Marion Island via the South African National Antarctic Programme (SANAP). This work was funded by a SANAP NRF grant (GUN2068301). My sincerest thanks and appreciation goes to my supervisors Armanda Bastos, Chris Chimimba and Steven Chown. Thank you for your mentorship, time, support, guidance, advice, input, encouragement and above all patience!!! Thank you for this opportunity to learn and grow. I want to thank Lindie Janse van Rensburg and Mark Keith for their assistance in the field. Lewis Davies provided access to material from Possession Island via the donation of several specimens to SLC, whilst material from Kerguelen Island was collected by Jacques Deere during a Scientific Committee on Antarctic Research (SCAR) exchange activity supported by the French Polar Institute (IPEV) and specifically Yves Frenot. I am indebted to Chris Lyal of the Natural History Museum London for providing dissection microscope photographs of the *B. parvulus* holotype and to Helene Brettschneider for sample sorting and SEM photography of specimens. I am grateful to Ute Kryger for abstract translation into German, Clarke Scholtz for assistance with *E. kucheli* species description and Anne Treasure for assistance with the species description of *B. huntleyi*. I would like to thank my parents for their love and support. They kept believing in me. I would like to thank my brother and grandmother for their continual interest and support. Also thank you to all my other family and friends that supported me through this adventure. Lastly I would like to thank my wife, Elizabeth, for all her love, encouragement and support. Together we are going on adventures and exploring the world.

Another adventure came to an end…..

Let the next adventure begin…..

Table of Contents

List of Figures and appendeces

Chapter 2

Chapter 3

Chapter 4

Chapter 5

List of Tables

Chapter2

Chapter 3

Chapter 4

Chapter 5

CHAPTER 1

General Introduction, Rationale and Key Questions

Mayr (1942) introduced the 'biological species concept' namely that "*species are groups of actually or potentially interbreeding natural populations that are reproductively isolated from other such groups*". Reproductive isolation is thus the *sine qua non* of a good species. The reproductive barriers that separate members of different species are divided into two groups, namely 'prezygotic isolating factors' (arising from mate discrimination, different habitat preferences, pollination by different insects, amongst others) and 'postzygotic isolating factors' (due to hybrid inviability and/or sterility). It is argued that reproductive isolation usually evolves in allopatry when different populations of the same species are isolated from each other by geographic barriers, and that allopatric speciation is the primary mode of speciation in nature (Futuyma & Mayer, 1980; Mayr, 1963; Coyne & Orr, 2004). Together with selection (Darwin, 1859) and genetic drift (Mayr, 1954), reproductive isolation creates and expands the morphological differences between closely related species (Mayr, 1954). Genetic drift is far more likely in island or peripheral populations founded by a few individuals and may allow rapid evolution to new fitness peaks, permitting the evolution of reproductive isolation between ancestral and founder populations (Mayr, 1954; Provine, 1989). Dobzhansky (1937) also proposed that when populations have diverged genetically to the extent that the offspring of within-population matings are more fit than hybrid offspring, selection favours an increase in non-random mating due to a preference for similar partners. This assortative mating then increases reproductive isolation by reducing the exchange of genes between the original populations, a process termed reinforcement. Support for reinforcement has been found in nature in flycatchers (Saetre et al., 1997).

It is also believed that speciation can occur between populations that occupy the same area, a process called sympatric speciation. Rice (1984) presented a model in which reproductive isolation and thus sympatric speciation can occur due to disruptive selection on habitat preference. Reproductive isolation has also been shown to occur by mate preference as seen in sticklebacks in Canadian lakes (Boughman, 2001). Dieckmann & Doebeli (1999) presented a model that shows that assortative mating due to differences in preference for a certain trait within a large species may split the species in two. Doebeli & Dieckmann (2003) provides another model for sympatric speciation where the outer ends of a species that occurs

over an environmental gradient adapt, over time, through internal competition to those circumstances and diverge from each other to form different species as selection in each part of the original species favours a different trait. Mutations in as little as one gene can cause reproductive isolation in some species as is seen in prezygotic isolation in snails where the direction in coiling can be changed by a single mutation, making copulation impossible (Orr, 1991). On the other hand it has been shown that at least 150 genes are responsible for inviability in hybrids between two chromosomal races of the grasshopper *Podisma pedestris* (Barton & Hewitt, 1981).

Another view on speciation is the phylogenetic species concept (Cracraft, 1983). Here a species is identified as an irreducible (basal) cluster of organisms, diagnosably distinct from other such clusters, and within which there is a parental pattern of ancestry and descent. Nixon & Wheeler (1990), identify a species as the smallest aggregation of populations (sexual) or lineages (asexual) diagnosable by a unique combination of character states in comparable individuals. Baum and Donoghue, (1995), on the other hand, identify a species as a basal group of organisms, whose genes all coalesce more recently with each other than with those of any organisms outside the group.

As isolated species such as those on oceanic islands often display more disparate morphological differences than related species on continents and as speciation on isolated islands often occurs very rapidly, different theories have been developed to explain how speciation has come about on these islands. One theory proposes that as an island is colonized by only a few founder individuals, they could experience large changes in their allele frequencies from random genetic drift. This may then explain rapid changes in morphology leading to reproductive isolation (Mayr, 1963; Carson & Templeton, 1984). Another theory proposes adaptive radiation. This is when a recent ancestor undergoes speciation and phenotypic adaptation which gives rise to an array of species exhibiting different morphological and physiological traits with which they can exploit a range of divergent environments (Barton & Charlesworth, 1984).

The Prince Edward Island Archipelago (PEIA) consists of two volcanic islands, Marion Island (MI) and Prince Edward Island (PEI), that lie approximately 1770 km south-east of Port Elizabeth, South Africa, the closest point to any continent (Hänel & Chown, 1999a). Together with Crozet, Kerguelen, Heard and MacDonald Islands, these islands form the South Indian Ocean Province (SIP). Marion Island (MI), the larger (290 km^2) and more south-westerly (46 54' S; 37 45' E) of the two islands is separated from the smaller (42 km^2) PEI (46 38' S; 37 57' E) by 19 km. While MI rises to a height of 1,230 m above sea level

(a.s.l.), PEI has a maximum elevation of 672 m (Verwoerd, 1971). Both islands are estimated to be approximately 500,000 years old (McDougall *et al*., 2001; Boelhouwers, 2008). The geographic isolation of the PEIA along with the strict control on human activities on these islands (PEIMPWG, 1996; Davies *et al*., 2007) make them an ideal natural laboratory that has been exploited for scientific research since the annexation of the islands by South Africa in 1948 (See Hänel & Chown 1999b and Chown & Froneman 2008 for more information).

The PEIA is especially well-situated to study climate change. The sea surface temperature around MI has increased by 1.4° C between 1949 and 1999 (Mélice *et al*., 2003) and the air temperature has increased by 1.2° C over the same period (Smith, 2002; le Roux & McGeoch, 2008). Daily maximum and minimum temperatures have also increased at a similar rate as mean temperature increases (le Roux & McGeoch, 2008). It has been suggested that MI may be a true sentinel for future changes in climate (Bergstorm & Chown, 1999). The small size of the PEIA provides an opportunity to study the effects of climate change on terrestrial and oceanic life. It has been noted, for instance, that the contribution of Antarctic zooplankton species in the region of the PEIA has decreased by some 20 % while the contribution of sub-tropical species has increased from 6 % to 26 % over the past two decades (Pakhomov *et al*., 2001).

Climatic changes also result in changes in interactions between indigenous and invasive species. Bergstorm & Chown (1999) have indicated that warmer climates increase the ease with which invasive alien organisms can become established on the PEIA. Although it was shown that most indigenous species on the PEIA are likely to survive an increase in temperature of several degrees (Slabber, 2005), these temperature increases will favour invasive species that are less tolerant of low temperatures than indigenous species (Slabber *et al*., 2007). Warmer climates will therefore, not only increase the ease with which introduced alien organisms can become established on the PEIA, but will also allow already established invasive species to expand their distributional ranges and to aggravate their effects on local species and ecosystems (Pakhomov & Chown, 2003).

A good example of an interaction between indigenous and invasive species is that between the invasive feral house mouse, *Mus musculus domesticus* (Jansen van Vuuren & Chown, 2007) and the weevils of the *Ectemnorhinus* group of genera (Kuschel & Chown, 1995). *Mus m. domesticus* was introduced to MI by sealers in the early 1800s (Hänel & Chown, 1999a), while PEI has remained mice-free. The introduced *Mus* feed on a variety of plants and invertebrates on MI, and especially on *Ectemnorhinus* weevils (Gleeson and van Rensburg, 1982; Smith *et al*., 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, leading 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 the body size of weevil species on MI relative to PEI, due to size-selective mice predation, as the body size frequency distributions of the *Ectemnorhinus* species differ considerably between the two islands (Chown and Smith, 1993).

The group of flightless weevils (Curculionidae: Coleoptera: Hexapoda) belonging to the *Ectemnorhinus* group of genera (Kuschel & Chown, 1995) or the tribe Ectemnorhinini (Alonso-Zarazaga & Lyal, 1999) is restricted to islands of the sub-Antarctic South Indian Ocean Province. This group of weevils comprises six genera and 36 flightless species (Kuschel & Chown, 1995), of which six occur on the Prince Edward Islands Archipelago. Of these six species, four, namely *Ectemnorhinus similis* (Waterhouse, 1885), *E. marioni* (Jeannel, 1940), *Bothrometopus parvulus* (Waterhouse, 1885) and *B. elongatus* (Jeannel, 1953), are endemic to the archipelago, with *B. randi* (Jeannel 1953) also being found on the Crozet archipelago to the east, and *Palirhoeus eatoni* (C.O. Waterhouse 1876) occurring on all SIP islands (Chown & Klok, 2001; Kuschel & Chown, 1995). Although the PEIA species were all described from MI, it has long been accepted that all also occur on PEI (Chown, 1992; Chown, 1994; Chown *et al*., 1998).

The *Ectemnorhinus* group of genera (Kuschel & Chown, 1995) has proven to be a taxonomically difficult group (Brown, 1964; Kuschel, 1970; Dreux & Voisin, 1989; Chown, 1991). Chown (1990) argued that the endemic *E. marioni* and *E. similis* are two morphologically similar, but ecologically distinct species. *Ectemnorhinus marioni* individuals are smaller in body size (3.77 mm – 7.79 mm; median: 5.53 mm) and feed on bryophytes while *E. similis* individuals are larger (4.51 mm – 8.69 mm, median: 6.44 mm) and feed mainly on angiosperms although bryophytes and 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). It has been suggested that *E. marioni* and *E. similis*, with their island wide distribution, 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). Endemic *B. parvulus* predominantly occurs in epilithic moss cushions from coastal

rock faces to high altitude fellfield and polar desert. The larvae and adults feed on algae, lichens, and bryophytes (Chown, 1989; 1992). Individuals may occur occasionally on *Azorella selago*, but feed on epiphytic algae and bryophytes, rather than on the plant itself (Chown, 1989; 1992). On both Marion and Prince Edward Islands, *B. parvulus* shows distinct variation in body size, associated both with elevation and habitat type, although differences among the two islands are not pronounced. *Bothrometopus parvulus* individuals are characterised by a compressed and carinate humeral area (Chown, 1992; Chown & Smith, 1993; Chown & Klok, 2003). *Bothrometopus elongatus* is the smallest weevil species present on Marion and Prince Edward Islands and adults are readily distinguished from other species based on the presence of long transberse hairs on the elytra and pronotum. Adults feed on lichen and epilitic moss and are restricted to the central highlands between 300 and 1000 m a.s.l. (Crafford *et al*., 1986; Chown, 1992). The largest weevil species on Marion and Prince Edward Islands, *B. randi*, occurs from sea level up to 1000 m a.s.l. on lichen-covered rocks. Adults and larvae feed on algae and lichen. Adults are distinguished from other species by a distinctive green elytra scale pattern (Crafford *et al*., 1986; Chown, 1992). *Palirhoeus eatoni* feed on marine algae and are restricted to the upper- to supra-littoral zone where they are regularly inundated and exposed to sea spray. Adults are distinguished from other species by the presence of a tarsal claw segment that is longer than the first three tarsal segments (Crafford *et al.*, 1986; Chown, 1992).

Studying the phylogenetics and biogeography of the different species within the *Ectemnorhinus* group of genera (Kuschel & Chown, 1995) will present unique opportunities to investigate the evolutionary history of the group as well as theories underpinning evolution. When comparing the known geological data with the biogeographical data, this will also provide us with insights into the history and colonization of the islands.

Relevance of this study

Until the present study, all studies of the *Ectemnorhinus* group of genera (Kuschel & Chown, 1995) from the PEIA relied primarily on morphology. No studies had been conducted to determine whether the morphological species classifications concur with results from molecular-based studies. It is also important to understand the within- and between-island biogeography of the species in order to maximise the conservation of the *Ectemnorhinus* group of genera. The *Ectemnorhinus* group of genera (Kuschel & Chown, 1995) is a preferred prey species of the invasive feral house mouse (Gleeson and van Rensburg, 1982; Smith *et al.* 2002) and decisions need to be made on how to effectively

protect endemic species from predation by invasive aliens. By protecting the *Ectemnorhinus* weevils from annihilation by invasive predators, the effect of climate change can be studied on these insects that might also provide valuable insights on how climate change may influence other ecosystems in the future. The Prince Edward Islands Management Plan (PEIMPWG, 1996), under which the PEIA is environmentally managed, is currently under revision (Chown *et al.*, 2006). However, there is always a critical need to improve this management plan (see de Villiers & Cooper, 2008).

The rationale of this study is thus to improve our knowledge on the species dynamics and biogeography of the *Ectemnorhinus* group of genera (Kuschel & Chown, 1995) in order to make scientifically based informed decisions on how to conserve this unique group of weevil species. The aims of this study were thus to resolve phylogenetic relationships of the *Ectemnorhinus*-group of taxa and to use historic population dynamics in order to elucidate phylogeograpic patterns. This information will serve to guide informed conservation recommendations.

Key research questions

The key research questions that will be addressed in the present study include:

***Chapter 2* - Molecular and morphometric assessment of the taxonomic status of *Ectemnorhinus* weevil species (Coleoptera: Curculionidae, Entiminae) from the sub-Antarctic Prince Edward Islands.**

Key research question:

Q1: What is the current taxonomic status of the *Ectemnorhinus* weevil species occurring on the Prince Edward Islands?

***Chapter 3* – The population dynamics of the *Ectemnorhinus* weevils from the Prince Edward Island Archipelago.**

Key research questions:

Q1: It is possible, with the use of molecular techniques, to distinguish between the different scenarios proposed in Chapter 2, for:

(i) The existence of a single *Ectemnorhinus* species on MI that is best explained by:

a) There were originally two species of *Ectemnorhinus* present on MI but one was lost, possibly through size selective predation by mice (Chown and Smith 1993; Smith et al. 2002) and rapid climatic changes (Smith, 2002).

or

b) The extreme morphological variation observed for *Ectemnorhinus* on MI was wrongly interpreted as indicating the presence of two species.

versus

(ii) two *Ectemnorhinus* species on PEI which were proposed to have arisin due either to:

a) Weevils on PEI having had longer exposure to vascular plants as an additional, more nutritious food source to bryophytes than those on MI, leading to divergence in sympatry according to the model of Rice (1984), resulting in two species: a smaller one with a preference for bryophytes and a larger one with a preference for angiosperms, as suggested by Chown (1990).

or

b) The weevils on PEI and MI diverged from each other allopatrically resulting in a MI *Ectemnorhinus* species and a PEI *Ectemnorhinus* species, with the subsequent colonization of PEI by the MI *Ectemnorhinus* species explaining the presence of two species on PEI.

Q2: How does the genetic history of the *Ectemnorhinus* weevils present on MI compare with the island's geological history?

***Chapter 4* – Cryptic species, phylogenetic complexity and the evolutionary history of the *Ectemnorhinus*-group in the sub-Antarctic, including a description of *Bothrometopus huntleyi n. sp*.**

Key research question:

Q1: What is the phylogenetic relationships among species from the genera *Palirhoeus, Bothrometopus* and *Ectemnorhinus* based on the material available for the group from Heard Island in the East through to the Prince Edward Islands in the west?

***Chapter 5* – Inter-island dispersal of flightless *Bothrometopus huntleyi* (Coleoptera: Curculionidae) from the sub-Antarctic Prince Edward Island Archipelago.**

Key research questions:

Q1: Are there differences among populations of the newly described *B. huntleyi* on MI and PEI?

Q2: Are there one or more species present, as has been recorded for the genus *Ectemnorhinus* on the islands (Grobler *et al*. 2006)?

References

Alonso-Zarazaga, M.A. & Lyal, C.H.C. 1999. *A World Catalogue of families and genera of Curculionoidea (Insecta: Coleoptera) excluding (Scolytidae and Platypodidae).* Entomopraxis: Barcelona, 315pp.

Barton, N.H. & Charlesworth, B. 1984. Genetic revolutions, founder effects, and speciation. *Annual Reviews of Ecology and Systematics* **15**, 133-164.

Barton, N.H. & Hewitt, G.M. 1981. The genetic basis of hybrid inviability between two chromosomal races of the grasshopper *Podisma pedestris*. *Heredity* **47**: 367-383.

Baum, D.A., & Donoghue, M.J. 1995. Choosing among alternative "phylogenetic" species concepts. *Systematic Botany* **20**: 560-573.

Bergstorm, D.M. & Chown, S.L. 1999. Life at the front: history, ecology, and change on Southern Ocean islands. *Trends in Ecology & Evolution* **14**: 472-477.

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.

Boughman, J.W. 2001. Evolutionary biology: Seeing red in speciation. *Nature* **411**: 944-948.

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

Carson, H.L. & Templeton A.R. 1984. Genetic revolutions in relation to speciation phenomena: the founding of new populations. *Annual Reviews of Ecology and Systematics* **15**:97-131.

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

Chown, S.L. 1990. Speciation in the sub-Antartica weevil genus *Dusmoecetes* Jeannel (Coleoptera Curculionidae). *Systematic Entomology* **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. 1992. A preliminary analysis of weevil assemblages in the sub-Antarctic: local and regional patterns. *Journal of Biogeography* **19**: 87-98.

Chown, S.L. 1994. Historical ecology of sub-Antarctic weevils: Patterns and processes on isolated islands. *Journal of Natural History*, **28**: 411-433.

Chown, S.L., Davies, S.J. & Joubert, L. 2006. Draft Prince Edward Islands Environmental Management Plan Version 0.1. DST-NRF Centre of Excellence for Invasion Biology, University of Stellenbosch, Stellenbosch, South Africa.

Chown, S.L., Gremmen, N.J.M. & Gaston, K.J. 1998. Ecological biogeography of southern ocean islands: Species area relationships, human impacts, and conservation. *American Naturalist* **152**: 562-575.

Chown, S.L. & Froneman, P.W. 2008. *eds. The Prince Edward Islands. Land-Sea Interactions in a Changing Ecosystem*. African Sun Media, Stellenbosch.

Chown, S.L. & Klok, C.J. 2001. First record of *Palirhoeus eatoni* (Coleoptera: Curculionidae) from sub-Antarctic Heard Island. *African Entomology* **9**: 193-194.

Chown, S.L. & Klok, C.J. 2003. Altitudinal body size clines: Latitudinal effects associated with changing seasonality. *Ecography* **26**: 445-455.

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.

Cracraft, J. 1983. Species concepts and speciation analysis. *Current Ornithology* **1**:159-187.

Crafford, J.E. & Chown, S.L. 1991. Comparative nutritional ecology of bryophyte and angiosperm feeders in a sub-Antarctic weevil species complex (Coleoptera: Curculionidae). *Ecological Entomology* **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. *South African Journal of Antarctic Research* **16**: 42-84.

Coyne, J.A. & Orr, H.A. 2004. *Speciation.* Sinauer Associates, Sunderland, MA.

Darwin, C. 1859. *On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life*, London: John Murray, retrieved 2009-01-09

Davies, S.J., Chown, S.L. & Joubert, L.J. 2007. Renewed management systems and provisions for South Africa's sub-Antarctic islands. *Papers and Proceedings of the Royal Society of Tasmania* **141**: 115-120.

De Villiers, M.S. & Cooper, J. 2008. Conservation and management. *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.

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.

Dieckmann, U. & Doebeli, M. 1999. On the origin of species by sympatric speciation. *Nature* **400**: 354-357.

Dobzhansky, T. 1937. *Genetics and the origin of species*. Columbia University Press, New York.

Doebeli, M. & Dieckmann, U. 2003. Speciation along environmental gradients. *Nature* **421**: 259-264.

Futuyma, D.J. & Mayer, G.C. 1980. Non-allopatric speciation in animals. *Systematic Zoology* **29**: 254–271.

Gleeson, J. P. & Van Rensburg, P.J.J. 1982. Feeding ecology of the house mouse *Mus musculus* on Marion Island. *South African Journal of Antarctic Research* **12**: 34-39.

Hänel, C. & Chown, S.L. 1999a. *An introductory guide to the Marion and Prince Edward Island Special Nature Reserves. Fifty years after annexation.* Department of Environmental Afairs and Tourism, Pretoria.

Hänel, C. & Chown, S.L. 1999b. Fifty years at Marion and Prince Edward Islands: A bibliography of scientific and popular literature. *South African Journal of Science* **95**: 87-112.

Jansen van Vuuren, B.J. & Chown, S.L. 2007. Genetic evidence confirms the origin of the house mouse on sub-Antarctic Marion Island. *Polar Biology* **30**: 327-332.

Jeannel, R. 1940. Coléoptères. Croisière du Bougainville aux îles australes francaises. *Mémoires du Muséum National d'Histoire Naturelle (N.S.)* **14**: 63-201.

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

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

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

Langegger, O. & Verwoerd, W.J. 1971. Topographic Survey In: Van Zinderen Bakker, E.M., Winterbottom, J.M. & R.A. Dyer. (eds) *Marion and Prince Edward Islands: Report on the South African Biological and Geological Expedition 1965-1966.* A.A. Balkeman, Cape Town, 32-39.

le Roux, P.C. & McGeoch, M.A. 2008. Changes in climate extremes, variability and signature on sub-Antarctic Marion Island. *Climatic Change* **86**: 309-329.

Li, P. & Bousquet, J. 1992. Relative-Rate test for nucleotide substitutions between two lineages. *Molecular Biology and Evolution* **9**: 1185-1189.

Mayr, E. 1942. *Systematics and the Origin of Species*. Columbia University Press, New York, NY.

Mayr, E. 1954. In *Evolution as a Process*, eds. Huxley, J., Hardy, A. C. & Ford, E. B. (Allen & Unwin, London), pp. 157–180.

Mayr, E. 1963. *Animal Species and Evolution.* Harvard University Press, Cambridge, MA.

McDougall, I., Verwoerd, W. & Chevallier, L. 2001. K-Ar geochronology of Marion Island, Southern Ocean. *Geological Magazine* **138**: 1-17.

Mélice, J.L., Lutjeharms, J.R.E., Rouault, M. & Ansorge, I.J. 2003. Sea-surface temperatures at the sub-Antarctic islands Marion and Gough during the past 50 years. *South African Journal of Science* **99**: 363-366.

Nixon, K.C. & Wheeler, Q.D. 1990. An amplification of the phylogenetic species concept. *Cladistics* **6**: 211-223.

Orr, H.A. 1991. Is single-gene speciation possible? *Evolution* **45**: 764-769.

Pakhomov, E.A., Froneman, P.W. & McQuaid, C.D. 2001. *The Prince Edward Islands live support system and variability of living resources in the Southern Ocean*. Final report submitted to South African Committee on Antarctic Reasearch.

Pakhomov, E.A. & Chown, S.L. 2003. The Prince Edward Islands: Southern Ocean oasis. *Ocean Yearbook* **17**: 348-379.

PEIMPWG (Prince Edward Islands Management Plan Working Group) 1996. *Prince Edward Islands Management Plan*. Department of Environmental Affairs & Tourism, Pretoria, pp. 1-64.

Provine, W.B. 1989. In *Genetics, Speciation, and the Founder Principle*, eds. Giddings, L. V., Kaneshiro, K. Y.&Anderson, W. W. (Oxford Univ. Press, New York), pp. 43–76

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

Rychlik, W. 1993. Selection of Primers for Polymerase Chain Reaction. *Methods in Molecular Biology.* Vol. 15: PCR Protocols: *Current Methods and Applications.* Edited by: B.A. White, Humana Press Inc., Totowa, NJ, p 31-40.

Sætre, G., Moum, T., Bures, S., Kral, M., Adamjan, M. & Moreno, J. 1997. A sexually selected character displacement in flycatchers reinforces premating isolation. *Nature* **387**: 589 - 592.

Slabber, S. 2005. *Physiological plasticity in arthropods from Marion Island: indigenous and alien species.* Ph.D. Thesis, University of Stellenbosch, South Africa.

Slabber, S., Worland, M.R., Leinaas, H.P. & Chown, S.L. 2007. Acclimation effects on thermal tolerance of springtails from Sub-Antarctic Marion Island: Indigenous and invasive species. *Journal of Insect Physiology* **53**: 113-125.

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

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

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, 40-62.

Watkins, B.P. & Cooper, J. 1986. Introduction, present status and control of alien species at the Prince Edward Islands, sub-Antarctic. *South African Journal of Antarctic Research* **16**: 86-94.

Waterhouse, C.O. 1876. Description of a new species of *Ectemnorhinus* from Kerguelen Island. *Entomologist's Monthly Magazine* **13**: 51-52.

Waterhouse, C.O. 1885. Description of two new Curculionidae (*Ectemnorhinus*) from Marion Islands. *Annals and Magazine of Natural History, London* **16**: 121-123.

In order to conserve, we need to know what we are conserving.

In order to make decisions we need to know what we are deciding about.

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)

The results presented here have been published in the *Journal of Zoological Systematics and Evolutionary Research* **44**, 200-211 (2006).

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 vestiture 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.

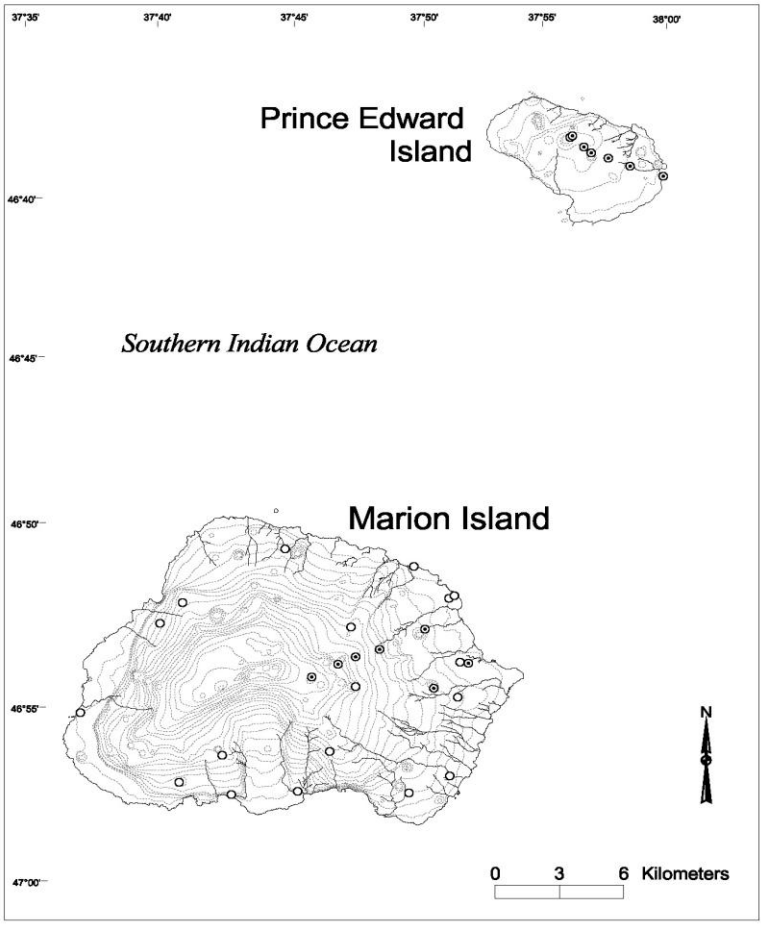


Fig. 1. Map indicating *Ectemnorhinus* weevil sampling localities on Marion Island and Prince Edward Island that correspond to the coordinates summarised in Table 1. Samples collected from all localities were included in the morphometric analyses while only those samples collected from the dotted localities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

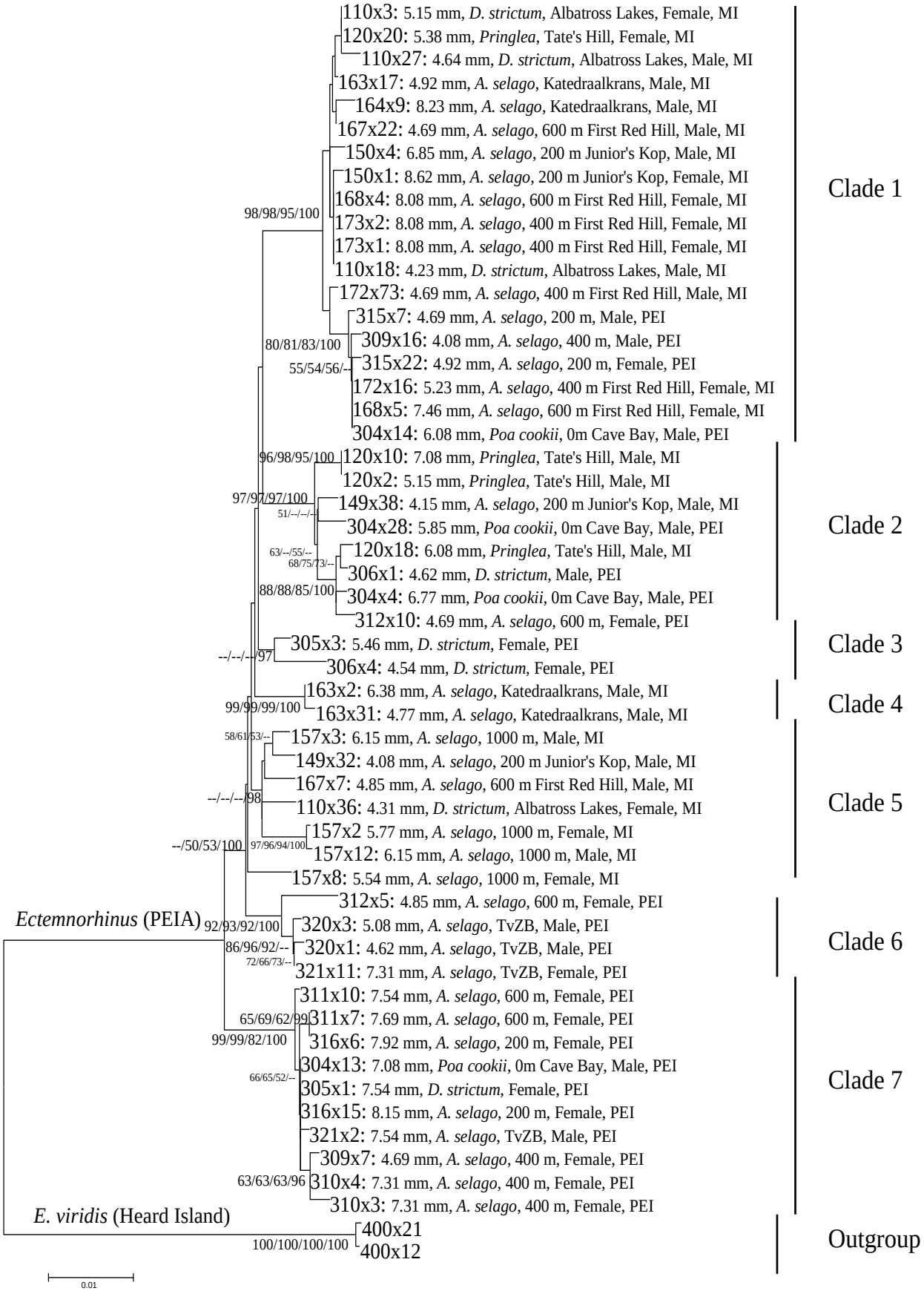
Molecular analyses

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. (Legend for Figure on page 27)


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
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
305x3: 5.46 mm, D. strictum, Female, PEI
306x4: 4.54 mm, D. strictum, Female, PEI
163x2: 6.38 mm, A. selago, Katedraalkrans, Male, MI
163x31: 4.77 mm, A. selago, Katedraalkrans, Male, MI
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
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
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
400x21
400x12
Ectemnorhinus (PEIA)
E. viridis (Heard Island)
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
0.01
Clade 1
Clade 2
Clade 3
Clade 4
Clade 5
Clade 6
Clade 7
Outgroup

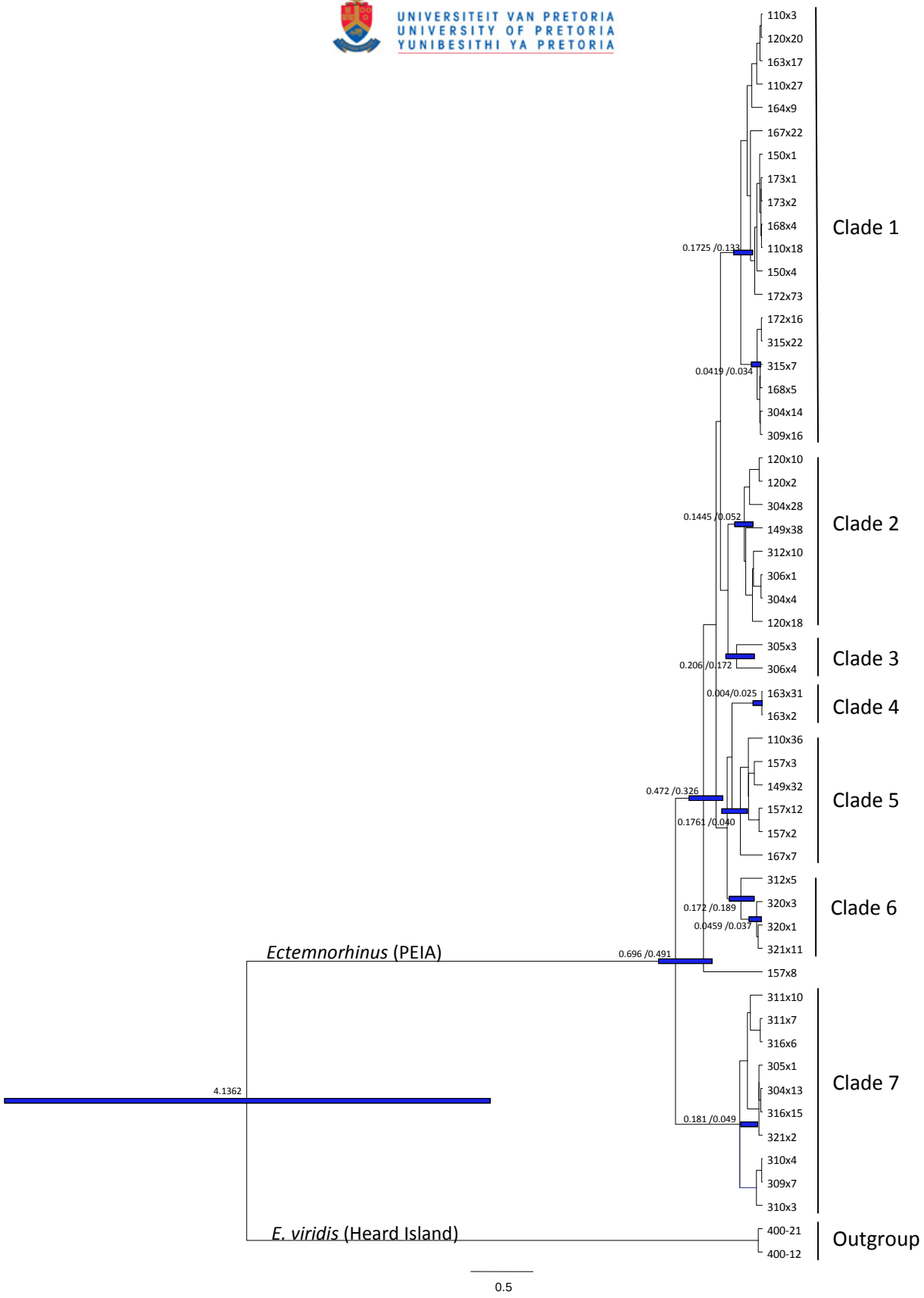


Fig. 3 Ultrametric tree obtained with BEAST. The numbers in the nodes (Beast estimates / uncorrected pairwise estimates) correspond to the estimated age in MY. Blue bars correspond to the 95 % confidence interval. A clock rate of 2.3 % sequence divergence per million years (MY) was used.

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).

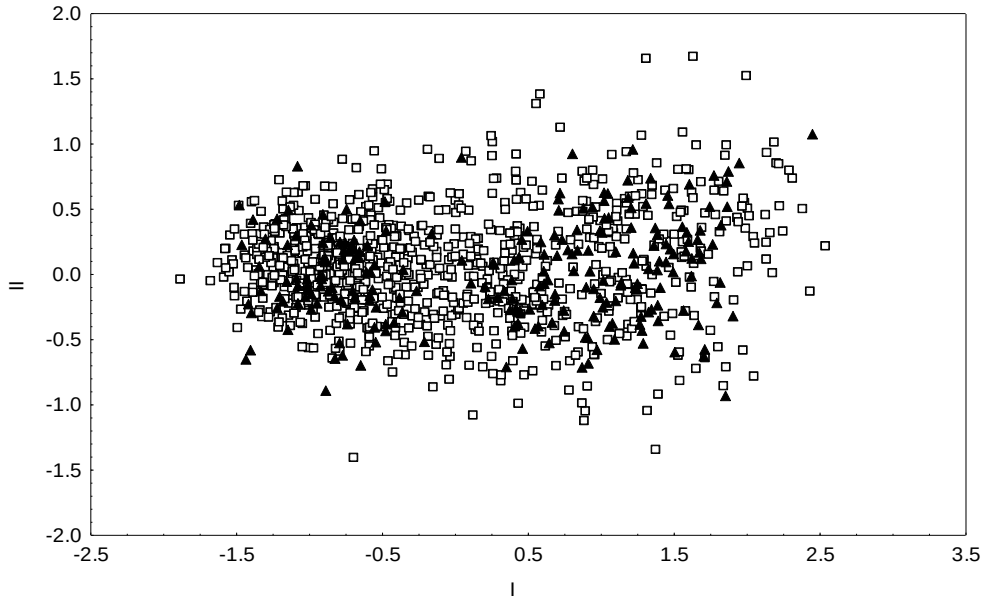


Fig. 4 Components I and II from a principal components analysis of *Ectemnorhinus* species collected from both Marion Island (open squares) and Prince Edward Island (closed triangles), indicating no distinct separation with reference to the two currently recognized *Ectemnorhinus* species nor with regard 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.

	a) Marion Island		b) Prince Edward Island		c) Marion and Prince Edward Island	
	Principal components		Principal components		Principal components	
*Variable	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 ($F_{(30,62)} = 4.34$, $P < 0.0001$).

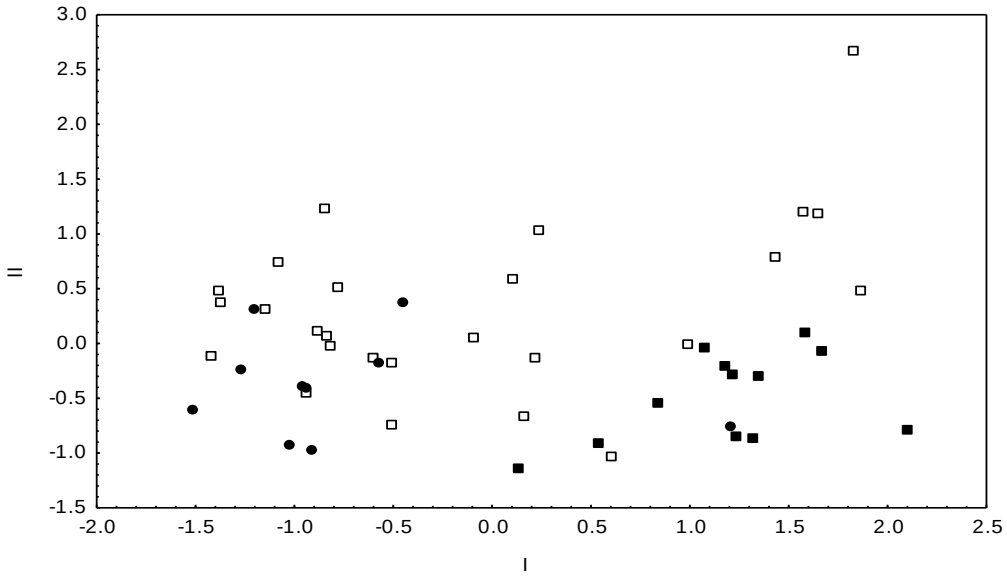


Fig. 5a Components I and II from a principal components analysis based on genetically identified individuals. No separation is observed in the Marion Island sample (open squares), while two groups are observed for samples collected on Prince Edward Island, *Ectemnorhinus* group A (closed circles; individuals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

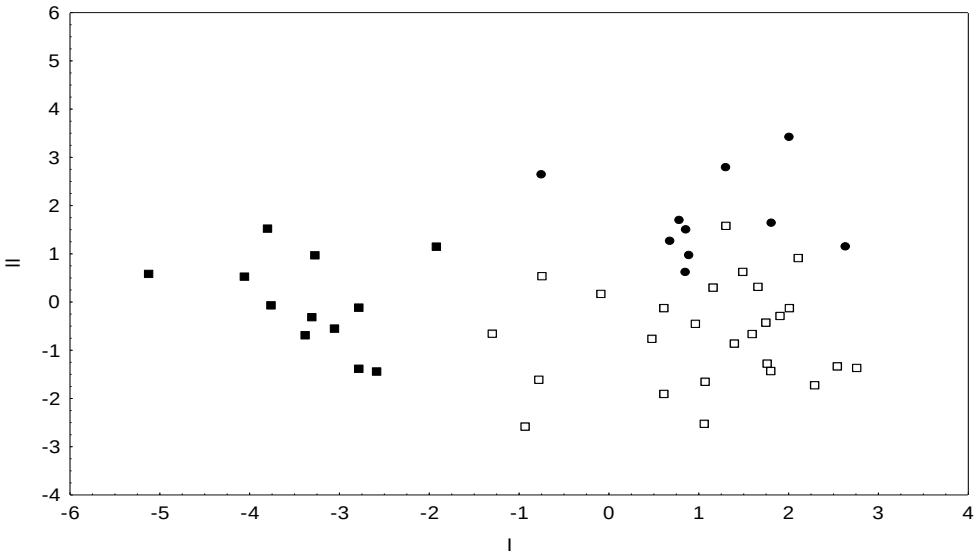


Fig. 5b Components I and II from a canonical variates analysis (CVA) showing a more pronounced separation of genetically identified individuals from Prince Edward Island, *Ectemnorhinus* group A (closed circles) and *Ectemnorhinus* group B (closed squares) with no separation in the Marion Island samples (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.

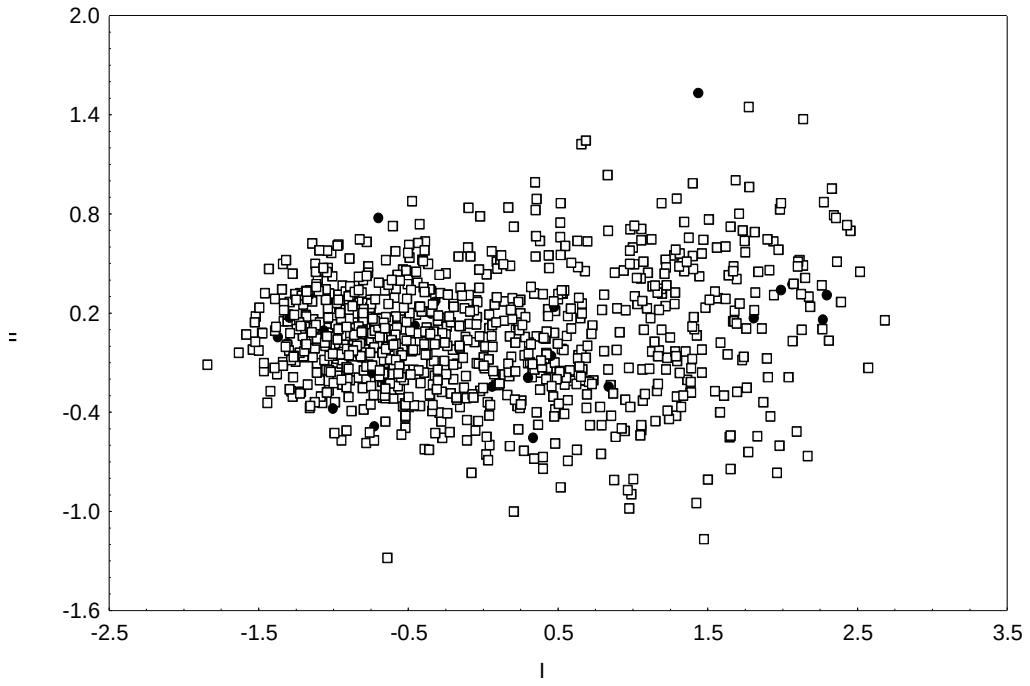


Fig. 6a Components I and II from a principal components analysis of *Ectemnorhinus* species collected from Marion Island, indicate no separation based on either size or shape variables. Genetically identified individuals are indicated by black circles.

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).

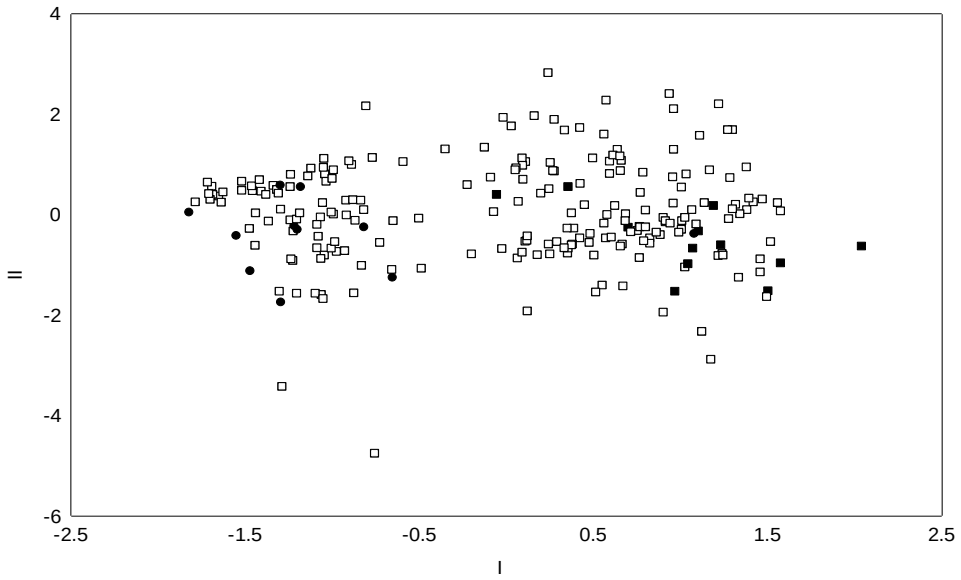


Fig. 6b Components I and II from a principal components analysis of *Ectemnorhinus* species collected from Prince Edward Island indicate separation between two group based on size variation. Genetically identified *Ectemnorhinus* group A (closed circles) and *Ectemnorhinus* group B (closed squares) are 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.

Acknowledgements

The authors wish to thank C. Lyal of the Natural History Museum for provision of the *Ectemnorhinus* lectotype image, and U. Kryger and C.H. Scholtz (University of Pretoria) for translation of the abstract and assistance with the species description, respectively. The Directorate Antarctica and Islands of the South African Department of Environmental Affairs and Tourism funded this work and provided logistic support at Marion Island via the South African National Antarctic Programme. This material is based upon work supported by the National Research Foundation South African National Antarctic Programme under Grant number GUN2068301. Any opinions, findings and conclusions or recommendations expressed in this material are those of the author(s) and therefore the NRF does not accept any liability in regard thereto.

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.

Results

Of the 187 sequences included in this study, 52 ingroup sequences as well as two outgroup sequences were generated in a previous study (Grobler *et al*., 2006; Chapter 2). The Genbank accession numbers for these previously characterized specimens are AY762267-AY762320. An additional 135 sequences were generated in this study and are deposited in Genbank under accession numbers JF327506-JF327640. Of the 187 sequences used in this study, 131 represent unique haplotypes.

Nested clade analysis

A single haplotype network (Fig. 5), based on the 95 % connection probability (≤ 13 steps), was estimated by TCS 1.13 (Clement *et al*., 2000). From the nested design presented in Fig. 6, the total cladogram can be divided into two 6-step clades, three 5-step clades, and six 4-step clades at the higher clade level. The six 4-step clades separate from each other by more than five steps, representing missing intermediate haplotypes as indicated in Fig. 7. Nested clade 4-4 which consists of *E. kucheli* (Grobler *et al*., 2006) was considered basal to the other 4-step clades because time of coalescence of these individuals preceded that of the other clades (Grobler *et al*., 2006; Chapter 2), and therefore, all higher-step clades were designated with this in mind.

The results of the nested cladistic analysis of geographical distance for the mtDNA data set from the PEIA are shown in Fig. 8. The contingency analyses (Templeton *et al*., 1995) detected significant geographical associations with the total cladogram, a 6-step clade, two 5-step clades, only one 4-step clade, and a single 1-step clade (Table 1). Significant geographical structure was thus mainly indicated for the higher clade levels. With the exception of nested clade 1-96, no significance at the 1-, 2- and 3-step levels were found. One clade of each of the 4-, 3-, and 2-step clades also had a lower significant geographical association. Table 2 presents the chain of inferences followed when the inference key was applied to the statistical results given in Fig. 7 as well as the resulting inferences obtained.

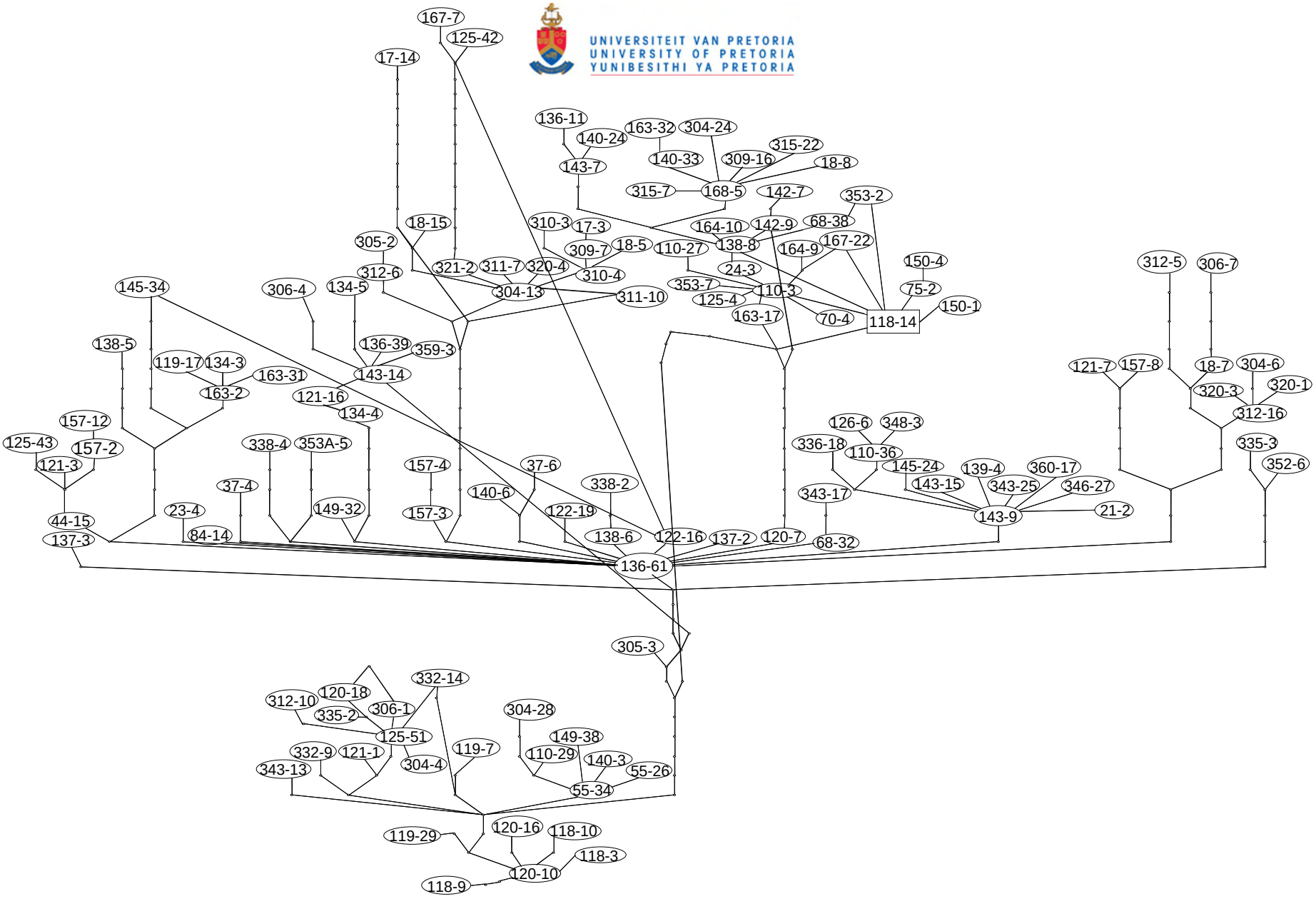


Fig. 5 Haplotype network based on 95% connection probability (≤ 13 steps) as estimated by TCS 1.13 (Clement et al., 2000).

Please find the figure on the disc in the envelope at the back

Fig. 6 Nested design according to previously described nesting rules of Templeton *et al.*, 1987 and Crandall, 1996.

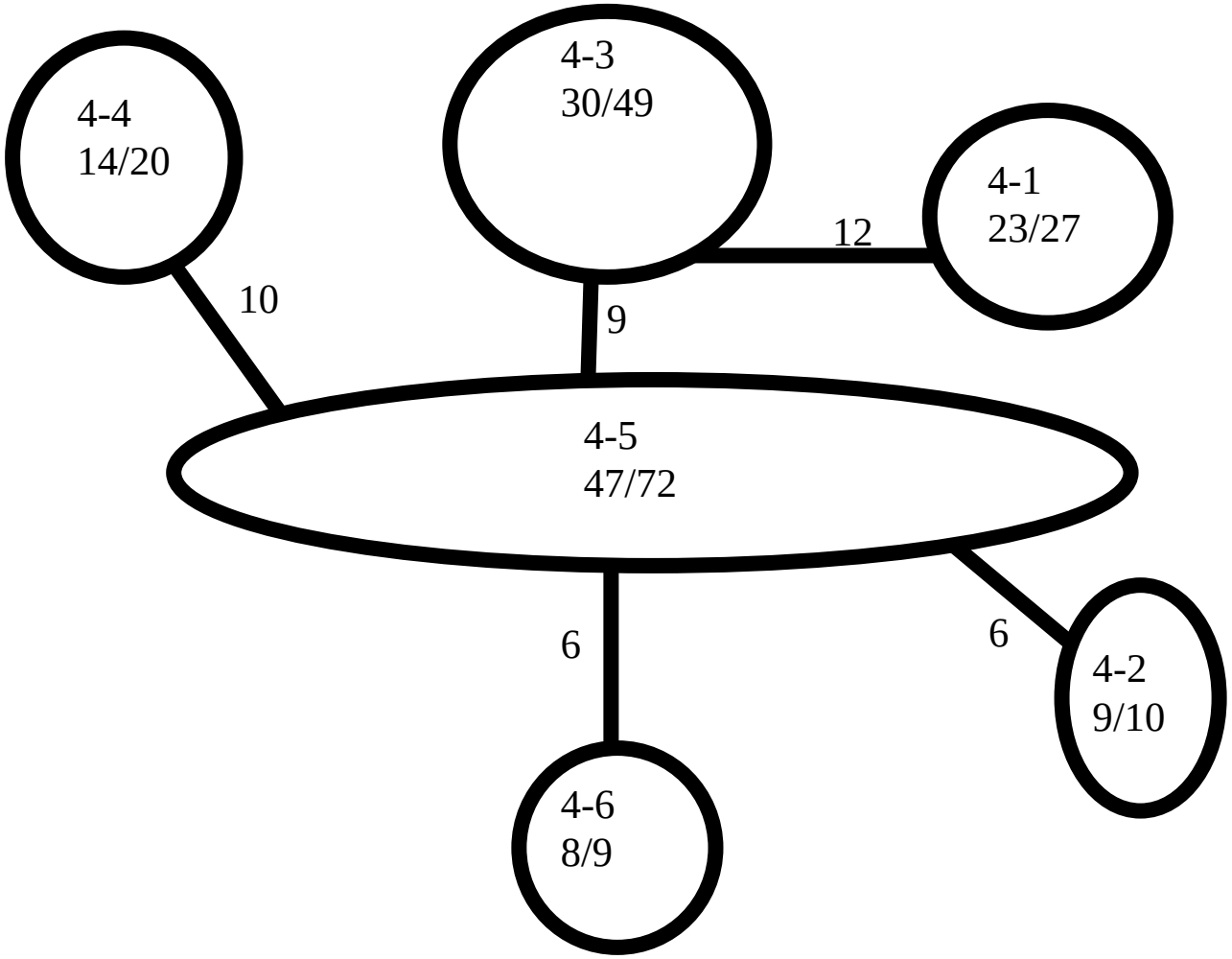


Fig. 7 Haplotype network of 187 *Ectemnorhinus* sequences. Sequences are grouped into six groups separated from each other by the number of missing haplotypes (as indicated on the lines). Within each circle is the designated name for the group followed by the number of unique haplotypes / total number of sequences / specimens characterized for each group.

Please find the figure on the disc in the envelope at the back

Fig. 8 Results of the nested clade analysis of the geographical distance for COI haplotypes of *Ectemnorhinus* weevils from the PEIA. Haplotypes (0-steps) are given at the top in bold. Interior haplotypes/clades are shaded. Higher level clade designations are indicated in bold as one moves down the figure. Boxed groupings indicate the nested structure. Following the number of any given clade is the clade (Dc) and nested clade (Dn) great circle distances. An 's' indicates that the distance is significantly small at the 5 % level and an "L" indicates that the distance is significantly large. For nested groupings that contain both tip and interior clades, the average differences of the distances between interior clades and tip clades within the nested group for clade distances, is indicated by the symbol '(T-1)c", with nested clade distances being indicated by the symbol '(T-1)n".

Table 1 Nested contingency analyses for geographical association showing the permutation chi-squared probabilities for geographical structure of the clades identified in Fig. 8 from 100 000 resamplings. Clades with a probability value less than 0.05 suggest significant geographical structure and are indicated with *. Clades with a probability value less than 0.1 are indicated with #.

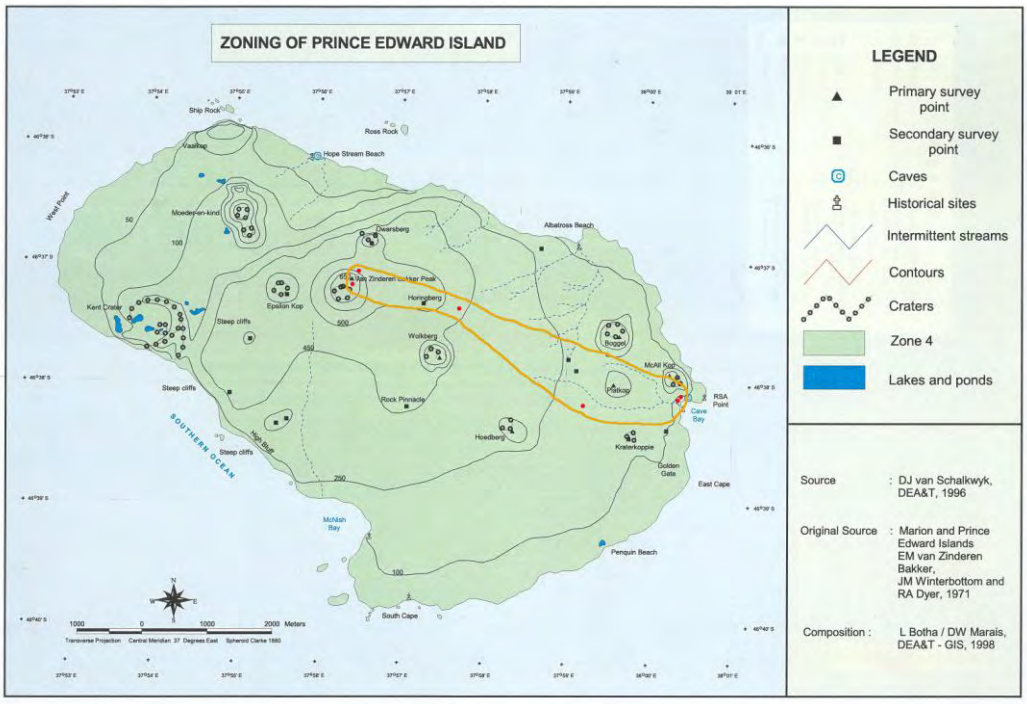
Clade	Permutational chi-square statistic	Probability
1-9	11.25	0.6860
1-12	1.3333	1.0000
1-14	15.0000	1.0000
1-15	2.0000	1.0000
1-17	2.0000	1.0000
1-18	34.0000	0.8300
1-20	3.0000	1.0000
1-34	2.0000	1.0000
1-38	0.7500	1.0000
1-43	10.0000	0.2010
1-56	2.0000	1.0000
1-60	1.3333	1.0000
1-67	10.0000	1.0000
1-71	75.4667	0.3210
1-79	2.0000	1.0000
1-80	5.9583	0.7680
1-81	10.0000	0.2900
1-82	2.0000	1.0000
1-83	34.0000	1.0000
1-85	2.0000	1.0000
1-87	3.0000	0.3220
1-89	21.3889	0.4830
1-91	5.0000	0.8370
1-92	2.0000	1.0000
1-93	3.0000	0.3340
1-96	62.2222	0.0410*
1-200	2.0000	1.0000
2-1	2.9167	0.6880
2-3	15.0000	1.0000
2-4	2.0000	1.0000
2-5	3.0000	1.0000
2-10	5.0000	0.4300
2-13	3.0000	1.0000
2-15	6.0000	0.3080
2-18	12.0000	0.0730#
2-19	4.0000	1.0000
2-20	12.0000	0.3810
2-22	17.0000	0.5780
2-23	2.0000	1.0000
2-27	10.0000	0.3380
2-29	3.2500	0.6120
2-31	18.0000	0.4910

Clade	Permutational chi-square statistic	Probability
2-33	153.3333	0.2680
2-34	30.6000	0.3640
2-35	11.2000	0.5500
2-36	4.9038	0.9120
2-37	11.0714	0.9880
3-2	1.2000	1.0000
3-3	13.3333	0.6430
3-4	19.2000	0.1400
3-6	13.9240	0.7950
3-7	35.1771	0.1100
3-8	0.6857	1.0000
3-9	29.8194	0.5160
3-10	20.0076	0.0950#
3-16	149.6400	0.4930
4-1	16.5515	0.2450
4-2	10.0000	0.1060
4-3	24.1412	0.1500
4-4	5.9649	0.8120
4-5	88.0677	0.0000*
4-6	3.9375	0.7870
5-1	100.0663	0.0020*
5-2	47.8767	0.0002*
6-1	71.2705	0.0000*
Total Cladogram	101.4970	0.0000*

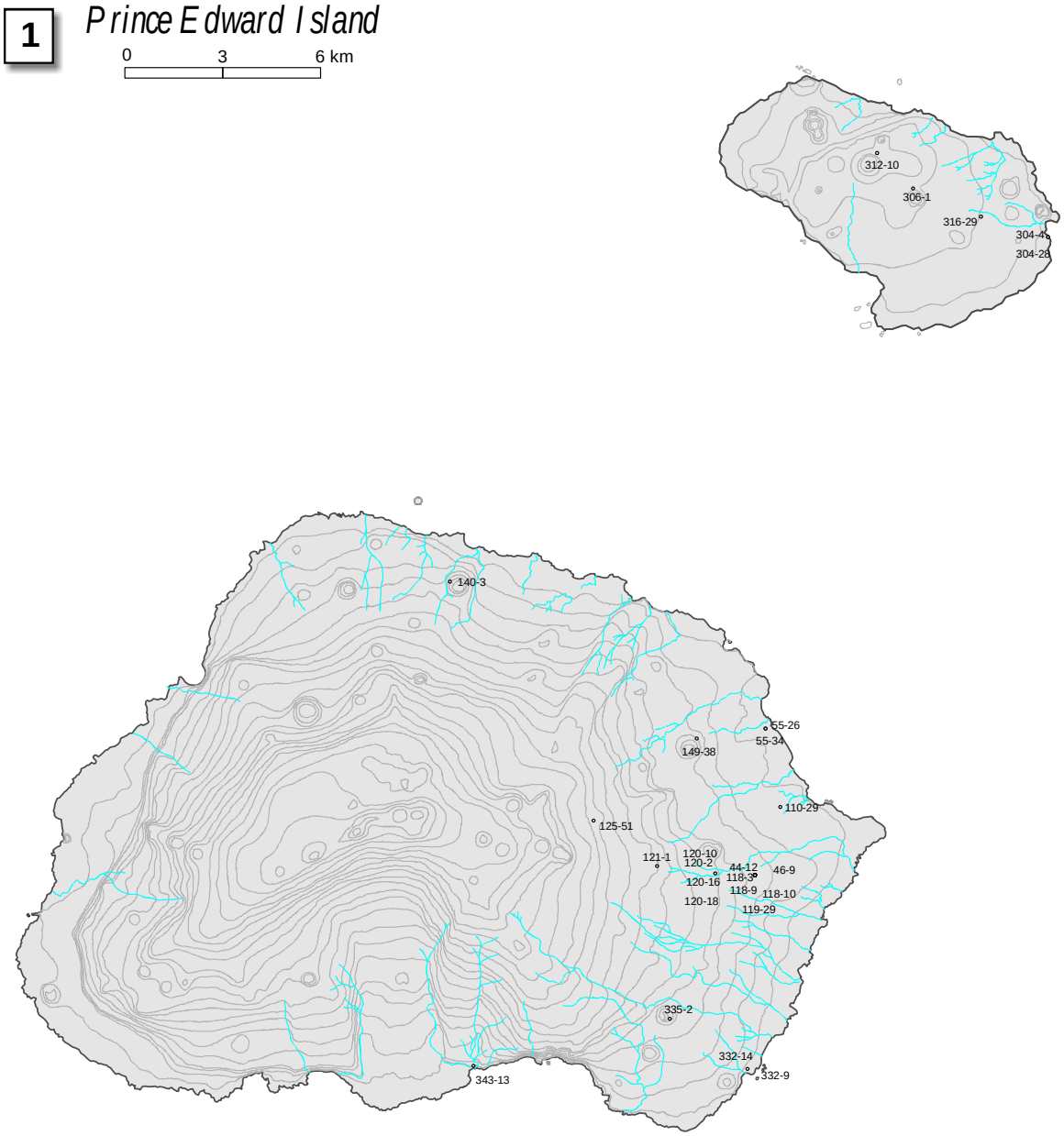
Table 2 Inference chain for the results of the *Ectemnorhinus* phylogeography as indicated by the nested clade analysis from the results given in Fig. 8 and Table 1. Only the clades that resulted in a rejection of the null hypothesis, i.e. that there are no significant relationships between genetic variation and respective geographical locations of haplotypes, are included. Clades marked with # were not significant at the 0.05 % level but were significant at the 0.1 % level.

Clade	Chain of inference	Inverence
1-96	1-2-3-5-6-7	Insufficient genetic resolution to discriminate between range expansion / colonization and restricted dispersal / gene flow. Restricted gene flow / dispersal but with some long distance dispersal.
2-18#	1- 2 – 11 - 12	Continuous range expansion
3-10#	1- 2 – 11 – 17 -	Inconclusive outcome
4-5	1-2-11-17-	Inconclusive outcome
5-1	1-2-11-12	Contiguous range expansion,
5-2	1-2-11-17-	Inconclusive outcome
6-1	1-2-11-17-4-	Restricted gene flow with isolation by distance

In order to gain further insight into the geographical structuring, clades were mapped onto the islands according to the sampling localities of the constituent haplotypes. Because of the very large dataset and the fact that the 4-step clades separate from each other by more than three missing haplotypes (Fig. 7) the nested clade diagram was partitioned into the six different 4-step clades to ensure manageable mapping and result presentation. The different maps for the individuals falling in each 4-step clade are presented in Figs. 8-13 with each being divided into six parts.



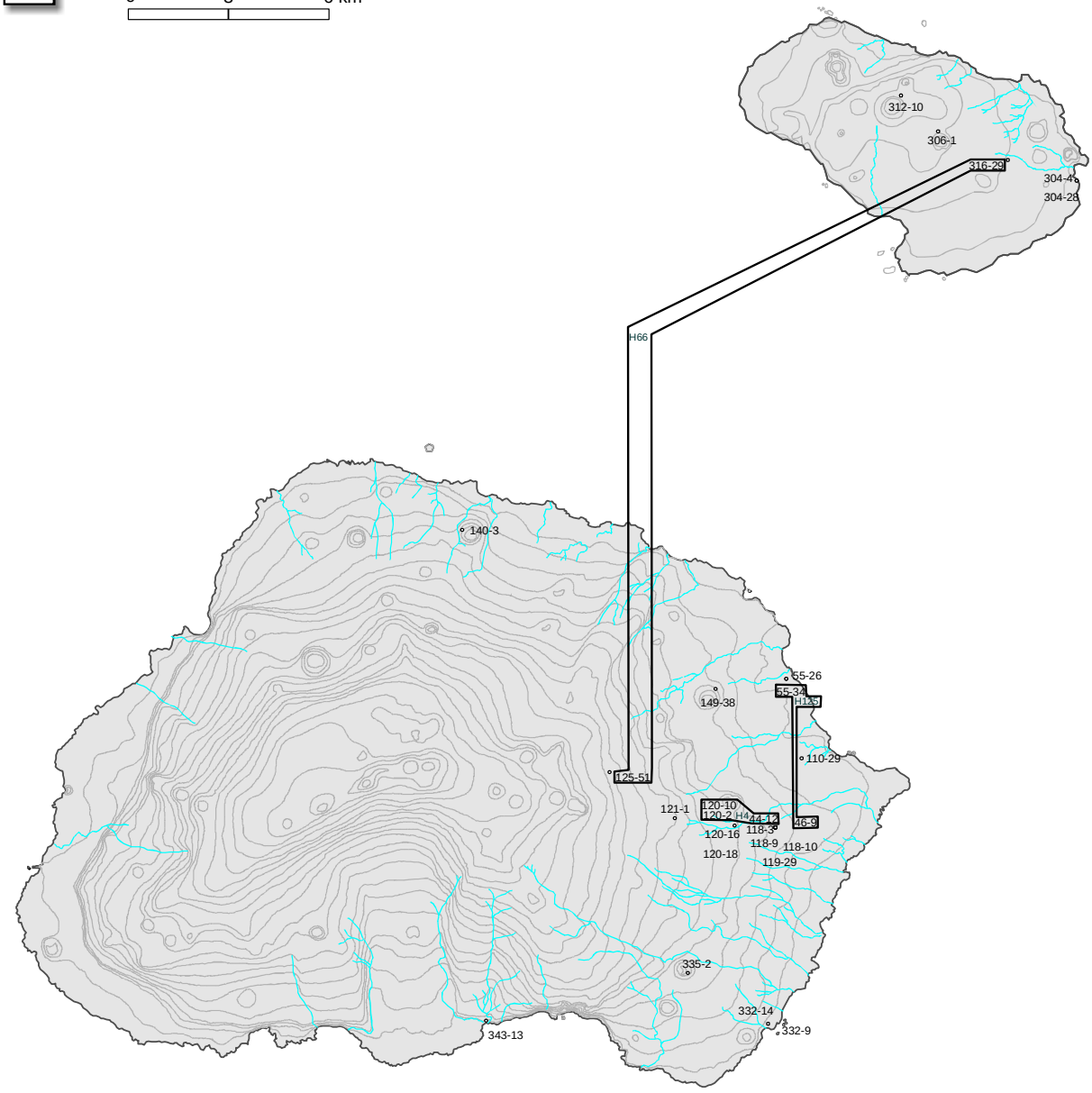
(a)


1
Prince Edward Island
0 3 6 km
312-10
306-1
316-29
304-4
304-28
N
140-3
55-26
55-34
149-38
110-29
125-51
121-1
120-10
120-2
44-12
46-9
118-3
120-16
118-9
118-10
120-18
119-29
335-2
332-14
332-9
343-13
1
Marion Island
0 3 6 km

(b)

1 *Prince Edward Island*

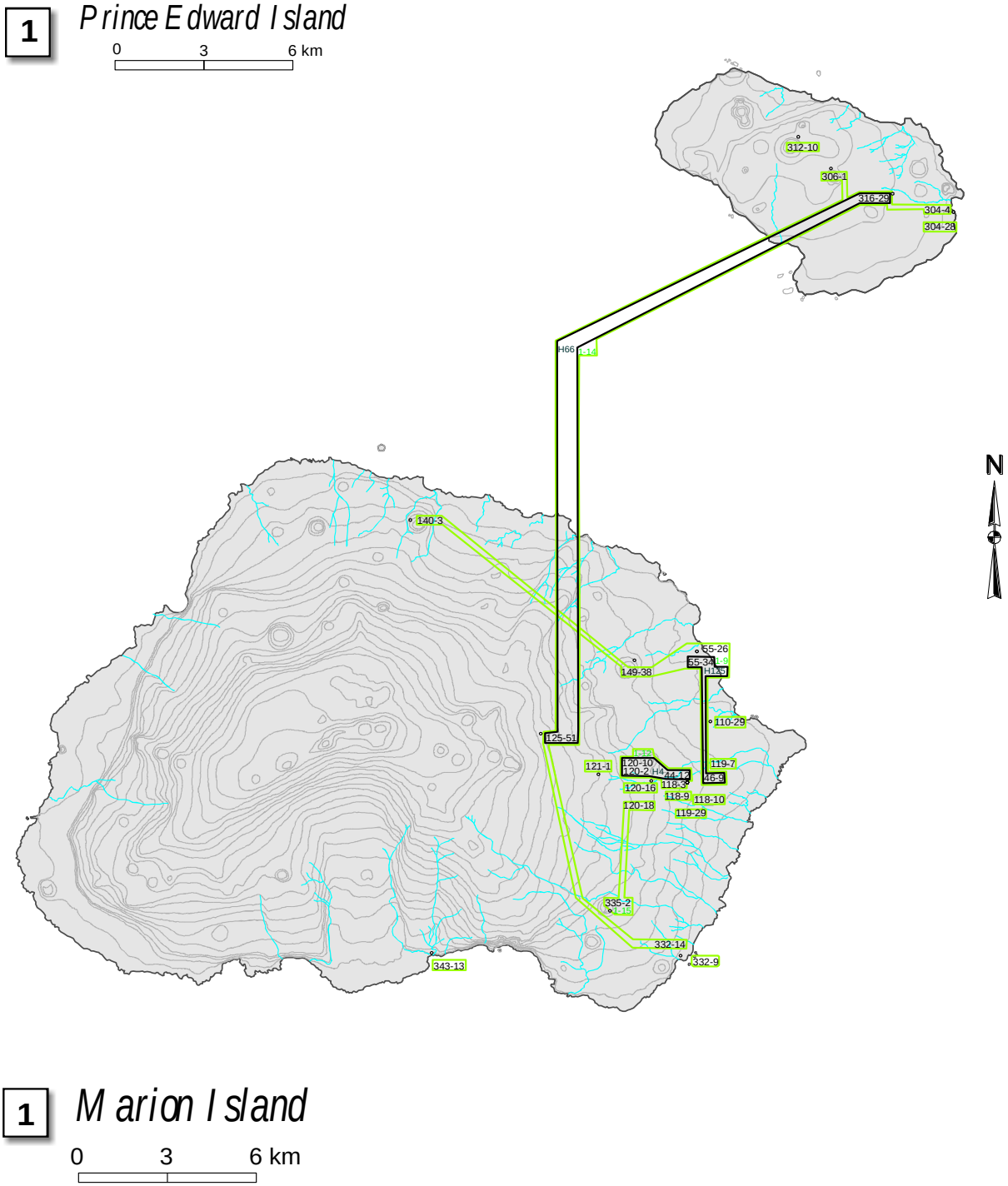
0 3 6 km



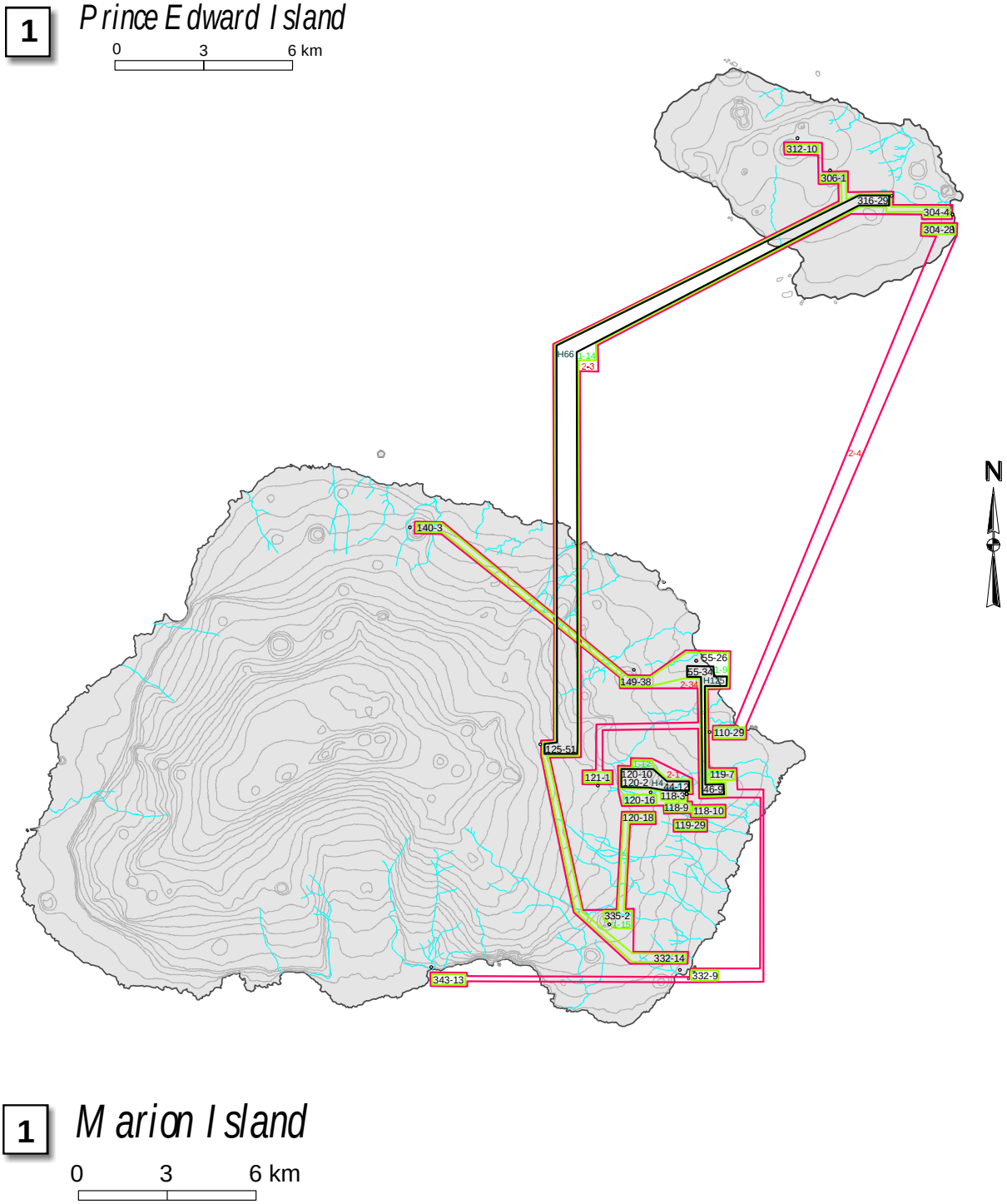
1 *Marion Island*

0 3 6 km

(c)


1
Prince Edward Island
0 3 6 km
312-10
306-1
316-29
304-4
304-28
H66
L14
140-3
149-38
55-26
55-34
L9
H15
110-29
125-51
121-1
120-10
120-2
H4
L12
44-12
46-9
119-7
118-3
120-16
118-9
118-10
120-18
119-29
335-2
L15
332-14
332-9
343-13
N
1
Marion Island
0 3 6 km

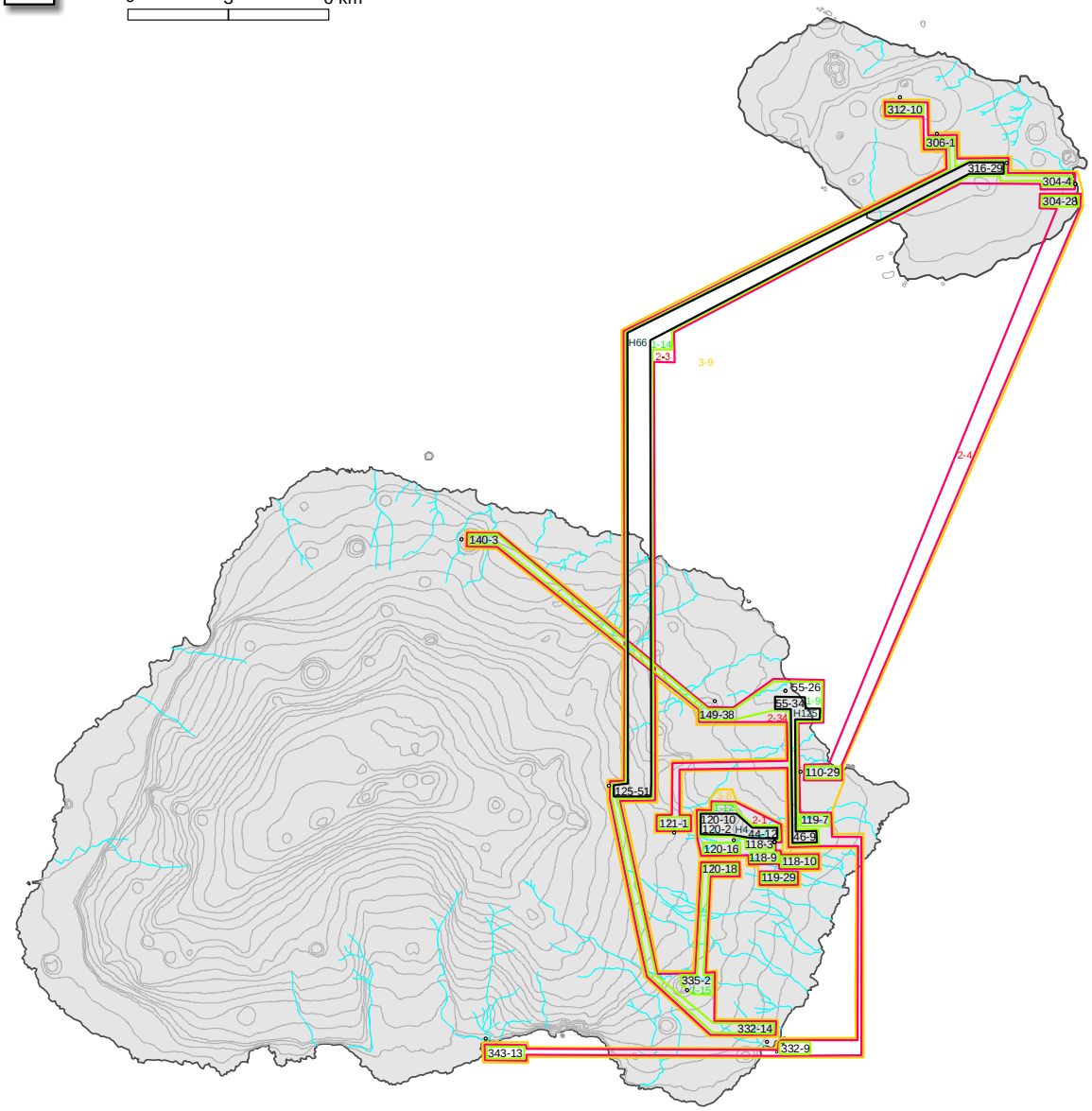
(d)


1
Prince Edward Island
0 3 6 km
312-10
306-1
316-29
304-4
304-28
H66
1-14
2-3
2-4
140-3
149-38
55-26
55-34
H55
2-24
110-29
125-51
121-1
120-10
120-2
H4
119-7
46-9
120-16
118-3
118-9
118-10
120-18
119-29
335-2
332-14
343-13
332-9
N
1
Marion Island
0 3 6 km

(e)

1 *Prince Edward Island*

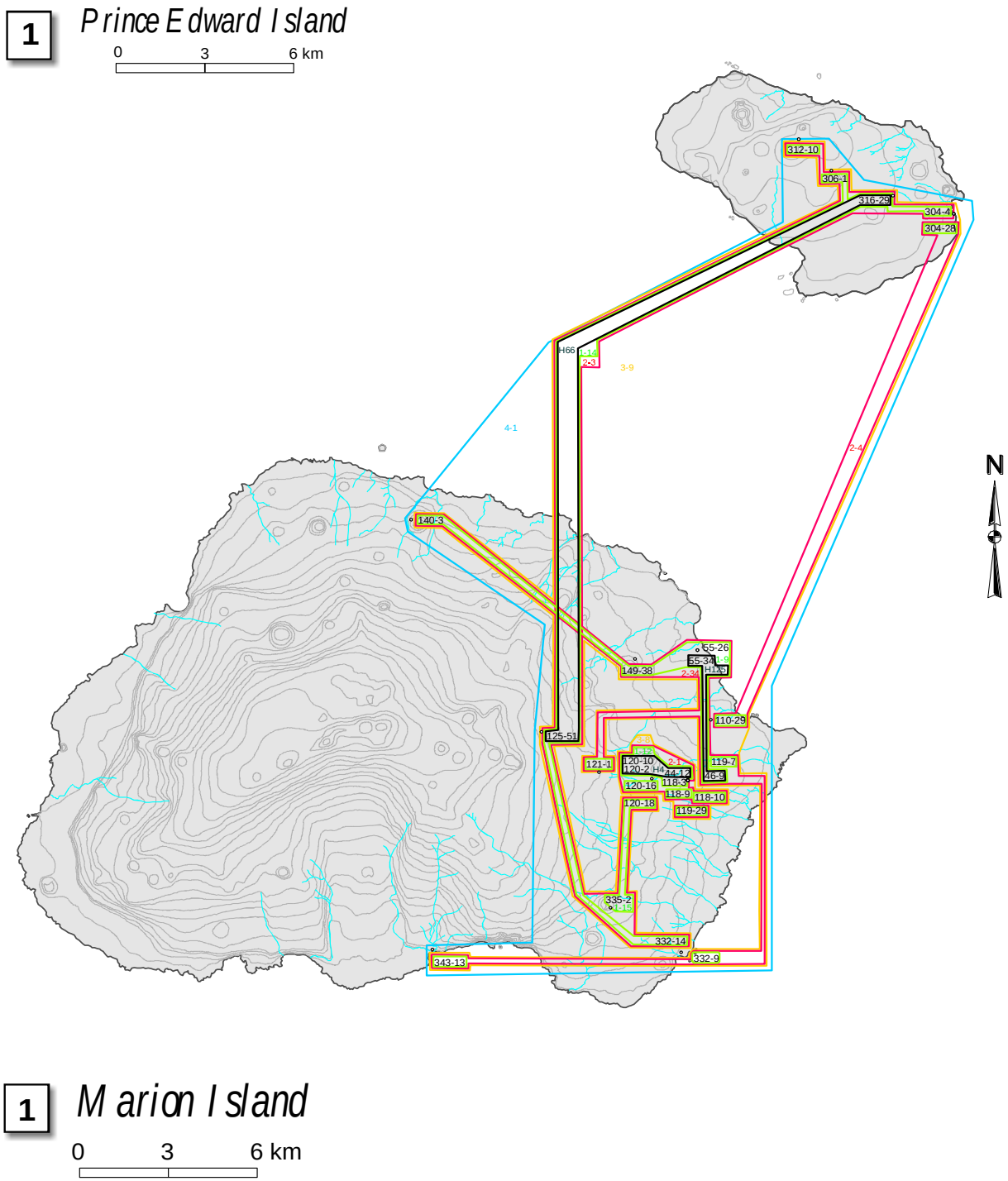
0 3 6 km



1 *Marion Island*

0 3 6 km

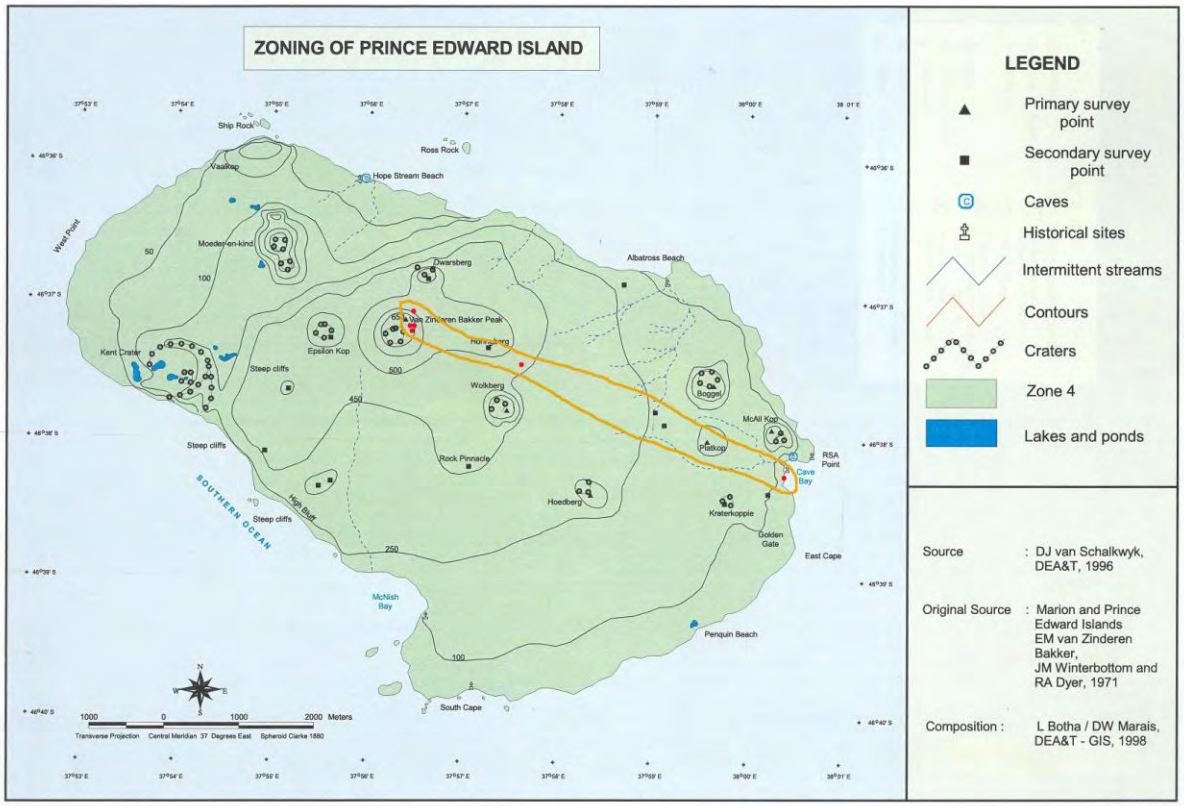
(f)



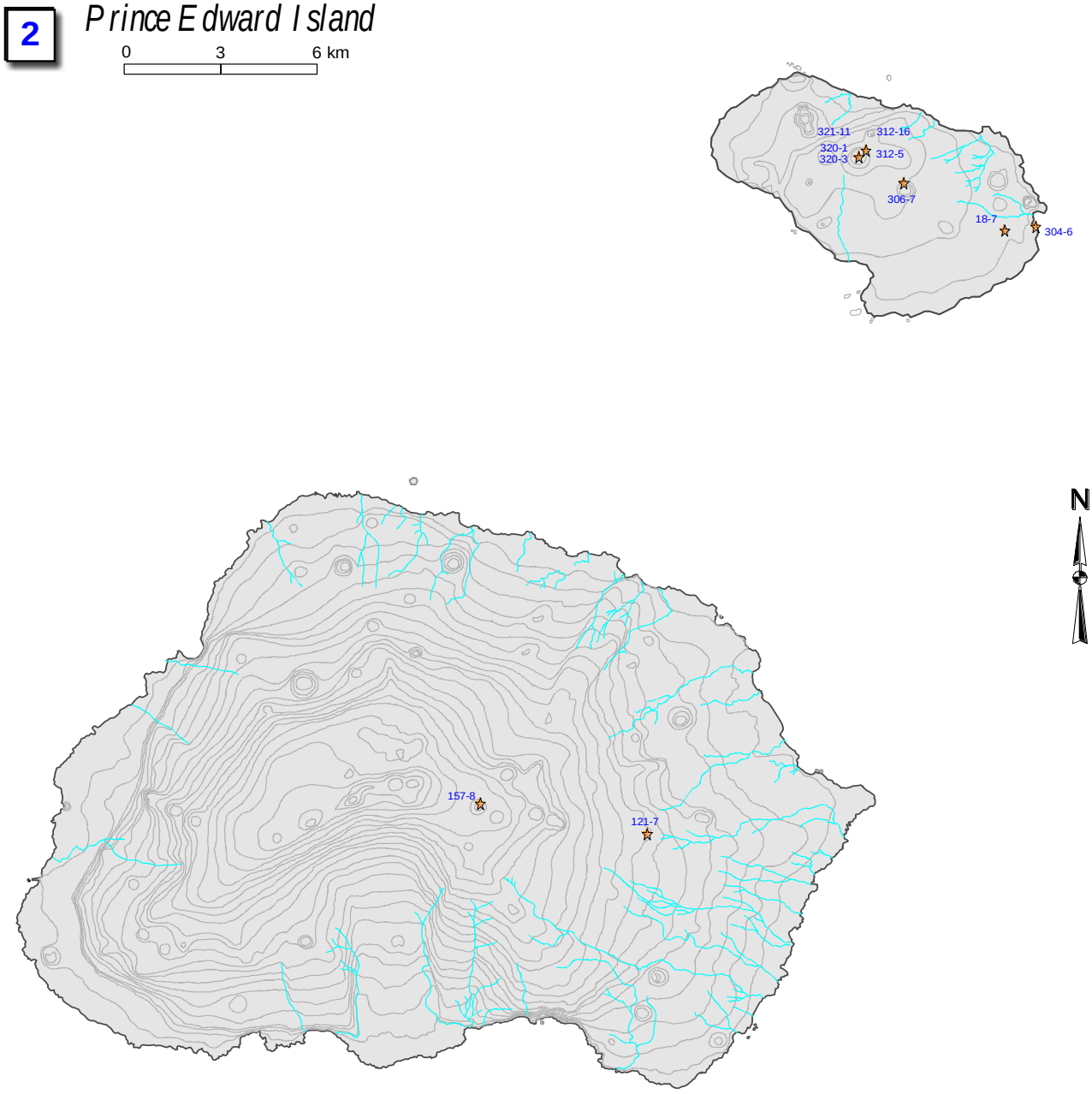
(g)

Fig. 9 *Ectemnorhinus* individuals that group into 4-step clade 4-1 mapped onto the islands according to a) sampling localities with place names for references, samples on Marion Island are indicated in green while those on Prince Edward Island are indicated in red b) sampling localities as used in the nested design c) those that share the same haplotypes nested into 0-step clades, the d) 1-step clades, e) 2-step clades f) 3 step clades and finally g) the complete 4-1 clade as depicted in Fig. 5.

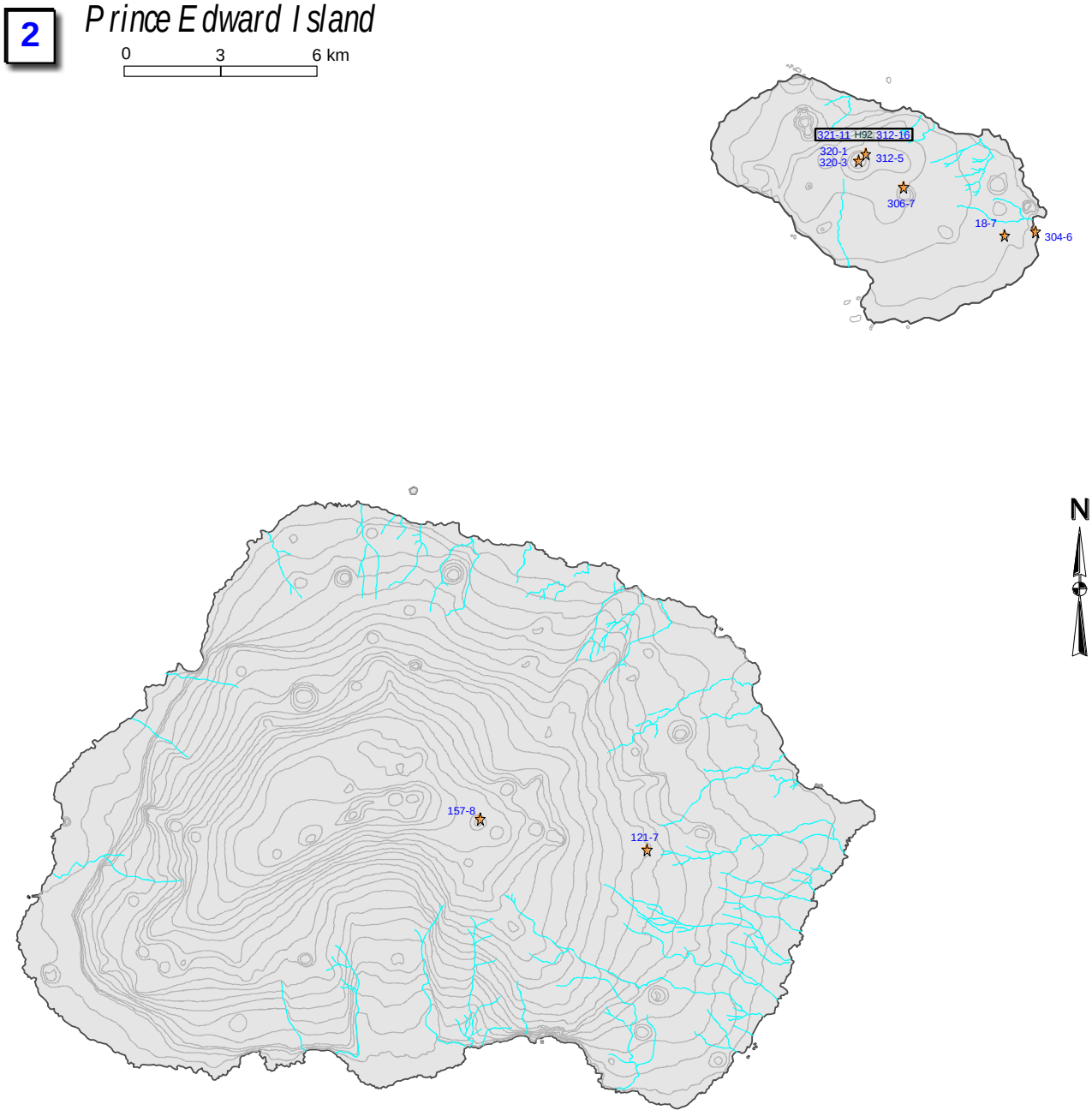

MARION ISLAND
WITH
MANAGEMENT ZONES
LEGEND
Wreck (of Soiglimt)
Historical sites
Primary survey point
Secondary survey point
Huts (zone 1)
Perennial stream
Intermittent stream
Contours
Craters
Caves
Waterfall
Pond
Zones
(Historical sites and Southern Grant Petrel and Gentoo Penguin colonies not included, due to scale of map in the case of the former and movement in the case of the latter)
1
2
3
4
Historical sites (zone 4)
Amendments:
C. Hänel
University of Pretoria
1998
Original Source:
Langenegger &
Verwoerd
1965
Map Composition:
D.W. Marais
DEA&T - GIS
1998
Transverse Projection Central Meridian 37 Degrees East Spheroid Clarke 1880


ZONING OF PRINCE EDWARD ISLAND
LEGEND
Primary survey point
Secondary survey point
Caves
Historical sites
Intermittent streams
Contours
Craters
Zone 4
Lakes and ponds
Source : DJ van Schalkwyk, DEA&T, 1996
Original Source : Marion and Prince Edward Islands EM van Zinderen Bakker, JM Winterbottom and RA Dyer, 1971
Composition : L Botha / DW Marais, DEA&T - GIS, 1998
Transverse Projection Central Meridian 37 Degrees East Spheroid Clarke 1880

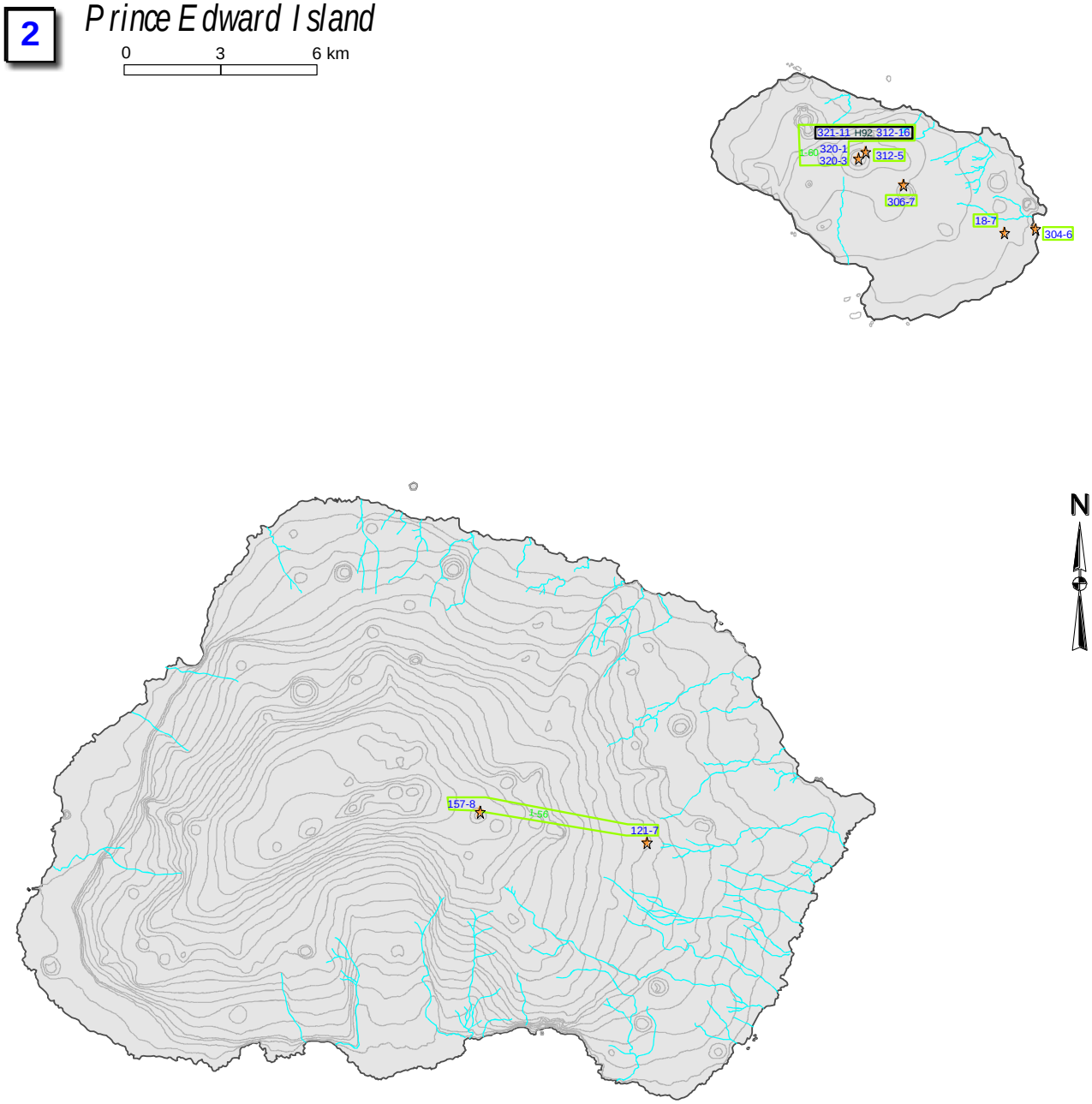
(a)


2
Prince Edward Island
0 3 6 km
321-11
312-16
320-1
320-3
312-5
306-7
18-7
304-6
N
157-8
121-7
2
Marion Island
0 3 6 km

(b)


2
Prince Edward Island
0 3 6 km
321-11 H92 312-16
320-1
320-3
312-5
306-7
18-7
304-6
N
157-8
121-7
2
Marion Island
0 3 6 km

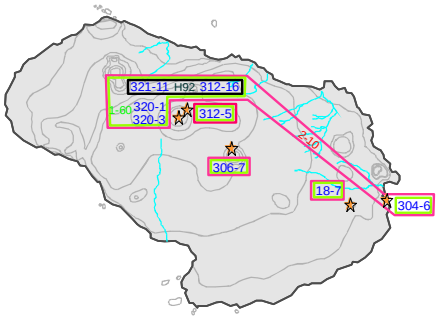
(c)

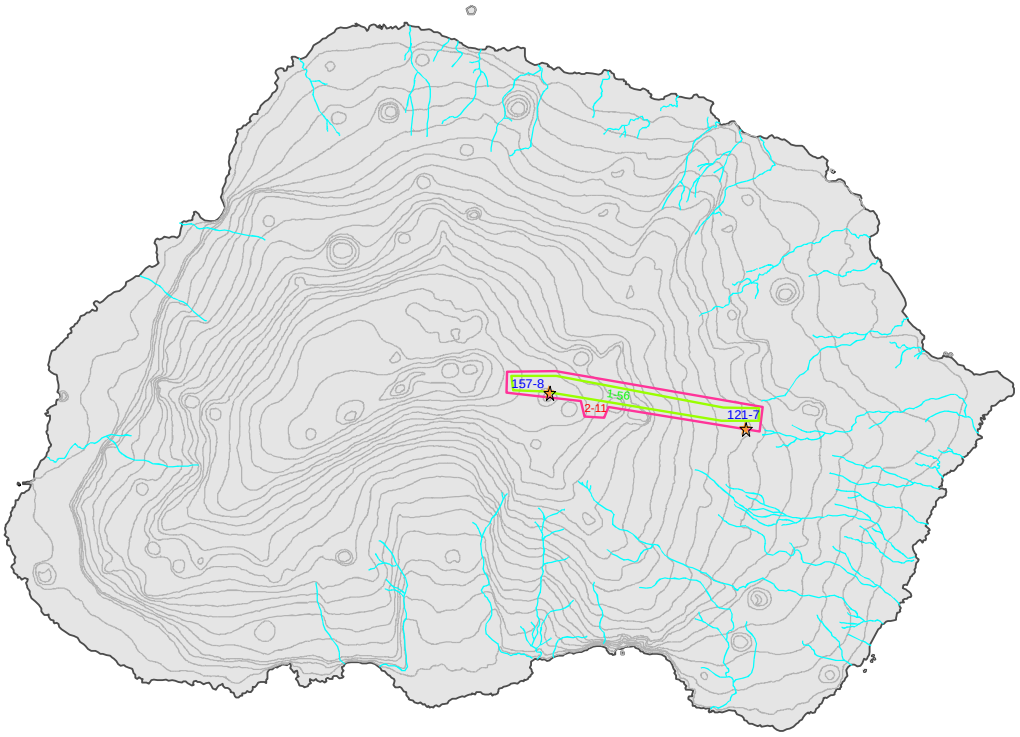

2
Prince Edward Island
0 3 6 km
321-11 H92 312-16
320-1
320-3
I-60
312-5
306-7
18-7
304-6
N
157-8
I-56
121-7
2
Marion Island
0 3 6 km

(d)

2 *Prince Edward Island*

0 3 6 km





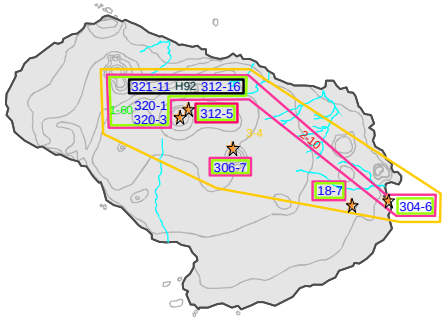
2 *Marion Island*

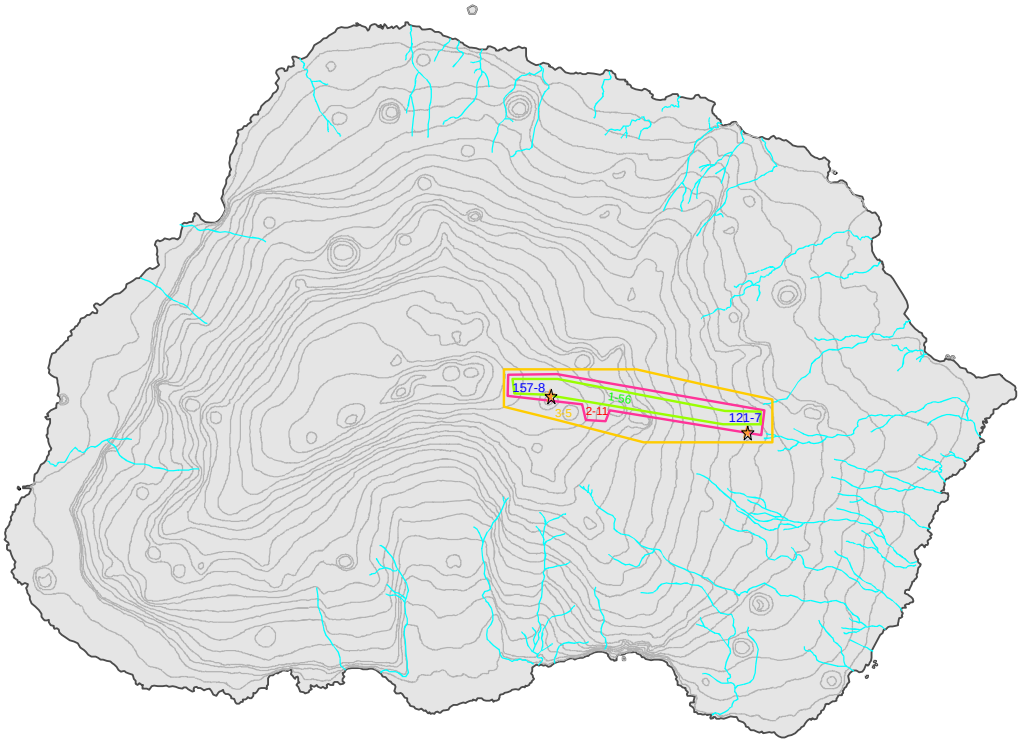
0 3 6 km

(e)

2 *Prince Edward Island*

0 3 6 km

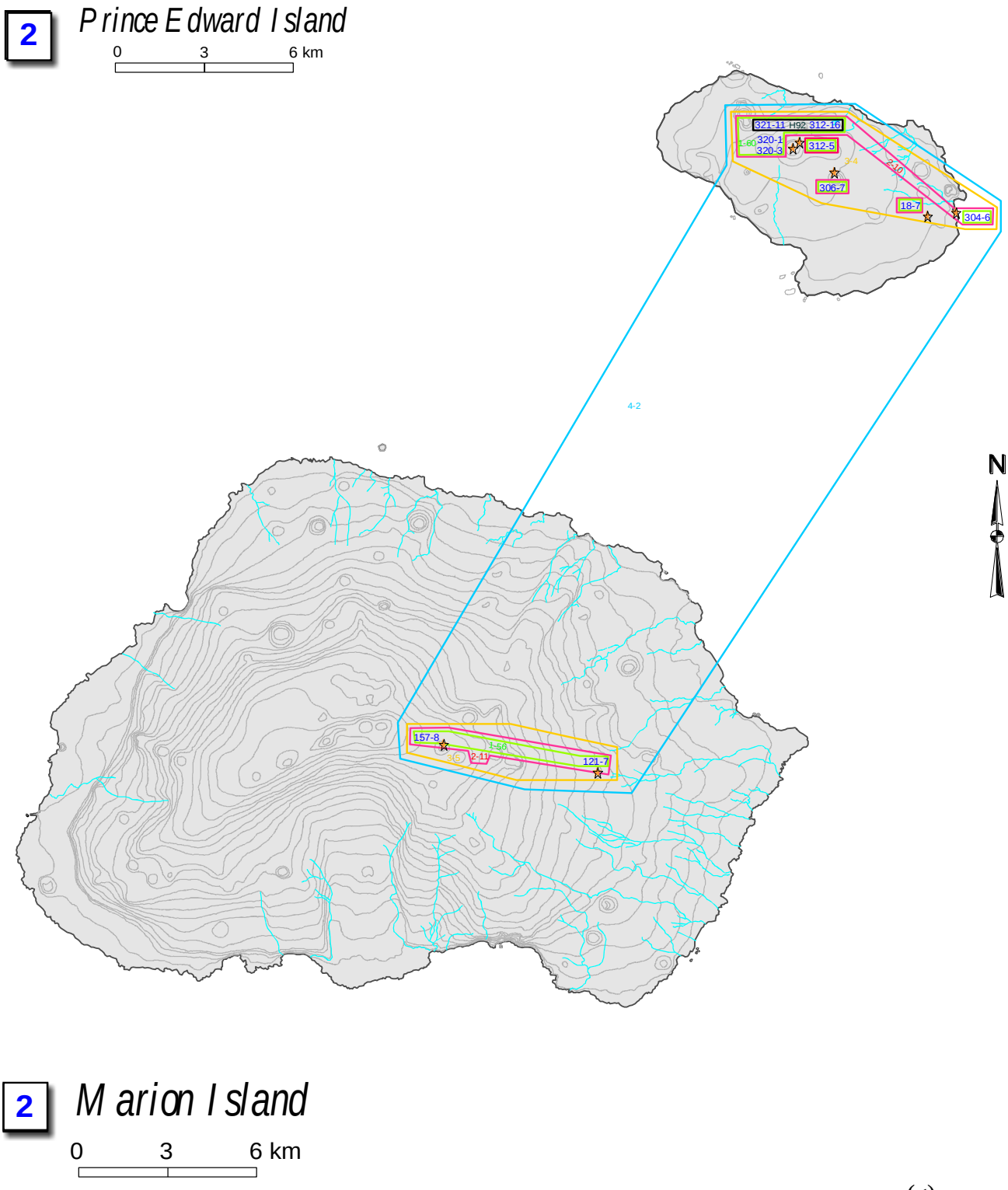




2 *Marion Island*

0 3 6 km

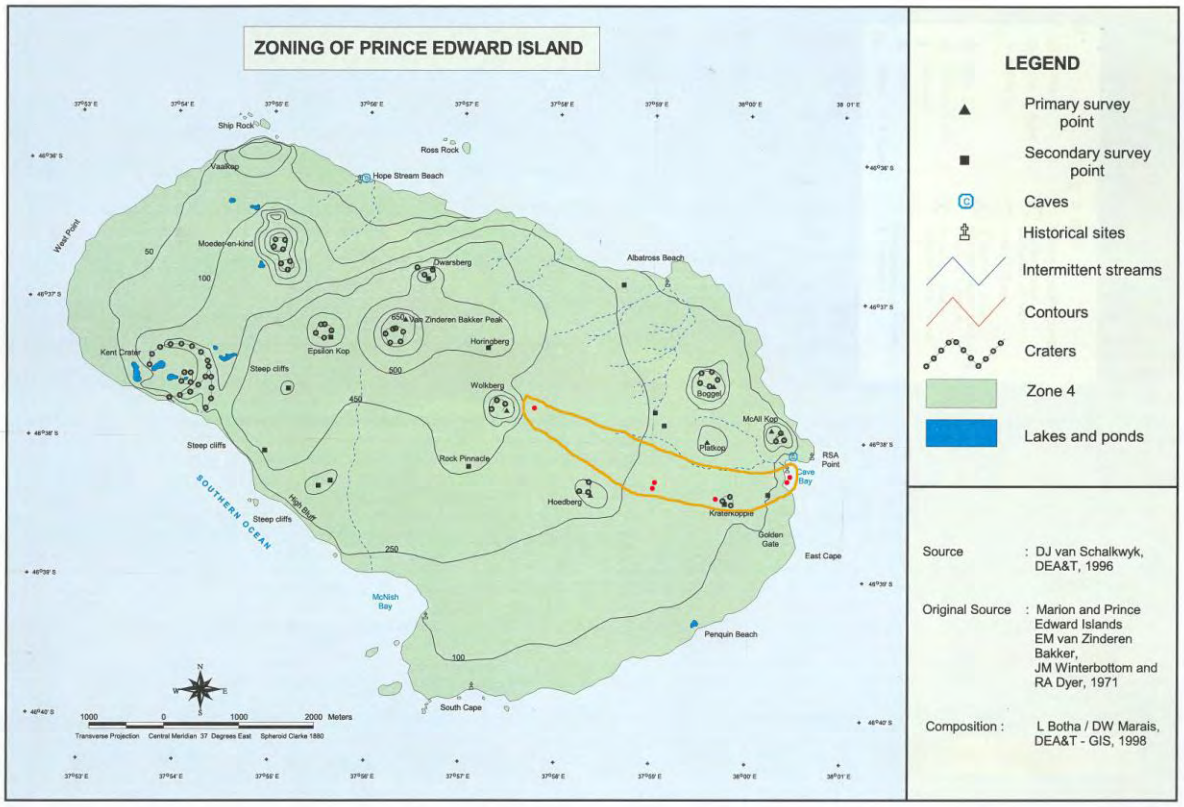
(f)



(g)

Fig. 10 *Ectemnorhinus* individuals that group into 4-step clade 4-2 mapped onto the islands according to a) sampling localities with place names for references, samples on Marion Island are indicated in green while those on Prince Edward Island are indicated in red b) sampling localities as used in the nested design c) those that share the same haplotypes nested into 0-step clades, the d) 1-step clades, e) 2-step clades f) 3 step clades and finally g) the complete 4-2 clade as depicted in Fig. 5.

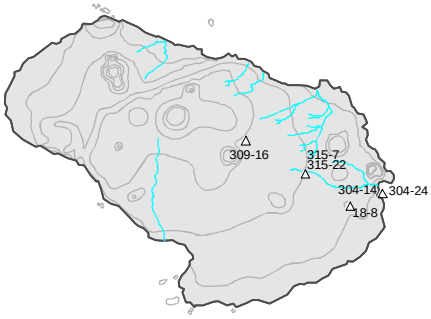

MARION ISLAND
WITH
MANAGEMENT ZONES
LEGEND
Wreck (of Soiglimt)
Historical sites
Primary survey point
Secondary survey point
Huts (zone 1)
Perennial stream
Intermittent stream
Contours
Craters
Caves
Waterfall
Pond
Zones
(Historical sites and Southern Grant Petrel and Gentoo Penguin colonies not included, due to scale of map in the case of the former and movement in the case of the latter)
1
2
3
4
Historical sites (zone 4)
Amendments:
C. Hänel
University of Pretoria
1998
Original Source:
Langenegger &
Verwoerd
1965
Map Composition:
D.W. Marais
DEA&T - GIS
1998
Transverse Projection Central Meridian 37 Degrees East Spheroid Clarke 1880

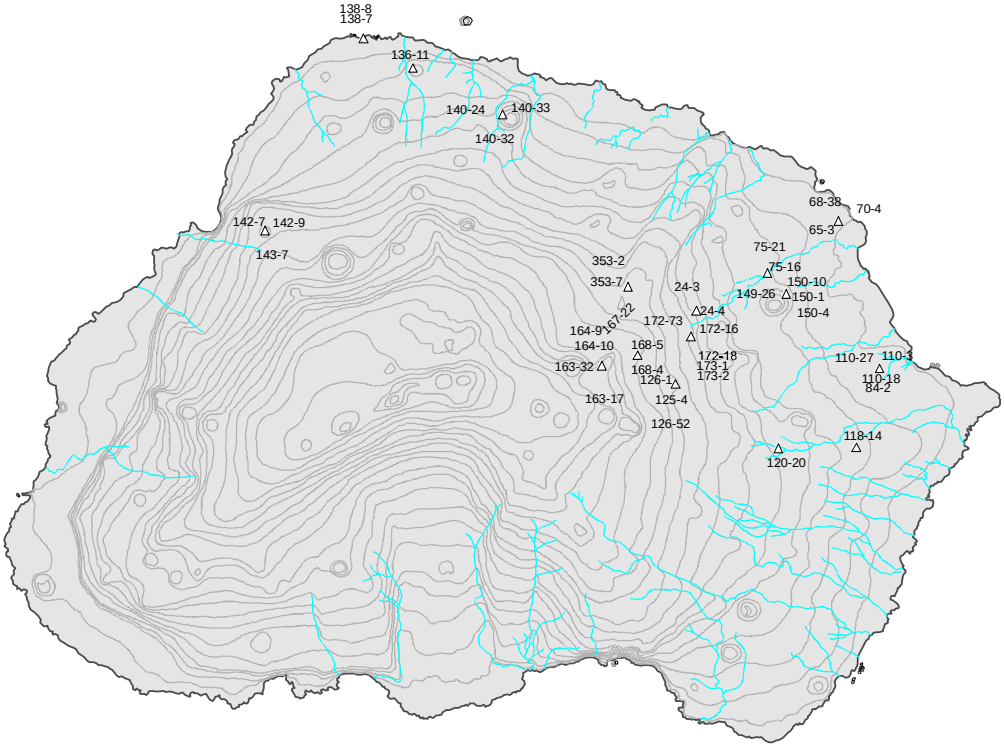

ZONING OF PRINCE EDWARD ISLAND
LEGEND
Primary survey point
Secondary survey point
Caves
Historical sites
Intermittent streams
Contours
Craters
Zone 4
Lakes and ponds
Source : DJ van Schalkwyk, DEA&T, 1996
Original Source : Marion and Prince Edward Islands EM van Zinderen Bakker, JM Winterbottom and RA Dyer, 1971
Composition : L Botha / DW Marais, DEA&T - GIS, 1998
Transverse Projection Central Meridian 37 Degrees East Spheroid Clarke 1880

(a)

3 *Prince Edward Island*

0 3 6 km





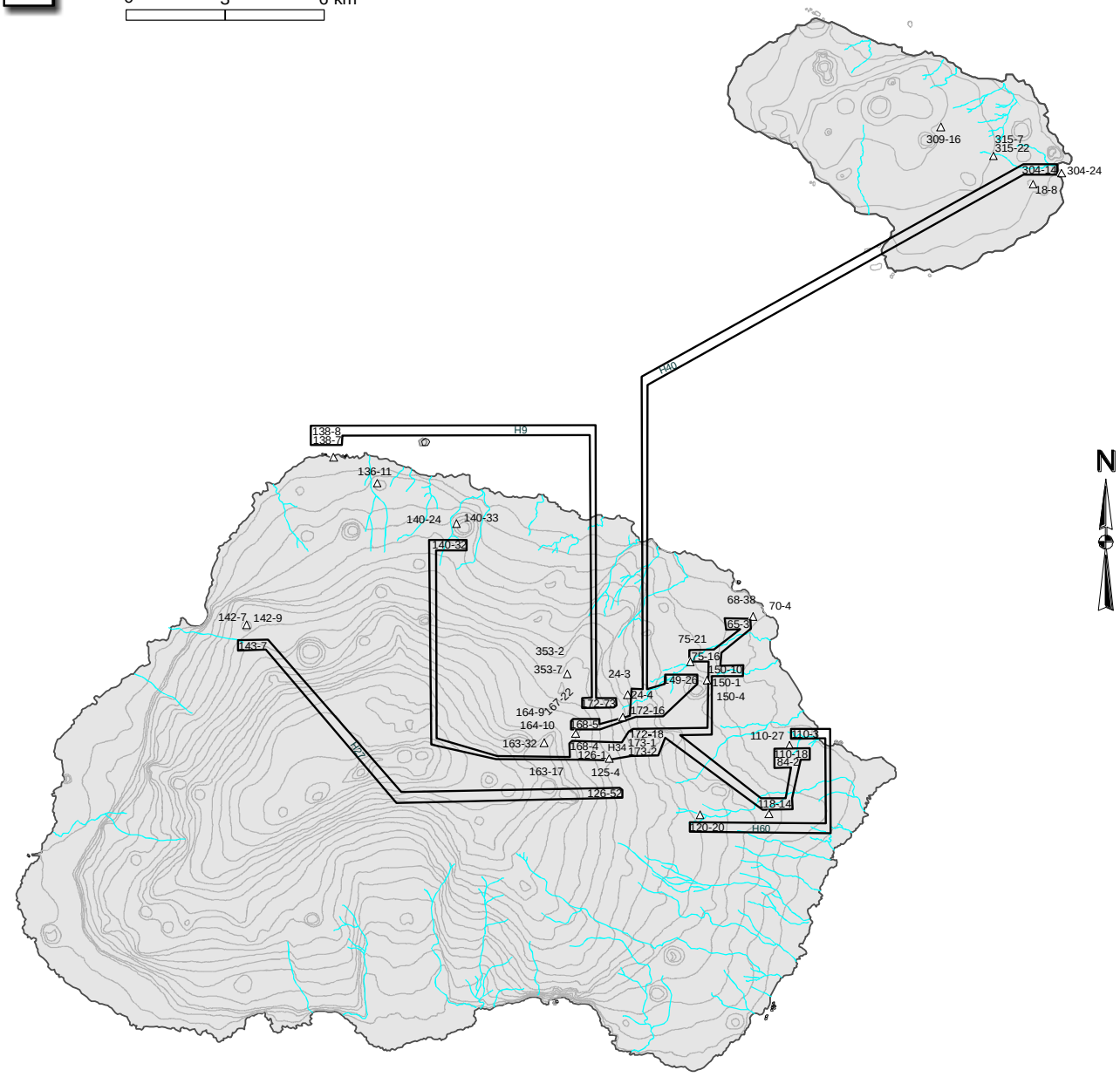
3 *Marion Island*

0 3 6 km

(b)

3 *Prince Edward Island*

0 3 6 km



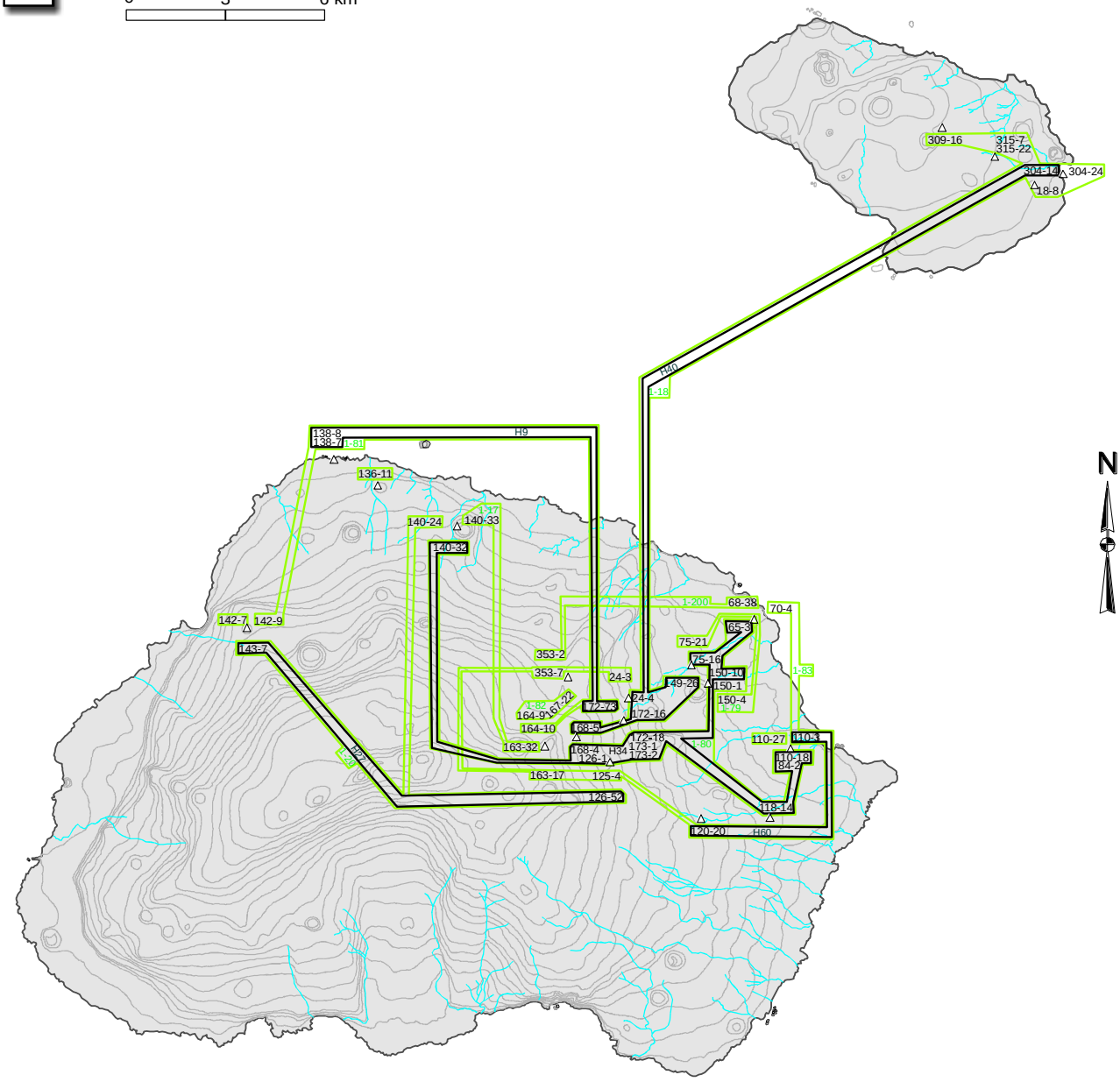
3 *Marion Island*

0 3 6 km

(c)

3 *Prince Edward Island*

0 3 6 km



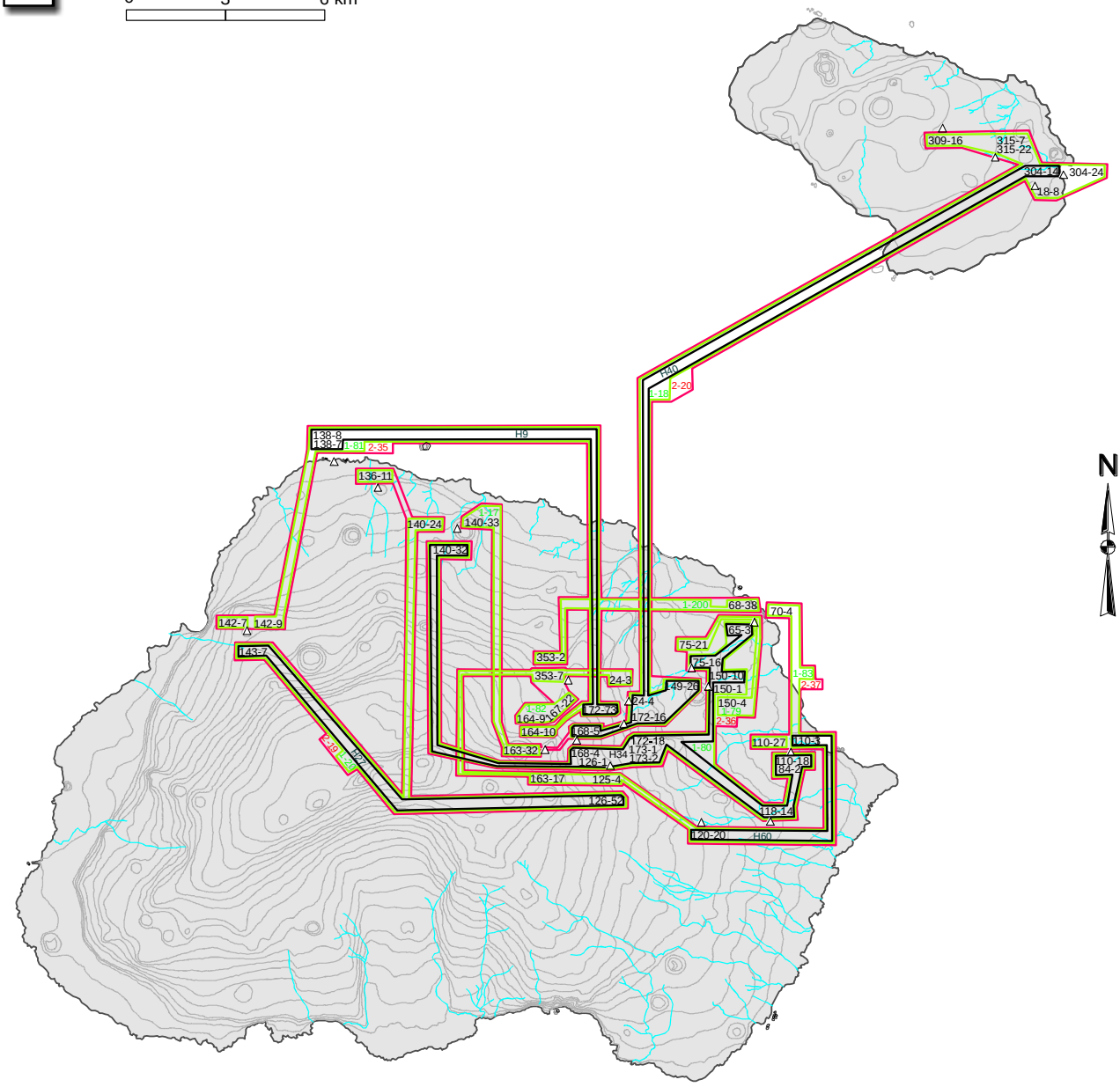
3 *Marion Island*

0 3 6 km

(d)

3 *Prince Edward Island*

0 3 6 km



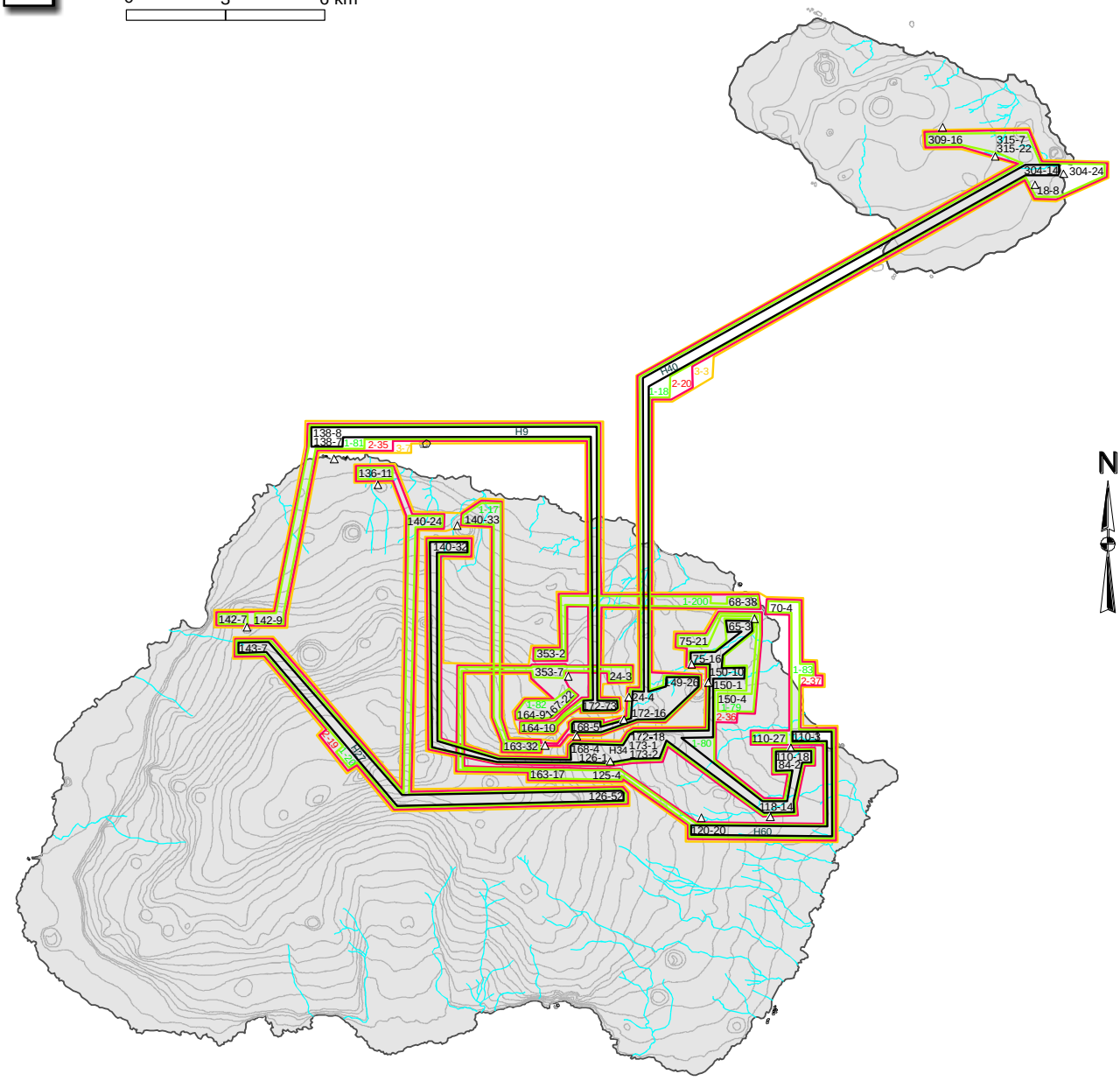
3 *Marion Island*

0 3 6 km

(e)

3 *Prince Edward Island*

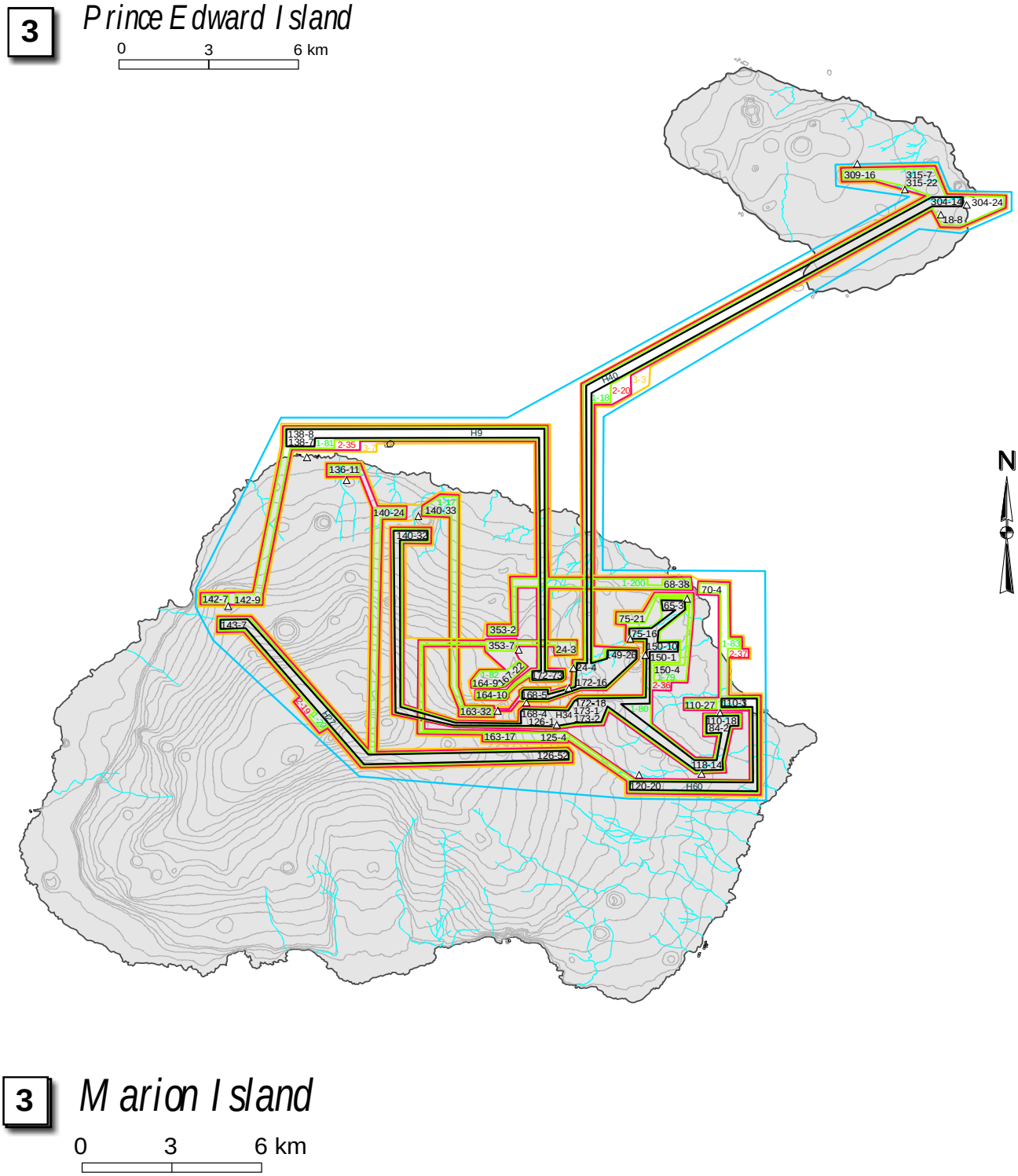
0 3 6 km



3 *Marion Island*

0 3 6 km

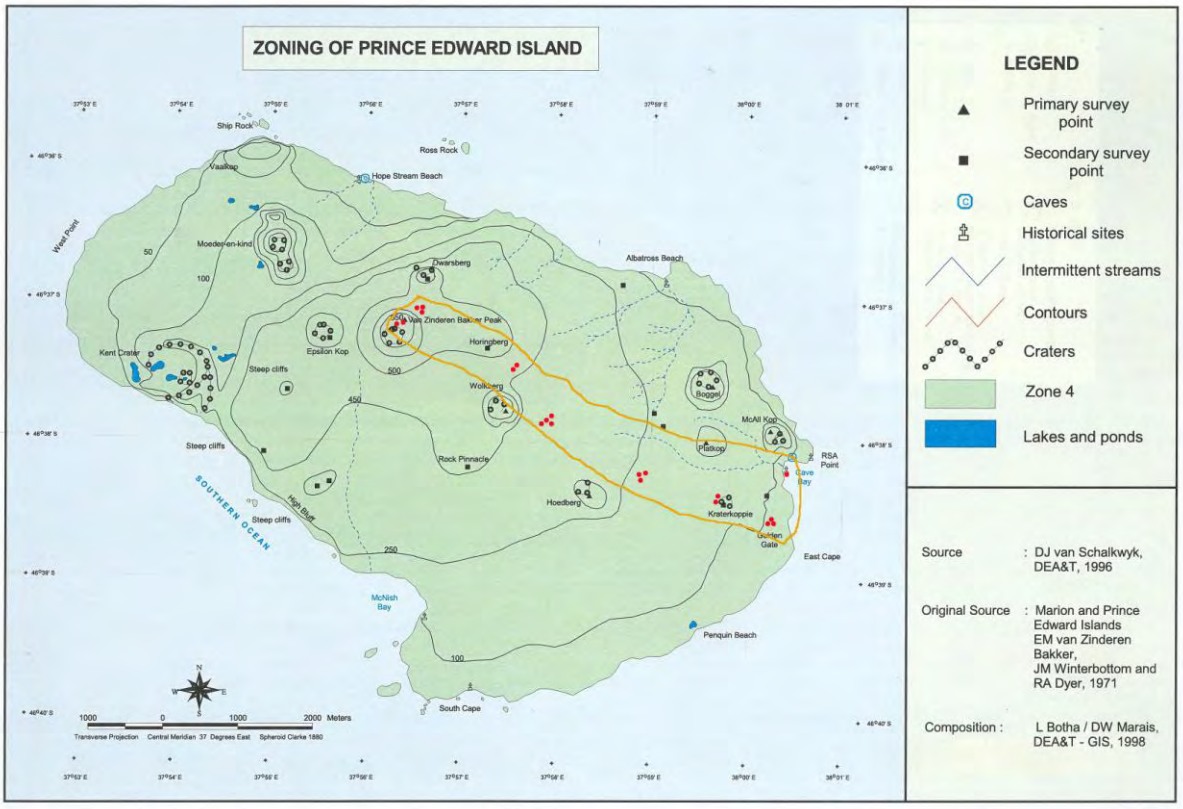
(f)



(g)

Fig. 11 *Ectemnorhinus* individuals that group into 4-step clade 4-3 mapped onto the islands according to a) sampling localities with place names for references, samples on Marion Island are indicated in green while those on Prince Edward Island are indicated in red b) sampling localities as used in the nested design c) those that share the same haplotypes nested into 0-step clades, the d) 1-step clades, e) 2-step clades f) 3 step clades and finally g) the complete 4-3 clade as depicted in Fig. 5.

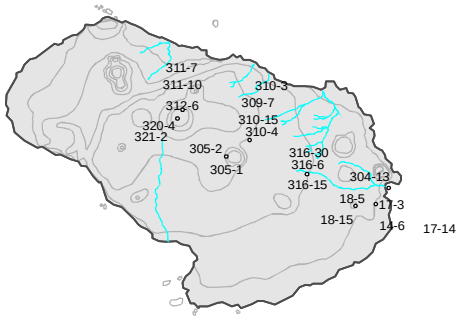

MARION ISLAND
WITH
MANAGEMENT ZONES
LEGEND
Wreck (of Soiglimt)
Historical sites
Primary survey point
Secondary survey point
Huts (zone 1)
Perennial stream
Intermittent stream
Contours
Craters
Caves
Waterfall
Pond
Zones
(Historical sites and Southern Grant Petrel and Gentoo Penguin colonies not included, due to scale of map in the case of the former and movement in the case of the latter)
1
2
3
4
Historical sites (zone 4)
Amendments:
C. Hänel
University of Pretoria
1998
Original Source:
Langenegger &
Verwoerd
1965
Map Composition:
D.W. Marais
DEA&T - GIS
1998
Transverse Projection Central Meridian 37 Degrees East Spheroid Clarke 1880

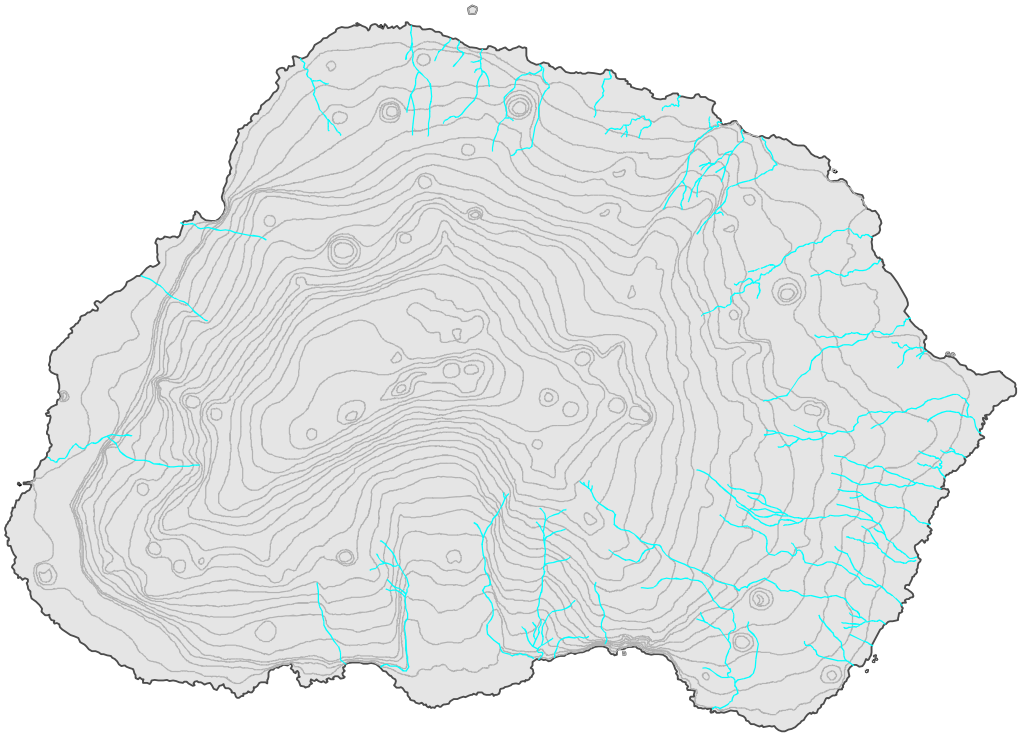

ZONING OF PRINCE EDWARD ISLAND
LEGEND
Primary survey point
Secondary survey point
Caves
Historical sites
Intermittent streams
Contours
Craters
Zone 4
Lakes and ponds
Source : DJ van Schalkwyk, DEA&T, 1996
Original Source : Marion and Prince Edward Islands EM van Zinderen Bakker, JM Winterbottom and RA Dyer, 1971
Composition : L Botha / DW Marais, DEA&T - GIS, 1998
Transverse Projection Central Meridian 37 Degrees East Spheroid Clarke 1880

(a)

4 *Prince Edward Island*

0 3 6 km





N

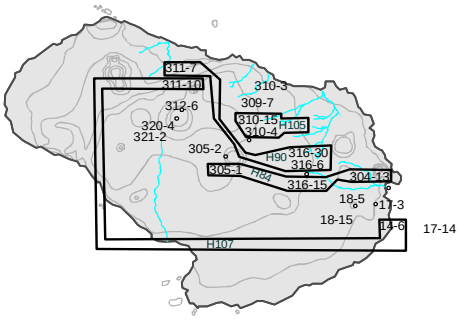
4 *Marion Island*

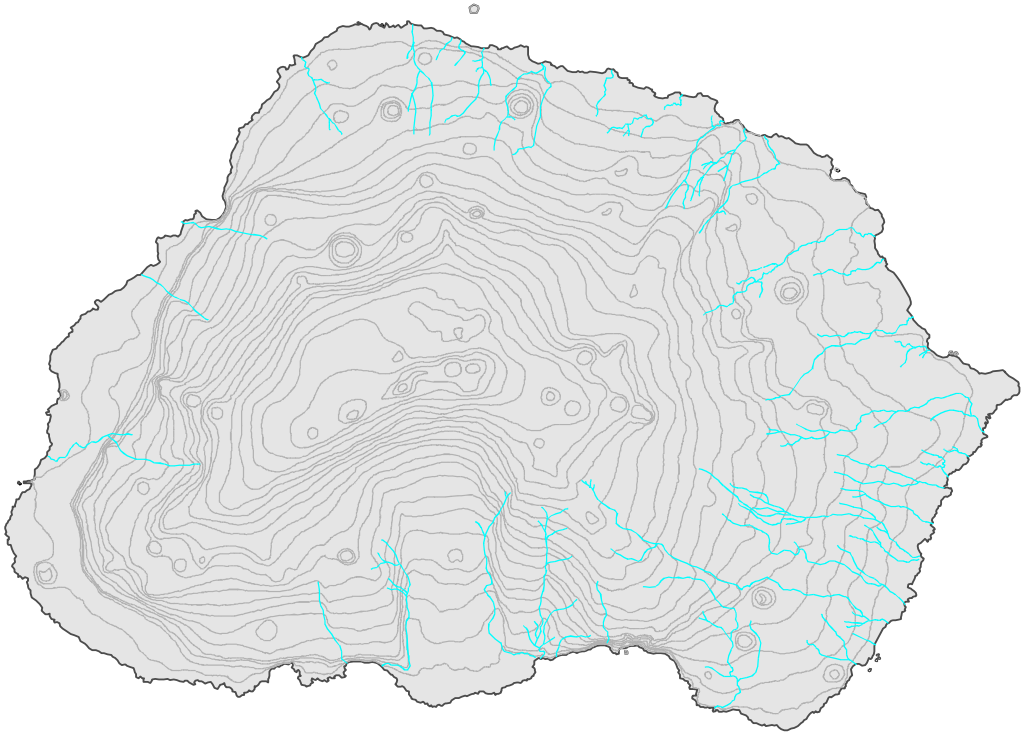
0 3 6 km

(b)

4 *Prince Edward Island*

0 3 6 km





N

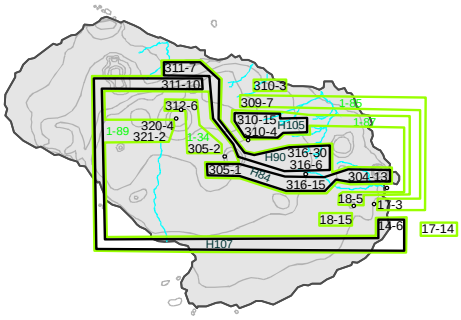
4 *Marion Island*

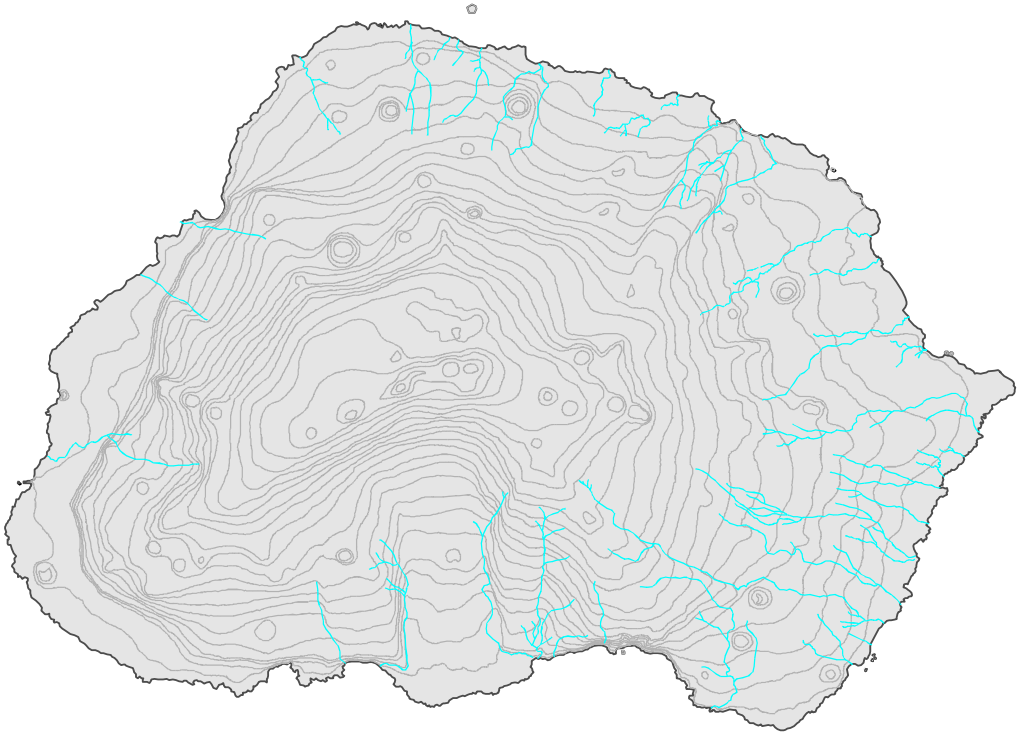
0 3 6 km

(c)

4 *Prince Edward Island*

0 3 6 km





N

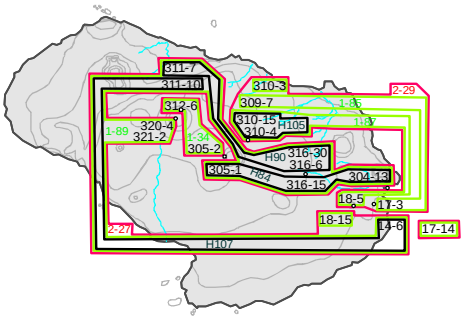
4 *Marion Island*

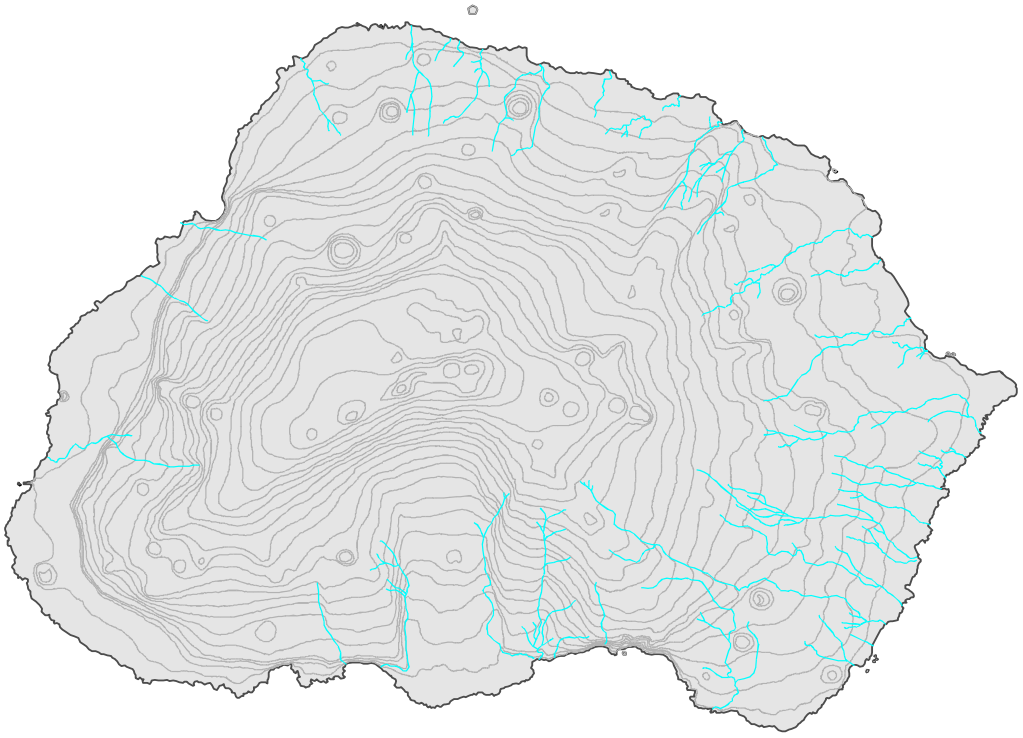
0 3 6 km

(d)

4 *Prince Edward Island*

0 3 6 km





N

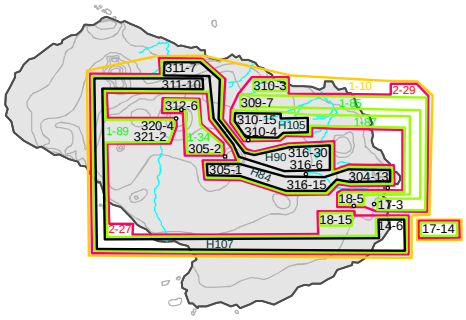
4 *Marion Island*

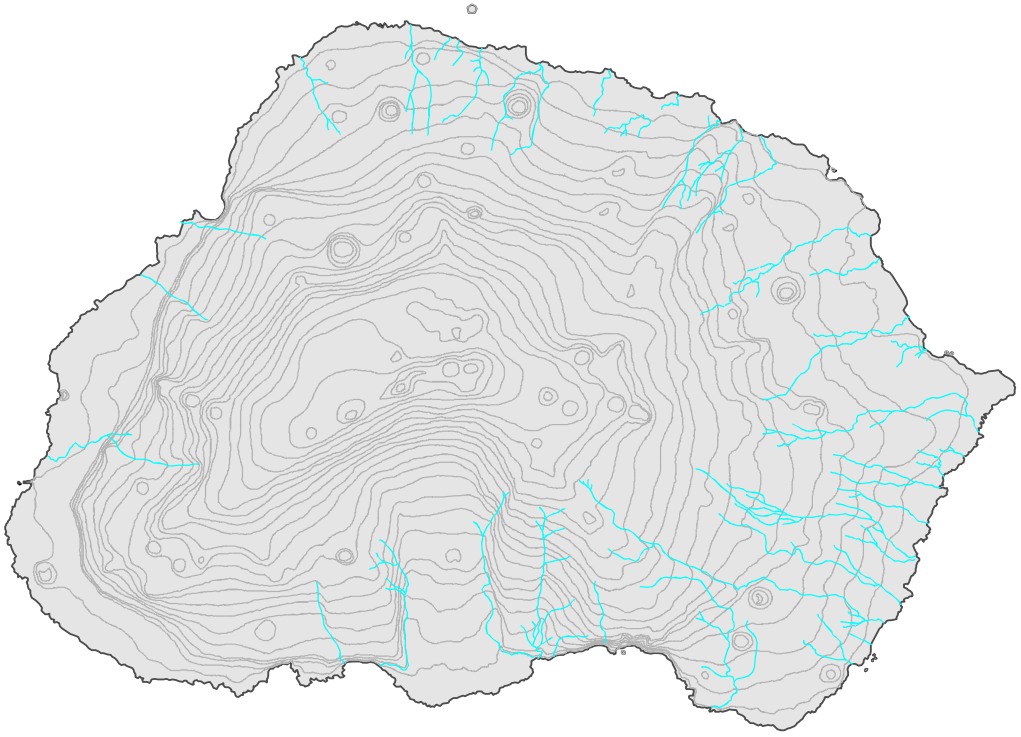
0 3 6 km

(e)

4 *Prince Edward Island*

0 3 6 km



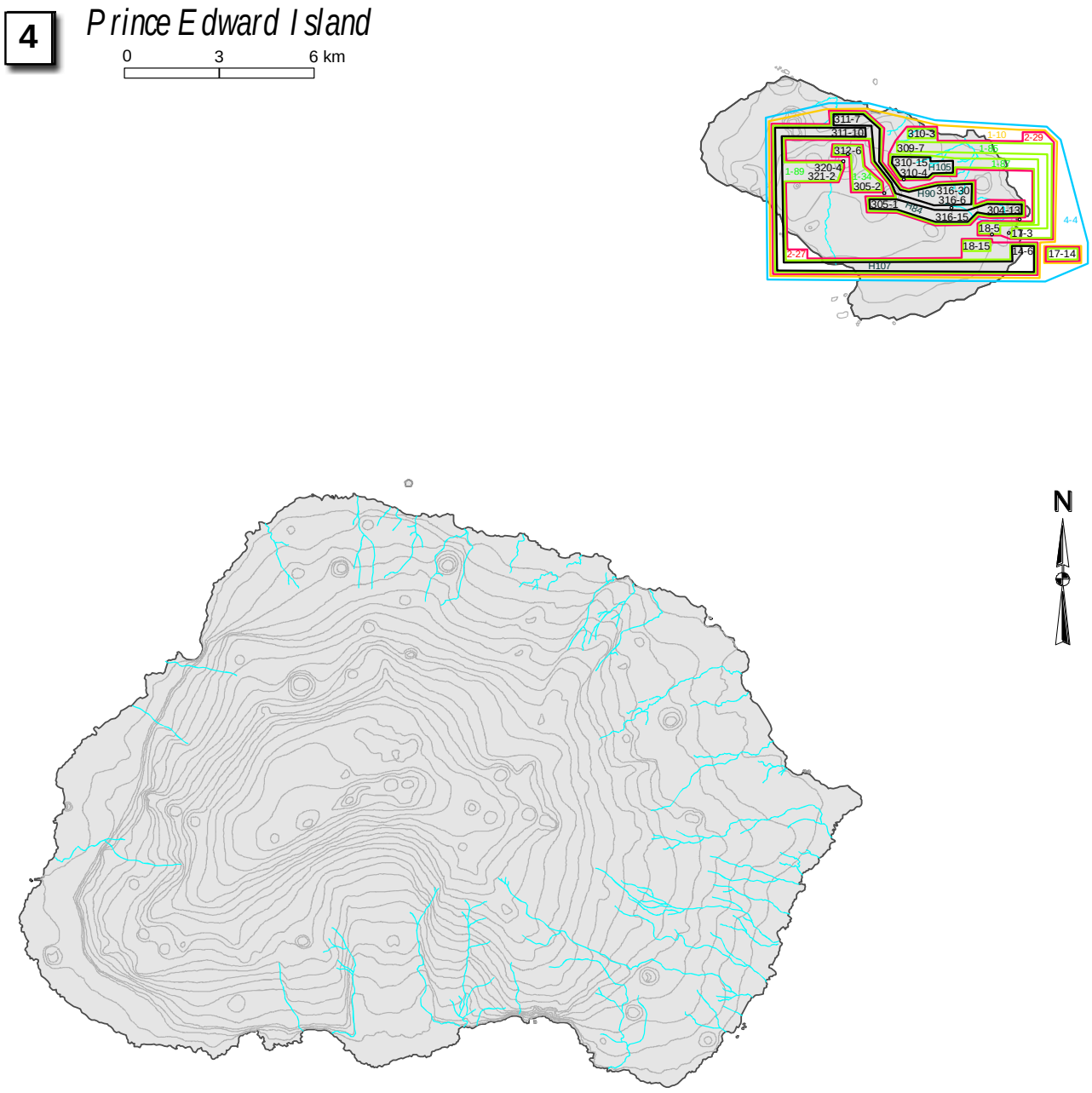


N

4 *Marion Island*

0 3 6 km

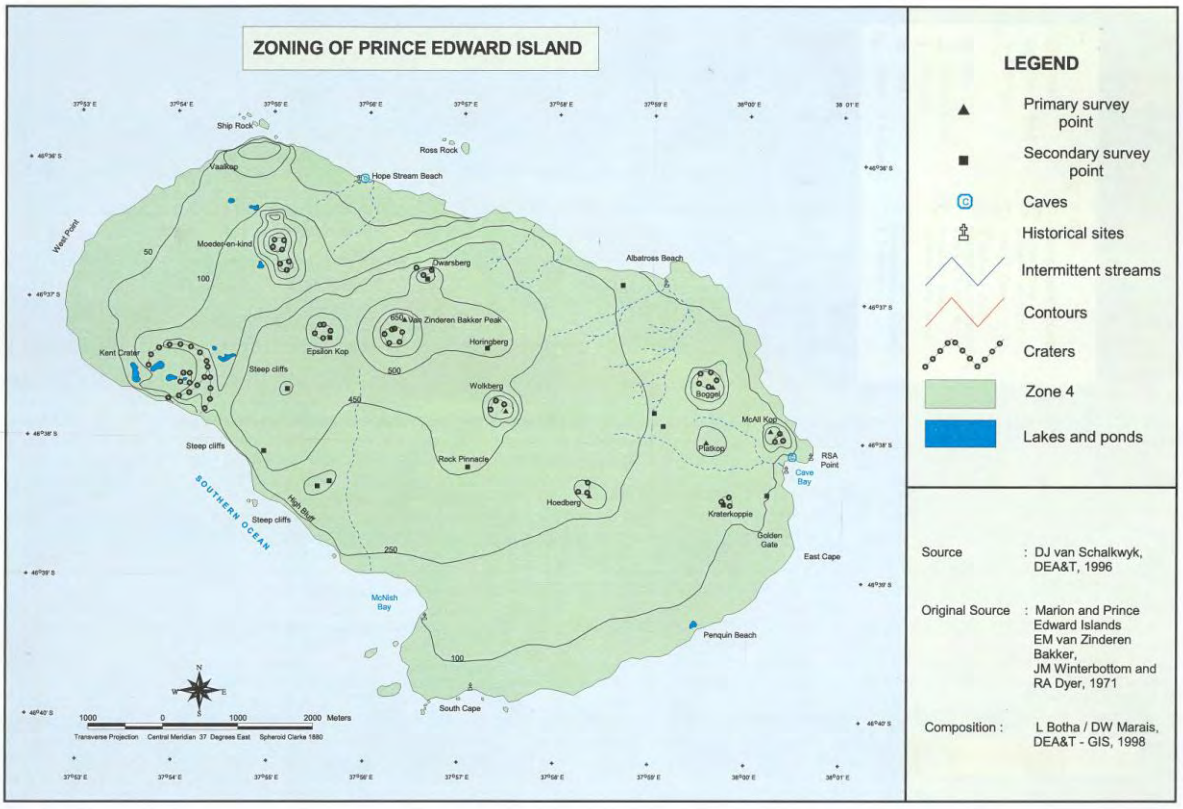
(f)



(g)

Fig. 12 *Ectemnorhinus* individuals that group into 4-step clade 4-4 mapped onto the islands according to a) sampling localities with place names for references, samples on Marion Island are indicated in green while those on Prince Edward Island are indicated in red b) sampling localities as used in the nested design c) those that share the same haplotypes nested into 0-step clades, the d) 1-step clades, e) 2-step clades f) 3 step clades and finally g) the complete 4-4 clade as depicted in Fig. 5.

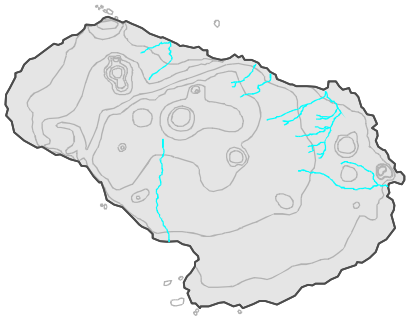

MARION ISLAND
WITH
MANAGEMENT ZONES
LEGEND
Wreck (of Soiglimt)
Historical sites
Primary survey point
Secondary survey point
Huts (zone 1)
Perennial stream
Intermittent stream
Contours
Craters
Caves
Waterfall
Pond
Zones
(Historical sites and Southern Grant Petrel and Gentoo Penguin colonies not included, due to scale of map in the case of the former and movement in the case of the latter)
1
2
3
4
Historical sites (zone 4)
Amendments:
C. Hänel
University of Pretoria
1998
Original Source:
Langenegger &
Verwoerd
1965
Map Composition:
D.W. Marais
DEA&T - GIS
1998
Transverse Projection Central Meridian 37 Degrees East Spheroid Clarke 1880

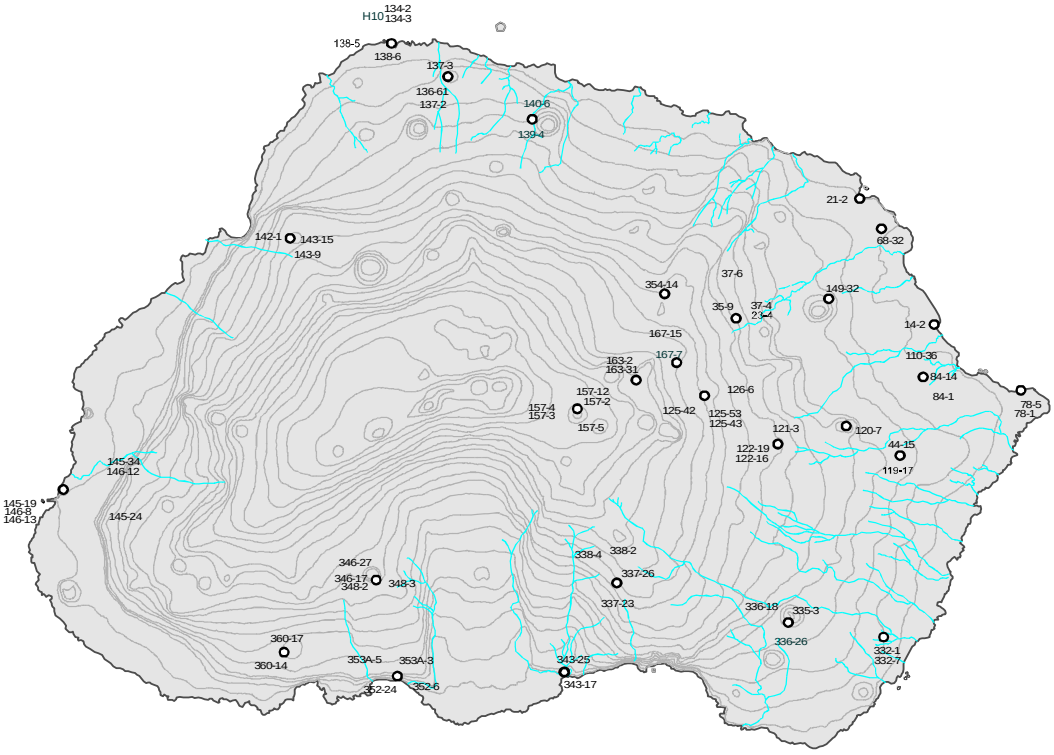

ZONING OF PRINCE EDWARD ISLAND
LEGEND
Primary survey point
Secondary survey point
Caves
Historical sites
Intermittent streams
Contours
Craters
Zone 4
Lakes and ponds
Source : DJ van Schalkwyk, DEA&T, 1996
Original Source : Marion and Prince Edward Islands EM van Zinderen Bakker, JM Winterbottom and RA Dyer, 1971
Composition : L Botha / DW Marais, DEA&T - GIS, 1998
Transverse Projection Central Meridian 37 Degrees East Spheroid Clarke 1880

(a)

5 *Prince Edward Island*

0 3 6 km





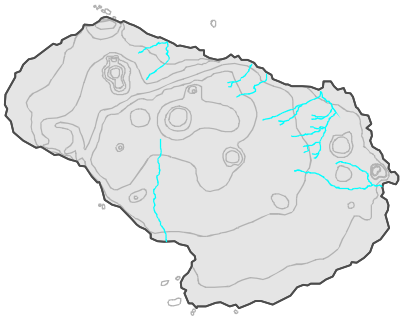
5 *Marion Island*

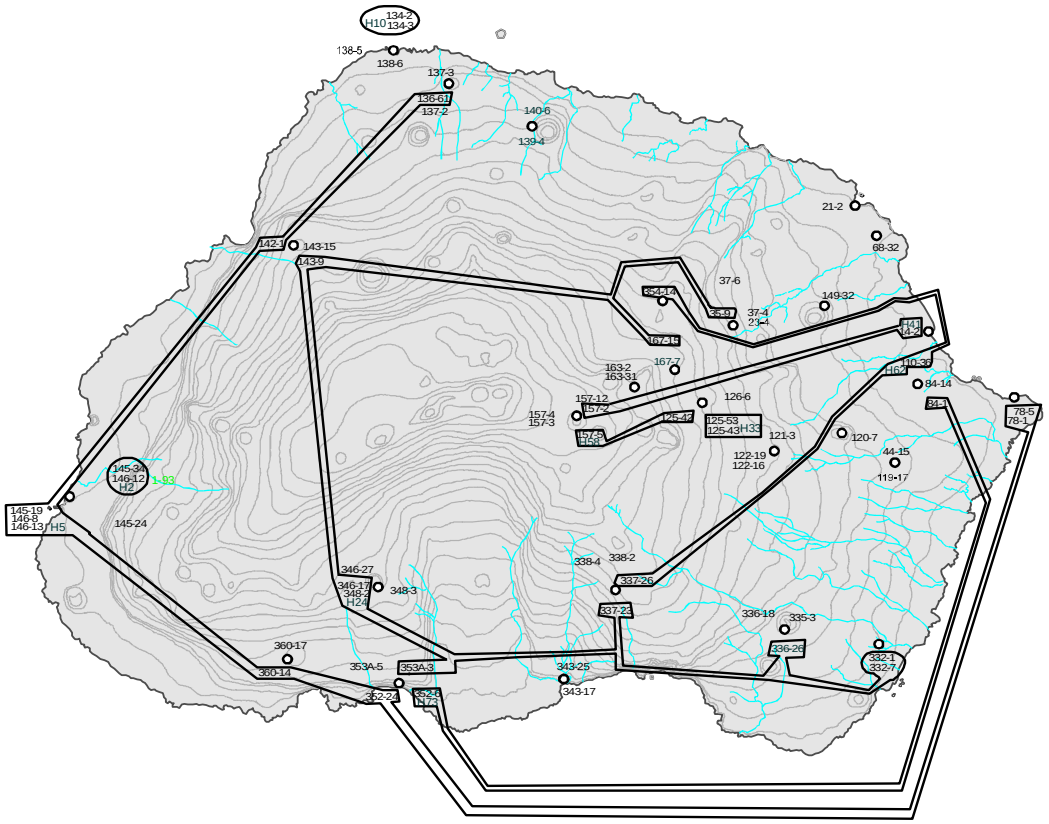
0 3 6 km

(b)

5 *Prince Edward Island*

0 3 6 km

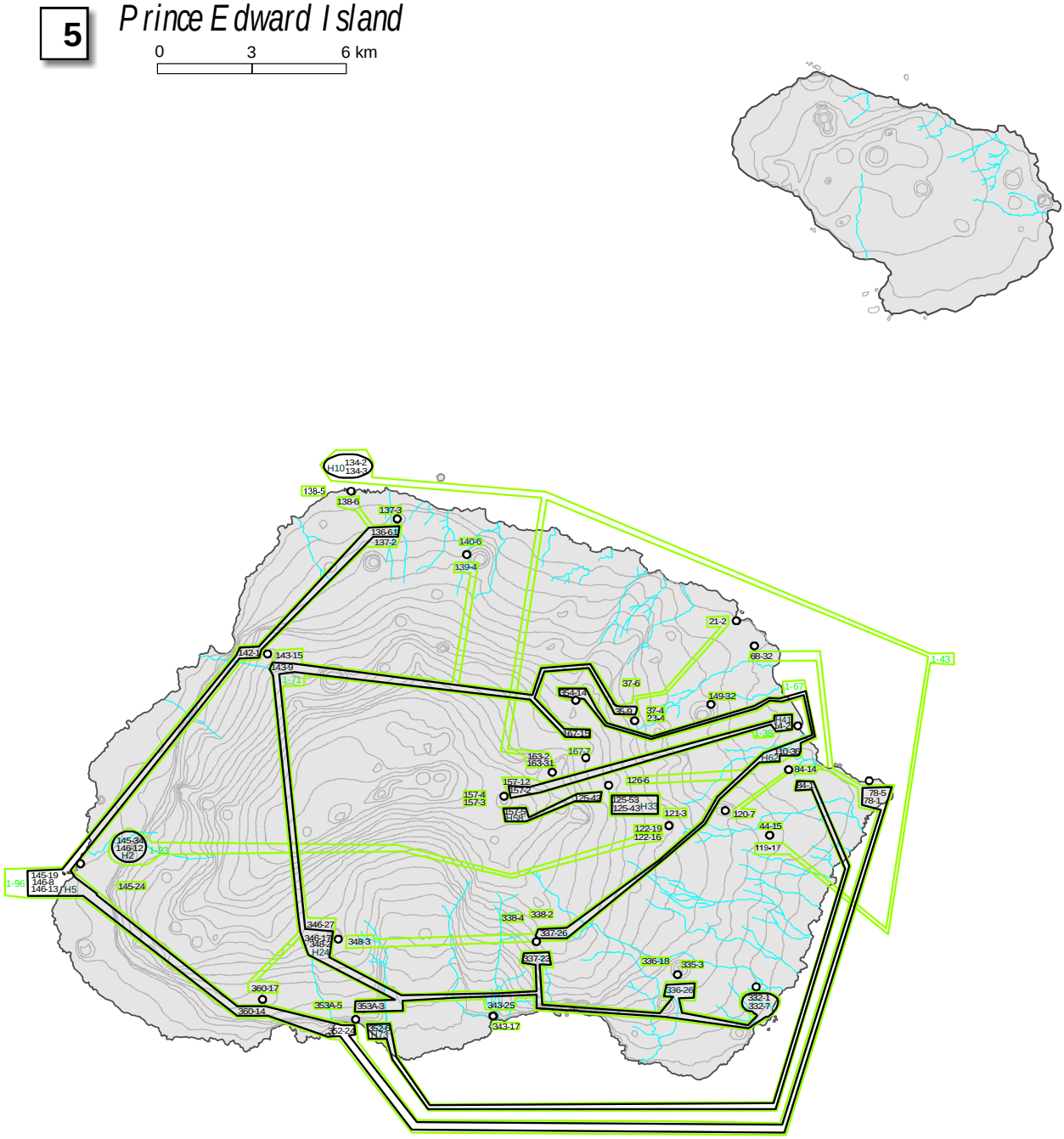




5 *Marion Island*

0 3 6 km

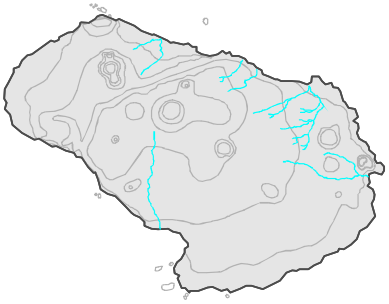
(c)

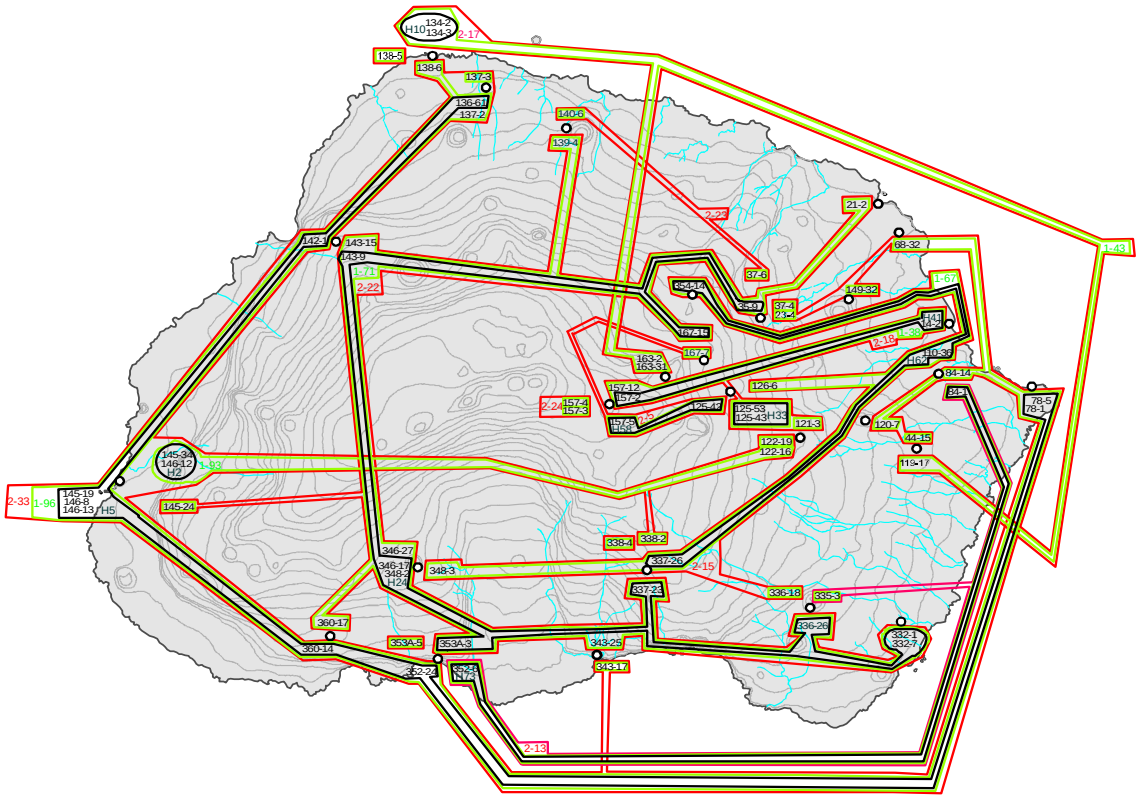


(d)

5 *Prince Edward Island*

0 3 6 km





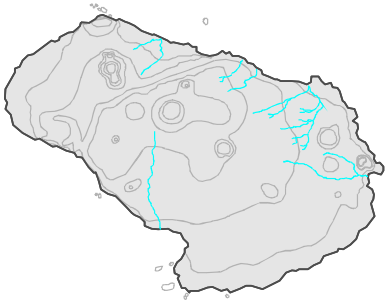
5 *Marion Island*

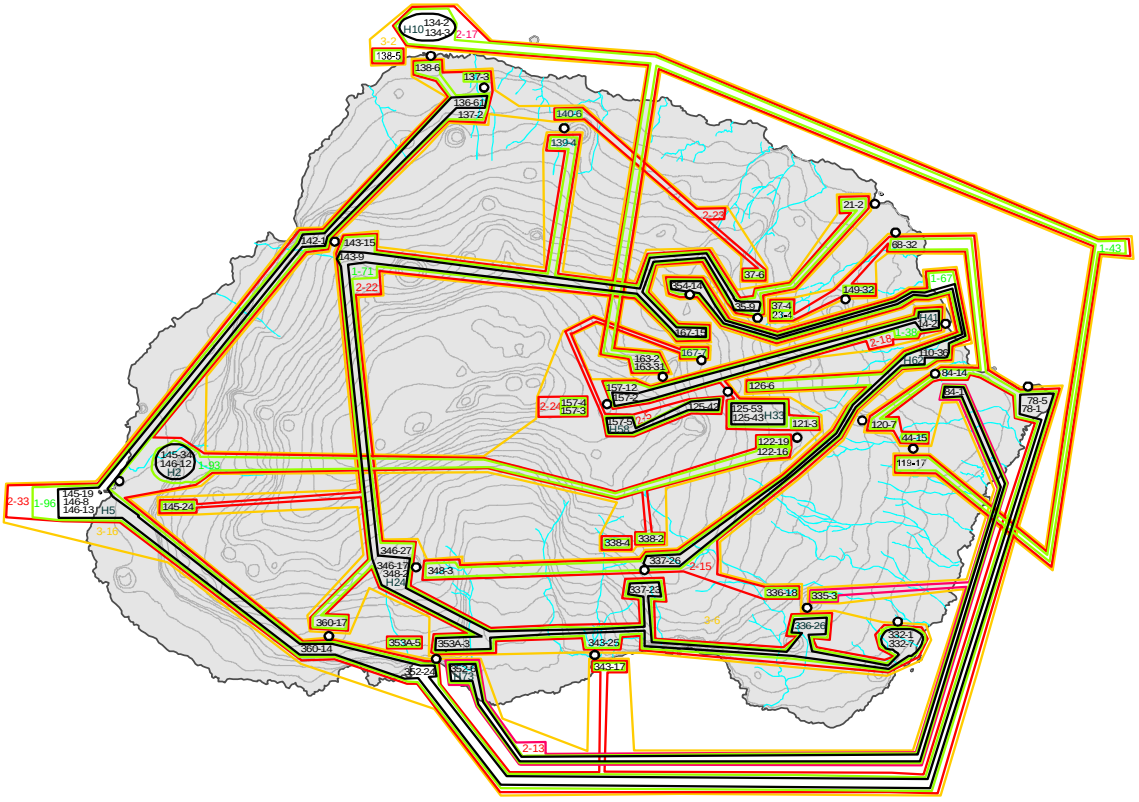
0 3 6 km

(e)

5 *Prince Edward Island*

0 3 6 km

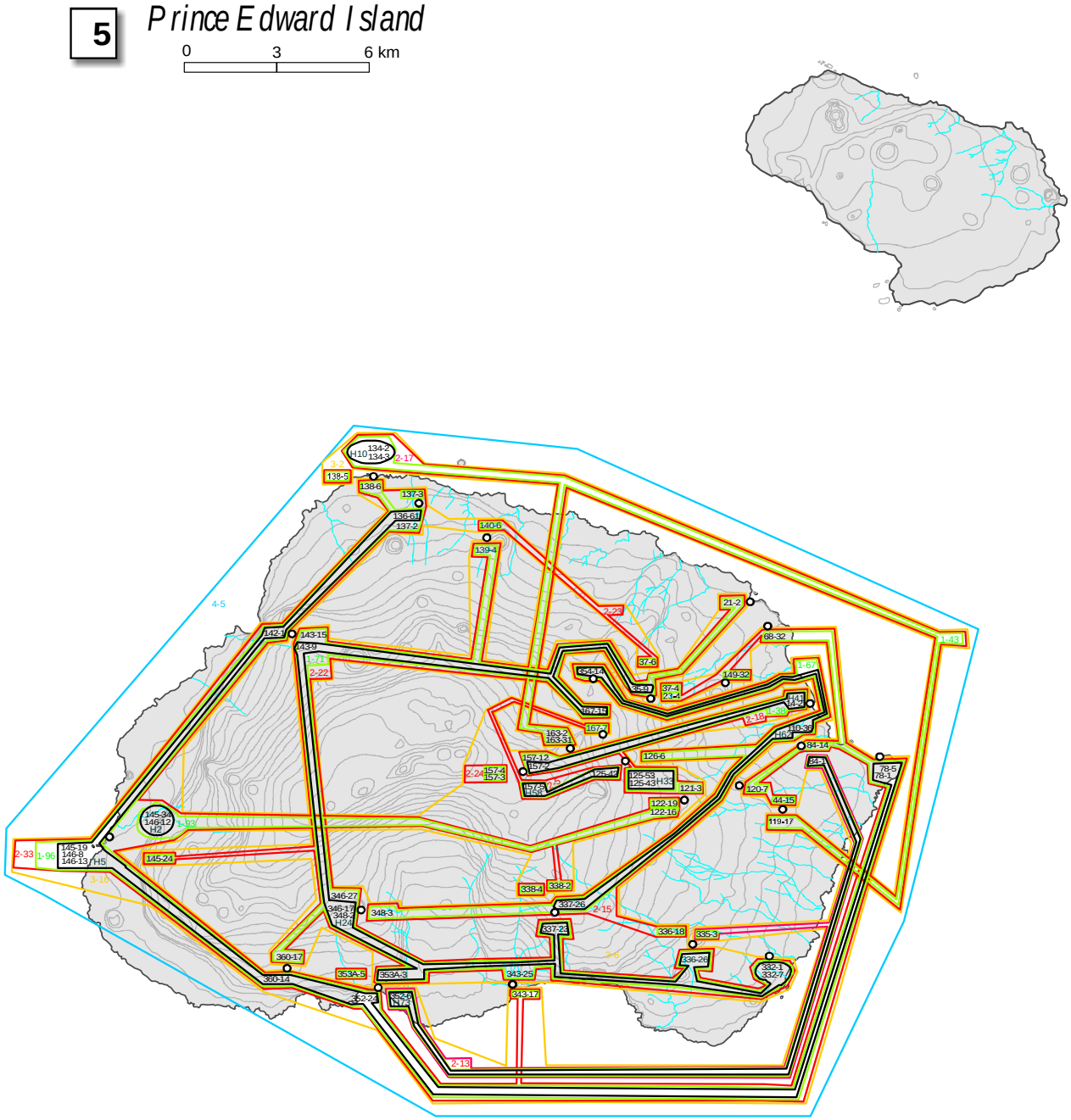




5 *Marion Island*

0 3 6 km

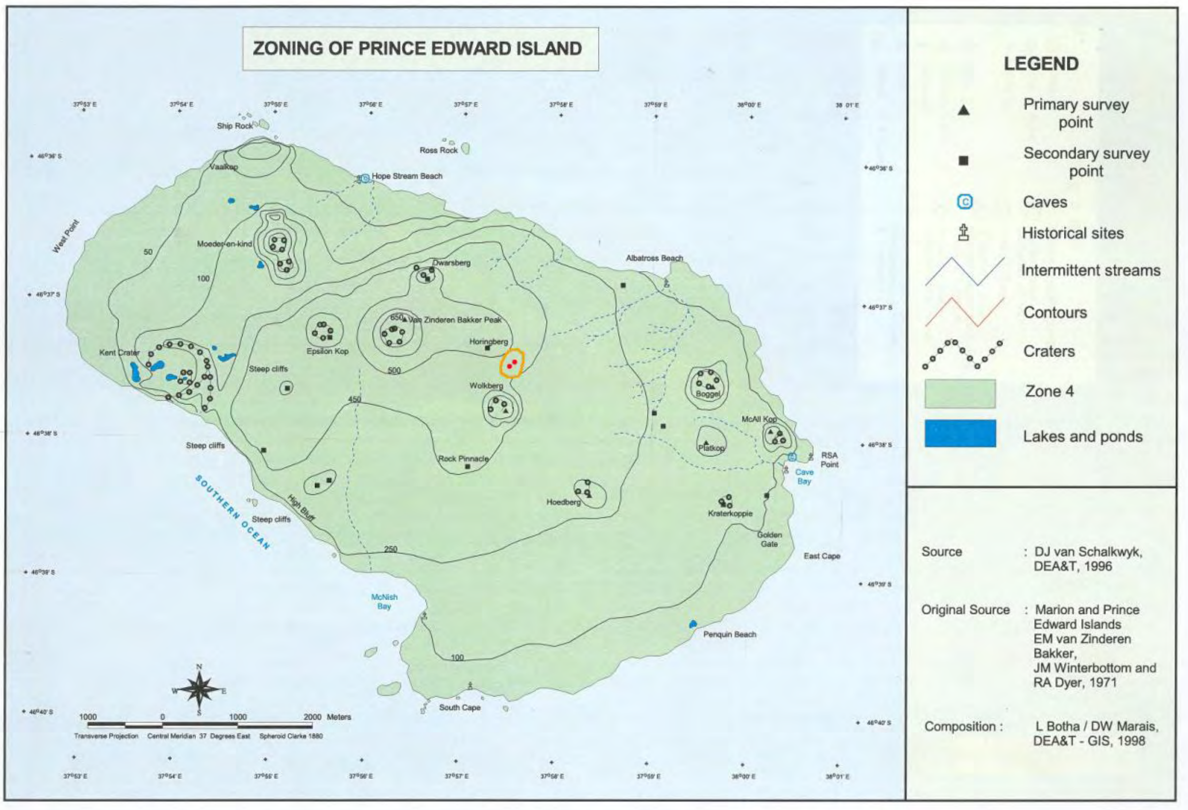
(f)



(g)

Fig. 13 *Ectemnorhinus* individuals that group into 4-step clade 4-5 mapped onto the islands according to a) sampling localities with place names for references, samples on Marion Island are indicated in green while those on Prince Edward Island are indicated in red b) sampling localities as used in the nested design c) those that share the same haplotypes nested into 0-step clades, the d) 1-step clades, e) 2-step clades f) 3 step clades and finally g) the complete 4-5 clade as depicted in Fig. 5.

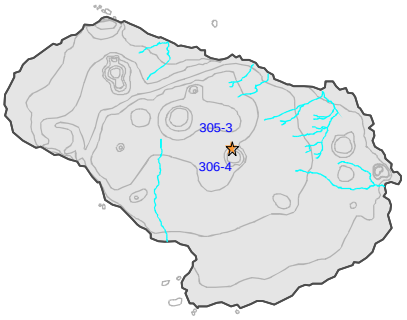

MARION ISLAND WITH MANAGEMENT ZONES
LEGEND
Wreck (of Solglimt)
Historical sites
Primary survey point
Secondary survey point
Huts (zone 1)
Perennial stream
Intermittent stream
Contours
Craters
Caves
Waterfall
Pond
Zones (Historical sites and Southern Grant Petrel and Gentoo Penguin colonies not included, due to scale of map in the case of the former and movement in the case of the latter)
Historical sites (zone 4)
Amendments: C. Hänel University of Pretoria 1998
Original Source: Langenegger & Verwoerd 1965
Map Composition: D.W. Marais DEA&T - GIS 1998
SOUTHERN OCEAN
CAPE DAVIS
CAPE CROZIER
CAPE HOOKER
EAST CAPE
Ice Plateau
STATE PRESIDENT SWART PEAK
Transverse Projection Central Meridian 37 Degrees East Spheroid Clarke 1880

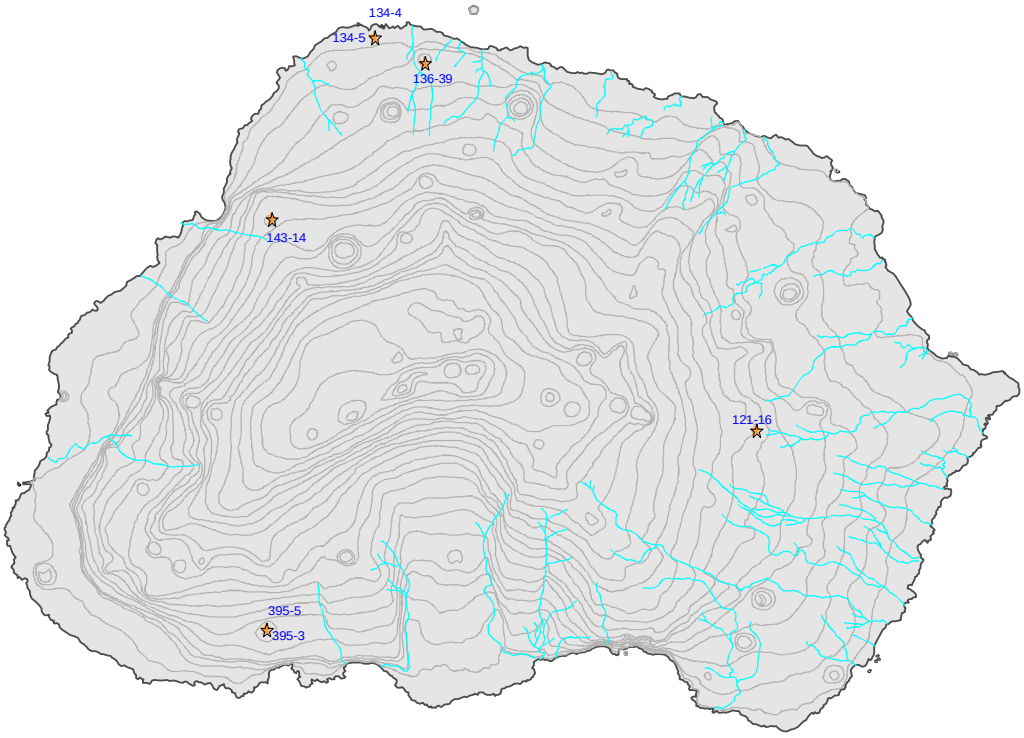

ZONING OF PRINCE EDWARD ISLAND
LEGEND
Primary survey point
Secondary survey point
Caves
Historical sites
Intermittent streams
Contours
Craters
Zone 4
Lakes and ponds
Source : DJ van Schalkwyk, DEA&T, 1996
Original Source : Marion and Prince Edward Islands EM van Zinderen Bakker, JM Winterbottom and RA Dyer, 1971
Composition : L Botha / DW Marais, DEA&T - GIS, 1998
SOUTHERN OCEAN
Transverse Projection Central Meridian 37 Degrees East Spheroid Clarke 1880

(a)

6 *Prince Edward Island*

0 3 6 km





N

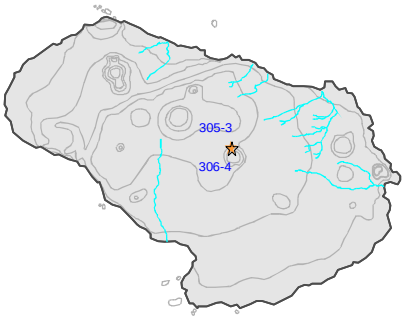
6 *Marion Island*

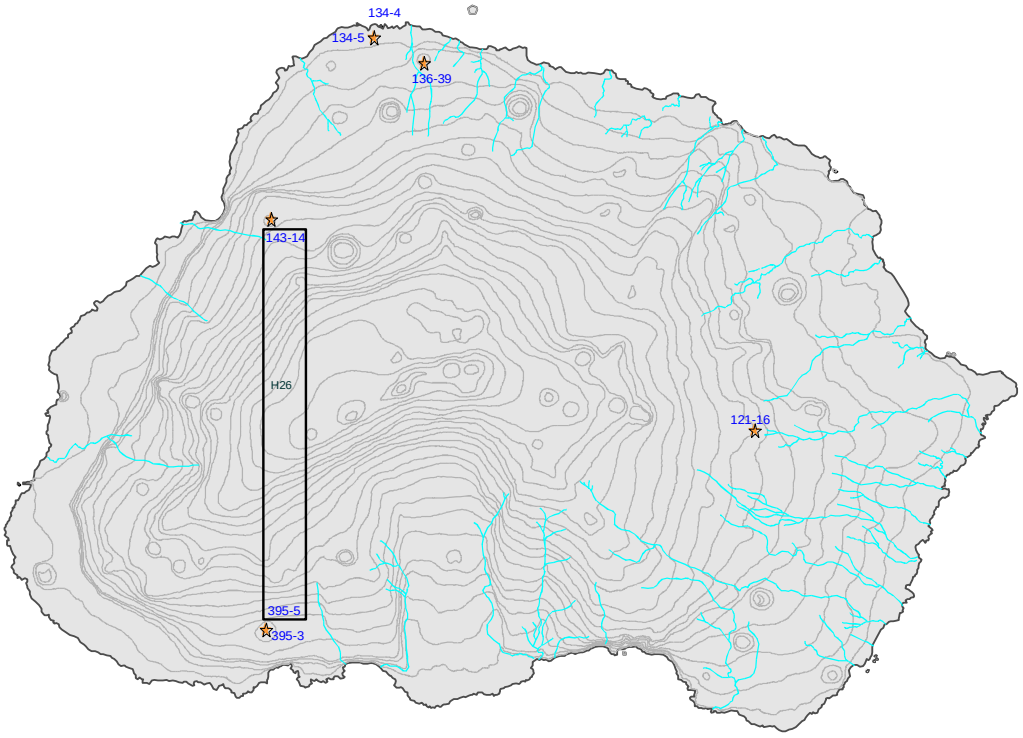
0 3 6 km

(b)

6 *Prince Edward Island*

0 3 6 km





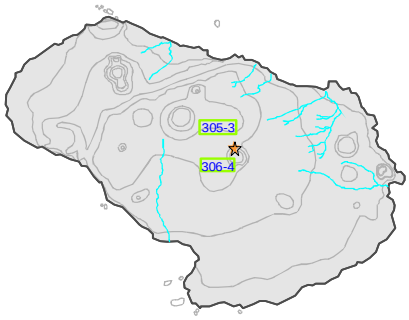
6 *Marion Island*

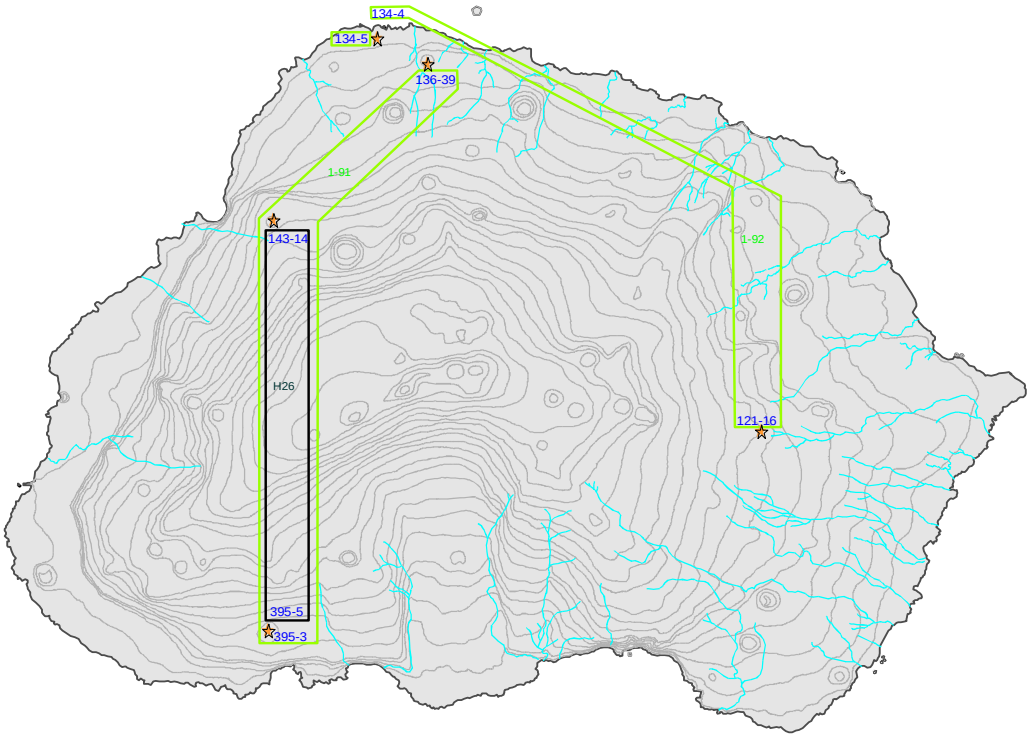
0 3 6 km

(c)

6 *Prince Edward Island*

0 3 6 km





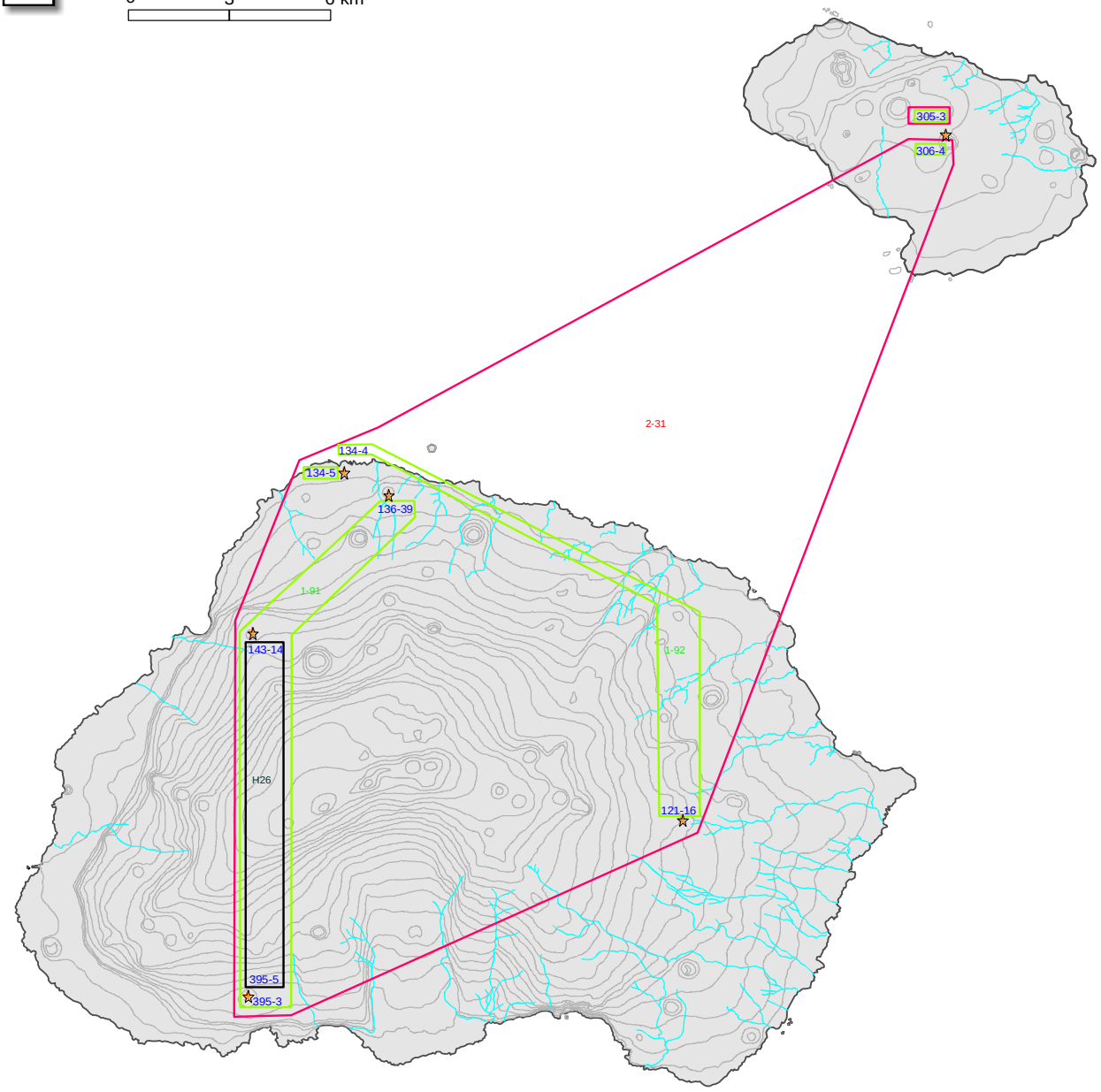
6 *Marion Island*

0 3 6 km

(d)

6 *Prince Edward Island*

0 3 6 km



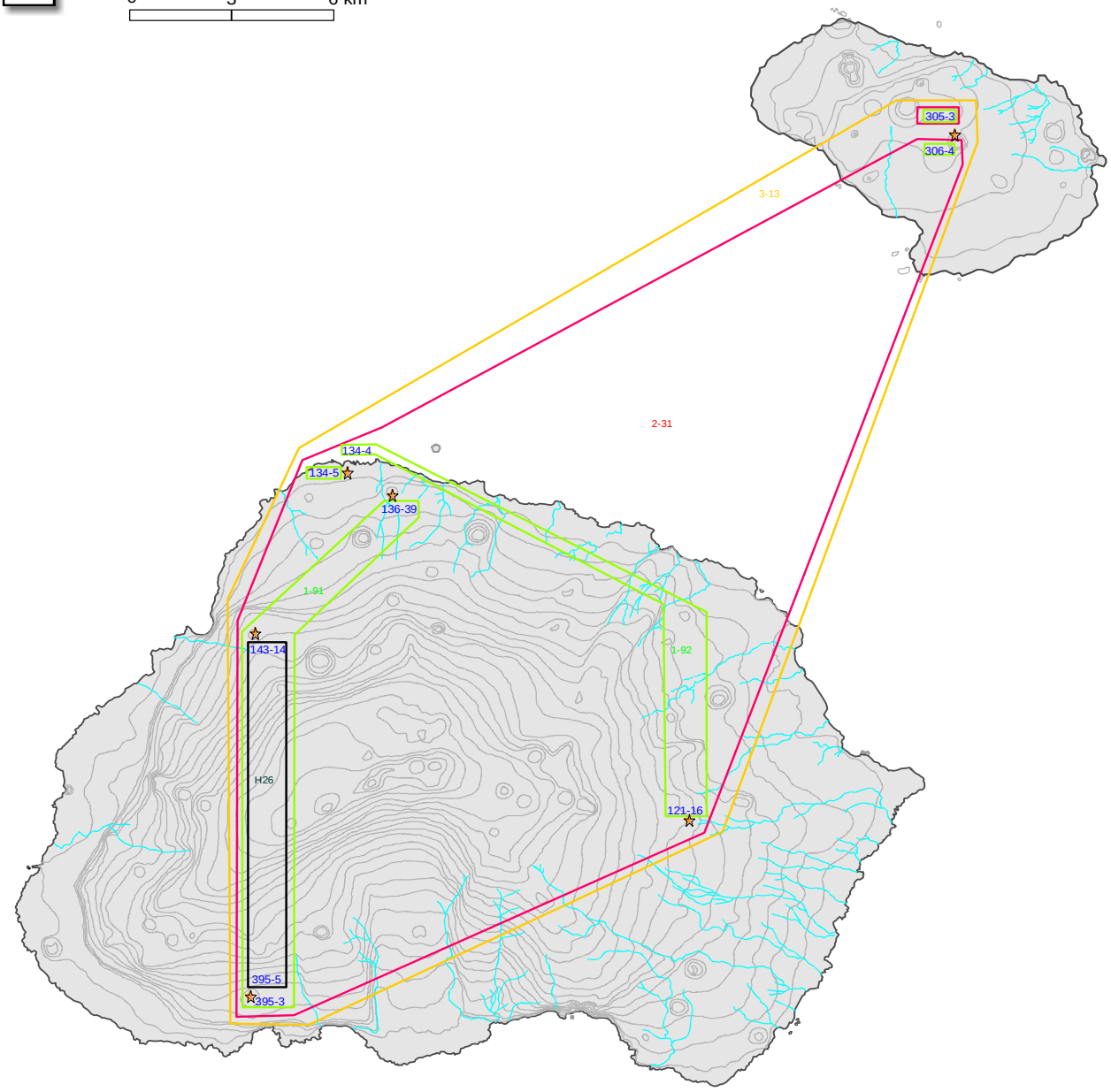
6 *Marion Island*

0 3 6 km

(e)

6 *Prince Edward Island*

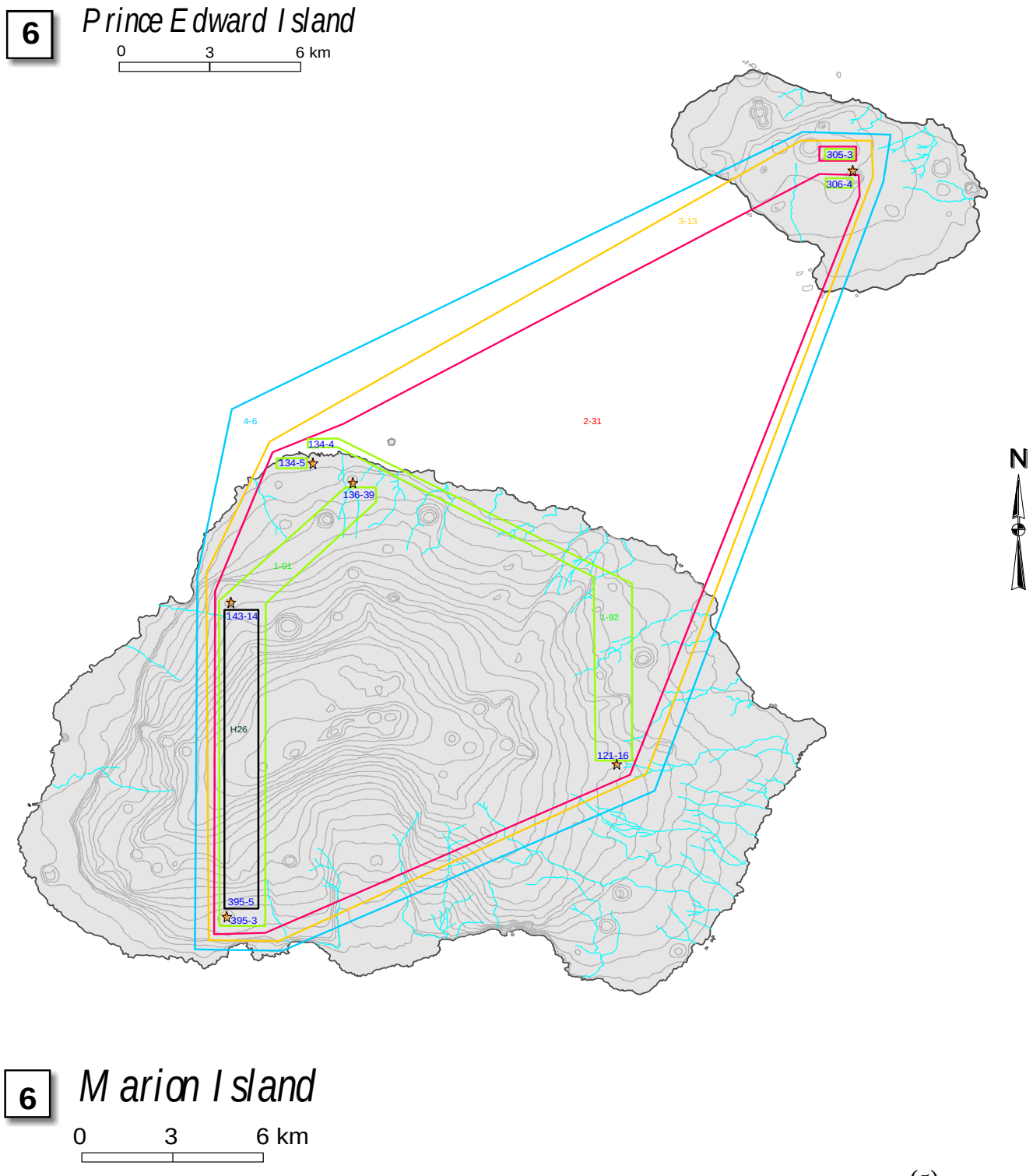
0 3 6 km



6 *Marion Island*

0 3 6 km

(f)



(g)

Fig. 14 *Ectemnorhinus* individuals that group into 4-step clade 4-6 mapped onto the islands according to a) sampling localities with place names for references, samples on Marion Island are indicated in green while those on Prince Edward Island are indicated in red b) sampling localities as used in the nested design c) those that share the same haplotypes nested into 0-step clades, the d) 1-step clades, e) 2-step clades f) 3 step clades and finally g) the complete 4-6 clade as depicted in Fig. 5.

Fig. 9a shows that the individuals included in nested clade 4-1 are located mainly to the eastern part of MI with some individuals occurring on PEI. Although 23 haplotypes were detected in clade 4-1 only three are represented by more than one individual (Fig. 9b) and only one of these haplotypes is shared between the two islands. These individuals group into 15 one-step clades (Fig. 9c), five two-step clades (Fig. 9d) and two 3-step clades (Fig. 9e). Nested clade 4-2 consists of just ten individuals the vast majority of which were collected from PEI (Fig. 10a). Within these ten individuals, nine different haplotypes were identified, with the two individuals sharing a haplotype occurring on PEI (Fig. 10b). The individuals that make up nested clade 4-2 group into six nested one-step clades (Fig. 10c), five nested 2-step clades (Fig. 10d) and two nested 3-step clades (Fig. 10e). It is important to note that the 3-step clades also separate the MI individuals from the PEI individuals. Nested clade 4-3 consists of 49 individuals that map mainly to the north-eastern side of MI, again with a few individuals occurring on PEI (Fig. 11a). In Fig. 11b, 30 different haplotypes are apparent with haplotype H34 occurring in 13 individuals. The nested design unfolds further into 12 one-step clades (Fig 11c), five two-step clades (Fig 11d) and two three-step clades (Fig. 11e). Fig. 12a shows that all 20 *E. kucheli* (Grobler *et al*., 2006) individuals of nested clade 4-4 map to PEI while Fig. 12b shows that they represent 14 different haplotypes. Seven 1-step clades (Fig. 12c), four 2-step clades (Fig. 12d) and two 3-step clades (Fig. 12e) all combine into nested clade 4-4 (Fig. 11f). Fig. 13a shows that nested clade 4-5 maps to the whole of MI and as the largest of the 4-step clades, consists of 72 individuals. Nested clade 4-5 has no representatives on PEI and the individuals therein can be grouped into 47 different haplotypes (Fig. 13b), 29 1-step clades (Fig. 13c), 12 2-step clades (Fig. 13d) and three 3-step clades (Fig. 13e). Four of the nine individuals making up nested clade 4-6 map to the north-western side of MI, two to the south-western side of MI, one to the central eastern side of MI and the last two to PEI (Fig. 14a). Only one of the eight haplotypes that occurred in more than one individual was shown to link the individuals from the north-western side of MI to those of the south-western side of MI (Fig. 14b). The remaining subclades of nested clade 4-6 consist of five one-step clades (Fig. 14c), two 2-step clades (Fig. 14d) and a single 3-step clade (Fig 14e).

Phylogenetic analyses

Table 3 shows the jModel Test results obtained for the whole data set, the two islands individually, as well as the different 4-step nested clades. Two phylogenetic trees containing all the individuals that are represented in the nested clade analyses are presented. Fig. 15 shows the tree obtained from the Minimum Evolution analyses while Fig. 16 shows the tree obtained from the Bayesian analyses. All the four-step nested clades are retained in the trees, except nested clade 4-2 and nested clade 4-5, which were each divided into 'a' and 'b' subclades. Individuals grouping in nested clades 4-1, 4-3 and 4-4 also group together in both trees with high levels of support. Individuals grouping together in nested clade 4-6 also group together in both trees. Although clade 4-6 does not have high bootstrap support in the Minimum Evolution tree (Fig. 15), it has 93 % support in the Bayesian tree (Fig. 16). Clade 4-2 in the nested design shows that sequences 121-7 and 157-8 (clade 2b) are from MI while the rest of the sequences (clade 2a) are from PEI and that the individuals from the different islands separates from each other even at the 3-step level (Fig. 10e). Both clades 2a and 2b have high levels of support in both trees (Figs. 15& 16). Nested clade 4-5 separates in both the Minimum Evolution (Fig. 15) and the Bayesian trees (Fig. 16) into clades 4-5a and 4-5b. Although neither clades 4-5a nor 4-5b have bootstrap support of more than 50% in the Minimum Evolution tree (Fig. 15), clade 4-5a has 94 % support and clade 4-5b has 85 % support in the Bayesian tree (Fig.16). The nested design (Fig. 6) shows that individuals grouping in clade 4-5b separate from those grouping in clade 4-5a by at least six missing haplotypes. Other 3-step nested clades are also easily recognisable in the trees. As the minimum evolution tree did not recover any of the internal nodes with high levels of support, this analysis gave no information about the relationship between the major clades. The Bayesian analyses (Fig. 16) on the other hand, clarifies the relationship between the major clades, where individuals grouping together in nested clade 4-4 are basal to the rest of the tree, as suggested by Grobler *et al*. (2006), and all the other clades also group together with 95% support.

Table 3. jModelTest results obtained for the different datasets. In each case the outgroup was included. I = Proportion of invariable sites and G= Gamma distribution shape parameter

Dataset	Model selected	Base frequencies				Substitution model						I	G
		A	C	G	T	A-C	A-G	A-T	C-G	C-T	G-T		
Total Cladogram	TIM3+I+G	0.3111	0.1671	0.1296	0.3921	2.7910	46.0397	1.0000	2.7910	15.6917	1.0000	0.4090	0.2410
MI dataset	TIM3+I+G	0.3129	0.1668	0.1291	0.3912	2.5888	38.6352	1.0000	2.5888	13.5356	1.0000	0.4360	0.2480
PEI dataset	TrN+I	0.3098	0.1682	0.1309	0.3911	1.0000	37.0083	1.0000	1.0000	10.9496	1.0000	0.8220	Equal rates for all sites
Nested clade 4-1	TIM1+I	0.3027	0.1671	0.1390	0.3913	1.0000	92.6399	5.0722	5.0722	29.0701	1.0000	0.8470	Equal rates for all sites
Nested clade 4-2	TPM2uf+I	0.3016	0.1653	0.1405	0.3927	9.4493	47.3177	9.4493	1.0000	47.3177	1.0000	0.7550	Equal rates for all sites
Nested clade 4-3	TPM1uf+I+G	0.3110	0.1597	0.1409	0.3883	1.0000	885.2974	59.7997	59.7997	885.2974	1.0000	0.6660	0.2320
Nested clade 4-4	TPM2uf+G	0.3014	0.1663	0.1426	0.3897	172.1505	1175.5851	172.1505	1.0000	1175.5851	1.0000	-	0.1560
Nested clade 4-5	HKY+I+G	0.3095	0.1672	0.1462	0.3772	Transitions/ Transversions = 14.0814				8.9150	1.0000	0.8540	0.4590
Nested clade 4-6	TPM2uf+G	0.3040	0.1655	0.1427	0.3878	216.8281	1008.4432	216.8281	1.0000	1008.4432	1.0000	-	0.1210

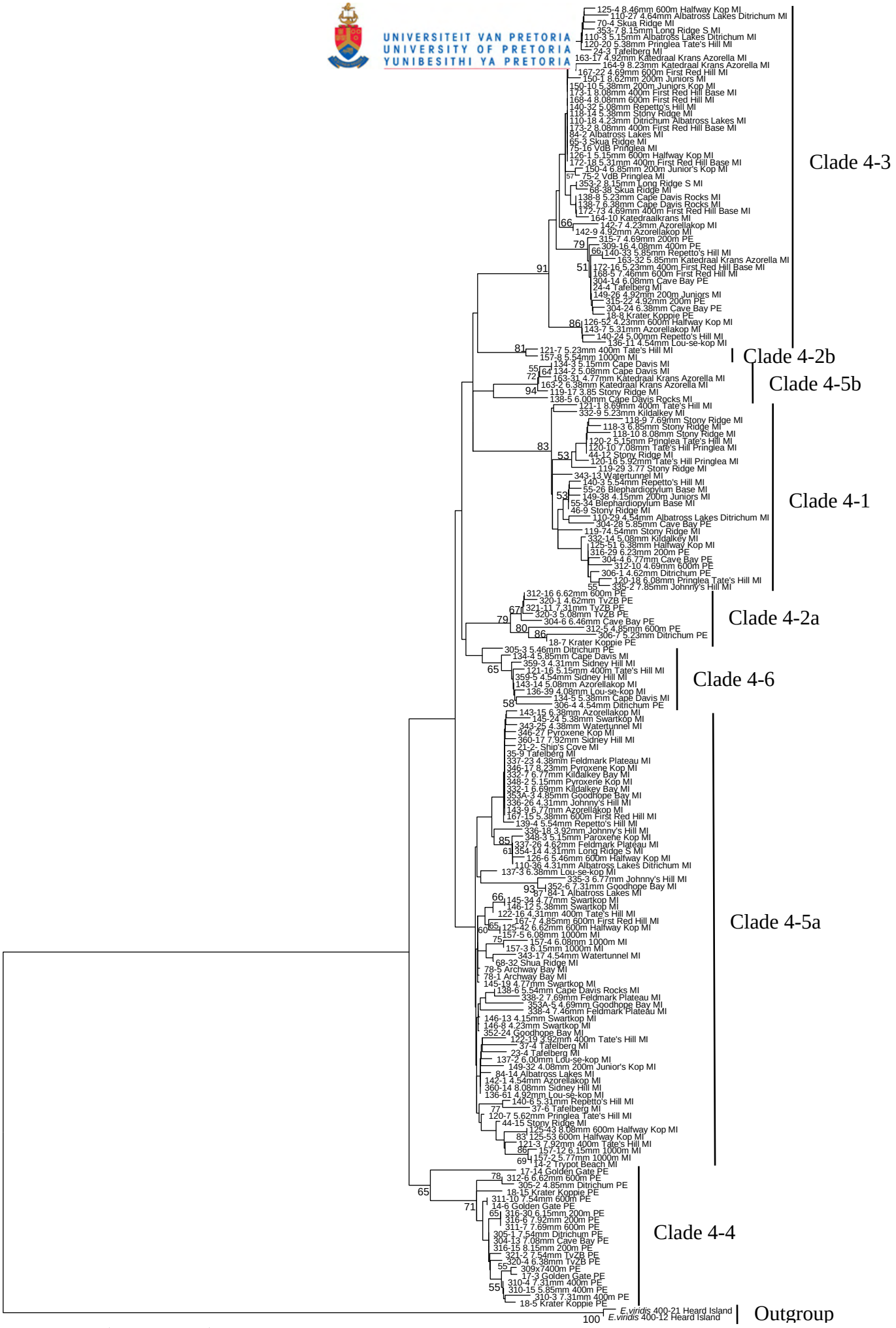
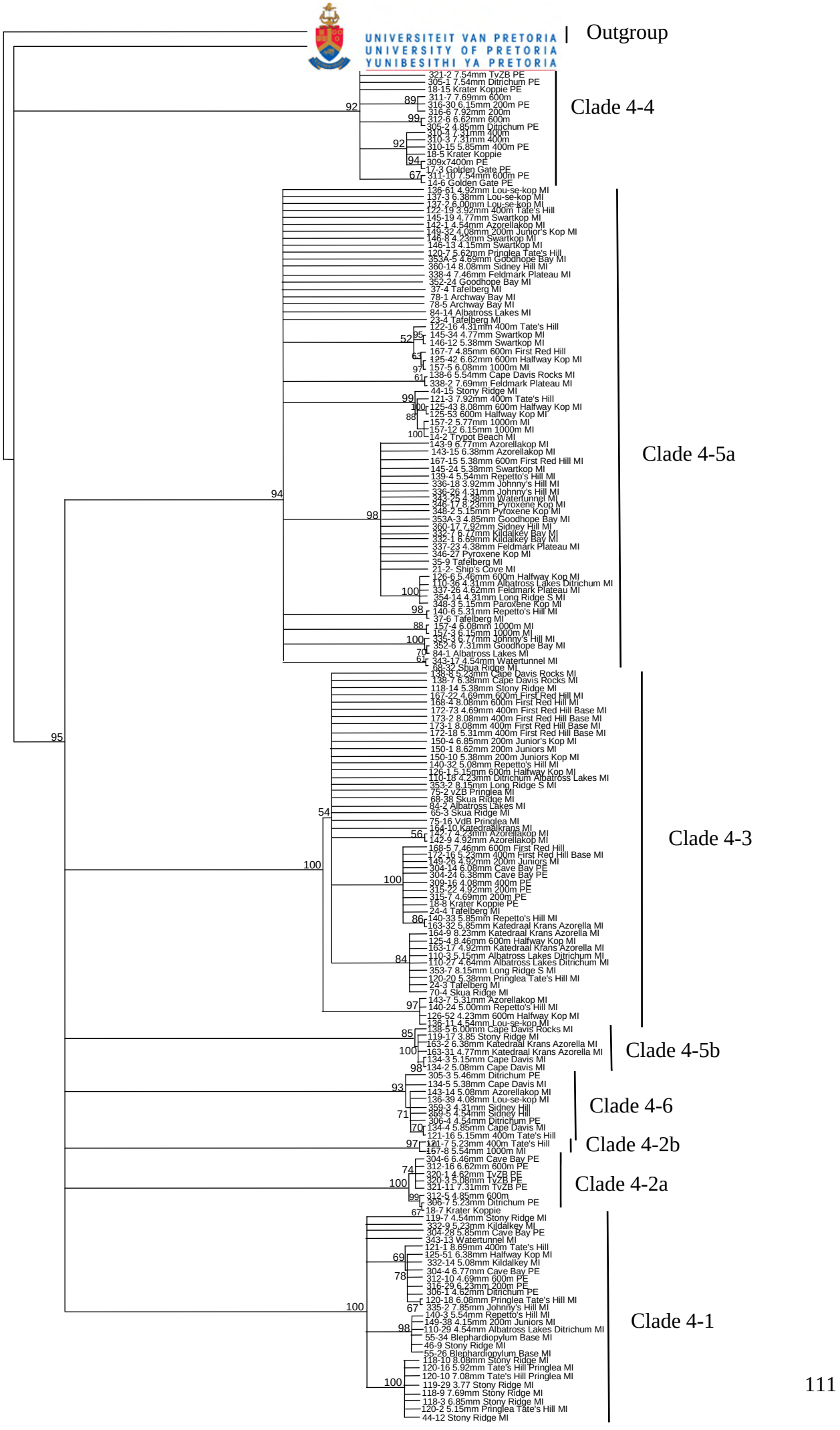

125-4 8.46mm 600m Halfway Kop MI
110-27 4.64mm Albatross Lakes Ditrichum MI
70-4 Skua Ridge MI
353-7 8.15mm Long Ridge S MI
110-3 5.15mm Albatross Lakes Ditrichum MI
120-20 5.38mm Pringlea Tate's Hill MI
24-3 Tafelberg MI
163-17 4.92mm Katedraal Krans Azorella MI
164-9 8.23mm Katedraal Krans Azorella MI
167-22 4.69mm 600m First Red Hill MI
150-1 8.62mm 200m Juniors MI
150-10 5.38mm 200m Juniors Kop MI
173-1 8.08mm 400m First Red Hill Base MI
168-4 8.08mm 600m First Red Hill MI
140-32 5.08mm Repetto's Hill MI
118-14 5.38mm Stony Ridge MI
110-18 4.23mm Ditrichum Albatross Lakes MI
173-2 8.08mm 400m First Red Hill Base MI
84-2 Albatross Lakes MI
65-3 Skua Ridge MI
75-16 VdB Pringlea MI
126-1 5.15mm 600m Halfway Kop MI
172-18 5.31mm 400m First Red Hill Base MI
150-4 6.85mm 200m Junior's Kop MI
75-2 VdB Pringlea MI
353-2 8.15mm Long Ridge S MI
68-38 Skua Ridge MI
138-8 5.23mm Cape Davis Rocks MI
138-7 6.38mm Cape Davis Rocks MI
172-73 4.69mm 400m First Red Hill Base MI
164-10 Katedraalkrans MI
142-7 4.23mm Azorellakop MI
142-9 4.92mm Azorellakop MI
315-7 4.69mm 200m PE
309-16 4.08mm 400m PE
140-33 5.85mm Repetto's Hill MI
163-32 5.85mm Katedraal Krans Azorella MI
172-16 5.23mm 400m First Red Hill Base MI
168-5 7.46mm 600m First Red Hill MI
304-14 6.08mm Cave Bay PE
24-4 Tafelberg MI
149-26 4.92mm 200m Juniors MI
315-22 4.92mm 200m PE
304-24 6.38mm Cave Bay PE
18-8 Krater Koppie PE
126-52 4.23mm 600m Halfway Kop MI
143-7 5.31mm Azorellakop MI
140-24 5.00mm Repetto's Hill MI
136-11 4.54mm Lou-se-kop MI
121-7 5.23mm 400m Tate's Hill MI
157-8 5.54mm 1000m MI
134-3 5.15mm Cape Davis MI
134-2 5.08mm Cape Davis MI
163-31 4.77mm Katedraal Krans Azorella MI
163-2 6.38mm Katedraal Krans Azorella MI
119-17 3.85 Stony Ridge MI
138-5 6.00mm Cape Davis Rocks MI
121-1 8.69mm 400m Tate's Hill MI
332-9 5.23mm Kildalkey MI
118-9 7.69mm Stony Ridge MI
118-3 6.85mm Stony Ridge MI
118-10 8.08mm Stony Ridge MI
120-2 5.15mm Pringlea Tate's Hill MI
120-10 7.08mm Tate's Hill Pringlea MI
44-12 Stony Ridge MI
120-16 5.92mm Tate's Hill Pringlea MI
119-29 3.77 Stony Ridge MI
343-13 Watertunnel MI
140-3 5.54mm Repetto's Hill MI
55-26 Blephardiopylum Base MI
149-38 4.15mm 200m Juniors MI
55-34 Blephardiopylum Base MI
46-9 Stony Ridge MI
110-29 4.54mm Albatross Lakes Ditrichum MI
304-28 5.85mm Cave Bay PE
119-74 5.54mm Stony Ridge MI
332-14 5.08mm Kildalkey MI
125-51 6.38mm Halfway Kop MI
316-29 6.23mm 200m PE
304-4 6.77mm Cave Bay PE
312-10 4.69mm 600m PE
306-1 4.62mm Ditrichum PE
120-18 6.08mm Pringlea Tate's Hill MI
335-2 7.85mm Johnny's Hill MI
312-16 6.62mm 600m PE
320-1 4.62mm TvZB PE
321-11 7.31mm TvZB PE
320-3 5.08mm TvZB PE
304-6 6.46mm Cave Bay PE
312-5 4.85mm 600m PE
306-7 5.23mm Ditrichum PE
18-7 Krater Koppie PE
305-3 5.46mm Ditrichum PE
134-4 5.85mm Cape Davis MI
359-3 4.31mm Sidney Hill MI
121-16 5.15mm 400m Tate's Hill MI
359-5 4.54mm Sidney Hill MI
143-14 5.08mm Azorellakop MI
136-39 4.08mm Lou-se-kop MI
134-5 5.38mm Cape Davis MI
306-4 4.54mm Ditrichum PE
143-15 6.38mm Azorellakop MI
145-24 5.38mm Swartkop MI
343-25 4.38mm Watertunnel MI
346-27 Pyroxene Kop MI
360-17 7.92mm Sidney Hill MI
21-2- Ship's Cove MI
35-9 Tafelberg MI
337-23 4.38mm Feldmark Plateau MI
346-17 8.23mm Pyroxene Kop MI
332-7 6.77mm Kildalkey Bay MI
348-2 5.15mm Pyroxene Kop MI
332-1 6.69mm Kildalkey Bay MI
353A-3 4.85mm Goodhope Bay MI
336-26 4.31mm Johnny's Hill MI
143-9 6.77mm Azorellakop MI
167-15 5.38mm 600m First Red Hill MI
139-4 5.54mm Repetto's Hill MI
336-18 3.92mm Johnny's Hill MI
348-3 5.15mm Paroxene Kop MI
337-26 4.62mm Feldmark Plateau MI
354-14 4.31mm Long Ridge S MI
126-6 5.46mm 600m Halfway Kop MI
110-36 4.31mm Albatross Lakes Ditrichum MI
137-3 6.38mm Lou-se-kop MI
335-3 6.77mm Johnny's Hill MI
352-6 7.31mm Goodhope Bay MI
84-1 Albatross Lakes MI
145-34 4.77mm Swartkop MI
146-12 5.38mm Swartkop MI
122-16 4.31mm 400m Tate's Hill MI
167-7 4.85mm 600m First Red Hill MI
125-42 6.62mm 600m Halfway Kop MI
157-5 6.08mm 1000m MI
157-4 6.08mm 1000m MI
157-3 6.15mm 1000m MI
343-17 4.54mm Watertunnel MI
68-32 Shua Ridge MI
78-5 Archway Bay MI
78-1 Archway Bay MI
145-19 4.77mm Swartkop MI
138-6 5.54mm Cape Davis Rocks MI
338-2 7.69mm Feldmark Plateau MI
353A-5 4.69mm Goodhope Bay MI
338-4 7.46mm Feldmark Plateau MI
146-13 4.15mm Swartkop MI
146-8 4.23mm Swartkop MI
352-24 Goodhope Bay MI
122-19 3.92mm 400m Tate's Hill MI
37-4 Tafelberg MI
23-4 Tafelberg MI
137-2 6.00mm Lou-se-kop MI
149-32 4.08mm 200m Junior's Kop MI
84-14 Albatross Lakes MI
142-1 4.54mm Azorellakop MI
360-14 8.08mm Sidney Hill MI
136-61 4.92mm Lou-se-kop MI
140-6 5.31mm Repetto's Hill MI
37-6 Tafelberg MI
120-7 5.62mm Pringlea Tate's Hill MI
44-15 Stony Ridge MI
125-43 8.08mm 600m Halfway Kop MI
83 125-53 600m Halfway Kop MI
121-3 7.92mm 400m Tate's Hill MI
157-12 6.15mm 1000m MI
157-2 5.77mm 1000m MI
14-2 Trypot Beach MI
17-14 Golden Gate PE
312-6 6.62mm 600m PE
305-2 4.85mm Ditrichum PE
18-15 Krater Koppie PE
311-10 7.54mm 600m PE
14-6 Golden Gate PE
316-30 6.15mm 200m PE
316-6 7.92mm 200m PE
311-7 7.69mm 600m PE
305-1 7.54mm Ditrichum PE
304-13 7.08mm Cave Bay PE
316-15 8.15mm 200m PE
321-2 7.54mm TvZB PE
320-4 6.38mm TvZB PE
309x7400m PE
17-3 Golden Gate PE
310-4 7.31mm 400m PE
310-15 5.85mm 400m PE
310-3 7.31mm 400m PE
18-5 Krater Koppie PE
E.viridis 400-21 Heard Island
E.viridis 400-12 Heard Island
Clade 4-3
Clade 4-2b
Clade 4-5b
Clade 4-1
Clade 4-2a
Clade 4-6
Clade 4-5a
Clade 4-4
Outgroup

Fig. 15 Minimum Evolution (ME) tree inferred using the Tamura-Nei distance correction algorithm. In each case, the sample number is given followed by the body length measurement, vegetation type it was collected from, locality it was collected from, sex and island of origin. 'MI' denotes Marion Island samples whilst Prince Edward Island samples are indicated by 'PEI'. Nodal support obtained following 100 000 bootstrap replications above 50% is indicated. TvZB indicates samples collected at the top of Van Zinderen Bakker Peak at an elevation of 672 m above sea level. (On page 109)

Fig. 16 Bayesian phylogenetic analyses. For each taxon, the sample number is given followed by the body length measurement, vegetation type it was collected from, locality it was collected from, sex and island of origin. 'MI' denotes Marion Island samples whilst Prince Edward Island samples are indicated by 'PEI'. TvZB indicates samples collected at the top of Van Zinderen Bakker Peak at an elevation of 672 m above sea level. (On page 111)


Outgroup
Clade 4-4
Clade 4-5a
Clade 4-3
Clade 4-5b
Clade 4-6
Clade 4-2b
Clade 4-2a
Clade 4-1

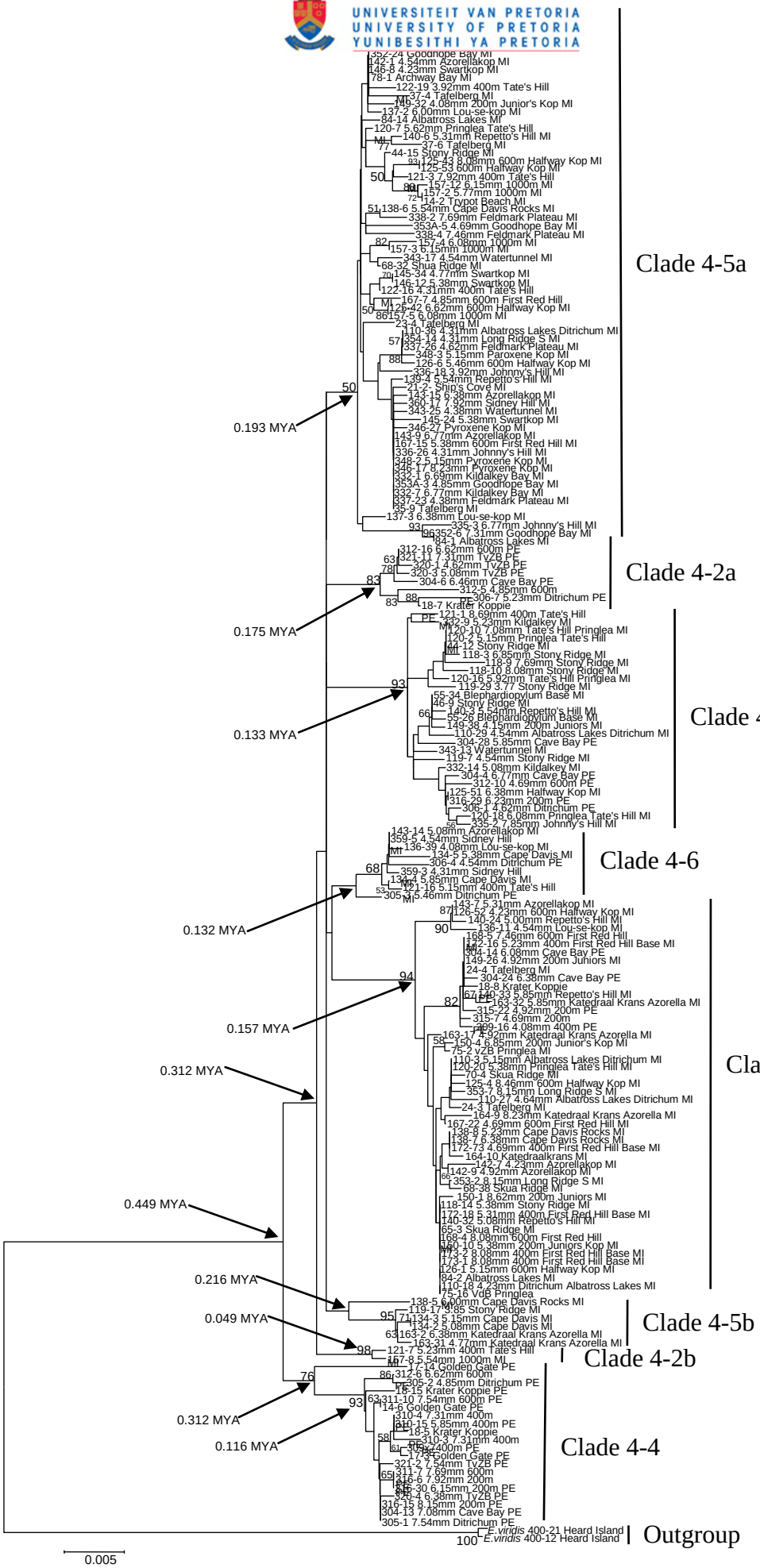
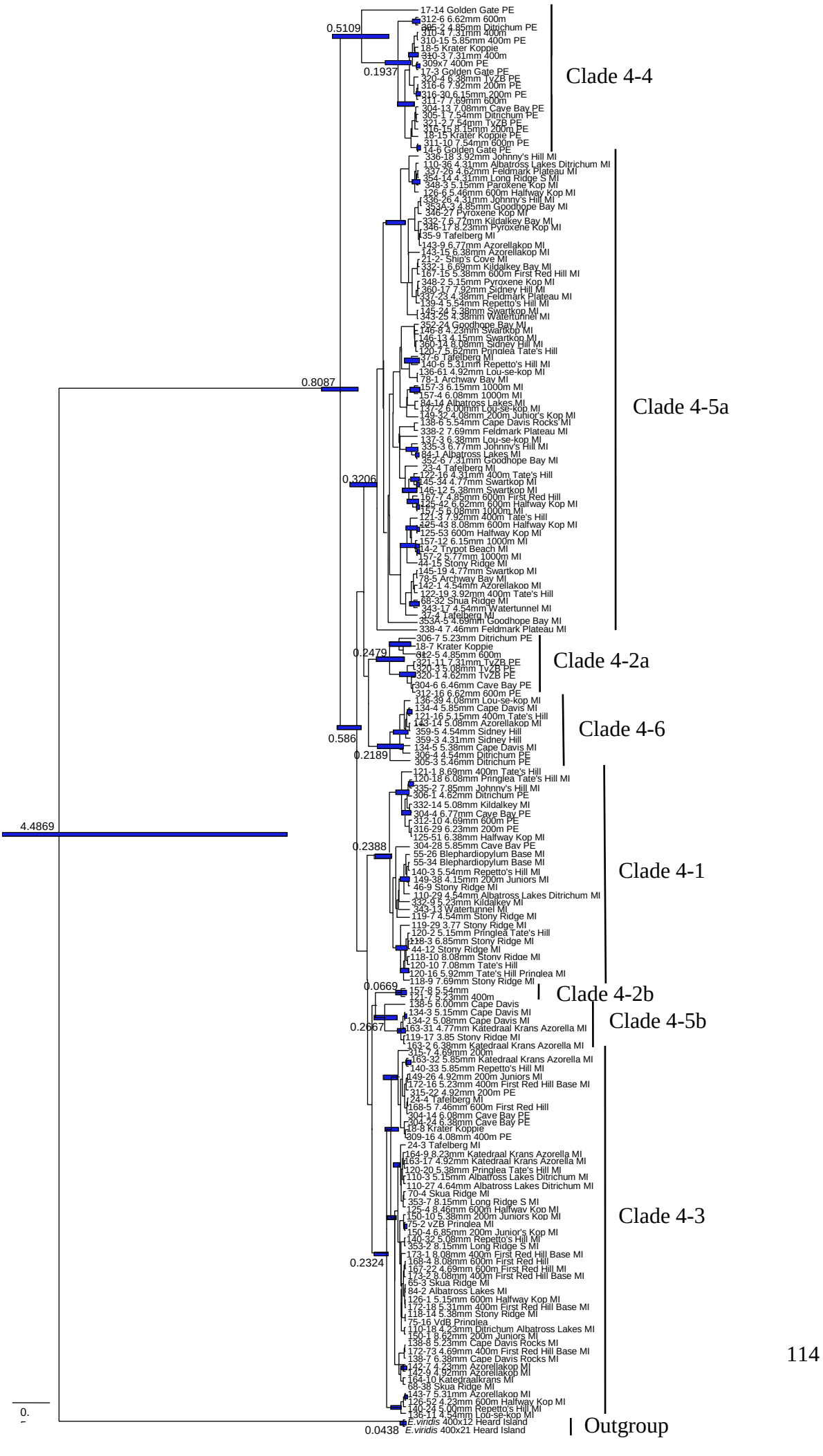

Clade 4-5a
Clade 4-2a
Clade 4-1
Clade 4-6
Clade 4-3
Clade 4-5b
Clade 4-2b
Clade 4-4
Outgroup
0.193 MYA
0.175 MYA
0.133 MYA
0.132 MYA
0.157 MYA
0.312 MYA
0.449 MYA
0.216 MYA
0.049 MYA
0.312 MYA
0.116 MYA
E.viridis 400-21 Heard Island
E.viridis 400-12 Heard Island
0.005

Fig. 17 Neighbour Joining (NJ) tree inferred using uncorrected p-distance values. For each taxon, the sample number is followed by the body length measurement, vegetation type it was collected from, locality it was collected from, sex, and its island of origin. 'MI' denotes Marion Island samples whilst Prince Edward Island samples are indicated by 'PEI'. Nodal support obtained following 100 000 bootstrap replications above 50% is indicated next to the relevant nodes. Time to lineage coalescence was estimated using a 2.3 % nucleotide sequence divergence per million years molecular clock calibration, based on the arthropod mtDNA survey of Brower (1994). Estimated time of coalescence is indicated by arrows. (On page 112)

Fig. 18 Ultrametric tree obtained with BEAST 1.6.1 (Drummond & Rambaut 2007) (On page 114). The numbers in the nodes 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), based on the arthropod mtDNA survey of Brower (1994), was used. The BEAST 1.6.1 (Drummond & Rambaut, 2007) statistics as analyzed with Tracer v1.5 (Drummond & Rambaut, 2007) are summarised in Table 4.


0.5109
0.1937
0.8087
0.3206
0.2479
0.586
0.2189
4.4869
0.2388
0.0669
0.2667
0.2324
0.0438
Clade 4-4
Clade 4-5a
Clade 4-2a
Clade 4-6
Clade 4-1
Clade 4-2b
Clade 4-5b
Clade 4-3
Outgroup
E.viridis 400x12 Heard Island
E.viridis 400x21 Heard Island

A neighbour joining p-distance tree with *E. viridis* as outgroup is presented in Fig. 17. The molecular clock, with a clock rate of 2.3 % sequence divergence per million years (MY) based on the arthropod mtDNA survey of Brower (1994), was utilised to determine the time when the individuals in each 4-step clade last shared a common ancestor so as to give a rough indication of when each of the 4-step clades originated on the PEIA. A Neighbour-Joining p-distance tree (Fig. 17) as well as an ultrametric tree obtained with BEAST (Fig. 18) to estimate times of coalescence were utilised. The results obtained are presented in Table 5. All BEAST estimates are older than those estimated by Neighbour-Joining uncorrected p-distance values. In each case, clade 4-4, representing *E. kucheli* (Grobler *et al*., 2006), exhibits to oldest coalescence times with nested clade 4-5, consisting of clades 4-5a and 4-5b, exhibiting the next oldest coalescence times. This supports the theory that nested clade 4-5 was the first to colonize Marion Island.

Table 4 BEAST 1.6.1 (Drummond & Rambaut, 2007) statistics as analyzed with Tracer v1.5 (Drummond & Rambaut, 2007).

Statistic	Mean	ESS
posterior	-3768.93	961.232
prior	-401.868	288.1
likelihood	-3367.062	1592.394
treeModel.rootHeight	4.593	13816.543
tmrca(4-1)	0.225	2918.564
tmrca(4-2a)	0.235	6186.949
tmrca(4-2b)	5.668E-2	11594.391
tmrca(4-3)	0.218	3832.055
tmrca(4-5a)	0.309	2837.015
tmrca(4-5b)	0.268	2842.287
tmrca(4-6)	0.21	4599.945
tmrca(Outgroup)	3.245E-2	4334.886
tmrca(4-4)	0.514	8447.389
constant.popSize	3.057	5423.428
ac	0.163	3522.169
ag	2.888	1715.709
at	8.15E-2	3099.201
cg	0.195	1447.834
gt	1.82E-2	2102.082
alpha	1.01	916.967
pInv	0.681	494.606
clock.rate	2.3E-2	-
treeLikelihood	-3367.062	1592.394
coalescent	-396.494	5675.378

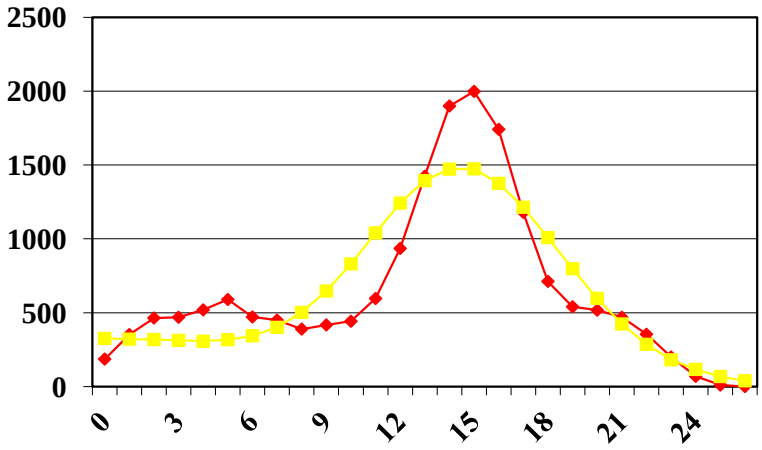
Table 5 Estimates of lineage coalescence for the major clades as determined by Neighbour-Joining (NJ) uncorrected p-distance values (Fig. 17) and BEAST (Fig. 18) using a clock rate of 2.3 % sequence divergence per million years (MY) based on the arthropod mtDNA survey of Brower (1994).

Clade	**NJ estimates**	**BEAST estimates**
Clade 4-1	0.133 MYA	0.2388 MYA
Clade 4-2a	0.175 MYA	0.2479 MYA
Clade 4-2b	0.049 MYA	0.0669 MYA
Clade 4-3	0.157 MYA	0.2324 MYA
Clade 4-4 (*E. kucheli*)	0.312 MYA	0.5109 MYA
Clade 4-5a	0.193 MYA	0.3206 MYA
Clade 4-5b	0.216 MYA	0.2667 MYA
Clade 4-6	0.132 MYA	0.2189 MYA

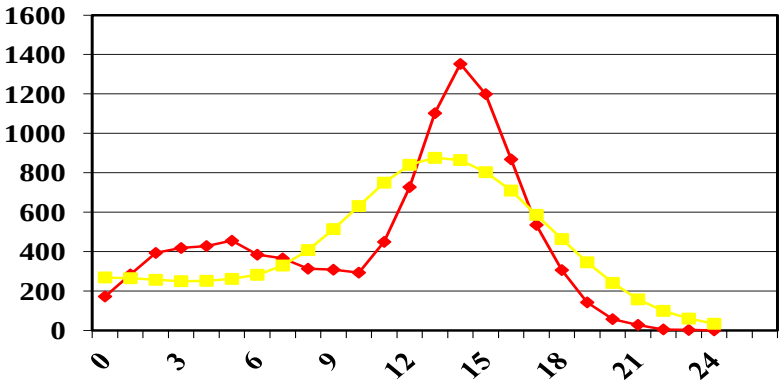
Mismatch analysis and population expansion

Fig. 19 a-j comprises of mismatch distributions under the sudden expansion model for the archipelago as a whole, each island on its own as well as the mismatch distributions of each of the eight discrete clades as identified in the phylogenetic trees (Figs. 15 & 16). Fig. 20 a-j, on the other hand, comprises the mismatch distributions under the spatial expansion model, of the archipelago as a whole, each island on its own as well as the mismatch distributions of each of the eight discrete clades as identified in the phylogenetic trees (Figs. 15 & 16). Both SSD and raggedness index values are shown in Table 6 under the sudden expansion model and in Table 7 under the spatial expansion model. None of these values shows any significant deviation from either the sudden expansion model or the spatial expansion model ($P > 0.05$), indicating a recent population expansion for each island as well as for each group on its own. In each case, demographic expansions predate range expansions, except in the cases of clades 4-2b and 4-6. The earliest demographic expansion for the different clade populations is estimated to have occurred more than 100 000 years ago (clades 4-1, 4-3 & 4-5a) with the most recent demographic expansion occurring more than 40 000 years ago (clades 4-b & 4-6). The earliest range expansions occurred approximately 170 000 years ago in clade 4-2b and the most recent approximately 40 000 years ago in clade 4-5b. Under the assumption of a purely demographic expansion, the populations represented by each clade were inferred to have expanded from small effective female population sizes to large effective female population sizes. Clades 4-5a & 4-6 needed larger initial effective female population sizes in order to explain demographic expansion. Under the assumption of a spatial expansion, populations represented in clades 4-5a, 4-3 & 4-2a required large effective female population sizes and no genetic exchange with neighboring populations to explain the inferred spatial expansion, while the population that is represented by clade 4-6 required a large effective female population size and very little genetic exchange with neighboring populations. The population represented by clade 4-5b required a small effective female population size and extensive genetic exchange with neighboring populations to explain the inferred spatial expansion. This is presumably due to individual 138-5 that may be from another population that was poorly sampled. Clade 4-5b also nested with clade 4-5a. Clade 4-4 also required a degree of genetic exchange with neighboring populations to explain the inferred spatial expansion, and this may possibly also be explained by individual 17-14 that is from another poorly sampled population.

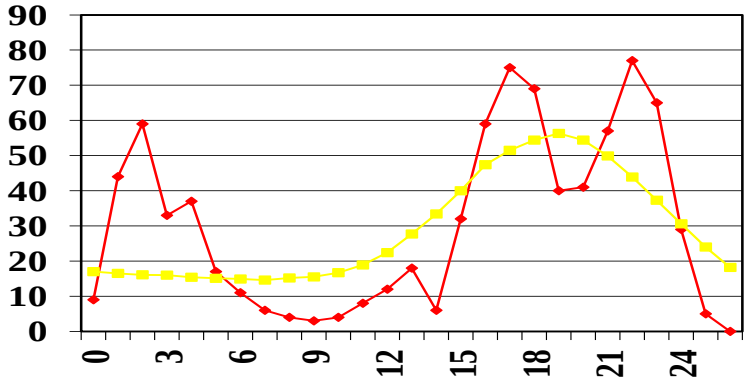
(a)


2500
2000
1500
1000
500
0
0 3 6 9 12 15 18 21 24

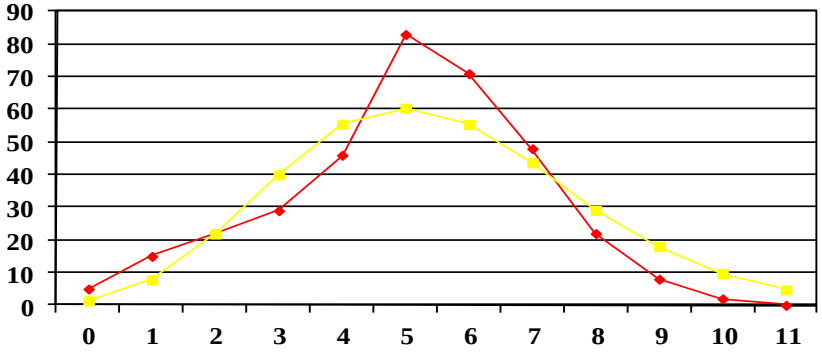
(b)


1600
1400
1200
1000
800
600
400
200
0
0 3 6 9 12 15 18 21 24

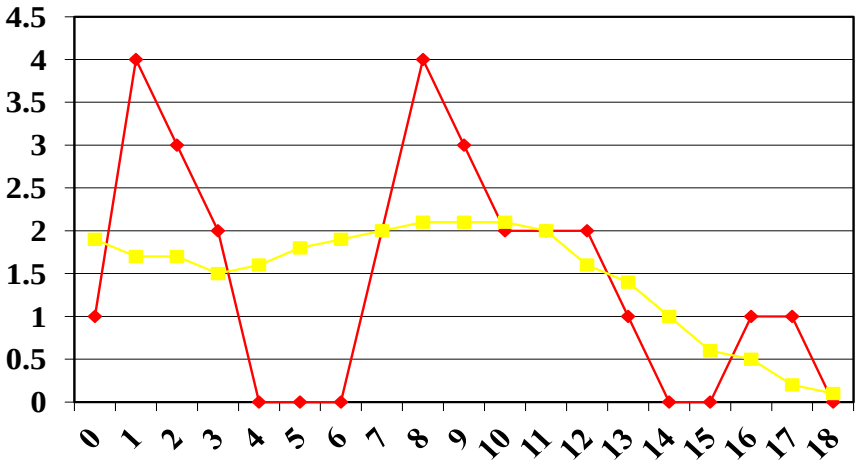
(c)


90
80
70
60
50
40
30
20
10
0
0 3 6 9 12 15 18 21 24

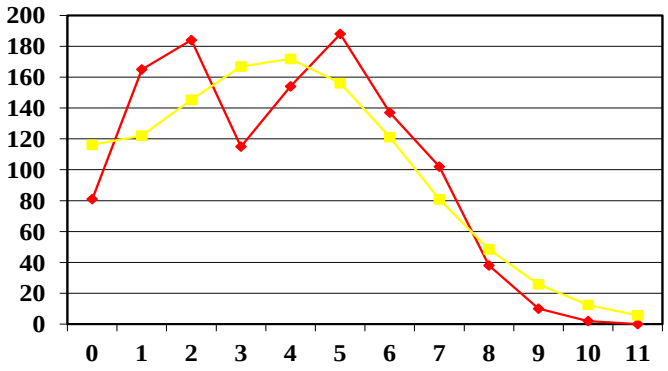
(d)


90
80
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10 11

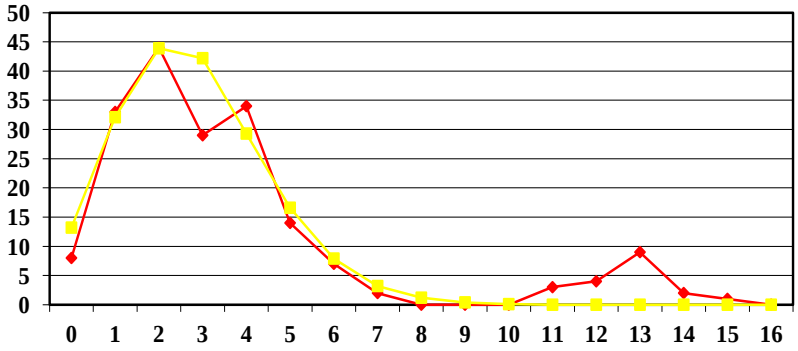
(e)


4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

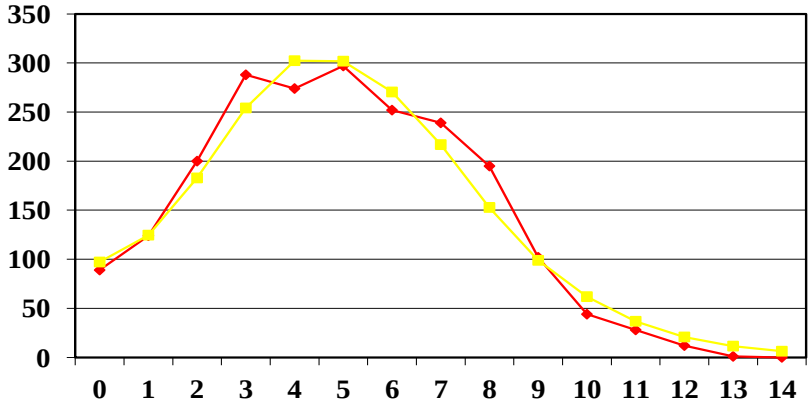
(f)


200
180
160
140
120
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10 11

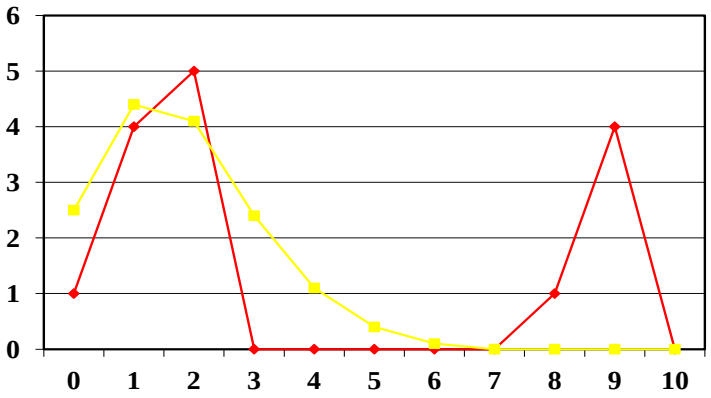
(g)


50
45
40
35
30
25
20
15
10
5
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

(h)


350
300
250
200
150
100
50
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14

(i)



(j)

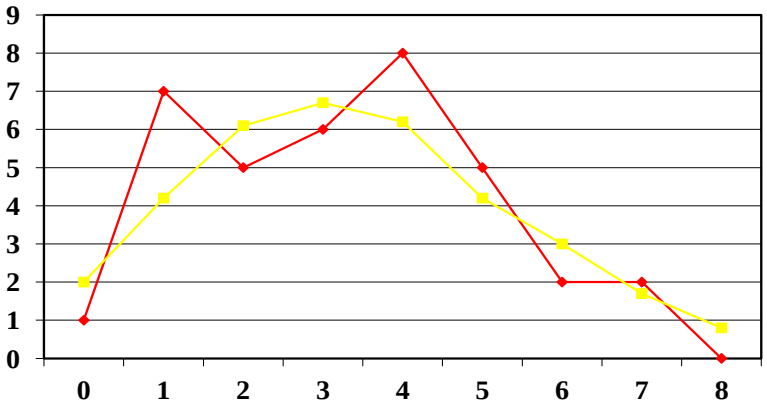
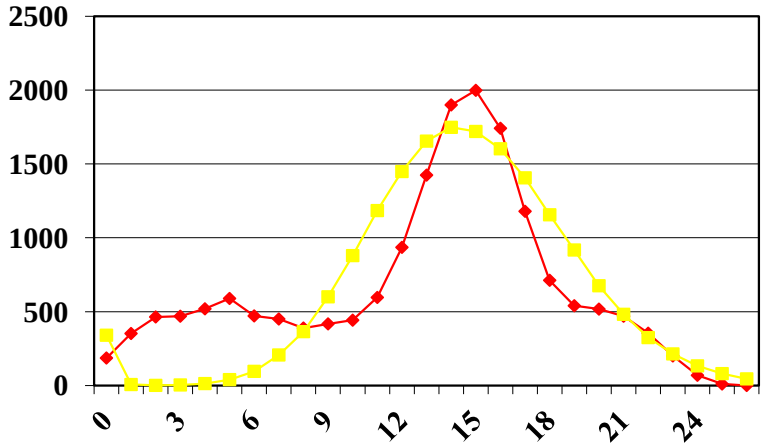


Fig. 19 Mismatch distribution estimated under the sudden expansion model for the different data sets: a) Prince Edward Islands Archipelago (PEIA), b) Marion Island (MI), c) Prince Edward Island (PEI), d) nested clade 4-1, e) clade 4-2a, f) nested clade 4-3, g) nested clade 4-4, h) clade 4-5a and i) clade 4-5b j) nested clade 4-6. The yellow line represents the simulated stepwise expansion model and the red line represents the observed data. No mismatch analyses were possible for clade 4-2b because of a small sample size.

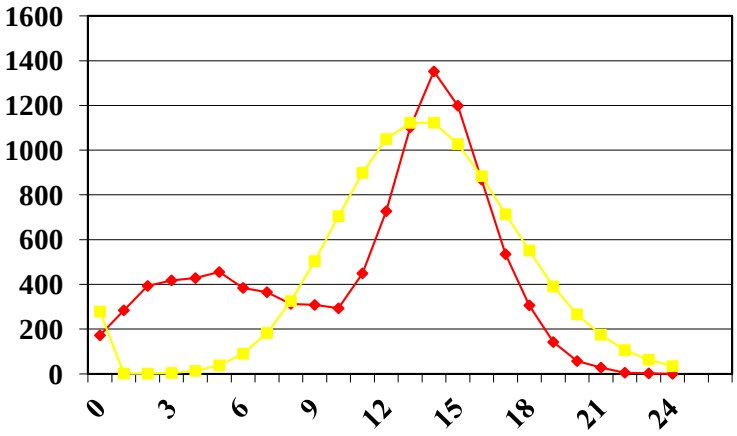
Table 6 Mismatch parameters estimated under the sudden expansion model: τ is the expansion parameter or date of the Growth or Decline measured in units of mutational time with $\tau = 2ut$ where u is the mutation rate and t is the time in generations. The timing of the most important expansion in each group ($t_{divergence}$) was calculated based on the equation $\tau = 2\mu t$. θ_0 and θ_1 are the substitution rates before and after expansion. SSD is the test of the validity of a stepwise expansion model based on the sum of the square deviations between the observed and the expected mismatch. The raggedness index quantifies the smoothness of the observed pairwise differences distribution. PEIA = Prince Edward Islands Archipelago; PEI = Prince Edward Island; MI = Marion Island. The significance of both SSD and the raggedness index are estimated with a parametric bootstrap approach (probability values: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Population	Mean number of differences	Mismatch observed variance	τ (alpha = 0.100 confidence values)	$t_{divergence}$ (years)	θ_0/ $N_{ft=0}$	θ_1/ $N_{ft=0}$	SSD	P(Sim. SSD ≥ Obs. SSD)	Raggedness index	P(Sim. Rag. ≥ Obs. Rag.)
PEIA	12.838	30.069	15.448 (10.840-18.299)	379464 (266273-449496)	0.004/ 98	52.266/ 1283861	0.0049468	0.179	0.00453067	0.367
PEI	14.609	59.701	20.406 (13.926-24.506)	501252 (342078-601965)	0.002/ 49	48.091/ 1181306	0.0127341	0.095	0.01198245	0.297
MI	11.087	25.953	14.458 (9.697-17.828)	355146 (238197-437926)	0.002/ 49	38.125/ 936502	0.0088584	0.131	0.00598689	0.414
4-1	5.057	3.894	5.494 (4.232-6.676)	134954 (103954-163989)	0.000/ 0	>99999.0/ $>2\times10^9$	0.0103789	0.112	0.02792997	0.177
4-2a	7.179	23.411	10.586 (4.113-15.930)	260034 (101031-391304)	0.000/ 0	13.398/ 329108	0.0290171	0.781	0.03698980	0.941
4-2b	2.000	0.0000	-	-	-	-	-	-	-	-
4-3	3.728	5.216	4.984 (2.090-7.809)	122426 (51338-191820)	0.032/ 786	9.736/ 239154	0.0071099	0.599	0.01708562	0.822
4-4	3.742	11.272	2.867 (1.539-3.842)	70424 (37803-94374)	0.002/ 49	69.531/ 1707958	0.0090510	0.313	0.04321330	0.465
4-5a	4.912	6.891	4.570 (2.711-8.467)	112257 (66592-207983)	1.106/ 27167	23.008/ 565168	0.0012460	0.865	0.00700866	0.950
4-5b	3.867	13.410	1.771(0.293-3.455)	43502 (7197-84868)	0.000/ 0	>99999.0/ $>2\times10^9$	0.1221409	0.119	0.27111111	0.252
4-6	3.278	3.349	3.033 (1.330-6.082)	47502 (32670-149398)	0.643/ 15794	31.055/ 762834	0.0126028	0.643	0.05169753	0.721

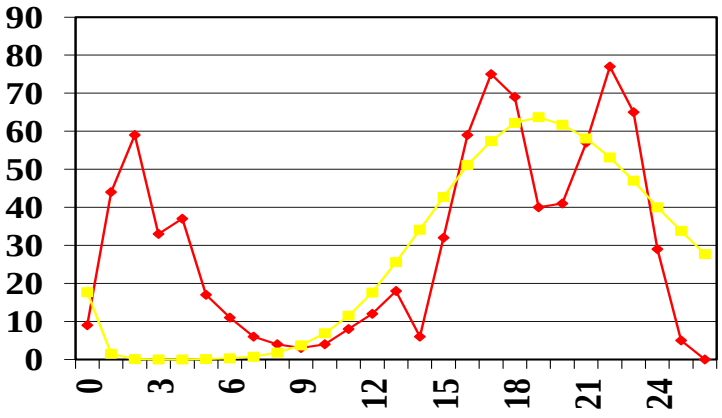
(a)


2500
2000
1500
1000
500
0
0 3 6 9 12 15 18 21 24

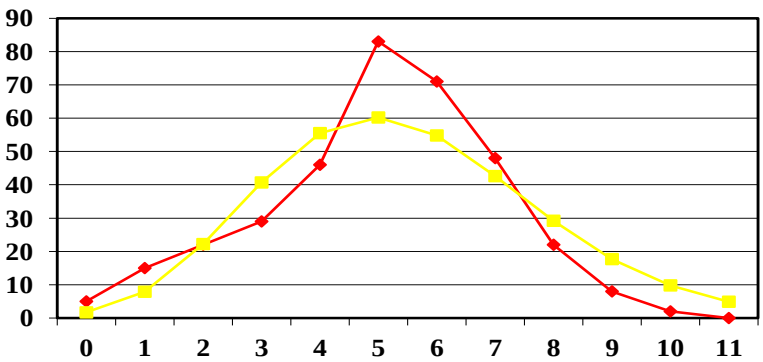
(b)


1600
1400
1200
1000
800
600
400
200
0
0 3 6 9 12 15 18 21 24

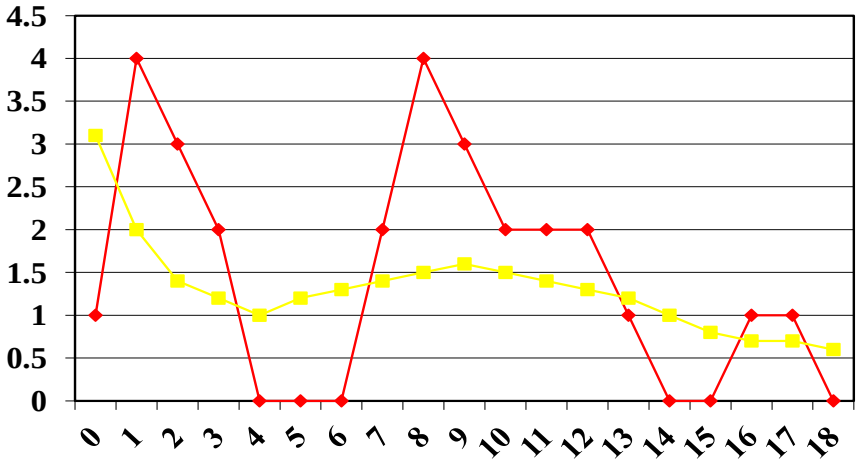
(c)


90
80
70
60
50
40
30
20
10
0
0 3 6 9 12 15 18 21 24

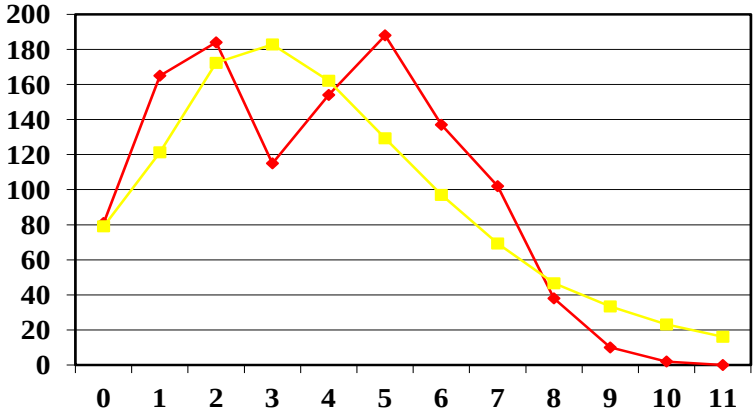
(d)


90
80
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10 11

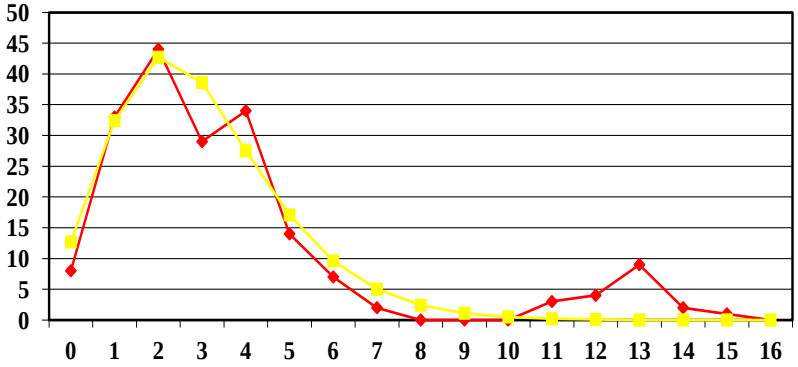
(e)


4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

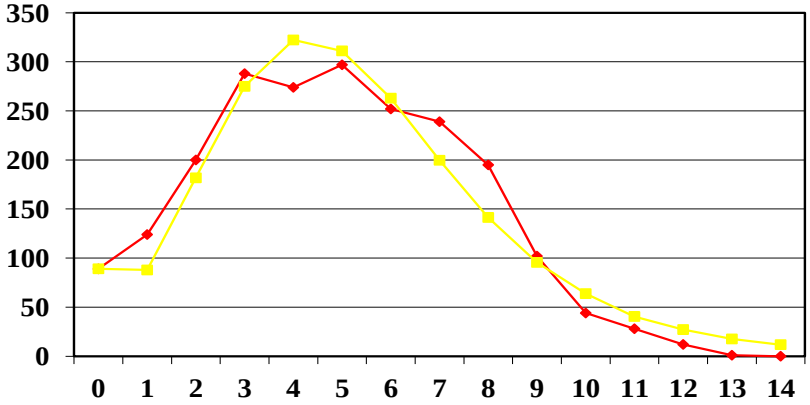
(f)


200
180
160
140
120
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10 11

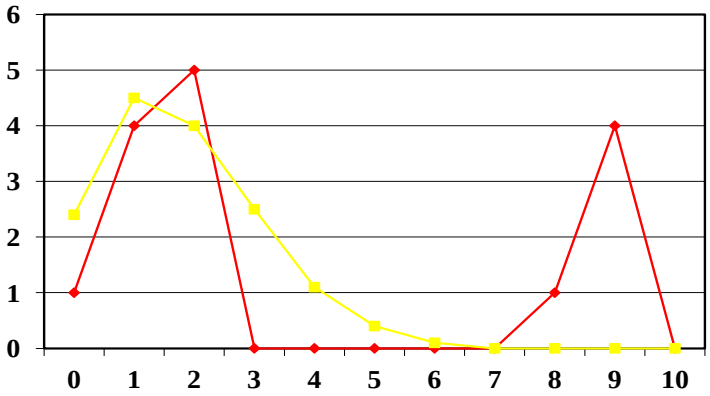
(g)


50
45
40
35
30
25
20
15
10
5
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

(h)


350
300
250
200
150
100
50
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14

(i)



(j)

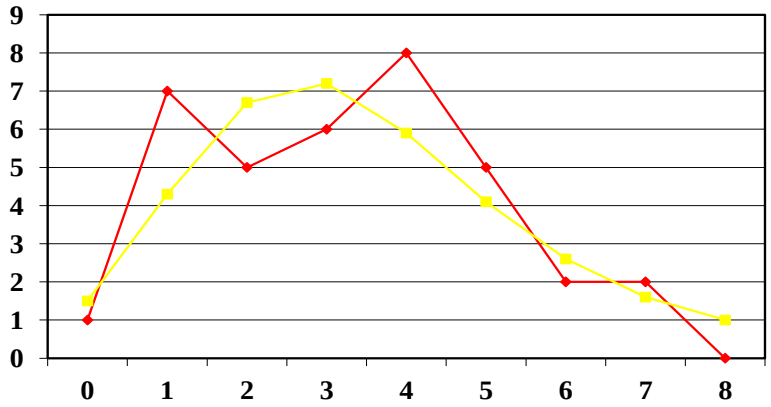


Fig. 20 Mismatch distribution estimated under the spatial expansion model for the different data sets: a) Prince Edward Islands Archipelago (PEIA), b) Marion Island (MI), c) Prince Edward Island (PEI), d) nested clade 4-1, e) clade 4-2a, f) nested clade 4-3, g) nested clade 4-4, h) clade 4-5a and i) clade 4-5b j) nested clade 4-6. The yellow line represents the simulated stepwise expansion model and the red line represents the observed data. No mismatch analyses were possible for clade 4-2b because of a small sample size.

Table 7 Mismatch parameters estimated under the spatial expansion model: τ is the expansion parameter or date of the Growth or Decline measured in units of mutational time with τ = 2*ut* where *u* is the mutation rate, and *t* is the time in generations. The timing of the most important expansion in each group ($t_{divergence}$) was calculated based on the equation τ = 2μ*t*. θ is ancestral population size and *M*= 2*Nm* is an indication of migration. *m* is the rate at which the sampled deme would exchange migrants with a unique population of infinite size. The effective female population size N_f = θ/2*u*. SSD is the test of the validity of a stepwise expansion model based on the sum of the square deviations between the observed and the expected mismatch. The raggedness index quantifies the smoothness of the observed pairwise differences distribution. PEIA = Prince Edward Islands Archipelago; PEI = Prince Edward Island; MI = Marion Island. The significance of both SSD and the raggedness index are estimated with a parametric bootstrap approach (probability values: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Population	Mean number of differences	Mismatch observed variance	τ (alpha = 0.100 confidence values)	$t_{divergence}$ (years)	θ / N_f	*M* / *m*	SSD	P(Sim. SSD ≥ Obs. SSD)	Raggedness index	P(Sim. Rag. ≥ Obs. Rag.)
PEIA	12.838	30.069	14.238 (11.912-16.261)	349742 (292606-399435)	0.884/ 21714.566	49.775/ 0.0011	0.009	0.007**	0.00453067	0.610
PEI	14.609	59.701	17.413 (14.268-21.705)	427732 (350478-533161)	3.507/ 86145.910	40.687/ 0.0002	0.019	0.000***	0.01198245	0.475
MI	11.087	25.953	13.889 (11.028-15.340)	341169 (270891-376811)	0.157/ 3856.546	37.661/ 0.0049	0.015	0.004**	0.00598689	0.685
4-1	5.057	3.894	5.494 (3.301-6.565)	134954 (81085-161262)	0.001/ 24.564	1003.383/ 20.4238	0.010	0.095	0.02792997	0.200
4-2a	7.179	23.411	6.946 (0.741-20.289)	170621 (18201-498378)	6.053/ 148685.83	2.053/ 0.000006	0.034	0.718	0.03698990	0.980
4-2b	2.000	0.0000	-	-	-	-	-	-	-	-
4-3	3.728	5.216	1.964 (0.810-5.255)	48243 (19896-129083)	2.608/ 64062.884	28.924/ 0.0002	0.011	0.271	0.01708562	0.740
4-4	3.742	11.272	2.025 (1.284-4.047)	49742 (31540-99410)	0.034/ 835.176	99999/ 59.867	0.008	0.339	0.04321330	0.473
4-5a	4.912	6.891	3.562 (2.332-6.298)	87496 (57283-154704)	1.923/ 47236.551	28.069/ 0.0003	0.002	0.747	0.00700866	0.966
4-5b	3.867	13.410	1.770 (0.732-3.267)	43478 (17980-80250)	0.001/ 24.564	99999/ 2035.479	0.122	0.057	0.27111111	0.222
4-6	3.278	3.349	2.414 (1.214-5.125)	59297 (29820-125890)	1.215/ 29845.247	99999/ 1.675	0.013	0.590	0.05169753	0.732

Table 8 Population expansion statistics showing Fu's Fs values (probability values: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$), Tajima's D as well as Tajima's estimations for θ. PEIA = Prince Edward Islands Archipelago; PEI = Prince Edward Island; MI = Marion Island.

Population	Fu's F_s test	P(sim_Fs ≤obs_Fs)	Fu's estimations for θ	Tajima's D	P(sim_D ≤obs_D)	Tajima's estimations for θ
Ectemnorhinus						
PEIA	-23.98497***	0.00100	12.83831	-1.38277	0.04600	12.83831
PE	-13.56464***	0.00100	14.60854	-0.33924	0.44300	14.60854
Marion	-24.22080***	0.00100	11.08654	-1.40322	0.04300	11.08654
4-1	-17.73110***	0.00000	5.05698	-1.37102	0.06600	5.05698
4-2a	-1.11105	0.21200	7.17857	-1.49761	0.05900	7.17857
4-2b	0.69315	0.36800	2	0	1	2
4-3	-24.46672***	0.00000	3.72789	-1.55217	0.04200	3.72789
4-4	-6.90308***	0.00100	3.74211	-1.62575	0.04000	3.74211
4-5a	-25.57643***	0.00000	4.91235	-2.10023	0.00200	4.91235
4-5b	-0.80758	0.22100	3.86667	-1.19369	0.13500	3.86667
4-6	-4.03444**	0.01000	3.27778	-1.74560	0.02700	3.27778

Fu's Fs statistic (Table 8) showed significant large negative values for most groups. Only clades 4-2a, 4-2b and 4-5b have no significant large negative values. A significant large negative Fs value is an indication of population expansion. The Fs values for clades 4-2a and 4-5b are still negative and thus show a tendency for population growth (Fu, 1997). The fact that clades 4-2a and 4-5b do not show significant Fs values may be due to some individuals in these clades being separated by up to seven missing haplotypes (Fig. 6). Clade 4-2b is the only clade with a positive Fs value, however as it comprises of only two individuals, it is not possible to compute any reliable statistics. Tajima's (1983) estimate for θ (Table 8) is based on the calculation of the mean number of pairwise differences of the sequences. Tajima's estimate of θ puts more weight on ancient mutations, therefore reflecting ancient population events (Fu, 1997).

Statistical analyses

Haplotype diversity (h) and nucleotide diversity (π) values for the PEIA as a whole, each island, and the different discrete clades as identified in the phylogenetic trees (Fig. 15 and Fig. 16) are summarised in Table 9. In every case, h is very close to 1. The number of haplotypes and parsimony informative sites are also summarised in Table 9. The number of haplotypes in the different clades as identified in the phylogenetic trees (Figs. 15 & 16) varied between 2 and 42, while the number of parsimony informative sites varied between 1 and 31.

Table 10 summarizes the population pairwise distance F_{ST} values as computed in Arlequin version 3.5.1.2 (Excoffier & Lischer, 2010) as well as the absolute number of migrants exchanged between two populations. This was computed between each of the eight discrete clades as identified in the phylogenetic trees (Figs. 15 & 16). F_{ST} assesses the variation in the subpopulations relative to that in the total population and has values between 0 and 1.0. An F_{ST} of zero means that all the subpopulations have the same gene frequencies while an F_{ST} of 1.0 means that the subpopulations have completely non-overlapping sets of alleles. Natural panmictic populations tend to have F_{ST} values that range between near zero up to just greater than 0.5. Values of F_{ST} above approximately 0.2 are considered "high". Consequently, all the Fst values in Table 10 can be considered "high" with the lowest value being 0.578 between genetically distinct clades 4-5a and 4-6. The only other F_{ST} values below 0.6 were those between genetically distinct clades 4-5a and 4-5b (F_{ST} = 0.580) and between genetically distinct clades 4-2a and 4-5a (F_{ST} = 0.583). In each case, the number of migrants exchanged between the distinct clades was less than one migrant per generation

with the estimate of 0.366 migrants per generation, between clades 4-5a and 4-6, being the highest estimate obtained. Thus, the higher the F_{ST} value the lower the migration rate. The Hudson *et al.*'s *(*1992) F_{ST} values as estimated in DNASP 5.1 (Librado & Rozas, 2009) and presented in Table 11 show a similar trend to the F_{ST} values presented in Table 10. All Hudson *et al.*'s (1992) F_{ST} values can again be considered high with the lowest F_{ST} values observed between clades 4-2a and 4-5a (F_{ST} = 0.56502) and between clades 4-5a and 4-5b (F_{ST} = 0.59530). The N_{ST} values in Table 12 (F_{ST} to which the correction of Jukes & Cantor (1969) has been applied) almost mirror the F_{ST} values in Table 11, with the differences being negligible. The number of pairwise differences between populations as presented in Table 13 reveal that the number of pairwise differences between populations correlates with the F_{ST} values found in the previous tables: The lower the F_{ST} values between two genetically distinct clades, the lower the number of pairwise differences between them. This is to be expected as fewer differences show a closer resemblance between populations whereas high F_{ST} values are an indication of differentiation between populations. Table 14 shows that the variation between the genetically distinct clades is more than double the variation within the clades. The overall fixation index of 0.73 is also very high. All F_{ST} values suggest that there are a low number of overlapping alleles between clades with less than one migrant between clades per generation. Given that these distinct clades overlap in geographical range, it may be possible that there is either some barrier that prevents interbreeding between individuals from different genetically identified clades, or that insufficient time has passed to eradicate genetic signatures of past isolation coupled with differentiation. Table 15 summarizes the tau values as well as their *P* values as calculated by Arlequin version 3.5.1.2 (Excoffier & Lischer, 2010) for divergence times between the different genetically identified clades. None of the tau values have *P* values under the 0.05 confidence level. Divergence times between clade 4-4, representing *E. kucheli* (Grobler *et al*., 2006), and the other clades are in each instance the oldest.

Table 9 Summary of mitochondrial DNA diversity. NH = number of unique haplotypes, h = haplotype diversity and π = nucleotide diversity. PEIA = Prince Edward Islands Archipelago; PEI = Prince Edward Island; MI = Marion Island.

Dataset (N)	NH	h	π	Parsimony informative sites
PEIA (*n* = 187)	131	0.9893	0.01451	84
MI (*n* = 146)	99	0.984	0.01253	69
PEI (*n* = 41)	34	0.989	0.01651	43
Clade 4-1 (*n* = 27)	23	0.986	0.00571	11
Clade 4-2a (*n* = 8)	7	0.964	0.00577	4
Clade 4-2b (*n* = 2)	2	1	0.00226	0
Clade 4-3 (*n* = 49)	30	0.931	0.00421	14
Clade 4-4 (*n* = 20)	14	0.958	0.00423	9
Clade 4-5a (*n* = 66)	42	0.959	0.00552	31
Clade 4-5b (*n* = 6)	5	0.933	0.00437	1
Clade 4-6 (*n* = 9)	8	0.972	0.00370	1

Table 10 Population pairwise distance F_{ST} values as computed in Arlequin version 3.5.1.2 (Excoffier & Lischer, 2010) using Tamura Nei (1993) distances and gamma as estimated by jModelTest 0.1.1 (Posada, 2008; Guindon & Gascuel, 2003). Pairwise F_{ST} values can be used as short-term genetic distances between populations (Reynolds, *et al*.; 1983; Slatkin, 1995). All *P* values estimated for F_{ST} values are significant at the 0.05 level. The value after the "/" is the *M* value (Slatkin, 1991) obtained in Arlequin version 3.5.1.2 (Excoffier & Lischer, 2010) where *M* is the absolute number of migrants exchanged between two populations.

	4-2a	4-2b	4-3	4-4	4-5a	4-5b	4-6
4-1	0.70819 / 0.20602	0.71085 / 0.20339	0.76985 / 0.14948	0.79697 / 0.12737	0.67993 / 0.23537	0.70290 / 0.21134	0.70031 / 0.21397
4-2a		0.62887 / 0.29508	0.78374 / 0.13797	0.78003 / 0.14100	0.58263 / 0.35818	0.67785 / 0.23763	0.64613 / 0.27384
4-2b			0.74562 / 0.17058	0.78705 / 0.13529	0.60757 / 0.32295	0.70664 / 0.20757	0.72504 / 0.18962
4-3				0.84003 / 0.09522	0.71118 / 0.20305	0.76549 / 0.15317	0.75907 / 0.15870
4-4					0.71450 / 0.19979	0.79462 / 0.12923	0.80109 / 0.12415
4-5a						0.57977 / 0.36241	0.57758 / 0.36567
4-5b							0.68816 / 0.22657

Table 11 Hudson *et al*.'s (1992) F_{ST} values as estimated in DNASP 5.1 (Librado & Rozas, 2009). An average F_{ST} value of 0.72745 was estimated as well as an average Nm (Number of migrants) of 0.09. Nm is based on the island model of population structure: Fst = 1 / (1 + 2Nm) (Wright, 1951).

	4-2a	4-2b	4-3	4-4	4-5a	4-5b	4-6
4-1	0.68663	0.77317	0.74400	0.78477	0.65725	0.70255	0.70519
4-2a		0.70232	0.74327	0.75402	0.56502	0.67169	0.63053
4-2b			0.78770	0.82056	0.69697	0.74857	0.75515
4-3				0.82590	0.70037	0.74500	0.75371
4-4					0.71837	0.78261	0.79474
4-5a						0.59430	0.60340
4-5b							0.66741

Table 12 Lynch and Crease's (1990) N_{ST} / Number of migrants (Nm) as estimated in DNASP 5.1 (Librado & Rozas, 2009). An average N_{ST} value of 0.72941 was estimated as well as an average Nm of 0.09. Nm is based on the island model of population structure: Nst = 1 / (1 + 2Nm) (Wright, 1951).

	4-2a	4-2b	4-3	4-4	4-5a	4-5b	4-6
4-1	0.68902	0.77505	0.74630	0.78712	0.65951	0.70452	0.70724
4-2a		0.70382	0.74548	0.75615	0.56682	0.67339	0.63219
4-2b			0.78924	0.82205	0.69848	0.74978	0.75654
4-3				0.82797	0.70238	0.74672	0.75553
4-4					0.72031	0.78437	0.79656
4-5a						0.59564	0.60496
4-5b							0.66869

Table 13 Above diagonal: Average number of pairwise differences between genetically distinct clades (PiXY). Diagonal elements (indicated with grey shading): Average number of pairwise differences within genetically distinct clades (PiX). Below diagonal: Corrected average pairwise difference (PiXY-(PiX+PiY)/2). All values were calculated in Arlequin version 3.5.1.2 (Excoffier & Lischer, 2010) according to Nei & Li (1979) using Tamura Nei (1993) distances and gamma as estimated by jModelTest 0.1.1 (Posada ,2008; Guindon & Gascuel, 2003).

	4-1	4-2a	4-2b	4-3	4-4	4-5a	4-5b	4-6
4-1	5.270	18.179	17.098	19.121	23.111	16.19	16.816	15.675
4-2a	12.847	5.393	12.863	19.240	19.992	12.461	15.043	12.263
4-2b	13.433	9.136	2.061	14.606	17.689	12.242	12.640	11.541
4-3	14.556	14.613	11.646	3.860	24.300	15.819	16.663	15.589
4-4	18.503	15.321	14.685	20.397	3.947	16.751	19.457	18.907
4-5a	10.995	7.195	8.642	11.320	12.208	5.139	11.708	11.111
4-5b	12.112	10.277	9.540	12.664	15.415	7.069	4.139	11.846
4-6	11.348	7.875	8.819	11.968	15.242	6.849	8.085	3.383

Table 14 AMOVA analyses using Tamura Nei (1993) distances and gamma as estimated by jModelTest 0.1.1 (Posada ,2008; Guindon & Gascuel, 2003) as calculated by Arlequin version 3.5.1.2 (Excoffier & Lischer, 2010). The eight genetically distinct clades were used as different populations. Va and F_{ST}: *P*(random value > observed value) = 0.00000, *P*(random value = observed value) = 0.00000 and *P*(random value ≥ observed Value) = 0.00000+-0.00000.

Source of variation	**Degrees of freedom**	**Sum of squares**	**Variance components**	**Percentage of variation**
Among clades	7	907.362	6.19684 Va	73.04
Within clades	179	409.447	2.28742 Vb	26.96
Total	186	1316.810	8.48425	
Fixation Index F_{ST}: 0.73039				

Table 15 Divergence times allowing for unequal population sizes (tau) / tau *P* values. All values were calculated in Arlequin, version 3.5.1.2 (Excoffier & Lischer 2010) using Tamura Nei (1993) distances and gamma as estimated by jModelTest 0.1.1 (Posada, 2008, Guindon & Gascuel, 2003). Time in years (*t*) was determined using the equation $\tau = 2ut$, where $u = m_T\mu$ (m_T is the number of nucleotides used and μ is the mutation rate per generation), and is shown under the grey-shaded diagonal.

	4-1	4-2a	4-2b	4-3	4-4	4-5a	4-5b	4-6
4-1		8.887 / 0.478	10.073 / 0.414	10.649 / 0.482	14.243 / 0.535	7.414 / 0.541	8.471 / 0.312	7.931 / 0.397
4-2a	218300		6.126 / 0.654	10.666 / 0.387	11.279 / 0.420	4.487 / 0.437	6.928 / 0.570	5.056 / 0.541
4-2b	247433	150478		8.909 / 0.336	11.789 / 0.212	5.777 / 0.424	6.974 / 0.470	6.459 / 0.368
4-3	261581	261999	218840		16.597 / 0.457	7.872 / 0.6115	9.267 / 0.596	8.835 / 0.481
4-4	349864	277057	289584	407688		8.612 / 0.536	11.740 / 0.601	11.826 / 0.468
4-5a	182117	110218	141906	193367	211545		4.472 / 0.453	4.312 / 0.483
4-5b	208081	170179	171309	227634	288381	109850		5.456 / 0.487
4-6	194816	124195	158658	217022	290493	105919	134012	

CHAPTER 3

The population dynamics of *Ectemnorhinus* weevils from the Prince Edward Island Archipelago

Abstract: *Ectemnorhinus* weevils were collected from diverse localities on the sub–Antarctic Marion (MI) and Prince Edward Island (PEI) of the Prince Edward Archipelago (PEAI). A COI gene phylogeny was inferred for the 187 specimens following nested clade analysis. The results of the nested clade analyses were mapped on the islands on the basis of weevil sampling locality. These results were interpreted with reference to known glacial margins, striae, and moraines of the last glacial maximus on Marion Island, as well as the geology of the islands. As no evidence of glaciation has been found on Prince Edward Island, emphasis was placed on Marion Island. Results indicate that *E. kucheli* originally colonized PEI and subsequently colonized MI before the last recorded glaciation on MI. *Ectemnorhinus similis* evolved in allopatry and almost reached extinction on MI during the last glaciation, being confined to an ice-free region in the south-western corner of the island. Once the ice began melting it appears that *E. similis* was able to re-colonize MI, most likely with the aid of the strong, frequent south-westerly winds. However, subsequent volcanic eruptions most likely pocketed the weevils across the island and the ensuing isolation was sufficient for differentiation of weevils, as a genetic signature of multiple genetically-identifiable populations is apparent. As conditions became favorable, weevils began to migrate in a mostly south-westerly direction consistent with the strong, frequent south-westerly winds. Although individuals from different genetically-identifiable lineages/populations currently have overlapping distributions, no gene flow is evident between them. The analyses also indicate that reverse migration of *E. similis* from MI to PEI occurred. With respect to *E. kucheli*, two genetically distinct populations were identified on PEI. In contrast, seven genetically-identifiable populations of *E. similis* are present on MI suggesting that the different geological histories of the islands underlie the genetic diversity and that volcanism in combination with glaciation have been strong evolutionary drivers on MI.

Key words: Weevils, *Ectemnorhinus*, Marion Island, Prince Edward Island, COI gene, phylogenetics, biogeography, glaciation, volcanism, population dynamics, conservation

Introduction

Molecular phylogenies provide a valuable, unbiased evaluation of morphological-based classification of the *Ectemnorhinus* weevils (Grobler. *et al*., 2010a). In a recent study, Grobler *et al.* (2006) revealed that there was no genetic support for the existence of two distinct weevil species on Marion Island. As a result, *Ectemnorhinus similis* (Waterhouse, 1885) and *E. marioni* (Jeannel, 1940) were synonymised (Grobler *et al*. 2006; Chapter 2) and only one *Ectemnorhinus* weevil species, *E. similis* (Waterhouse, 1885), is presently recognised on Marion Island (MI), while two species, *E. similis* (Waterhouse, 1885) and *Ectemnorhinus kucheli* (Grobler, 2006) occur on Prince Edward Island (PEI). These results contrast markedly with what was originally expected as there was substantial support (Chown, 1990; Kuschel & Chown, 1995) for the presence of two size-discrete species, the larger *E. similis* (Waterhouse, 1885) and the smaller *E. marioni* (Jeannel, 1940) on MI. Due to the presence of weevils with the same morphology and body size classes on PEI, the presence of both species was inferred for this island, however, no formal taxonomic work had been performed on the weevils from PEI prior to the molecular study (Grobler *et al.* 2006), in which two possible reasons for the presence of just one species of *Ectemnorhinus* on MI were proposed, *viz*.: 1) There was originally two species of *Ectemnorhinus* present on MI, but that one of these species became locally extinct, possibly through body-size selective predation by mice (Chown & Smith 1993; Smith *et al.* 2002) and/or rapid climatic changes (Smith, 2002). Both factors could for example, remove the body size-induced reproductive barrier that was proposed on grounds of the significant relationship between female and male body size in *in-copula* pairs as observed by Chown (1990); or 2) That only one *Ectemnorhinus* species was ever present on MI and that the extreme morphological variation observed for *Ectemnorhinus* on the island was wrongly interpreted as indicating the presence of two species. Two possible reasons for the presence of two species of *Ectemnorhinus* on PEI were also proposed, *viz*.: 1) That because of the difference in glaciation histories, MI was extensively glaciated while PEI was not (Verwoerd, 1971), weevils on PEI may have had longer exposure to vascular plants as an additional, more nutritious food source to bryophytes than those on MI and may have diverged sympatrically according to the model of Rice (1984) into two species, a smaller one with a preference for bryophytes and a larger one with a preference for angiosperms, as suggested by Chown (1990); or 2) That the weevils on PEI and MI diverged from each other allopatrically resulting in an MI and PEI *Ectemnorhinus* species, with the subsequent

colonization of PEI by the MI *Ectemnorhinus* species, resulting in the presence of the two species seen on PEI today (Grobler *et al*., 2006).

In order to distinguish between these scenarios, a population-level study was undertaken in which genetic characterization of the COI gene of weevils sampled from across the islands, was performed. Both population genetics and biogeography were assessed in an attempt to gain insights into the complex and dynamic history of the *Ectemnorhinus* weevils occurring on the Prince Edward Islands archipelago (PEIA). Figure 1 shows the PEIA in the geographical context of the nearest land masses.

The results obtained in this study are in turn interpreted with reference to the geological history of the PEIA. Both MI and PEI are volcanic islands with MI being the tip of an active oceanic interplate volcano rising more than 3500 m from the ocean floor (Mahoney *et. al.*, 1992). The earliest sub-aerial eruptions occurred at least 450 000 years ago making MI at least 0.5 million years old (McDougall *et al*., 2001). The volcanic activity on MI is still active with recent eruptions being reported in 1980 (Verwoerd *et al.*, 1981) and 2004 (Meiklejohn & Hedding, 2005). Another small eruption consisting of gas only occurred in 2005 (I. Meiklejohn, pers. com.)

Hall (1981; 1982) suggested that MI went through three major glaciations while McDougall *et al*. (2001), on the basis of additional geo-chronological data, suggested that MI had at least five (possibly eight) different cold periods as well as eight different volcanic ages (Fig. 1) with some volcanic ages accompanying major glaciations (Fig. 2). Rapid deglaciation of MI at the end of the Last Glacial Maximum was proposed to have triggered the latest volcanic events (Hall, 1978; 1982; 2004). These so-called 'younger black lavas' have not been subjected to K-Ar dating as yet (McDougall *et al*., 2001). It was suggested that the rapid deglaciation of MI at the end of the Last Glacial Maximum started at 17-18 ka BP (Bianchi & Gersonde 2004). During the last glaciations, MI experienced a mean annual decrease in temperature of between 3º C and 6º C (Van Zinderen-Bakker, 1973; Hall, 1980; 1982) due to the fact that the Polar Front moved northwards to encompass MI and put the island within a zone of more southerly winds that were colder than those experienced at present (Hall, 1978). This drop in temperature initiated glaciation by causing the annual precipitation to fall in the form of snow rather than rain (Hall, 1978). As the glacier cover on MI was maintained by precipitation rather than by cold temperatures as is the case on Antarctica, a slight rise in temperature changed the precipitation back from snow to rain. The glaciers which were now cut off from their source in combination with higher temperatures led to rapid melting of the ice on MI despite the extensive cover (Hall, 1978). The lower possible limit of the palaeo-

snowline at maximum glacial conditions was 250 m and the upper was 550 m (Hall, 1978). Hall (1978) further hypothesised that the true snowline was most likely at 550 m. If this was true, it could maintain plant growth in unglaciated locations. The fact that plant life was not eradicated during glaciation, but that it survived in ice-free locations on MI has also been suggested by Van Zinderen-Bakker (1973) from pollen evidence. Hall (1978) further proposed that the snow cover was responsible for placing pressure on MI that resulted in the island being pushed under the water with a subsequent rise in sea levels of 3 m, 6 m and 10 to 11 m at different stages. The geological history of MI is summarised schematically in Figs. 3 and 4 in terms of volcanism and glaciation, respectively. It has been suggested that the Feldmark Plateau area on MI either remained ice-free through much of the last glacial maximum, or alternatively, the area was de-glaciated before other areas (Boelhouwers *et. al*., 2008). Boelhouwers *et. al*. (2008) also suggested that parts of Long Ridge may have remained ice-free during the Last Glacial Maximum.

Materials and methods

Genetic sampling

Ectemnorhinus weevil specimens were collected during three consecutive years (April 2001 – April 2003) from 30 localities encompassing the entire sub-Antarctic MI and from eight localities on PEI during April 2003 (see Appendix 1). All specimens were collected by hand and preserved in absolute ethanol.

Mitochondrial NA amplification and sequencing

DNA from each individual was extracted from a leg which, following removal from ethanol was washed and rehydrated in distilled water for 10 minutes prior to being frozen in liquid nitrogen and ground using a pestle. DNA was extracted using the High Pure PCR Template Preparation Kit from Roche Applied Science according to the supplier's procedure for isolation of nucleic acids from mammalian tissue. The only adaptation to the protocol was that the proteinase K digestion was performed for a minimum of 24 h instead of the 1 h digestion recommended for mammalian tissue by the supplier. MI-specific COI primers, GF-1858 and GR1-2938 (Grobler *et al*., 2006) were used to amplify a 1059 bp PCR product under the following reaction conditions: 200 ng of template DNA was added to 1×Buffer, 0.2 mM dNTP, 0.4 µM of each primer and 1 U *Taq* polymerase in a final volume of 50 µl. A

typical thermal cycling profile consisted of an initial denaturation step at 94º C for 90 s, followed by 40 cycles of 94º C for 22 s, 46º C for 30 s and 72º C for 1 min, and a final extension step of 1 min at 72º C.

Fig. 1 The Prince Edward Archipelago in the geographical context of the nearest land masses.

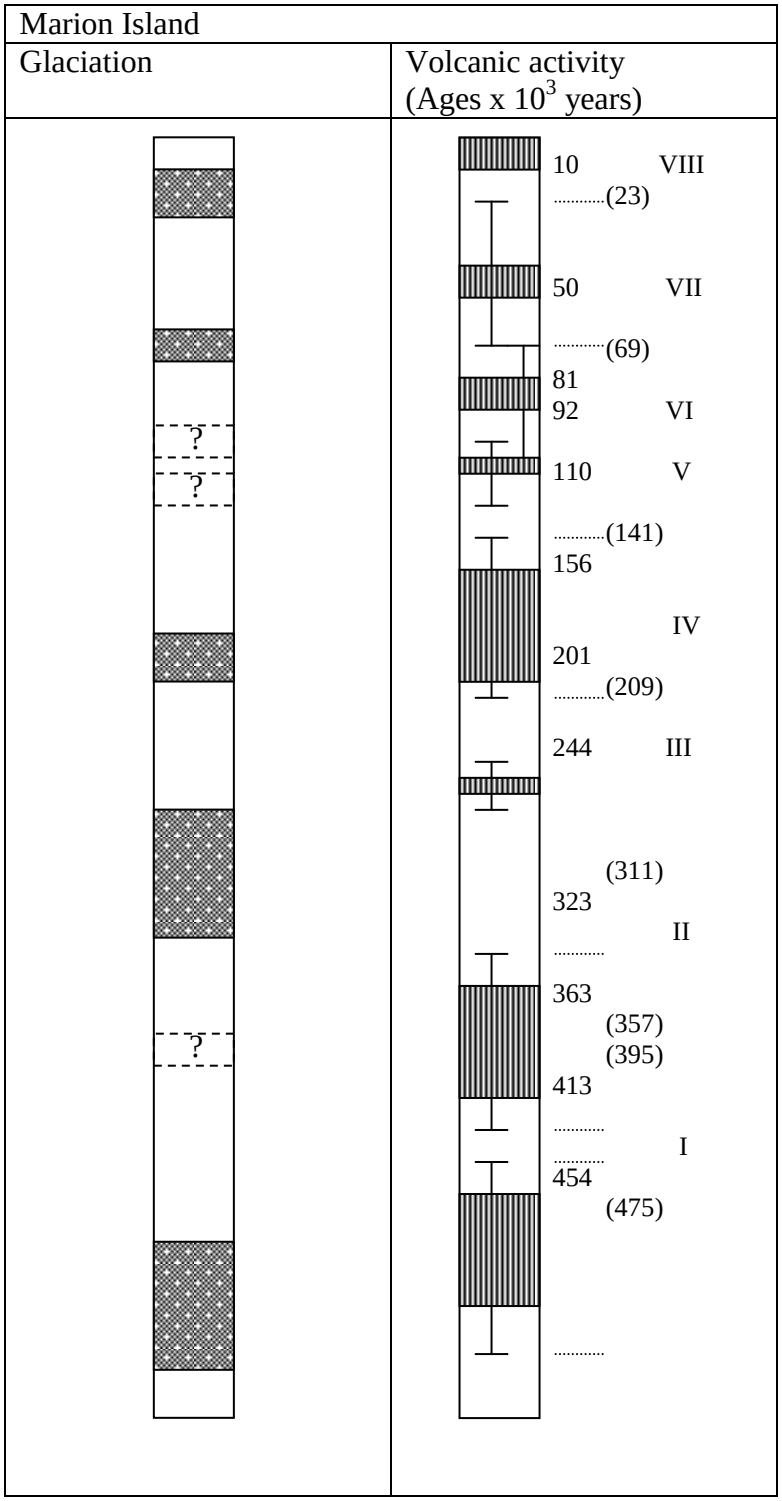


Fig. 2 Glacial and volcanic chronology from Marion Island (After McDougall *et al.,* 2001).

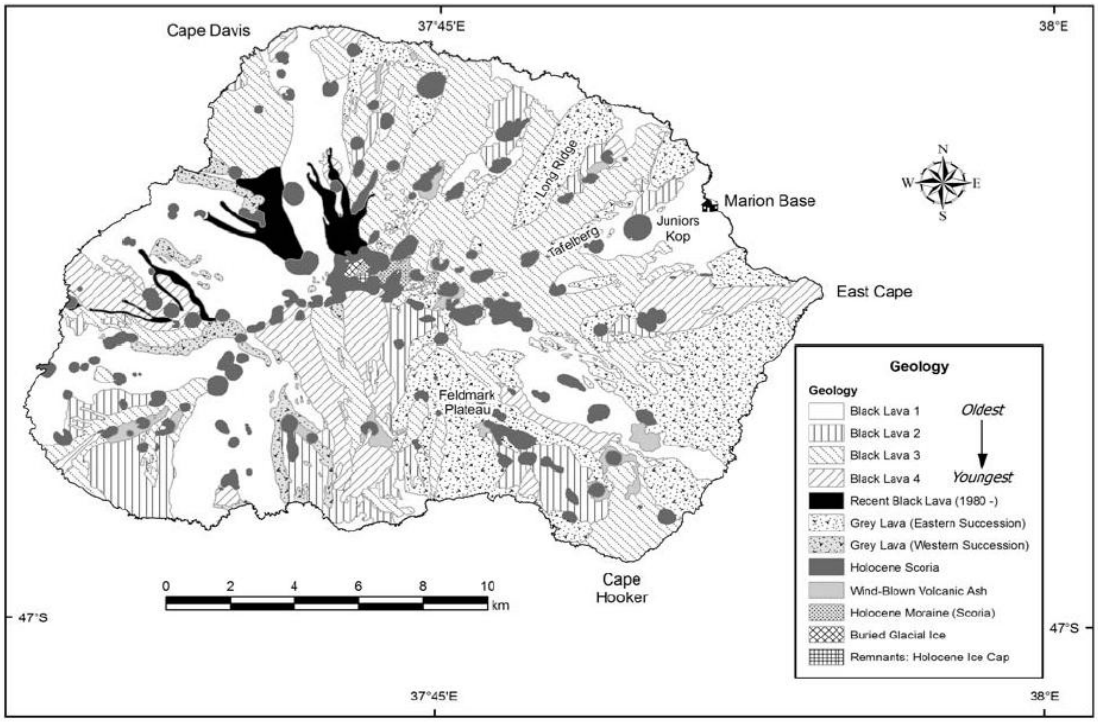


Fig. 3 Simplified geology of Marion Island (Modified after Verwoerd, 1971; Chevallier 1986; Chevallier *et al.*, 1992; McDougal *et al.*, 2001) as depicted in Boelhouwers *et al.* (2008).

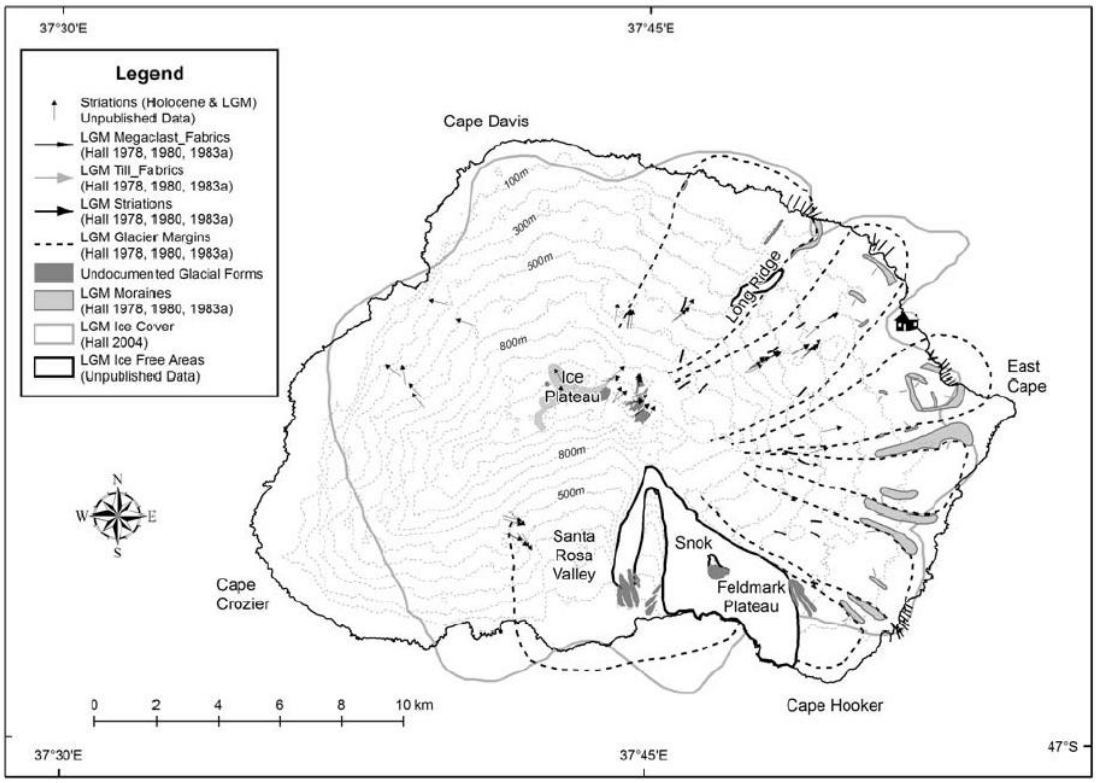


Fig. 4 Glacial margins, striae, and moraines as identified by Hall (1978) and a proposed local cirque near Snok as depicted in Boelhouwers *et al.* (2008).

The PCR product was purified directly from the tube using a Roche High Pure PCR Product Purification Kit. DNA sequences were determined by automated cycle sequencing on an ABI PRISM™ 3100 Analyser using the ABI PRISM Big Dye™ Terminator V3.0 sequencing standard. Primers GF5-1940 and GR5-2935 (Grobler *et al*., 2006) were used in all cycle-sequencing reactions following a thermal profile of 96º C for 10 s, 46º C for 8 s and 60º C for 4 min, repeated 25 times. The sequences were viewed and edited in Chromas 1.43 (McCarthy, 1996-1997) and aligned with DAPSA 4.9 (Harley, 2000).

Nested clade analyses

Although some criticism has been levelled against nested clade analyses (see Knowles & Maddison, 2002; Knowles, 2008), these concerns have been addressed (Templeton, 2008) and the method and its inferences remain valuable for understanding population dynamics. The program TCS 1.13 (Clement *et al*., 2000) was therefore used to generate a haplotype cladogram displaying the number of base pair differences between haplotypes. TCS 1.13 (Clement *et al*., 2000) incorporates the cladogram estimation algorithm described by Templeton *et al*. (1992) and provides 95 % parsimoniously plausible branch connections between the different haplotypes. Clades were nested by hand according to previously described nesting rules (Crandall, 1996; Templeton *et al*., 1987). Ambiguous connections were dealt with as outlined in Templeton & Sing (1993) as well as in Crandall & Templeton (1993). Geodis 2.0 (Posada *et al*., 2000) was used to test for significantly large and small clade (D_C) and nested clade (D_N) distances for clades and tip/interior contrasts when both genetic and geographical variations were present in the nested clade. D_C represents the mean distance between members of a clade and the geographical centre of that clade, providing an estimate of how geographically widespread the clade is. D_N represents the mean distance between the members of a particular clade and the geographical centre of all clades with which it has been grouped, including itself. This is a measure of the geographical isolation of a clade relative to the nested clade as a whole (Brown *et al*., 2002). Permutation analyses of all clades were based on 100 000 replicates in Geodis 2.0 (Posada *et al*., 2000). The inference key as obtained from http://darwin.uvigo.es/download/geodisKey_14Jul04.pdf was used to interpret the results obtained from Geodis 2.0 (Posada *et al*., 2000).

Phylogenetic analyses

jModelTest 0.1.1 (Posada, 2008; Guindon & Gascuel, 2003) was used to identify the model of evolution that best fits the data with parameters identified under the Akaike Information

Criterion (AIC; Akaike, 1974). The Minimum Evolution (Rzhetsky & Nei, 1992) algorithm in MEGA version 4 (Tamura et al. 2007) was used to construct phylogenies with node support assessed by 100 000 bootstrap replications. Minimum Evolution (Rzhetsky & Nei, 1992) trees were constructed using the Tamura-Nei (1993) distance correction algorithm and gamma distributions as estimated by jModelTest 0.1.1 (Posada, 2008; Guindon & Gascuel 2003). Bayesian phylogenetic analyses using MrBayes version 3.1.2 (Huelsenbeck & Ronquist, 2001) were performed on the complete dataset with the model and parameters recovered by jModelTest 0.1.1 (Posada, 2008; Guindon & Gascuel, 2003) being used to guide the setting of priors. The analyses were initiated with random starting trees and run for 10 000 000 generations with Markov chains sampled every 2000 generations. Of the 5000 trees obtained, 1250 were discarded as "burn-in". 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, calculating and comparing log likelihood scores with and without the molecular clock enforced. A Neighbor Joining (NJ; Saitou and Nei, 1987) tree using uncorrelated p-distance values, with node support assessed by 100 000 bootstrap replications, was drawn in MEGA version 4 (Tamura *et al*., 2007) and 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). In addition, a BEAST analysis performed using BEAST 1.6.1 (Drummond & Rambaut, 2007) was used to obtain an ultrametric tree using Bayesian Monte Carlo Markov chain (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 divergence times were estimated using the 2.3 % nucleotide sequence divergence per million years, estimated from an arthropod mtDNA survey (Brower, 1994) and the appropriate parameter-rich model of evolution (Papadopoulou *et al*. 2010) selected under the AIC in jModelTest 0.1.1 (Posada, 2008; Guindon & Gascuel, 2003) using a strict molecular clock model. The results of two independent runs of 20 000 000 generations each with Markov chains sampled every 1000 generations, were merged and analyzed with Tracer v1.5 and TreeAnnotator v1.6.1 (Drummond & Rambaut, 2007). Of the 40 000 trees obtained, 5000 were discarded as "burn-in". Trees were rooted with *Ectemnorhinus viridis* (Waterhouse, 1853) from Heard Island since it has been shown to be closely related to the *Ectemnorhinus* weevils from the PEIA (Grobler et al. 2011a).

Mismatch analyses

Mismatch analysis provides information about demographic processes such as the magnitude and timing of population expansion based on the observed nucleotide pairwise distribution in the demographic mismatch analysis (Rogers & Harpending, 1992). It also characterizes the effect of range expansion independently of demographic expansion in the spatial mismatch analysis (Ray *et al*.. 2003; Excoffier, 2004). Pairwise mismatch distributions where the observed pairwise mismatch distributions were fitted to a stepwise expansion model by a generalized least square procedure as implemented in Arlequin version 3.5.1.2 (Excoffier & Lischer, 2010) were generated. The validity of a stepwise expansion model for the data was tested by MCMC simulations (1000 steps) with Arlequin version 3.5.1.2 (Excoffier & Lischer, 2010). A unimodal distribution is considered a signature of a population expansion, although it is not possible to establish whether it is a consequence of either demographic or spatial processes (Excoffier, 2004). A constant size population is expected to show a ragged, multimodal distribution, while an expanding population shows a smooth unimodal distribution. In each case, the raggedness index assesses the match of the real data to the model. A non-significant raggedness index indicates a relatively good fit of the data to a model of population expansion. The overall validity of the estimated expansion model was tested by comparing the distribution of the test statistic sum of squared differences (SSD), between the observed and the estimated mismatch distribution using a bootstrap approach. Evidence for departure from the estimated expansion model is given by significant SSD values. This can include either an expanding or a stationary population (Excoffier & Schneider, 1999). Population spatial expansion occurs if the range of a population is initially restricted to a very small area, and then the range of the population increases over time and over space. The resulting population becomes generally sub-divided in the sense that individuals will tend to mate with geographically close individuals rather than remote individuals. In the specific case of a range expansion, preferential reproduction with neighbouring individuals (local demes) should lead to some level of population structure while the local deme should nevertheless exhibit a unimodal mismatch distribution. Range expansion may thus produce the same genetic signature as a demographic expansion (Excoffier, 2004). The time of the main expansion in generations (t) was estimated from the equation $\tau = 2ut$ where $u = m_T\mu$ (m_T being the number of nucleotides used and μ being the mutation rate per generation) using the moment estimator of time to the expansion (τ) computed in Arlequin version 3.5.1.2 (Excoffier & Lischer, 2010) and a mutation rate of 2.3

% nucleotide sequence divergence per million years for arthropod mtDNA (Brower, 1994). As the weevils complete one generation in one-year (Chown & Scholtz, 1989), *t* could also be used as the time of the main expansion in years. Thus $t = \tau / 2(0.000000023 \times 885 \times 1) = \tau / 0.00004071$. For the spatial expansion the tree parameters τ, $\theta = \theta_0 = \theta_1$ (assuming that $N = N_0$), and $M = 2Nm$ were estimated and the rate at which the sampled deme would exchange migrants with a unique population of infinite size (m) was calculated. The analysis of spatial mismatch, based on the assumption of no demographic expansion within the sampled deme, provides an estimate ($N_f = \theta/2u$) of the effective female population size necessary to explain the mismatch distribution uniquely in terms of range expansion (Excoffier, 2004). Thus $N_f = \theta/2(0.000000023 \times 885 \times 1) = \theta/0.00004071$. Arlequin version 3.5.1.2 (Excoffier & Lischer 2010) was utilized to carry out Fu's (1997) F_S test of neutrality as well as to estimate Tajima's *D* and the essential population parameter θ (Tajima 1983).

Statistical analyses

Haplotype diversity (h) and nucleotide diversity (π) were estimated in DNASP 5.1 (Librado & Rozas, 2009). π is the average number of nucleotide differences between two sequences from one population (Nei & Li, 1979). Genetic structure was investigated with Arlequin version 3.5.1.2 (Excoffier & Lischer, 2010) as well as with DNASP 5.1 (Librado & Rozas 2009). Genetic structure analyses include F_{ST} values (fixation indexes), N_{ST} values as well as the number of migrants per generation. N_{ST} (Lynch & Crease, 1990) is almost the same as F_{ST} (Hudson *et al*., 1992), except that for N_{ST}, the correction of Jukes & Cantor (1969) has been applied. The larger the F_{ST} / N_{ST} values the larger the genetic variation between the sub-populations in terms of the total population (Holsinger & Weir, 2009). The average number of pairwise differences between populations (Pi_{XY}), the average number of pairwise differences within each population (Pi_X) as well as the corrected average pairwise difference between populations (Pi_{XY}-(Pi_X+Pi_Y)/2) were also calculated using Arlequin version 3.5.1.2 (Excoffier & Lischer, 2010). Analysis of molecular variance (AMOVA; Excoffier *et al.*, 1992) was utilised to investigate the population structure by calculating the among-population and within-population variation when assessing all individuals sequenced (Excoffier & Lischer, 2010). An overall fixation index was also uncovered by the AMOVA analysis which was performed using Arlequin version 3.5.1.2 (Excoffier & Lischer, 2010). Divergence times (tau) allowing for unequal population sizes between genetically distinct clades was also calculated using Arlequin version 3.5.1.2 (Excoffier & Lischer, 2010) .

Discussion

Phylogeography

In order to gain insights into the patterns of the *Ectemnorhinus* population dynamics on the PEIA it is important to first consider the results of both the nested clade analyses as well as the phylogenetic analyses. These results are also meaningful if interpreted in context of Figs. 9–14 where the haplotype network was superimposed on the island maps according to sampling localities. As a single haplotype network was obtained, it allowed the evaluation of each sample relative to the rest.

In Fig. 12, the individuals that form nested clade 4-4 (Figs. 6 & 8) are mapped according to their sampling localities. All the individuals of nested clade 4-4 map to PEI and the individuals in this clade have been designated *E. kucheli* (Grobler *et al*., 2006). This clade has also been designated the base of the nested clade analyses (Figs. 6–8) as it has been shown to be older than the other clades (Grobler *et al*., 2006). In this study, nested clade 4-4 was found to be the most basal clade in the Bayesian tree with all other clades grouping together with 92% support (Fig. 16). Of all the nested clades for which time to lineage

coalescence was calculated, nested clade 4-4 gave the oldest time of approximately between 0.312 MYA (Fig. 17) and 0.5109 MYA (Fig. 18), indicating that it was the first to have colonised the PEIA. The Tau values (Table 15) indicate that in each case, the pairwise divergence times from nested clade 4-4 are the oldest. These results provide support for the decision to use nested clade 4-4 as the most basal clade in the nested clade analyses. Although nested clade 3-10 was one of the few nested clades that showed a significant relationship between genetic variation and the respective geographical locations of the haplotypes (Table 1), the nested clade analyses could not provide a conclusive outcome (Table 2). However, when the individuals of nested clade 4-4 were mapped according to sampling locality, it was evident that the whole sampling area on PEI was represented (Fig. 12a). Even the four haplotypes that have more than one representative span large areas of the sampling transect (Fig. 12b). When we consider Figs. 12c–f and compare it to the PEI volcanological map (Fig. 3) it is possible that the younger Holocene black basaltic lavas were colonised from the older Pleistocene grey lavas to the northeast. It is interesting to note that individual 17-14, collected from the Golden Gate area, is separated from the remainder of nested clade 4-4 individuals by eight steps / missing haplotypes (Fig. 6).This individual is possibly a representative of a population that was isolated by the younger black lavas east of Karterkoppie from other con-specifics inhabiting the older grey lavas (Fig. 3). Individual 17-14 appears to represent a discrete sub-population of *E. kucheli* on PEI that is less abundant than the main population.

As *E. kucheli* was the first species to colonise the PEIA at PEI, Grobler *et al.* (2006) suggested that the presence of *E. similis* on MI could perhaps be explained by the initial colonisation of MI by PEI *E. kucheli* followed by differentiation into a new species *E. similis* and the extinction of the ancient colonisers.

In so far as the population dynamics of *E. similis* on MI is concerned, nested clade 4-4 is only connected with nested clade 4-5 (Figs. 6– 8). This suggests that the individuals within nested clade 4-5 are the closest relatives to the original migrants to MI. The phylogenetic analyses (Figs. 15 & 16) show that nested clade 4-5 separates into two distinct clades, clade 4-5a & 4-5b. Although both clades have less than 50 % bootstrap support in the ME analyses (Fig. 15), clade 4-5a has 94 % support in the Bayesian analyses, while clade 4-5b has 85 % support (Fig. 16). Both clades form monophyletic groups within *E. similis* in the Bayesian analyses (Fig. 17). With no significant support for any of the internal nodes, the ME analyses (Fig. 15) provide no indication of the internal structuring of the clades. The nested design shows that clade 4-5b separates from clade 4-5a as clade 4-5b is enveloped in nested clade 3-

2 (Figs. 6 & 8), the individuals in clade 4-5a separated from those in clade 4-5b by six and eight missing haplotypes (Fig. 6). The time of lineage coalescence of clades 4-5a and 4-5b indicate that clade 4-5a arose between 0.193 MYA (Fig. 17) and 0.3206 MYA (Fig. 18), while clade 4-5b arose approximately between 0.216 MYA (Fig. 17) and 0.226 MYA. Clade 4-5a is also the second oldest clade according to the BEAST analyses (Fig. 18) making it the oldest on MI and indicating it as the original colonizer of MI from PEI. In Fig. 17, clade 4-5b appears to be older than clade 4-5a. The examination of both in the nested design (Fig. 6) indicates that there are seven missing haplotypes between members of clade 4-5b (nested clade 3-2). This very high number of missing haplotypes between members of the same clade may considerably distort the evolutionary scenario given that more complex population dynamics may be playing a role. It is thus plausible to assume that clade 4-5a was the original *E. similis* on MI. It is possible that clade 4-5b diverged from clade 4-5a and this is discussed in greater detail below.

Three of the eight nested clades that showed significant relationships between genetic variation and the respective geographical locations of the haplotypes (Table 1) fall within nested clade 4-5. In Fig. 13 where the sampling localities of all the individuals that form part of nested clade 4-5 have been mapped, it was found that they all map to MI with no representatives on PEI (Fig. 13a). In Fig. 13b, the eight haplotypes that consist of more than one representative have been indicated. H5 and H24 can be identified as two major haplotypes (Fig. 6). H5 consists of nine individuals of which three were collected from Swartkop. The remaining individuals that form part of H5 were either sampled at the coast or near to it, both in a northern and southern to western direction (Fig. 13b). H24 consists of ten individuals and forms a similar path around MI as H5 except that H5 seems to be distributed more widely distributed across the bottom of the eastern escarpment while H24 occurs across the top of the eastern escarpment (Fig. 13a). Although H5 and H24 are both part of clade 4-5a (Fig. 15 and Fig 16) it seems that they were at least partially divided by the shear steepness of the eastern escarpment. The other haplotypes that have more than one representative map primarily to the western side of MI, with the exception of H2 that is also located at Swartkop and H10 that forms part of clade 4-5b (Figs. 15 & 16). At the 1-step level (Figs. 6, 8 & 13c), nested clade 1-96 represents one of the nested clades that have a significant relationship between its genetic variation and the respective geographical locations of its haplotypes at the 0.05 % level of significance (Table 1). Table 2 indicates restricted gene flow / dispersal, with some long distance dispersal, as a likely explanation. At the 2-step level (Fig. 13d), nested clade 2-18 shows a significant relationship between genetic variation and geographical

locations of its haplotypes at the 0.1 % level of significance (Table 1) consistent with continuous range expansion (Table 2). The tracing of the nested succession through to Fig. 13e shows that all the individuals in nested clade 4-5 can be divided into three 3-step nested clades (Figs. 6, 8 & 13e). Of the 3-step nested clades, nested clades 3-16 and 3-6 form part of clade 4-5a (Figs. 15 & 16) while nested clade 3-2 correlates with clade 4-5b (Figs. 15 & 16). Both nested clades 3-16 and 3-6 appear to form a ring around the island. The map of the glacial history of MI (Fig. 4) indicates that the south-western part of the island was not glaciated during the last glacial maximum and that this ice-free area occurred below as well as above the western escarpment. The remainder of MI was completely covered in ice, except for two possible sites at Feldmark Plateau and Long Ridge (Fig. 4). At the end of the last glacial maximum, the ice cover on MI contracted and the coastal areas became exposed first. It is likely that the extensive ice coverage during the last glacial maximum, all weevils on MI except those on the south western side of the island, succumbed. As the ice melted, the coastal areas became exposed and available for re-colonisation by plants as well as weevils. From this initial re-colonisation it appears that the weevils moved in both a westerly and easterly direction along the coast, recolonising the remainder of the island with the help of the strong, frequent winds from the SW, as opposed to the weaker infrequent winds from the NE (Schulze, 1971). Early Holocene post-glacial volcanism (Hall, 1978; 1982; 2004) may also have provided the impetus for weevil migration from the south-western side of the island to other available habitats in the east and north. The population that survived the glaciation in the south-western corner of the island also survived the extensive post-glacial volcanism of this region with the ancestors of nested clade 3-16 being protected at Coldridge and the ancestors of nested clade 3-6 being protected at the Pyroxene Kop area (Fig. 3). The fact that MI rises 1280 m above sea level (Langenegger & Verwoerd, 1971) and that large parts of the higher altitudes were still mostly under ice until recent years (Sumner *et al.*, 2004) explains the west-east lower-altitude movement of weevils around the island rather than traversing the high altitude section of the island. Although not traversed, some higher altitudes sites were colonised by the weevils from clade 4-5a again most likely with the assistance of the strong, frequent south-westerly winds (Schulze, 1971). The large degree of 3rd-stage Holocene post-glacial volcanism to the north and east would have hampered re-colonisation from the north-eastern and eastern sides of MI (Fig. 3).

Clade 4-5b does not group with clade 4-5a in the phylogenetic analyses (Figs. 15 & 16) although the nested analyses (Fig. 6) show that it links only with the individuals from clade 4-5a and was also included in nested clade 4-5. Fig. 13e shows that clade 4-5b (nested

clade 3-2) seems to have been isolated in the northern part of the island from the rest of nested clade 4-5 and spread from there to the south and east. The ancestors of the weevils that today comprise clade 4-5b may have emanated from the weevils that were isolated in the south-western corner of MI as the ice cover began to retract. Holocene post-glacial volcanism (Hall, 1978; 1982; 2004) in this area may then have isolated the migrants on this part of the island into a small population (Fig. 3), allowing it only to expand to the rest of the island after the black lavas became hospitable. The only evidence for the expansion of this population in this study is in an eastern direction that again correlates with the strong, frequent south-westerly winds (Schulze, 1971). Nested clade 4-5 (Figs. 6, 8 & 13f,) shows a significant relationships between its genetic variation and the respective geographical locations of its haplotypes at the 0.05 % level of significance (Table 1) although the nested clade analyses showed no conclusive evidence for this (Table 2). This may be due to the close proximity of the sampling localities.

Clade 4-6 forms a monophyletic clade in both phylogenetic analyses with less than 50 % bootstrap support in the ME tree (Fig. 15) and 93 % support in the Bayesian tree (Fig. 16). The monophyletic status of this clade is also confirmed by the nested clade analyses where the individuals nesting in nested clade 4-6 are separated by three missing haplotyes from nested clade 4-5 and by 7 missing haplotypes from nested clade 4-1 (Figs. 6 & 7). No significant relationships between the genetic variation and the respective geographical locations of the haplotypes were shown for any of the nested clades (Table 1). Examination of the distribution and subsequent relationship between the individuals on the maps (Fig. 14), reveals only a single haplotype with more than one representative (Fig. 14b). It is possible that clade 4-6 may have been part of the weevils that migrated west-wards from the south-westerly glaciations-isolated clade 4-5a after the ice retracted. The most probable explanation is that it was isolated from the rest of the weevils in the Sidney area as the area to the south of Sidney was sheltered from the lava from the Holocene post-glacial volcanism (Fig. 3). Its small population size explains the low number of specimens collected. When the lava became hospitable again this population expanded north-wards (Fig. 14) and probably also west-wards with the aid of the strong, frequent south-westerly winds (Schulze, 1971). However, no samples were collected west of Sidney to substantiate this and it is also possible that a large amount of younger lava flow in the Santa Rosa area would have impeded a west-ward migration (Fig. 3).

Figs. 6 & 7 show that nested clade 4-1 is more closely connected to nested clade 4-6 than to any other clade. Although none of the clades nested within nested clade 4-1 show any

significant geographical association (Table 1), when individuals comprising nested clade 4-1 (Figs. 3 & 5) are mapped according to their sampling localities (Fig. 9a), the MI portion of samples map mainly to the eastern side of MI and the PEI portion of the samples are distributed along almost the whole of the gradient sampled. Nested clade 4-1 forms a monophyletic clade in both phylogenetic analyses with 83 % bootstrap support in the ME tree (Fig. 15) and 100 % support in the Bayesian tree (Fig. 16). The monophyletic status of this clade is also confirmed by the nested clade analyses where the individuals nesting in nested clade 4-1 are separated from the rest of haplotype network by seven missing haplotypes from the split with clade 4-6 (Figs. 6 & 7). Fig. 9b shows that three sets of identical haplotypes within the nested clade 4-1: H4 that has two individuals from Tate's Hill and one individual from Stony Ridge, H125 that links the MI meteorological base at Transvaal Cove with Stony ridge and H66 that forms a link between Halfway Kop on MI with PEI. The tracing of the nested diagram further through Fig. 9c – 9e shows its development into two 3-step nested clades. Nested clade 3-8 consists of eight individuals that all come from Stony Ridge and Tate's Hill. Nested clade 3-9 on the other hand, is geographically not as well structured. Nested clade 3-9 at the lower levels shows that it consists of three 2-step nested clades (Fig. 9d). Nested clade 2-4 consists of two individuals connecting the Albatross Lakes area of MI with PEI, nested clade 2-3 consists of eight individuals connecting Halfway Kop with Jonny's Hill, Kildalkey Bay, Tate's Hill as well as the rest of the individuals from PEI and nested clade 2-34 that consists of nine individuals that connects Transvaal Cove with Junior's Kop, Repetto's Hill, Tate's Hill, Kildalkey Bay and Watertunnel. Past geographic events in this region suggest that the whole eastern region, with the possible exception of the Feldmark Plateau area, was covered in ice during the last glacial maximum (Fig. 4). When the ice retracted, the coastal parts of MI were available for re-colonization by weevils from the western side of the island. First stage Holocene post-glacial volcanism (Hall, 1978; 1982; 2004) that covered the area south of Skua Ridge to the north of Macaroni Bay as well as south of Kerguelen Rise (Fig. 3) may have been responsible for the isolation of the population that is currently found in nested clade 4-1 (Fig. 9f). Subsequent Holocene post-glacial volcanism (Hall, 1978; 1982; 2004) likely underlies the formation of the different 3-step nested clades shown in Fig. 9e. Individuals from nested clade 3-8 were isolated at the Tate's Hill - Stony Ridge area by the 3rd- and 4th-stage Holocene post-glacial black lava (Fig. 3) from the individuals in nested clade 3-9. A question that arises is why no individuals of nested clade 4-1 occur to the west and only a few to the north? Western migration may have been prevented firstly by the 3rd- and 4th-stage Holocene post-glacial black lava that links

Green Hill with Johnny's Hill and north-west from there as well as the extensive 3rd- and 4th-stage Holocene post-glacial black lava in the Santa Rosa valley (Fig. 3). North-ward expansion was most possibly hampered firstly by the 1st-stage Holocene post-glacial black lava that covers the whole area south of Skua Ridge to the north of Macaroni Bay, and secondly by the subsequent 3rd- and 4th-stage Holocene post-glacial black lava that extends west-wards from East Cape as well as the other more extensive 3rd-stage Holocene post-glacial black lava to the north (Fig. 3).

The nested design (Fig. 6) shows that nested clade 4-3 has connections with both nested clade 4-5 and nested clade 4-1. There are nine missing haplotypes between nested clade 4-3 and nested clade 4-5 and 12 missing haplotypes between nested clade 4-3 and nested clade 4-1. The fact that nested clade 4-3 connects with both nested clade 4-5 and nested clade 4-1 suggests that it separated from both at some stage in the past. The individuals that comprise nested clade 4-3 group together as clade 4-3 in the ME analyses (Fig. 15), with 91 % bootstrap support, as well as in the Bayesian analyses (Fig. 16), with 100 % support. In both the ME analyses (Fig. 15) and the Bayesian analyses (Fig. 16) some internal nodes with high support are present. Although none of the clades nested within nested clade 4-3 show any significant geographical association (Table 1), when the individuals that form part of nested clade 4-3 were mapped according to their sampling localities, the MI portion of samples map mainly to the north-eastern side of MI (Fig. 11a). Clade 4-3 also has some representatives on PEI although none of the samples collected from the higher altitudes on PEI fall into this group. When the haplotypes containing more than one representative were included (Fig. 11b), five different groupings were revealed. Of these groupings, H34 with 12 different representatives is the most prominent. One of these haplotypes connects the weevils from MI to the weevils from PEI. The tracing of nested design from Fig. 11c to 11f and its comparison to the geographical history of the area with reference to the volcanological map of MI (Fig. 3) shows that the north-eastern side of MI is very fractured by Holocene post-glacial black lavas from all four stages. The same boundary that hampered the north-wards spread of nested clade 4-1, namely the 1st-stage Holocene post-glacial black lava that covers the whole area south of Skua Ridge to the north of Macaroni Bay (Fig. 3) as well as the subsequent 3rd- and 4th-stage Holocene post-glacial black lava extending west-wards from East Cape (Fig. 3) also seems to have restricted the south-ward movement of individuals of nested clade 4-3. The northern migration of individuals from nested clade 4-1 (Fig. 9a) might have been aided by the strong, frequent south-westerly winds (Schulze, 1971) while the southern migration of individuals from

nested clade 4-3 (Fig. 11a) might have been hampered by the strong, frequent south-westerly winds (Schulze, 1971). The east-ward migration of nested clade 4-3 may have been hampered firstly by the 3rd-stage Holocene post-glacial black lava found between Ned's Kop and the north of Ship's Cove (Fig. 3) and further by the 3rd-stage Holocene post-glacial black lavas west of Long Ridge and those east of Repetto's Hill (Fig. 3). The fact that clade 4-3 has so many internal clades with high support (Figs. 15 & 16) may be due to the north-eastern side of MI being very fractured by Holocene post-glacial black lavas from all four stages. This may have resulted in fragmentation of the original population that groups into clade 4-3 (Figs. 15 & Fig. 16) into small isolated pockets that started to diverge from each other. The original boundaries of these small isolated pockets may have been formed by the 1st-stage Holocene post-glacial black lavas (Fig. 3), but are at present obscured by the subsequent Holocene post-glacial black lavas (Fig. 3). The older grey basaltic lavas that can be observed at present, as well as northeast of, Tafelberg may be possible sites for their survival during the 1st-stage Holocene post-glacial black lavas (Fig. 3).

The individuals that form part of nested clade 4-2 in the phylogenetic analyses are, in both the ME analyses (Fig. 15) and the Bayesian analyses (Fig. 16), divided into two different clades, namely, clade 4-2a and 4-2b. Both clades have high support in both phylogenetic analyses (Figs. 15 & 16). Nested clade 4-2 in the nested design (Fig. 6) shows that there are seven missing haplotypes between nested clade 1-56 that corresponds to clade 4-2b, and nested clade 3-4 that corresponds to clade 4-2a. Noteworthy is that there are also seven missing haplotypes between some of the individuals within clade 4-2a and nested clade 3-4 (Fig. 6). Nested clade 4-2 is also one of the clades that show a significant relationship between its genetic variation and the respective geographical locations of its haplotypes at the 0.1 % level of significance (Table 1). The nested clade analyses suggest allopatric fragmentation for this relationship (Table 2). Fig. 10, where the individuals from nested clade 4-2 were mapped according to their sampling localities, show that all the individuals that belong to clade 4-2b map to MI while those that belong to clade 4-2a map to PEI and that they are so divided up to the 3-step level (Fig. 10a–e). Allopatric fragmentation as suggested by the nested clade analyses (Table 2) is thus evident. No conclusions can be drawn from the ME tree as to the relationship between clade 4-2a and clade 4-2b with the rest of the clades, due to lack of support for the internal nodes (Fig. 15). Nonetheless, the Bayesian analyses (Fig. 16) show that both clade 4-2a and clade 4-2b group within *E. similis* rather than with *E. kucheli* and the nested design (Fig. 6) shows that nested clade 4-2 only has ties with nested clade 4-5 and none with nested clade 4-4, strongly suggesting an MI rather than a PEI origin.

Patterns of mtDNA variation

All methods of calculating F_{ST} values (Tables 10 & Table 11) gave very high F_{ST} values for all the pairwise comparisons when they were calculated among the different clades as identified in Figs. 15 and 16. The number of migrants between these clades is also in each case less than one migrant per generation (Table 10). Similar results were obtained from the N_{ST} values (Table 12). From Table 14, the among-clade variation was 2.71 times higher than the within-clade variation, while the fixation index is also very high being above 70 %. These results suggest that the isolation of the different clades, as discussed previously, was sufficiently to prevent viable gene flow between clades. The first major isolation was when MI was colonised from PEI. This separation by distance and the ensuing allopatric speciation resulted in the emergence of *E. kucheli* on PEI and *E. similis* on MI. The last glacial maximum as well as the subsequent Holocene post-glacial volcanism was responsible for pocketing the *E. similis* weevils on MI in small populations that correspond to the different clades that are currently present on the island. In light of the evidence provided by the F_{ST} values, it is suggested that the separation of the original populations occurred to such an extent and for sufficient time that they diverged into the different populations that are currently detectable. Athough they presently have a sympatric distribution, the distinct genetic signature has been retained and there is no noticeable gene flow between them.

Population expansion

Clear indications for population growth were supported by several lines of evidence, including genetic diversity statistics, mismatch analyses and neutrality tests for *E. kucheli* on PEI as well as for all the genetically-identifiable populations of *E. similis* on MI. For all groups, the nucleotide diversity was relatively low and the haplotype diversity was extremely high (Table 9), suggesting a population expansion. The neutrality tests also show strong support for population expansion of *E. kucheli* on PEI as well as for each of the genetically-identifiable populations of *E. similis* on MI. Instances where a weak signal was obtained, eg. clades 4-2a and clade 4-5b, are likely attributable to small sample sizes and the large numbers of missing haplotypes. Further evidence for both demographic expansions and range expansions were obtained from the mismatch analyses (Tables 6 & 7) and in each case, except in the cases of clades 4-2b and 4-6, demographic expansions predates range expansions. This makes sense in the light of the geographical history as major expansion was necessary for each genetically-identifiable population to cover its present geographical range after its original isolation by the Holocene post-glacial volcanism.

Conclusions

The present study suggests that PEI was the first of the two islands of the PEIA to be colonized by *Ectemnorhinus* weevils, and a time of coalescence of approximately 0.312 MYA (Fig. 17) and 0.5109 MYA (Fig. 18) was estimated for this event. No evidence of glaciation was found on PEI. If indeed PEI was not glaciated, it may explain the survival of *Ectemnorhinus* weevils through the glaciation stages suggested by McDougall *et al.* (2001) as indicated in Fig. 1a. The PEI population then acted as the source population for the colonization of MI by *Ectemnorhinus* weevils some time before the last glaciation, approximately 10 000 to 35 000 years ago (Fig. 2). This would give the weevils an approximate maximum of 30 000 years, the amount of time between the last glaciation and the one before, to colonize the whole of MI (Fig. 2). The current data suggest that strong, frequent winds from the SW as opposed to the weaker infrequent winds from the NE (Schulze, 1971) hampered the movement of weevils from east to west. Sufficient time for this east-west movement was thus available. The separation by distance of the PEI *Ectemnorhinus* weevils from those on MI then gave rise to two species by allopatric speciation, namely *E. kucheli* on PEI and *E. similis* on MI. During the last glaciation MI was extensively glaciated with only the south-western corner of the island being ice-free (Fig. 4). The extensive glaciation of MI may have resulted in the extinction of *E. similis* on MI except for the populations occurring on the ice-free south-western corner of the island. The weevils that at present group in clade 4-5 (Figs. 15 & 16) appear to be represent the relict population of the original MI colonizers that were able to migrate, despite the resistance of the strong, frequent south-western winds (Schulze, 1971), to the south-western corner of MI. This may also explain why clade 4-5 is the MI clade that is most closely related to *E. kucheli* on PEI (Table 15) despite the presence of geographically closer clades on the eastern side of MI. Date estimates obtained by the molecular clock should always be interpreted with caution as it is a subject of considerable debate (Graur & Martin, 2004). In this case, it is even more so as the time of coalescence for the individuals that comprise clade 4-5a was calculated from weevils that firstly originated from PEI. If the current observation of very high numbers of unique haplotypes in each clade is taken into account, the same trend may most possibly have been the case on MI before the last glaciation. Estimates from the Bayesian analyses may be over-inflated due to insufficient time for fixation of mutations. At the end of the last glacial maximum, when the ice started to melt, the coastal areas of MI emerged first from beneath the ice and were available for the re-colonization by the weevils. The movements of the weevils that were isolated in the south-western corner of MI, along the coastal areas of the

island, may have been assisted by the strong, frequent south-westerly winds (Schulze, 1971), and may have occurred over a very short time span. Evidence for this short time span comes from the observation that weevils that are members of clade 4-5 are currently found over the entire MI. Subsequent Holocene post-glacial volcanism (Hall, 1978; 1982; 2004) was then responsible for the fragmentation of the new migrants, resulting in small pockets of weevils across the island, surrounded by fresh, uninhabitable lava. As with the weevils that were isolated in the south-western corner of MI by the ice, these pockets of weevils, isolated by the lava, were not necessarily very closely related. The main pockets of isolated weevils correlate with the different clades that are identifiable at present: clade 4-5b that was isolated in the north of MI in the Cape Davis area, clade 4-3 that was isolated in the extensively fragmented north-east of MI, clade 4-1 that was isolated in the east of MI in the Tate's Hill – Stony Ridge area, clade 4-5 was again isolated by the lava in the south-western side of the island, clade 4-6 that was isolated in the Sidney Hill area and clade 4-2b that consists of a very small population of which sample size in the present study is too small for valid conclusions. The time it took for the different populations of isolated weevils to recover from the devastation of the lava as well as the time it took for the lava that isolated the weevils in small populations to become inhabitable for re-colonization by weevils was sufficient for the weevils in each population to diverge from those in the other populations. When the Holocene black lavas became re-colonizeable, the weevils from the different isolated populations may have again migrated to the rest of the island. The population that in the present study was recognised as clade 4-5a may again have spread over the entire MI with the assistance of the strong, frequent south-westerly winds (Schulze, 1971). The population that in the present study was denoted clade 4-1 was hampered by the strong, frequent south-westerly winds (Schulze, 1971) in their south-ward and west-ward migration but were much freer to disperse to the north. The 3rd- and 4th-stage Holocene post-glacial black lava (Fig. 3) may to some extent also have hampered both the east-ward and the north-ward migration of this population. The population that was recognised as clade 4-3 in the present study, like clade 4-1, was hampered by the strong, frequent south-westerly winds (Schulze, 1971) in their south-ward migration. Both west-ward and south-ward migration may also have been hampered by 3rd- and 4th-stage Holocene post-glacial black lava (Fig. 3). The population that was recognised as clade 4-6 in the present study was a very small population, and its east-ward migration may most likely have been blocked by the 3rd- and 4th-stage Holocene post-glacial black lava at Santa Rosa valley (Fig. 3). Only north-ward migration was thus possible for members of this population. The strong, frequent south-westerly winds (Schulze, 1971) as

well as the large amount of 3rdstage Holocene post-glacial black lava north of Triegaardt Bay may have prevented the westward movement of individuals from the population that was recognised as clade 4-5a in the present study. Currently, members of the different genetically-identifiable populations occur sympatrically, and in some cases even on the same plant, but with no noticeable geneflow being detected between them. It is thus suggested that the time of isolation, before the Holocene post-glacial black lavas became hospitable was sufficiently long and the populations sufficiently small that a number of genetically-discrete populations arose. The present study recognised two discrete populations of *E. kucheli* on PEI and seven of *E. similis* on MI. The fact that the living conditions and food available for all the different isolated populations were identical may explain why members of the different genetically-discrete populations are morphologically indistinct. It is suggested that breeding experiments between individuals from the different genetically-discrete populations should be undertaken to determine whether they are able to interbreed and if not, to determine whether there are some external factor(s) that may be preventing geneflow between them. This study supports Grobler *et al*. (2011b) who emphasized the need for the prevention of anthropogenic geneflow between MI and PEI in order to not interfere with the natural population dynamics. The current practice of limiting visits to PEI should be maintained as well as the strict provisions for quarantine for such visits. The presence of numerous genetically-discrete populations of *E. similis* on MI that do not occur on Prince Edward Island and that are under threat via increased size-selective predation by the house mouse, *Mus musculus domesticus* (Chown & Smith, 1993), underscores the urgency for control of mice on Marion Island before these genetically-discrete populations become extinct.

References

Akaike, H. 1974. A new look at the statistical model identification. *IEEE Transactions on Automatic Control* 19:716-723.

Bianchi, C. & Gersonde, R. 2004. Climate evolution at the last deglaciation: the role of the Southern Ocean. *Earth and Planetary Science Letters* 228: 407-424.

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.

Brower, A.V.Z. 1994. Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. *Proceedings of the National Academy of Sciences of the United States of America* 91: 6491-6495.

Brown, R.P., Suárez, N.M. & Pestano J. 2002. The Atlas mountains as a biogeographical divide in North-West Africa: evidence from mtDNA evolution in the Agamid lizard *Agama impalearis*. *Molecular Phylogenetics and Evolution* 24: 324-332.

Chevallier, L. 1986. Tectonics of Marion and Prince Edward Volcanoes (Indian Ocean): results of regional control and edifice dynamics. *Tectonophysics* 124: 155-175.

Chevallier, L., Verwoerd, W.J., Bova, P., Stettler, E., du Plessis, A., du Plessis, J.G., Fernandez, L.M. & Nel, M. 1992. Volcanological features and preliminary geophysical investigations on Marion Island. *South African Journal of Antarctic Research* 22: 15-35.

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

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.

Clement, M., Posada. D. & Crandall, K.A. 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology* 9: 1657-1660.

Crandall, K.A. 1996. Multiple Interspecies Transmissions of Humans and Simian T-Cell Leukemia/Lymphoma Virus Type I sequences. *Molecular Biology and Evolution* 13: 115-131.

Crandall, K.A. & Templeton, A.R. 1993. Empirical Tests of Some Predictions From Coalescent Theory With Applications to Intraspecific Phylogeny Reconstruction. *Genetics* 134: 959-969.

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

Excoffier, L. 2004. Patterns of DNA sequence diversity and genetic structure after a range expansion: lessons from the infinite-island model. *Molecular Ecology* 13: 853–864.

Excoffier, L., Smouse, P. & Quattro, J. 1992 Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics* 131:479-491.

Excoffier, L. & Lischer, H.E.L. 2010. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* 10: 564-567.

Excoffier, L. & Schneider, S. 1999. Why hunter-gatherer populations do not show signs of Pleistocene demographic expansions. *Proceedings of the National Academy of Sciences of the United States of America* 96: 10597-10602.

Felsenstein, J. 1981. Evolutionary trees from DNA sequences: A maximum likelihood approach. *Journal of Molecular Evolution* 17: 368-376.

Felsenstein, J. 1988. Phylogenies from molecular sequences: Inference and reliability. *Annual Reviews in Genetics* 22: 521-565.

Fu, Y.X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* 143: 557-570.

Graur, D. & Martin, W. 2004. Reading the entrails of chickens: molecular timescales of evolution and the illusion of precision. *Trends in Genetics* 20: 80-86.

Grobler, G.C., Janse van Rensburg, L., Bastos, A.D.S., Chimimba, C.T. & Chown, S.L. 2006. Molecular and morphometric assessment of the taxonomic status of *Ectemnorhinus* weevil species (Coleoptera: Curculionidae, Entiminae) from the sub-Antarctic Prince Edward Islands. *Journal of Zoological Systematics and Evolutionary Research* 44: 200-211.

Grobler, G.C., Bastos, A.D.S., Treasure, A. & Chown, S.L. 2011. Cryptic species, biogeographic complexity and evolutionary history of the *Ectemnorhinus*-group in the sub-Antarctic, including a description of *Bothrometopus huntleyi*, n. sp. *Antarctic Science* 23: 211-224.

Grobler, G.C., L., Bastos, A.D.S., Chimimba, C.T. & Chown, S.L. 2011. Inter-island dispersal of flightless *Bothrometopus huntleyi* (Coleoptera: Curculionidae) from the sub-Antarctic Prince Edward Island Archipelago. *Antarctic Science* 23: 225-234.

Guindon, S. & Gascuel, O. 2003. A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. *Systematic Biology* 52: 696-704.

Hall, K.J. 1978. Evidence for Quaternary glaciation of Marion Island (Sub-Antarctic) and some implications. In Van Zinderen Bakker E.M. (ed.) *Antarctic Glacial History and World Palaeo-environments.* A.A. Balkema, Rotterdam, pp. 137-147.

Hall, K. 1980. Late glacial ice cover and palaeotemperatures on sub-Antarctic Marion Island. *Palaeogeography, Palaeoclimatology and Palaeoecology* 29: 243-259.

Hall, K. 1981. Quantitative analysis of till lithology on Marion Island. *South African Journal of Science* 77: 86-90.

Hall, K. 1982. Rapid deglaciation as an initiator of volcanic activity: an hypothesis. *Earth Surface Processes and Landforms* 7: 45-51.

Hall, K. 2004. Quaternary glaciation of the sub-Antarctic Islands. In Ehlers J. & Gibbard P.I. (Eds.). *Quaternary Glaciations - Extent and Chronology, Part III*. Elsevier, Amsterdam, pp. 339-345.

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

Holsinger, K.E & Weir, B.S. 2009. Genetics in geographically structured populations: defining, estimating and interpreting FST. *Nature Reviews Genetics*, 10: 639-650.

Hudson, R.R., Slatkin, M. & Maddison W.P. 1992. Estimation of levels of gene flow from DNA sequence data. *Genetic*s 132: 583-589.

Huelsenbeck, J.P. & Ronquist, F. 2001. MRBAYES: Bayesian inference of phylogeny. *Bioinformatics*, 17: 754-755.

Jeannel, R. 1940. Coléoptères. Croisière du Bougainville aux îles australes francaises. *Mémoires du Muséum National d'Histoire Naturelle (N.S.)* 14: 63-201.

Jukes, T.H. & Cantor, C.R. 1969. Evolution of protein molecules. In Munro, H.N., editor, *Mammalian Protein Metabolism*, pp. 21-132, Academic Press, New York.

Knowles, L.L. 2008. Why does a method that fails continue to be used? *Evolution* 62: 2713–2717.

Knowles, L.L., & Maddison, W.P. 2002. Statistical phylogeography. *Molecular Ecology* 11: 2623–2635.

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.

Kuschel, G. & Chown, S.L. 1995. Phylogeny and Systematics of the *Ectemnorhinus*-group of Genera (Insecta: Coleoptera). *Invertebrate Taxonomy* 9: 841-863.

Langenegger, O. & Verwoerd, W.J. 1971. Topographic Survey. Report on the South African biological and geological expedition 1965-1966, In: *Marion and Prince Edward Islands*, van Zinderen Bakker EM, Winterbottom JM, Dryer RA (eds). Cape Town, A. A. Balkema, pp 32-39.

Li, P. & Bousquet, J. 1992. Relative-Rate test for nucleotide substitutions between two lineages. *Molecular Biology and Evolution* 9: 1185-1189.

Librado, P. & Rozas, J. 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25: 1451-1452.

Lynch, M. & Crease T.J. 1990. The analysis of population survey data on DNA sequence variation. *Molecular Biology and Evolution* 7: 377-394.

Mahoney, J., Le Roux, A.P., Peng, Z., Fisher, R.L., & Natland, J.H. 1992. Southwestern limits of Indian Ocean ridge mantle and the origin of low $^{206}Pb/^{204}Pb$ mid-ocean ridge basalt: Isotope systematics of the central Southwest Indian Ridge (17º-50ºE). *Journal of Geophysical Research* 97: 19771-19790.

McCarthy, C. 1996-1997. School of Biomolecular and Biomedical Science, Faculty of Science and Technology, Griffith University, Brisbane, Queensland, Australia.McDougall, I., Verwoerd, W. & Chevallier, L. 2001. K-Ar geochronology of Marion Island, Southern Ocean. *Geological Magazine* 138: 1-17.

McDougall, I., Verwoerd, W. & Chevallier, L. 2001. K-Ar geochronology of Marion Island, Southern Ocean. *Geological Magazine*, 138, 1-17.

Meiklejohn, K.I. & Hedding, D.W. 2005. Marion Island. In: Wunderman, R., Venzke, E. & Mayberry, G. (Eds.). *Bulletin of the Global Volcanism Network*, 30: 16.

Nei, M & Li, W.H. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences of the United States of America* 76: 5269-5273.

Papadopoulou, A., Anastasiou, I. & Vogler, A.P. 2010. Revisiting the molecular clock: the mid-Aegean trench calibration. *Molecular Biology and Evolution* 27: 1659-1672.

Posada, D. 2008. jModelTest: Phylogenetic Model Averaging. *Molecular Biology and Evolution* 25: 1253-1256.

Posada, D., Crandall, K.A. & Templeton, A.R. 2000. GeoDis: a program for the cladistic nested analysis of the geographical distribution of genetic haplotypes. *Molecular Ecology* 9: 487-488.

Ray, N., Currat, M. & Excoffier, L. Intra-Deme Molecular Diversity in Spatially Expanding Populations. *Molecular Biology and Evolution* 20: 76–86.

Reynolds, J., Weir, B.S. & Cockerham, C.C. 1983. Estimation of the coancestry coefficient: basis for a short-term genetic distance. *Genetics* 105: 767-779.

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

Rogers, R.A. & Harpending, H. 1992. Population Growth Makes Waves in the Distrubution of Pairwise Genetic Differences. *Molecular Biology and Evolution* 9: 552-569.

Rzhetsky, A. & Nei, M. 1992. A simple method for estimating and testing minimum-evolution trees. *Molecular Biology and Evolution* 9: 945-967.

Saitou, N. & Neim M. 1987. The Neighbor-joining Method: A New Method for Reconstructing Phylogenetic Trees. *Molecular Biology and Evolution* 4: 406-425.

Schulze, B.R. 1971. The climate of Marion Island. 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. 16-31.

Slatkin, M. 1991. Inbreeding coefficients and coalescence times. *Genetic Research, Cambridge* 58: 167-175.

Slatkin, M. 1995. A measure of population subdivision based on microsatellite allele frequencies. *Genetics* 139: 457-462.

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

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

Sumner, P.D., Meiklejohn, K.I., Boelhouwers, J.C. & Hedding, D.W. 2004. Climate change melts Marion Island's snow and ice. *South African Journal of Science* 100: 1-4.

Tamura, K. & Nei, M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* 10: 512-526.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* 24: 1596-1599.

Tajima, F. 1983. Evolutionary relationship of DNA sequences in finite populations. *Genetics* 105: 437-460.

Templeton, A.R., 2008. Why does a method that fails continue to be used? The answer. *Evolution* 63: 807–812.

Templeton, A.R., Boerwinkle, E. & Sing, C.F. 1987. A cladistic analyses of phenotypic associations with haplotypes inferred from restriction endonuclease mapping. I. Basic theory and an analysis af alcohol dehydrogenase activity in Droaophila. *Genetics* 117: 343-351.

Templeton, A.R., Crandall, K.A. & Sing, C.F. 1992. A caldistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping. III. Cladogram estimation. *Genetics* 132: 619-633.

Templeton, A.R., Routman, E. & Phillips, C.A. 1995. Separating population structure from population history: a cladistic analysis of the geographical distribution of mitochondrial DNA haplotypes in the Tiger Salamander, *Ambysoma Tigrinum*. *Genetics*, 134: 659-669.

Templeton, A.R. & Sing, C.F. 1993. A Cladistic Analyses of Phenotypic Associations with Haplotypes Inferred from Restriction Endonuclease Mapping. IV. Nested Analyses with Cladogram Uncertainty and Recombination. *Genetics* 134: 659- 669.

Van Zinderen Bakker, E.M. 1973. The glaciation(s) of Marion Island (sub-Antarctic). In *Palaeoecology of Africa and the Surrounding Islands and Antarctica*, vol. 8, ed. van Zinderen Bakker, E.M., pp. 161-178.

Verwoerd, W.J. 1971. Geology. Report on the South African biological and geological expedition 1965-1966, In: *Marion and Prince Edward Islands*, van Zinderen Bakker, E.M., Winterbottom, J.M. & Dryer, R.A. (eds). Cape Town, A. A. Balkema, pp 40-62.

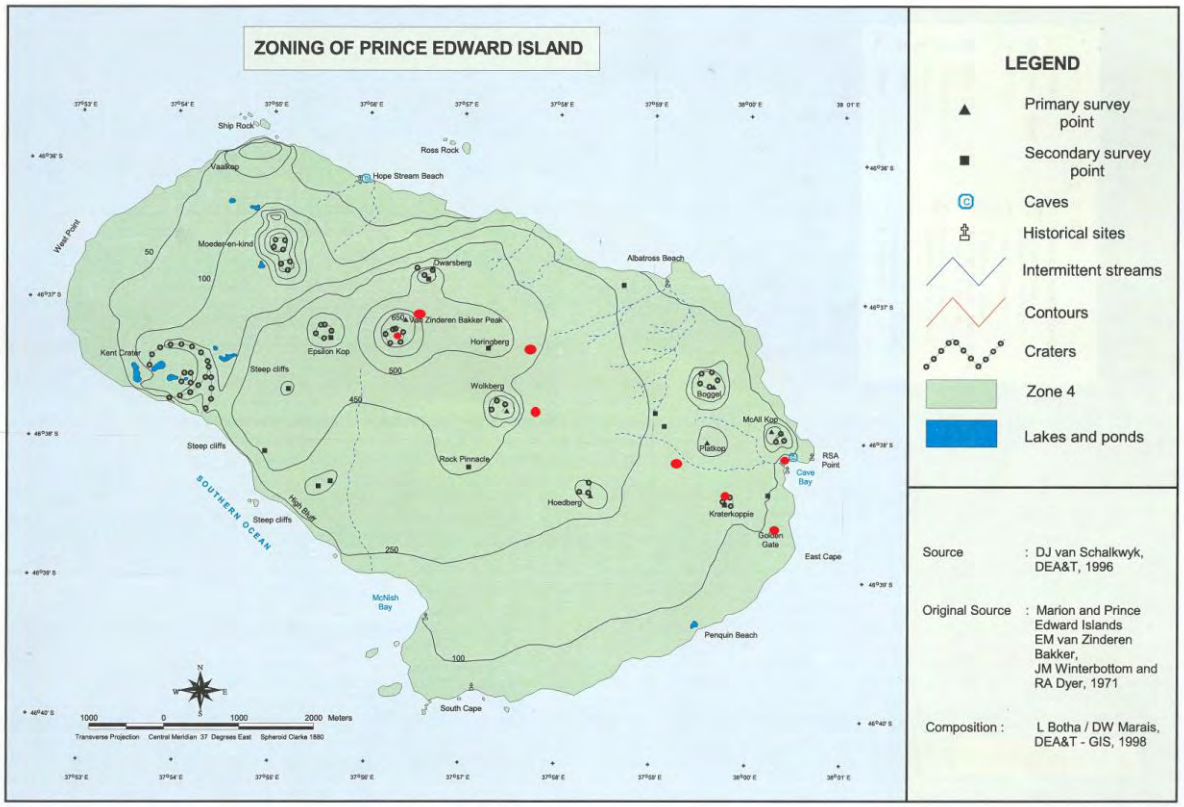
Verwoerd, W.J., Russell, S., & Berruti, A. 1981. 1980 Volcanic eruption reported on Marion Island. *Earth and Planetary Science Letters* 54: 153-156.

Waterhouse, G.R. 1853. Descriptions of new genera and species of Culculionides. *Transactions of the Entomological Society of London* 2: 172-207.

Waterhouse, C.O. 1885. Description of two new Curculionidae (*Ectemnorhinus*) from Marion Islands. *Annals and Magazine of Natural History, London* 16: 121-123.

Wright, S. 1951. The genetical structure of populations. *Annals of Eugenics* 15: 323-354.

Appendix 1: Map showing sample sites on Marion Island, in green, and on Prince Edward Island, in red.



Appendix II: Summary of the sampling localities on Marion (MI) and Prince Edward Islands (PEI) from which the genetically characterised specimens included in this study were collected.

Island	Sampling Locality (a.s.l)	Geographic coordinates	Voucher	Species	GenBank
MI	Halfway Kop (600 m)	S 46º54'06'' E 37º47'83''	125-4	*E. similis*	JF327538
MI	Halfway Kop (600 m)	S 46º54'06'' E 37º47'83''	126-1	*E. similis*	JF327559
MI	Halfway Kop (600 m)	S 46º54'06'' E 37º47'83''	126-52	*E. similis*	JF327560
MI	Halfway Kop (600 m)	S 46º54'06'' E 37º47'83''	125-51	*E. similis*	JF327566
MI	Halfway Kop (600 m)	S 46º54'06'' E 37º47'83''	126-6	*E. similis*	JF327561
MI	Halfway Kop (600 m)	S 46º54'06'' E 37º47'83''	125-42	*E. similis*	JF327562
MI	Halfway Kop (600 m)	S 46º54'06'' E 37º47'83''	125-43	*E. similis*	JF327539
MI	Halfway Kop (600 m)	S 46º54'06'' E 37º47'83''	125-53	*E. similis*	JF327630
MI	Albatros Lakes (50 m)	S 46º53'49.2'' E 37º51'55''	110-27	*E. similis*	AY762288
MI	Albatros Lakes (50 m)	S 46º53'49.2'' E 37º51'55''	110-3	*E. similis*	AY762287
MI	Albatros Lakes (50 m)	S 46º53'49.2'' E 37º51'55''	110-18	*E. similis*	AY762294
MI	Albatros Lakes (50 m)	S 46º53'49.2'' E 37º51'55''	84-2	*E. similis*	JF327626
MI	Albatros Lakes (50 m)	S 46º53'49.2'' E 37º51'55''	110-29	*E. similis*	JF327563
MI	Albatros Lakes (50 m)	S 46º53'49.2'' E 37º51'55''	110-36	*E. similis*	AY762289
MI	Albatros Lakes (50 m)	S 46º53'49.2'' E 37º51'55''	84-1	*E. similis*	JF327616
MI	Albatros Lakes (50 m)	S 46º53'49.2'' E 37º51'55''	84-14	*E. similis*	JF327609
MI	Skua Ridge (100 m)	S 46º51'50'' E 37º51'486''	70-4	*E. similis*	JF327624
MI	Skua Ridge (100 m)	S 46º51'50'' E 37º51'486''	65-3	*E. similis*	JF327627
MI	Skua Ridge (100 m)	S 46º51'50'' E 37º51'486''	68-38	*E. similis*	JF327625
MI	Skua Ridge (100 m)	S 46º51'50'' E 37º51'486''	68-32	*E. similis*	JF327607
MI	Long Ridge South (450 m)	S 46°52'45'' E 37°47'00''	353-7	*E. similis*	JF327592
MI	Long Ridge South (450 m)	S 46°52'45'' E 37°47'00''	353-2	*E. similis*	JF327591
MI	Long Ridge South (450 m)	S 46°52'45'' E 37°47'00''	354-14	*E. similis*	JF327593
MI	Tate's Hill (400m)	S 46º54'36'' E 37º50'28.68''	120-20	*E. similis*	AY762293
MI	Tate's Hill (400m)	S 46º54'36'' E 37º50'28.68''	121-7	*E. similis*	JF327526
MI	Tate's Hill (400m)	S 46º54'36'' E 37º50'28.68''	121-1	*E. similis*	JF327525
MI	Tate's Hill (400m)	S 46º54'36'' E 37º50'28.68''	120-2	*E. similis*	AY762290
MI	Tate's Hill (400m)	S 46º54'36'' E 37º50'28.68''	120-10	*E. similis*	AY762267
MI	Tate's Hill (400m)	S 46º54'36'' E 37º50'28.68''	120-16	*E. similis*	JF327508
MI	Tate's Hill (400m)	S 46º54'36'' E 37º50'28.68''	120-18	*E. similis*	AY762291
MI	Tate's Hill (400m)	S 46º54'36'' E 37º50'28.68''	121-16	*E. similis*	JF327523
MI	Tate's Hill (400m)	S 46º54'36'' E 37º50'28.68''	122-16	*E. similis*	JF327522
MI	Tate's Hill (400m)	S 46º54'36'' E 37º50'28.68''	122-19	*E. similis*	JF327521
MI	Tate's Hill (400m)	S 46º54'36'' E 37º50'28.68''	120-7	*E. similis*	JF327564
MI	Tate's Hill (400m)	S 46º54'36'' E 37º50'28.68''	121-3	*E. similis*	JF327524
MI	Tafelberg (250 m)	S 46°53'03.5'' E 37°48'20.1''	24-3	*E. similis*	JF327623
MI	Tafelberg (250 m)	S 46°53'03.5'' E 37°48'20.1''	24-4	*E. similis*	JF327621
MI	Tafelberg (250 m)	S 46°53'03.5'' E 37°48'20.1''	35-9	*E. similis*	JF327610
MI	Tafelberg (250 m)	S 46°53'03.5'' E 37°48'20.1''	37-4	*E. similis*	JF327605
MI	Tafelberg (250 m)	S 46°53'03.5'' E 37°48'20.1''	23-4	*E. similis*	JF327613
MI	Tafelberg (250 m)	S 46°53'03.5'' E 37°48'20.1''	37-6	*E. similis*	JF327612
MI	Katedraal Krans (800 m)	S 46°53'89.6'' E 37°46'48.2''	163-17	*E. similis*	AY762269
MI	Katedraal Krans (800 m)	S 46°53'89.6'' E 37°46'48.2''	167-22	*E. similis*	AY762272
MI	Katedraal Krans (800 m)	S 46°53'89.6'' E 37°46'48.2''	164-10	*E. similis*	JF327629
MI	Katedraal Krans (800 m)	S 46°53'89.6'' E 37°46'48.2''	164-9	*E. similis*	AY762268

Island	Sampling Locality (a.s.l)	Geographic coordinates	Voucher	Species	GenBank
MI	Katedraal Krans (800 m)	S 46°53’89.6’’ E 37°46’48.2’’	163-32	*E. similis*	JF327541
MI	Katedraal Krans (800 m)	S 46°53’89.6’’ E 37°46’48.2’’	163-31	*E. similis*	AY762292
MI	Katedraal Krans (800 m)	S 46°53’89.6’’ E 37°46’48.2’’	163-2	*E. similis*	AY762270
MI	Junior's Kop (200 m)	S 46°52’79.4’’ E 37°50’08.3’’	150-1	*E. similis*	AY762285
MI	Junior's Kop (200 m)	S 46°52’79.4’’ E 37°50’08.3’’	150-10	*E. similis*	JF327546
MI	Junior's Kop (200 m)	S 46°52’79.4’’ E 37°50’08.3’’	150-4	*E. similis*	AY762284
MI	Junior's Kop (200 m)	S 46°52’79.4’’ E 37°50’08.3’’	149-26	*E. similis*	JF327545
MI	Junior's Kop (200 m)	S 46°52’79.4’’ E 37°50’08.3’’	149-38	*E. similis*	AY762286
MI	Junior's Kop (200 m)	S 46°52’79.4’’ E 37°50’08.3’’	149-32	*E. similis*	AY762283
MI	First Red Hill (400 m)	S 46°53’41.2’’ E 37°48’21’’	173-1	*E. similis*	AY762281
MI	First Red Hill (400 m)	S 46°53’41.2’’ E 37°48’21’’	173-2	*E. similis*	AY762280
MI	First Red Hill (400 m)	S 46°53’41.2’’ E 37°48’21’’	172-18	*E. similis*	JF327544
MI	First Red Hill (400 m)	S 46°53’41.2’’ E 37°48’21’’	172-73	*E. similis*	AY762279
MI	First Red Hill (400 m)	S 46°53’41.2’’ E 37°48’21’’	172-16	*E. similis*	AY762282
MI	First Red Hill (600 m)	S 46º53’39.82’’ E 37º47’12.48’’	168-4	*E. similis*	AY762274
MI	First Red Hill (600 m)	S 46º53’39.82’’ E 37º47’12.48’’	168-5	*E. similis*	AY762273
MI	First Red Hill (600 m)	S 46º53’39.82’’ E 37º47’12.48’’	167-15	*E. similis*	JF327542
MI	First Red Hill (600 m)	S 46º53’39.82’’ E 37º47’12.48’’	167-7	*E. similis*	AY762271
MI	Repetto’s Hill (300 m)	S 46º50’35.7’’ E 37º44’21.8’’	140-32	*E. similis*	JF327550
MI	Repetto’s Hill (300 m)	S 46º50’35.7’’ E 37º44’21.8’’	140-33	*E. similis*	JF327536
MI	Repetto’s Hill (300 m)	S 46º50’35.7’’ E 37º44’21.8’’	140-24	*E. similis*	JF327557
MI	Repetto’s Hill (300 m)	S 46º50’35.7’’ E 37º44’21.8’’	140-3	*E. similis*	JF327537
MI	Repetto’s Hill (300 m)	S 46º50’35.7’’ E 37º44’21.8’’	139-4	*E. similis*	JF327558
MI	Repetto’s Hill (300 m)	S 46º50’35.7’’ E 37º44’21.8’’	140-6	*E. similis*	JF327535
MI	Stony Ridge (150 m)	S 46°54’88.1’’ E 37°51’48.4’’	118-14	*E. similis*	JF327540
MI	Stony Ridge (150 m)	S 46°54’88.1’’ E 37°51’48.4’’	119-17	*E. similis*	JF327517
MI	Stony Ridge (150 m)	S 46°54’88.1’’ E 37°51’48.4’’	118-9	*E. similis*	JF327519
MI	Stony Ridge (150 m)	S 46°54’88.1’’ E 37°51’48.4’’	118-3	*E. similis*	JF327551
MI	Stony Ridge (150 m)	S 46°54’88.1’’ E 37°51’48.4’’	118-10	*E. similis*	JF327506
MI	Stony Ridge (150 m)	S 46°54’88.1’’ E 37°51’48.4’’	44-12	*E. similis*	JF327617
MI	Stony Ridge (150 m)	S 46°54’88.1’’ E 37°51’48.4’’	119-29	*E. similis*	JF327518
MI	Stony Ridge (150 m)	S 46°54’88.1’’ E 37°51’48.4’’	46-9	*E. similis*	JF327619
MI	Stony Ridge (150 m)	S 46°54’88.1’’ E 37°51’48.4’’	119-7	*E. similis*	JF327520
MI	Stony Ridge (150 m)	S 46°54’88.1’’ E 37°51’48.4’’	44-15	*E. similis*	JF327614
MI	Van Den Boogaard (150 m)	S 46º52’46.3’’ E 37º49’58.1’’	75-16	*E. similis*	JF327628
MI	Van Den Boogaard (150 m)	S 46º52’46.3’’ E 37º49’58.1’’	75-2	*E. similis*	JF327622
MI	Lou-se-kop (150 m)	S 46º49’49.44’’ E 37º42’47.52’’	136-11	*E. similis*	JF327565
MI	Lou-se-kop (150 m)	S 46º49’49.44’’ E 37º42’47.52’’	136-39	*E. similis*	JF327554
MI	Lou-se-kop (150 m)	S 46º49’49.44’’ E 37º42’47.52’’	137-3	*E. similis*	JF327510
MI	Lou-se-kop (150 m)	S 46º49’49.44’’ E 37º42’47.52’’	137-2	*E. similis*	JF327511
MI	Lou-se-kop (150 m)	S 46º49’49.44’’ E 37º42’47.52’’	136-61	*E. similis*	JF327509
MI	Johnny’s Hill (300 m)	S 46º57’50’’ E 37º49’30’’	335-2	*E. similis*	JF327568
MI	Johnny’s Hill (300 m)	S 46º57’50’’ E 37º49’30’’	336-26	*E. similis*	JF327571
MI	Johnny’s Hill (300 m)	S 46º57’50’’ E 37º49’30’’	336-18	*E. similis*	JF327570
MI	Johnny’s Hill (300 m)	S 46º57’50’’ E 37º49’30’’	335-3	*E. similis*	JF327569
MI	Cape Davis (0 m)	S 46°49’41.2’’ E 37°41’83.3’’	138-8	*E. similis*	JF327513
MI	Cape Davis (0 m)	S 46°49’41.2’’ E 37°41’83.3’’	138-7	*E. similis*	JF327527

Island	Sampling Locality (a.s.l)	Geographic coordinates	Voucher	Species	GenBank
MI	Cape Davis (0 m)	S 46°49'41.2'' E 37°41'83.3''	134-3	*E. similis*	JF327514
MI	Cape Davis (0 m)	S 46°49'41.2'' E 37°41'83.3''	134-2	*E. similis*	JF327553
MI	Cape Davis (0 m)	S 46°49'41.2'' E 37°41'83.3''	138-5	*E. similis*	JF327552
MI	Cape Davis (0 m)	S 46°49'41.2'' E 37°41'83.3''	134-4	*E. similis*	JF327515
MI	Cape Davis (0 m)	S 46°49'41.2'' E 37°41'83.3''	134-5	*E. similis*	JF327516
MI	Cape Davis (0 m)	S 46°49'41.2'' E 37°41'83.3''	138-6	*E. similis*	JF327512
MI	Azorellakop (450 m)	S 46º52'2.1'' E 37º39'57.3''	142-7	*E. similis*	JF327530
MI	Azorellakop (450 m)	S 46º52'2.1'' E 37º39'57.3''	142-9	*E. similis*	JF327556
MI	Azorellakop (450 m)	S 46º52'2.1'' E 37º39'57.3''	143-7	*E. similis*	JF327534
MI	Azorellakop (450 m)	S 46º52'2.1'' E 37º39'57.3''	143-14	*E. similis*	JF327533
MI	Azorellakop (450 m)	S 46º52'2.1'' E 37º39'57.3''	143-15	*E. similis*	JF327532
MI	Azorellakop (450 m)	S 46º52'2.1'' E 37º39'57.3''	143-9	*E. similis*	JF327531
MI	Azorellakop (450 m)	S 46º52'2.1'' E 37º39'57.3''	142-1	*E. similis*	JF327529
MI	Ice Plateau (1000 m)	S 46º54'17.4'' E 37º45'22.5''	157-8	*E. similis*	AY762276
MI	Ice Plateau (1000 m)	S 46º54'17.4'' E 37º45'22.5''	157-5	*E. similis*	JF327567
MI	Ice Plateau (1000 m)	S 46º54'17.4'' E 37º45'22.5''	157-4	*E. similis*	JF327543
MI	Ice Plateau (1000 m)	S 46º54'17.4'' E 37º45'22.5''	157-3	*E. similis*	AY762278
MI	Ice Plateau (1000 m)	S 46º54'17.4'' E 37º45'22.5''	157-12	*E. similis*	AY762277
MI	Ice Plateau (1000 m)	S 46º54'17.4'' E 37º45'22.5''	157-2	*E. similis*	AY762275
MI	Kildalkey Bay (0 m)	S 46°57'38.3'' E 37°51'22.2''	332-9	*E. similis*	JF327585
MI	Kildalkey Bay (0 m)	S 46°57'38.3'' E 37°51'22.2''	332-14	*E. similis*	JF327586
MI	Kildalkey Bay (0 m)	S 46°57'38.3'' E 37°51'22.2''	332-7	*E. similis*	JF327583
MI	Kildalkey Bay (0 m)	S 46°57'38.3'' E 37°51'22.2''	332-1	*E. similis*	JF327584
MI	Water Tunnel (0 m)	S 46°57'49.2'' E 37°44'50.44''	343-13	*E. similis*	JF327602
MI	Water Tunnel (0 m)	S 46°57'49.2'' E 37°44'50.44''	343-25	*E. similis*	JF327573
MI	Water Tunnel (0 m)	S 46°57'49.2'' E 37°44'50.44''	343-17	*E. similis*	JF327572
MI	Transvaal Cove Base (0 m)	S 46º52'36.6'' E 37º51'35.94''	55-26	*E. similis*	JF327620
MI	Transvaal Cove Base (0 m)	S 46º52'36.6'' E 37º51'35.94''	55-34	*E. similis*	JF327618
MI	Sidney Hill (300 m)	S 46º57'34.7'' E 37º39'53.2''	359-3	*E. similis*	JF327579
MI	Sidney Hill (300 m)	S 46º57'34.7'' E 37º39'53.2''	360-17	*E. similis*	JF327581
MI	Sidney Hill (300 m)	S 46º57'34.7'' E 37º39'53.2''	359-5	*E. similis*	JF327580
MI	Sidney Hill (300 m)	S 46º57'34.7'' E 37º39'53.2''	360-14	*E. similis*	JF327582
MI	Swartkop (50 m)	S 46º55'26.94'' E 37º35'43.86''	145-24	*E. similis*	JF327555
MI	Swartkop (50 m)	S 46º55'26.94'' E 37º35'43.86''	145-34	*E. similis*	JF327507
MI	Swartkop (50 m)	S 46º55'26.94'' E 37º35'43.86''	146-12	*E. similis*	JF327549
MI	Swartkop (50 m)	S 46º55'26.94'' E 37º35'43.86''	145-19	*E. similis*	JF327528
MI	Swartkop (50 m)	S 46º55'26.94'' E 37º35'43.86''	146-13	*E. similis*	JF327548
MI	Swartkop (50 m)	S 46º55'26.94'' E 37º35'43.86''	146-8	*E. similis*	JF327547
MI	Feldmark Plateau (600 m)	S 46°56'35'' E 37°46'10''	337-23	*E. similis*	JF327587
MI	Feldmark Plateau (600 m)	S 46°56'35'' E 37°46'10''	337-26	*E. similis*	JF327588
MI	Feldmark Plateau (600 m)	S 46°56'35'' E 37°46'10''	338-2	*E. similis*	JF327589
MI	Feldmark Plateau (600 m)	S 46°56'35'' E 37°46'10''	338-4	*E. similis*	JF327590
MI	Pyroxene Kop (600 m)	S 46°56'43.4'' E 37°41'40.5''	346-27	*E. similis*	JF327600
MI	Pyroxene Kop (600 m)	S 46°56'43.4'' E 37°41'40.5''	346-17	*E. similis*	JF327574
MI	Pyroxene Kop (600 m)	S 46°56'43.4'' E 37°41'40.5''	348-2	*E. similis*	JF327575
MI	Pyroxene Kop (600 m)	S 46°56'43.4'' E 37°41'40.5''	348-3	*E. similis*	JF327594
MI	Goodhope Bay (0 m)	S 46°57'55.9'' E 37°42'04.4''	353A-3	*E. similis*	JF327577

Island	Sampling Locality (a.s.l)	Geographic coordinates	Voucher	Species	GenBank
MI	Goodhope Bay (0 m)	S 46°57'55.9'' E 37°42'04.4''	352-6	*E. similis*	JF327576
MI	Goodhope Bay (0 m)	S 46°57'55.9'' E 37°42'04.4''	353A-5	*E. similis*	JF327578
MI	Goodhope Bay (0 m)	S 46°57'55.9'' E 37°42'04.4''	352-24	*E. similis*	JF327601
MI	Ship's Cove (0 m)	S 46°51'41'' E 37°50'66''	21-2	*E. similis*	JF327611
MI	Trypot Beach (0 m)	S 46°53'05.2'' E 37°52'06''	14-2	*E. similis*	JF327615
MI	Archway Bay (0 m)	S 46º53'56.9'' E 37º53'45''	78-5	*E. similis*	JF327608
MI	Archway Bay (0 m)	S 46º53'56.9'' E 37º53'45''	78-1	*E. similis*	JF327606
PEI	Cave Bay (0 m)	S 46º38'45.12'' E 37º59'46.8''	304-14	*E. similis*	AY762298
PEI	Cave Bay (0 m)	S 46º38'45.12'' E 37º59'46.8''	304-24	*E. similis*	JF327595
PEI	Cave Bay (0 m)	S 46º38'45.12'' E 37º59'46.8''	304-28	*E. similis*	AY762299
PEI	Cave Bay (0 m)	S 46º38'45.12'' E 37º59'46.8''	304-4	*E. similis*	AY762301
PEI	Cave Bay (0 m)	S 46º38'45.12'' E 37º59'46.8''	304-6	*E. similis*	JF327596
PEI	Cave Bay (0 m)	S 46º38'45.12'' E 37º59'46.8''	304-13	*E. kucheli*	AY762312
PEI	(200 m)	S 46º38'27.42'' E 37º58'23.76''	315-7	*E. similis*	AY762295
PEI	(200 m)	S 46º38'27.42'' E 37º58'23.76''	315-22	*E. similis*	AY762297
PEI	(200 m)	S 46º38'27.42'' E 37º58'23.76''	316-29	*E. similis*	JF327598
PEI	(200 m)	S 46º38'27.42'' E 37º58'23.76''	316-30	*E. kucheli*	JF327637
PEI	(200 m)	S 46º38'27.42'' E 37º58'23.76''	316-6	*E. kucheli*	AY762311
PEI	(200 m)	S 46º38'27.42'' E 37º58'23.76''	316-15	*E. kucheli*	AY762314
PEI	(400 m)	S 46º38'12.66'' E 37º57'28.92''	309-16	*E. similis*	AY762296
PEI	(400 m)	S 46º38'12.66'' E 37º57'28.92''	309-7	*E. kucheli*	AY762316
PEI	(400 m)	S 46º38'12.66'' E 37º57'28.92''	310-4	*E. kucheli*	AY762317
PEI	(400 m)	S 46º38'12.66'' E 37º57'28.92''	310-15	*E. kucheli*	JF327640
PEI	(400 m)	S 46º38'12.66'' E 37º57'28.92''	310-3	*E. kucheli*	AY762318
PEI	(600 m)	S 46º37'31.98'' E 37º55'59.1''	312-10	*E. similis*	AY762302
PEI	(600 m)	S 46º37'31.98'' E 37º55'59.1''	312-16	*E. similis*	JF327597
PEI	(600 m)	S 46º37'31.98'' E 37º55'59.1''	312-5	*E. similis*	AY762305
PEI	(600 m)	S 46º37'31.98'' E 37º55'59.1''	312-6	*E. kucheli*	JF327636
PEI	(600 m)	S 46º37'31.98'' E 37º55'59.1''	311-10	*E. kucheli*	AY762309
PEI	(600 m)	S 46º37'31.98'' E 37º55'59.1''	311-7	*E. kucheli*	AY762310
PEI	TvZB (672 m)	S 46º37'35.4'' E 37º55'53.46''	320-1	*E. similis*	AY762307
PEI	TvZB (672 m)	S 46º37'35.4'' E 37º55'53.46''	321-11	*E. similis*	AY762308
PEI	TvZB (672 m)	S 46º37'35.4'' E 37º55'53.46''	320-3	*E. similis*	AY762306
PEI	TvZB (672 m)	S 46º37'35.4'' E 37º55'53.46''	321-2	*E. kucheli*	AY762315
PEI	TvZB (672 m)	S 46º37'35.4'' E 37º55'53.46''	320-4	*E. kucheli*	JF327638
PEI	Ditrichum (450 m)	S 46º38'3.42'' E 37º56'46.26''	306-1	*E. similis*	AY762300
PEI	Ditrichum (450 m)	S 46º38'3.42'' E 37º56'46.26''	306-7	*E. similis*	JF327599
PEI	Ditrichum (450 m)	S 46º38'3.42'' E 37º56'46.26''	305-3	*E. similis*	AY762303
PEI	Ditrichum (450 m)	S 46º38'3.42'' E 37º56'46.26''	306-4	*E. similis*	AY762304
PEI	Ditrichum (450 m)	S 46º38'3.42'' E 37º56'46.26''	305-2	*E. kucheli*	JF327639
PEI	Ditrichum (450 m)	S 46º38'3.42'' E 37º56'46.26''	305-1	*E. kucheli*	AY762313
PEI	Krater Koppie (150 m)	S 46º38'24.36'' E 37º59'46.92''	18-8	*E. similis*	JF327604
PEI	Krater Koppie (150 m)	S 46º38'24.36'' E 37º59'46.92''	18-7	*E. similis*	JF327603
PEI	Krater Koppie (150 m)	S 46º38'24.36'' E 37º59'46.92''	18-15	*E. kucheli*	JF327631
PEI	Krater Koppie (150 m)	S 46º38'24.36'' E 37º59'46.92''	18-5	*E. kucheli*	JF327635
PEI	Golden Gate (50 m)	S 46º38'35.7'' E 38º00'16.38''	17-14	*E. kucheli*	JF327634
PEI	Golden Gate (50 m)	S 46º38'35.7'' E 38º00'16.38''	14-6	*E. kucheli*	JF327632

Island	Sampling Locality (a.s.l)	Geographic coordinates	Voucher	Species	GenBank
PEI	Golden Gate (50 m)	S 46º38'35.7'' E 38º00'16.38''	17-3	*E. kucheli*	JF327633

CHAPTER 4

Cryptic species, biogeographic complexity and the evolutionary history of the *Ectemnorhinus*-group in the sub-Antarctic, including a description of *Bothrometopus huntleyi*, *n. sp*.

G.C. GROBLER[1], A.D.S. BASTOS[1], A.M. TREASURE[2] & S.L. CHOWN[2,*]

[1]*Department of Zoology and Entomology, University of Pretoria, Pretoria 0001, South Africa*
[2]*Centre for Invasion Biology, Department of Botany and Zoology, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa*

The results presented here have been published in *Antarctic Science* 23, 211-224 (2011).

Abstract: The biogeography of the South Indian Ocean Province (SIP) biotas has long been controversial. Much of the discussion has been based on interpretation of species distributions, based on morphological or anatomical delimitations. However, molecular phylogenetic approaches elsewhere have recently shown that interpretations based solely on morphological data may be misleading. Nonetheless, few studies have employed molecular phylogenetic approaches to understand the biogeography of the SIP biotas. We do so here for the *Ectemnorhinus*-group of genera, a monophyletic unit of weevils endemic to the region. We use mitochondrial cytochrome oxidase I DNA sequence data to reconstruct relationships among 13 species and 22 populations in the genera *Palirhoeus*, *Bothrometopus* and *Ectemnorhinus*. On the basis of this analysis we find little support for separating the genus *Palirhoeus* from *Bothrometopus*, and little support for the morphologically-based species groups currently recognized within *Bothrometopus*. Using a molecular clock we show that dispersal among islands likely took place against the prevailing wind direction. These data also support a previous hypothesis of radiation of the epilithic genera *Bothrometopus* and *Palirhoeus* during the Pliocene/early Pleistocene, but reject the hypothesis that the genus *Ectemnorhinus* radiated following the last glacial maximum. We show that *Bothrometopus parvulus* (C.O. Waterhouse) on the Prince Edward Islands comprises two species that are not sister taxa. We name the second species *Bothrometopus huntleyi* n. sp. and provide a description thereof.

Key words: Coleoptera, Curculionidae, dispersal, evolution, phylogeny, Southern Ocean islands, speciation

Introduction

The evolutionary history and biogeography of the sub-Antarctic islands have long been the topics of both interest and controversy. Since the biotas of the region were first described in the 1800s, many hypotheses have been proposed concerning the origins thereof and the biogeographic relationships among the various islands in the region (e.g. Gressitt 1970, Udvardy 1987, Chown 1990a, 1994, Pugh 2004, Greve *et al.* 2005, Michaux & Leschen 2005, Van der Putten *et al.* 2010). More generally, the geological history of the Kerguelen Plateau and the role it might have played in influencing distributions among the continents has also featured prominently in debates about the biogeographic history of the southern hemisphere (reviewed in Ali & Aitchison 2009).

Much of the discussion of sub-Antarctic biogeography has, to date, centred on assessments of species distributions based primarily on either phylogenetic analyses or presence/absence data using morphological or anatomical species delimitations (e.g. Gressitt 1970, Kuschel & Chown 1995, Pugh 2004, Greve *et al.* 2005). Indeed, even the most recent assessments, though clearly providing modern geological interpretations and contexts (e.g. Craig *et al.* 2003, Michaux & Leschen 2005, Pugh & Convey 2008, Van der Putten *et al.* 2010) still rely heavily on such approaches. Whilst these works have provided a range of important insights (see Chown *et al.* 1998, Craig *et al.* 2003, Van de Vijver *et al.* 2005) they are also limited, and modern, molecular approaches have shown how misleading interpretations, founded solely on morphologically-based distributional data, may be. In particular, they have demonstrated that dispersal across the southern hemisphere has been much more common than previously thought (e.g. De Quieroz 2005, Sanmartin *et al.* 2007). In addition to providing a means for dating significant biogeographic events, molecular studies also bring additional data to bear on hypotheses of relationships among taxa and areas (Sanmartin *et al.* 2007). Such information is particularly useful where analyses of morphological variation might be confounded by cryptic species or substantial environmental influences (see De Wever *et al.* 2009, Torricelli *et al.* 2010).

Despite the benefits that molecular approaches bring to investigations of biogeography and evolutionary history of any region and its biota, few such investigations have focussed on terrestrial taxa. The most common investigations are those of relationships among marine species and populations across the region (Thornhill *et al.* 2008, Fraser *et al.* 2009, Wilson *et al.* 2009), and for terrestrial groups among plant taxa from New Zealand and its sub-Antarctic islands (see Michaux & Leschen 2005). Several studies have also sought to explore the

phylogeography of particular species typically on a single island or archipelago (Skotnicki *et al.* 2004, Grobler *et al.* 2006, Mortimer & Jansen van Vuuren 2007, Myburgh *et al.* 2007, Mortimer *et al.* 2008, McGaughran *et al.* 2010a) or relationships among populations or species on the Antarctic Peninsula and Scotia Arc islands (Allegrucci *et al.* 2006, McGaughran *et al.* 2010b). By contrast, investigations of terrestrial taxa across one or more sub-Antarctic archipelagos are limited to springtails (Stevens *et al.* 2006), ameronothroid mites (Mortimer *et al.* 2010), and the Antarctic hair grass (van de Wouw *et al.* 2007). This situation is particularly concerning given the considerable change in perspective on the evolution and biogeography of both Antarctic and sub-Antarctic groups that has resulted from molecular approaches (reviewed in Chown & Convey 2007), and the controversy surrounding the origins of many of the groups endemic to the sub-Antarctic islands (Jeannel 1964, Udvardy 1987, Chown 1994, Van de Vijver *et al.* 2005, Van der Putten *et al.* 2010).

Such controversy about origins and species relationships has been a feature of investigations of the *Ectemnorhinus*-group of genera, a monophyletic unit of weevils (Kuschel & Chown 1995) restricted to the South Indian Ocean Province (or Kerguelen Biogeographic province) of the sub-Antarctic (reviewed in Chown 1992, 1994). Although the group is small by comparison with other taxa in the Curculionidae, it is one of the most speciose monophyletic taxa in the South Indian Ocean Province (Chown 1989), providing an ideal group with which to investigate biogeographic hypotheses in the region. Thus, we provide an analysis of phylogenetic relationships among species from the genera *Palirhoeus*, *Bothrometopus* and *Ectemnorhinus*, based on the material available from Heard Island in the east to the Prince Edward Islands in the west. Whilst this study does not comprise a complete analysis of the six genera and 36 species of the group (= Ectemnorhinini (Kuschel & Chown 1995, Alonso-Zarazaga & Lyal 1999, Grobler *et al.* 2006)), it does provide a strong argument for reconsideration of the species in the group and its evolution, and, as a consequence the need for additional molecular-based investigations of taxa endemic to the sub-Antarctic.

Materials and methods

Study animals and sites

The *Ectemnorhinus*-group of genera (Kuschel & Chown 1995) is confined to the South Indian Ocean Province Islands, and is thought to be most closely related to the genera *Oclandius* and *Heterexis* from the New Zealand sub-Antarctic islands (Kuschel & Chown 1995). The systematics of the group has been controversial, especially the status of species within the genera, the genera that are valid, and the evolutionary and biogeographic relationships among these taxa (Dreux & Voisin 1987, 1989, Kuschel 1971, 1991, Kuschel & Chown 1995). All of this work has been based on morphological assignments of individuals to species and subsequent assessments of the ecological characteristics and geographic distributions of these species (reviewed in Chown 1994). However, the systematic complexity of the group given its morphological variability suggests that interpretations of the systematic, biogeography and evolutionary history of the group would benefit considerably from, and likely be substantially altered by, the inclusion of molecular data.

One recent approach of this kind has shown that this is indeed the case, demonstrating that the genus *Ectemnorhinus* on the Prince Edward Islands does indeed comprise two species, though not as originally envisaged (c.f. Kuschel 1971, Chown 1990b). *Ectemnorhinus similis* (= *E. marioni* junior synonym) is found on both islands, whereas *E. kuscheli* Grobler *et al.*, is found on Prince Edward Island only (Grobler *et al.* 2006). Such complexity is perhaps not unexpected given the extent of variation within the genus *Ectemnorhinus*, and the intricacy of the ecological situation on the Prince Edward islands, where individuals of the genus *Ectemnorhinus* are a preferred prey item of introduced house mice present on Marion, but not on Prince Edward Island (Chown & Smith 1993). However, both a revision of the *Bothrometopus* species on Possession Island (Chown & Kuschel 1994) and a recent assessment of the phylogeography of the species found on the Prince Edward Islands (Grobler *et al.* 2006, 2011) suggested that cryptic species and complicated evolutionary relationships may also be a feature of other genera in the *Ectemnorhinus* group. We explore this question here.

The geological and glacial histories of the South Indian Ocean Province islands have been summarized (e.g. Hall 2002, Boelhouwers *et al.* 2008, Van der Putten *et al.* 2010) and their contemporary climatic characteristics (generally cool and oceanic) and nature of their ecosystems have also been reviewed in a range of studies (e.g. Frenot *et al.* 2001, Chown *et*

al. 1998). The islands vary in age from 0.5 million years (m.y.) for Marion Island to approximately 40 m.y. for the Kerguelen archipelago, with substantial variation within archipelagos in terms of age, history and extent of glaciation. Perhaps the most enigmatic of the groups in terms of its biogeography is the Crozet archipelago (Jeannel 1964, Chown 1994, Van der Putten *et al.* 2010), owing to a complex geological history.

Taxon sampling, genetic characterization and phylogenetic analysis

For this study we focussed on the genera *Palirhoeus* Kuschel, *Bothrometopus* Jeannel, and *Ectemnorhinus* G.R. Waterhouse. Whilst material of the genera *Canonopsis* C.O. Waterhouse and *Christensenia* Brinck were available, we were unable to obtain DNA in condition that was suitable for sequencing. We obtained sequence data from approximately half of the total number of species in the three genera and what we thought initially was 12 species and 20 populations representing all of the major archipelagos, but which following analysis turned out to be 13 species from 22 populations (Table 1).

The most comprehensive sampling was undertaken on the most readily accessible Prince Edward Islands (see also Grobler *et al.* 2006, 2011). For an outgroup, we used two COI gene sequences from *Sitona discoideus* (Curculionidae: Etiminae; Genbank accession numbers EF118292 and EF118299) from Norfolk Island, Australia (Vink & Phillips 2007).

DNA from each individual was extracted from a leg which, following removal from ethanol was washed and rehydrated in distilled water for 10 minutes prior to being frozen in liquid nitrogen and ground in individual Eppendorf tubes using an Eppendorf pestle. DNA was extracted using the High Pure PCR Template Preparation Kit (Roche Applied Science) using the supplier's procedure for isolation of nucleic acids from mammalian tissue with modification to the proteinase K tissue lysis incubation step which was performed for 24 h instead of the recommended 1 h for mammalian tissue.

Taxon-specific COI primers, GF5-1940 and GR5-2935 (Grobler *et al.* 2006), were used to amplify a 996 bp PCR product under previously described reaction conditions (Grobler *et al.* 2006) using a thermal cycling profile comprising an initial denaturation step at 94ºC for 90 s, followed by 40 cycles of 94ºC for 22 s, 46ºC for 30 s and 72ºC for 1 min and concluding with a final extension step of 1 min at 72ºC. PCR products of the correct size were purified directly from the tube using a Roche High Pure PCR Product Purification Kit. DNA sequences were determined by automated cycle sequencing reactions run on an ABI PRISM™ 3100 Analyser and generated using the ABI PRISM Big Dye™ Terminator V3.0 sequencing standard

TABLE 1. Summary of the sampling localities from which the genetically characterised specimens included in this study were collected.

Species	Sampling Locality (a.s.l)	Geographic coordinates	Number of specimens per locality
B. parvulus	Ship's Cove MI (0 m)	S 46°51'41'' E 37°50'66''	5
	Trypot Beach MI (0 m)	S 46°53'05.2'' E 37°52'06''	1
	Goodhope Bay MI (0 m)	S 46°57'55.9'' E 37°42'04.4''	2
	Cape Davis MI (0 m)	S 46°49'41.2'' E 37°41'83.3''	3
	Kildalkey Bay MI (0 m)	S 46°57'38.3'' E 37°51'22.2''	3
	McAll Coast PEI (0 m)	NA	3
B. randi	Ship's Cove MI (0 m)	S 46°51'41'' E 37°50'66''	1
	Trypot Beach MI (0 m)	S 46°53'05.2'' E 37°52'06''	1
	Water Tunnel MI (0 m)	S 46°57'49.2'' E 37°44'50.44''	1
	Goodhope Bay MI (0 m)	S 46°57'55.9'' E 37°42'04.4''	1
	Long Ridge South MI (450 m)	S 46°52'45'' E 37°47'00''	1
	Katedraal Krans MI (800 m)	S 46°53'89.6'' E 37°46'48.2''	1
	Tafelberg MI (250 m)	S 46°53'03.5'' E 37°48'20.1''	1
	Feldmark Plateau MI (600 m)	S 46°56'35'' E 37°46'10''	1
	Pyroxene Kop MI (600 m)	S 46°56'43.4'' E 37°41'40.5''	2
	Cave Bay PEI (0 m)	S 46°38'75.2'' E 37°59'78''	1
B. huntleyi	Ship's Cove MI (0 m)	S 46°51'41'' E 37°50'66''	2
	Kildalkey Bay MI (0 m)	S 46°57'38.3'' E 37°51'22.2''	1
	Trypot Beach MI (0 m)	S 46°53'05.2'' E 37°52'06''	1
	First Red Hill MI (400 m)	S 46°53'41.2'' E 37°48'21''	2
	Junior's Kop MI (200 m)	S 46°52'79.4'' E 37°50'08.3''	1
	Feldmark Plateau MI (600 m)	S 46°56'35'' E 37°46'10''	1
	Cave Bay PEI (0 m)	S 46°38'75.2'' E 37°59'78''	4
	PEI (400m)	S 46°38'21.1'' E 37°57'48.2''	1
	Top of VZB PEI (672 m)	S 46°37'59'' E 37°55'89.1''	1
B. elongatus	Tafelberg MI (250 m)	S 46°53'03.5'' E 37°48'20.1''	1
	Stony Ridge MI (150 m)	S 46°54'88.1'' E 37°51'48.4''	1
	Long Ridge South MI (450 m)	S 46°52'45'' E 37°47'00''	1
	PEI (600m)	S 46°37'53.3'' E 37°55'98.5''	2
B. fasciatus	Possession Island*	S 46°25'33.9" E 51° 51'38.2"	2
B. gracilipes	Heard Island*	S 53°01'09.4" E 73°23'30.5"	2
B. angusticollis	Kerguelen Island*	S 49°21'05.7" E 70°13'09.4"	2
B. sulcatus	Kerguelen Island*	S 49°21'05.7" E 70°13'09.4"	5
B. brevis	Kerguelen Island*	S 49°21'05.7" E 70°13'09.4"	7
	Heard Island*	S 53°01'09.4" E 73°23'30.5"	2
E. similis	Junior's Kop MI (200 m)	S 46°52'79.4'' E 37°50'08.3''	1
	Ice Plateau MI (1000 m)	S 46°54'29'' E 37°45'37.5''	1
	Cave Bay PEI (0 m)	S 46°38'75.2'' E 37°59'78''	2
E. kuscheli	Cave Bay PEI (0 m)	S 46°38'75.2'' E 37°59'78''	1
	PEI (400 m)	S 46°38'21.1'' E 37°57'48.2''	1
E. viridis	Heard Island*	S 53°01'09.4" E 73°23'30.5"	2
	Kerguelen Island*	S 49°21'05.7" E 70°13'09.4"	1
P. eatoni	Ship's Cove MI (0 m)	S 46°51'41'' E 37°50'66''	3
	Kildalkey Bay MI (0 m)	S 46°57'38.3'' E 37°51'22.2''	1
	Trypot Beach MI (0 m)	S 46°53'05.2'' E 37°52'06''	1
	Cave Bay PEI (0 m)	S 46°38'75.2'' E 37°59'78''	2
	Heard Island*	S 53°01'09.4" E 73°23'30.5"	1

VZB: Van Zinderen Bakker Peak; *: Geographic coordinates given for the scientific stations on Kerguelen and Possession Islands and for Atlas Cove on Heard Island.

(Applied Biosystems). The sequences were viewed, edited and aligned using the alignment explorer function incorporated within the MEGA4 programme (Tamura *et al.* 2007).

Neighbor-Joining (NJ) and Minimum Evolution (ME) algorithms in MEGA4 (Tamura *et al.* 2007) were used to construct distance trees. Bayesian inference (BI) using MrBayes version 3.1.2 (Ronquist & Huelsenbeck 2003) was performed with the model and parameters estimated in jModelTest 0.1.1 (Guindon & Gascuel 2003, Posada 2008) under the Akaike Information Criterion (AIC). The analysis was initiated with random starting trees with four parallel runs for 10,000,000 generations using one cold and three heated Markov chains using the default heating setting. The Markov chains were sampled every 1000 generations. Tracer plots were visually inspected and tracer diagnostics (standard deviation of split frequencies, effective sample size), as implemented in MrBayes and Tracer v1.4 (Drummond & Rambaut 2007) were checked to ensure that the Markov chain had reached stationarity. Of the 10,000 trees obtained 2,000 were discarded as "burn-in" and the trees were summarized using an 'all-compatible' consensus. Maximum parsimony (MP) analyses were performed in PAUP* (Swofford 2003). Starting trees were obtained by closest stepwise addition and heuristic searches were performed using the tree-bisection reconnection (TBR) branch swapping algorithm. Characters were unordered and assigned equal weights in the initial analysis, and subsequently reweighted using the rescaled consistency (RC) index as detailed previously by Farris (1969). Nodal support was assessed by 100 bootstrap replicates.

Haplotype (h) and nucleotide diversities (π) were estimated in DNASP 5.00.07 (Librado & Rozas 2009). To obtain more accurate divergence estimates for the older splits, the standard 2.3% nucleotide sequence divergence per million years estimate (Brower 1994) was used in combination with a model of sequence evolution that corrects for multiple hits and accounts for rate heterogeneity (Papadopoulou *et al.* 2010). We therefore retained and imposed the original 2.3 % estimate as it was shown to correspond well with the mean mtDNA divergence rate obtained for Aegean tenebrionids (2.23% and 2.39% $m.y^{-1}$) when using the GTR+Γ+I model under a strict and relaxed clock, respectively (Papadopoulou *et al.* 2010). 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, MP and BI analyses were constrained to be monophyletic and the HKY+I+Γ model identified in jModelTest 0.1.1 (Posada 2008, Guindon & Gascuel 2003) under the AIC was enforced using a strict molecular clock model. The results of two independent runs were merged and analyzed with Tracer v1.4 and TreeAnnotator v1.4.7 (Drummond & Rambaut 2007).

Table 2. Mean p-distance values between and within (indicated by grey shading) species complexes estimated in MEGA version 4 (Tamura *et al.* 2007) and expressed as a percentage.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
B. parvulus (**1**)	1.0 %												
B. huntleyi (**2**)	9.0 %	1.3 %											
B. randi (**3**)	7.4 %	8.2 %	0.4 %										
B. elongates (**4**)	8.9 %	8.3 %	9.2 %	1.4 %									
B. gracilipes (**5**)	6.9 %	8.0 %	8.2 %	8.0 %	0.1 %								
B. brevis (**6**)	9.2 %	5.1 %	8.6 %	8.1 %	8.3 %	0.7 %							
B. fasciatus (**7**)	8.5 %	8.3 %	8.5 %	6.3%	8.3 %	7.8 %	0.8 %						
B. angusticollis (**8**)	7.6 %	7.0 %	8.6 %	7.4 %	5.5 %	7.3 %	7.0 %	0.3 %					
B.sulcatus (**9**)	7.9 %	7.9 %	8.4 %	8.0 %	5.5 %	8.0 %	7.4 %	1.8 %	0.8 %				
E. similis (**10**)	13.1 %	12.1 %	11.9 %	12.2 %	11.4 %	12.5 %	12.3 %	10.8 %	11.0 %	1.5 %			
E. kuscheli (**11**)	12.8 %	11.9 %	11.8 %	12.0 %	10.8 %	11.7 %	11.9 %	10.8 %	11.0 %	2.3 %	0.2 %		
E. viridis (**12**)	11.0 %	10.2 %	10.9 %	11.1 %	10.2 %	11.4 %	11.3 %	10.5 %	10.6 %	7.6 %	7.4 %	0.2 %	
P. eatoni (**13**)	8.0 %	7.4 %	7.6 %	7.9 %	7.5 %	7.3 %	8.3%	7.4 %	8.0 %	11.8 %	11.3 %	9.4 %	1.2 %

Results

Genetic characterization and Phylogenetic analyses

All sequences used in our final dataset were 885 bp in length and correspond to nucleotide positions 514 to 1399 of the COI gene. All novel sequences have been deposited in the Genbank database under accession numbers: GQ856478-80, GQ856482-8, GQ856490-1, GQ856493-GQ856500 and GU947664-GU947703, and were complemented with nucleotide sequence entries from two other studies, *viz.* AY762278, AY762285, AY762298-9, AY762317-20 (Grobler *et al.* 2006) GQ131943, GQ131946, GQ131952, GQ131954-5, GQ131961, GQ131967, GQ131979, GQ131997, GQ131999, GQ132004, GQ132006, GQ132009, GQ132012-4 (Grobler *et al.* 2011).

Of the 885 sequenced sites 592 were conserved across all 86 specimens in the dataset. Of the 293 variable sites 277 sites were parsimony informative and 159 of the latter were assigned weights other than one after rescaled consistency index (RCI) character reweighting. Parsimony analyses with equal weighted characters recovered 92 trees with a length of 779 and homoplasy indexes of: CI = 0.485; RI = 0.898 and RCI = 0.435. The analysis in which characters were RCI reweighted also recovered 92 trees, all 342.97 in length, with homoplasy indexes of: CI = 0.672, RI = 0.935 and RCI = 0.629.

The HKY+I+Γ model of sequence evolution selected under the AIC in jModelTest 0.1.1 (Guindon & Gascuel 2003, Posada 2008) recovered a transition transversion ratio of 4.4317, a gamma distribution shape parameter (Γ) of 1.000, proportion of invariable sites (I) = 0.6020 and base frequencies of A = 0.3462, C = 0.1528, G = 0.1012 and T = 0.3998 (% AT = 74.60%).

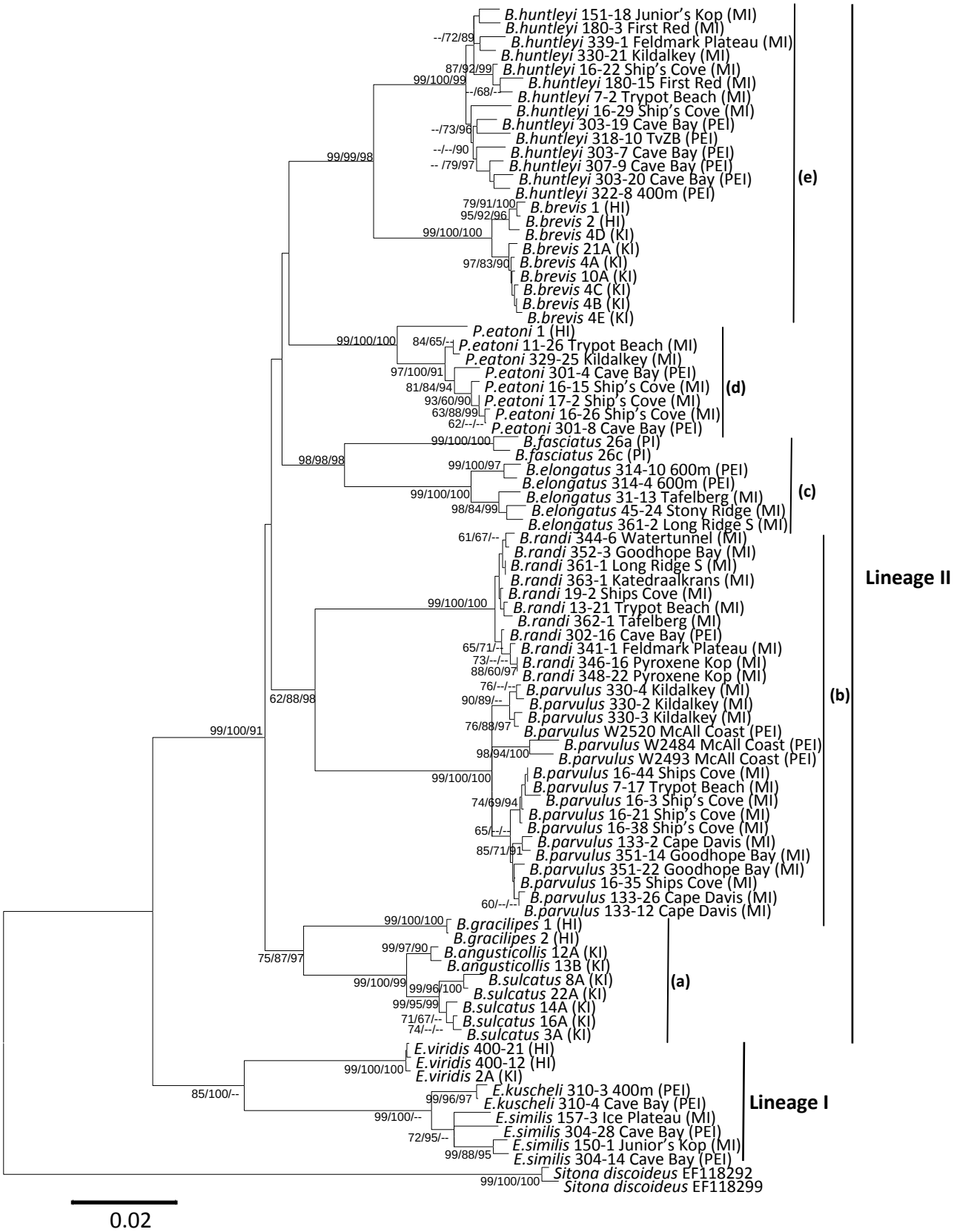
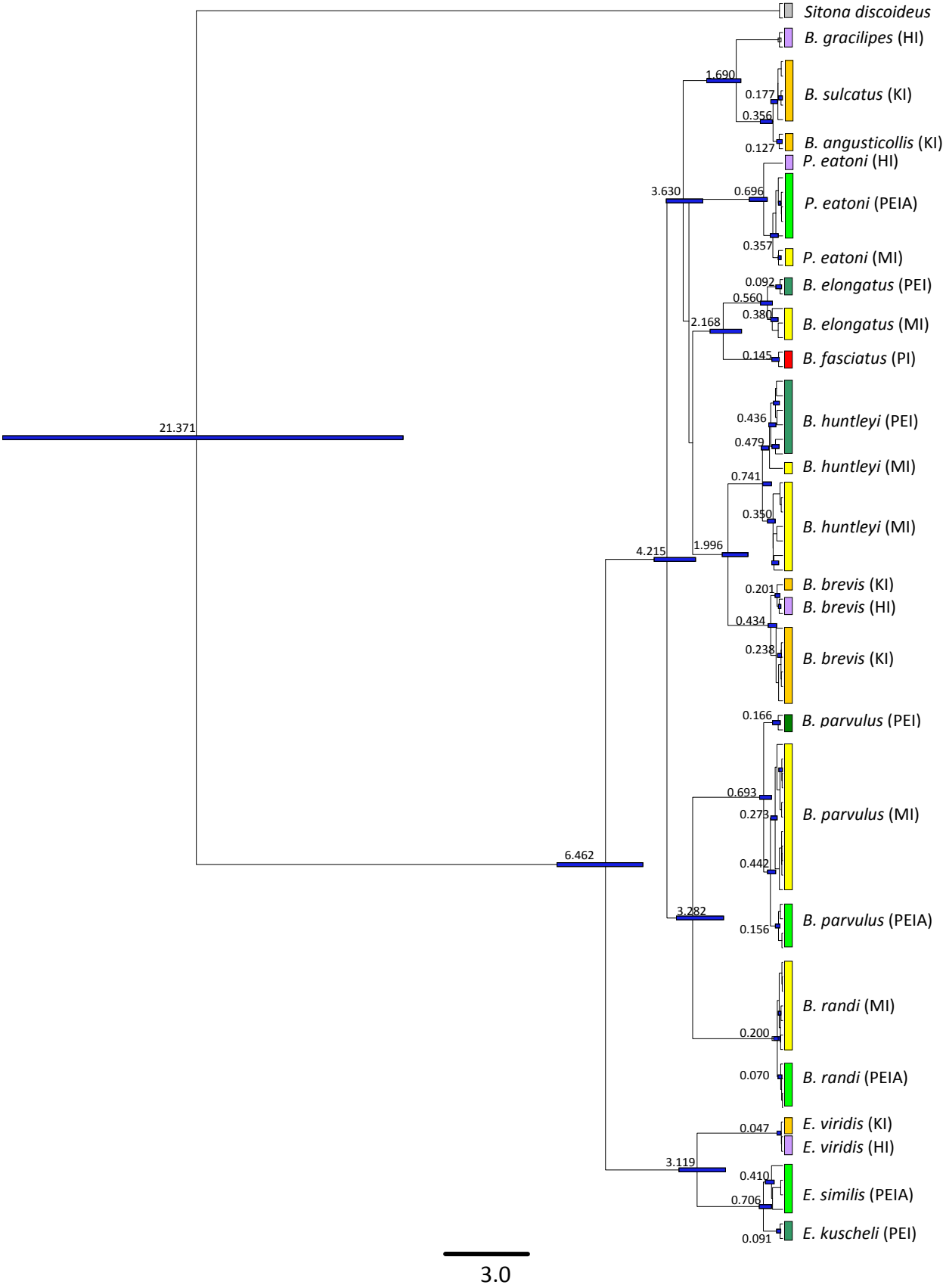

B.huntleyi 151-18 Junior's Kop (MI)
B.huntleyi 180-3 First Red (MI)
B.huntleyi 339-1 Feldmark Plateau (MI)
B.huntleyi 330-21 Kildalkey (MI)
B.huntleyi 16-22 Ship's Cove (MI)
B.huntleyi 180-15 First Red (MI)
B.huntleyi 7-2 Trypot Beach (MI)
B.huntleyi 16-29 Ship's Cove (MI)
B.huntleyi 303-19 Cave Bay (PEI)
B.huntleyi 318-10 TvZB (PEI)
B.huntleyi 303-7 Cave Bay (PEI)
B.huntleyi 307-9 Cave Bay (PEI)
B.huntleyi 303-20 Cave Bay (PEI)
B.huntleyi 322-8 400m (PEI)
B.brevis 1 (HI)
B.brevis 2 (HI)
B.brevis 4D (KI)
B.brevis 21A (KI)
B.brevis 4A (KI)
B.brevis 10A (KI)
B.brevis 4C (KI)
B.brevis 4B (KI)
B.brevis 4E (KI)
P.eatoni 1 (HI)
P.eatoni 11-26 Trypot Beach (MI)
P.eatoni 329-25 Kildalkey (MI)
P.eatoni 301-4 Cave Bay (PEI)
P.eatoni 16-15 Ship's Cove (MI)
P.eatoni 17-2 Ship's Cove (MI)
P.eatoni 16-26 Ship's Cove (MI)
P.eatoni 301-8 Cave Bay (PEI)
B.fasciatus 26a (PI)
B.fasciatus 26c (PI)
B.elongatus 314-10 600m (PEI)
B.elongatus 314-4 600m (PEI)
B.elongatus 31-13 Tafelberg (MI)
B.elongatus 45-24 Stony Ridge (MI)
B.elongatus 361-2 Long Ridge S (MI)
B.randi 344-6 Watertunnel (MI)
B.randi 352-3 Goodhope Bay (MI)
B.randi 361-1 Long Ridge S (MI)
B.randi 363-1 Katedraalkrans (MI)
B.randi 19-2 Ships Cove (MI)
B.randi 13-21 Trypot Beach (MI)
B.randi 362-1 Tafelberg (MI)
B.randi 302-16 Cave Bay (PEI)
B.randi 341-1 Feldmark Plateau (MI)
B.randi 346-16 Pyroxene Kop (MI)
B.randi 348-22 Pyroxene Kop (MI)
B.parvulus 330-4 Kildalkey (MI)
B.parvulus 330-2 Kildalkey (MI)
B.parvulus 330-3 Kildalkey (MI)
B.parvulus W2520 McAll Coast (PEI)
B.parvulus W2484 McAll Coast (PEI)
B.parvulus W2493 McAll Coast (PEI)
B.parvulus 16-44 Ships Cove (MI)
B.parvulus 7-17 Trypot Beach (MI)
B.parvulus 16-3 Ship's Cove (MI)
B.parvulus 16-21 Ship's Cove (MI)
B.parvulus 16-38 Ship's Cove (MI)
B.parvulus 133-2 Cape Davis (MI)
B.parvulus 351-14 Goodhope Bay (MI)
B.parvulus 351-22 Goodhope Bay (MI)
B.parvulus 16-35 Ships Cove (MI)
B.parvulus 133-26 Cape Davis (MI)
B.parvulus 133-12 Cape Davis (MI)
B.gracilipes 1 (HI)
B.gracilipes 2 (HI)
B.angusticollis 12A (KI)
B.angusticollis 13B (KI)
B.sulcatus 8A (KI)
B.sulcatus 22A (KI)
B.sulcatus 14A (KI)
B.sulcatus 16A (KI)
B.sulcatus 3A (KI)
E.viridis 400-21 (HI)
E.viridis 400-12 (HI)
E.viridis 2A (KI)
E.kuscheli 310-3 400m (PEI)
E.kuscheli 310-4 Cave Bay (PEI)
E.similis 157-3 Ice Plateau (MI)
E.similis 304-28 Cave Bay (PEI)
E.similis 150-1 Junior's Kop (MI)
E.similis 304-14 Cave Bay (PEI)
Sitona discoideus EF118292
Sitona discoideus EF118299
(e)
(d)
(c)
(b)
(a)
Lineage II
Lineage I
0.02

Fig. 1. Minimum Evolution (ME) tree of 13 species from the *Ectemnorhinus*-group of genera based on 885 nucleotides of the mitochondrial cytochrome oxidase I (COI) gene. Each taxon label contains the species designation, sample number, sampling locality, and island of origin. Nodal support values obtained from 10,000 bootstrap replications (ME), 100 bootstrap replications from Maximum Parsimony (MP) and posterior support from Bayesian Inference (BI) analyses, expressed as percentages and denoted ME/MP/BI on each node. '--' indicates support values < 65 (for ME and MP) and <90 (for BI). The scale indicates the number of nucleotide substitutions. Islands are abbreviated as follows: Marion Island (MI), Prince Edward Island (PEI), The Prince Edward Island Archipelago (PIEA), Heard Island (HI), Kerguelen Island (KI) and Possession Island (PI). (On page 167)

Fig. 2. Ultrametric tree obtained with BEAST with a clock rate of 2.3 % sequence divergence per million years. The topology was constrained to retain monophyletic lineages recovered across all methods of inference (i.e. NJ, MP and BI). The numbers in the nodes correspond to the estimated age in million years, and the blue bars to the 95 % confidence interval. The scale indicates change in million years. Islands are abbreviated as follows: Marion Island (MI), Prince Edward Island (PEI), The Prince Edward Island Archipelago (PIEA), Heard Island (HI), Kerguelen Island (KI) and Possession Island (PI). (On page 169)


Sitona discoideus
B. gracilipes (HI)
1.690
0.177
B. sulcatus (KI)
0.356
B. angusticollis (KI)
0.127
P. eatoni (HI)
3.630
0.696
P. eatoni (PEIA)
0.357
P. eatoni (MI)
0.092
B. elongatus (PEI)
0.560
0.380
B. elongatus (MI)
2.168
0.145
B. fasciatus (PI)
21.371
0.436
B. huntleyi (PEI)
0.479
B. huntleyi (MI)
0.741
0.350
B. huntleyi (MI)
4.215
1.996
0.201
B. brevis (KI)
B. brevis (HI)
0.434
0.238
B. brevis (KI)
0.166
B. parvulus (PEI)
0.693
0.273
B. parvulus (MI)
6.462
0.442
3.282
0.156
B. parvulus (PEIA)
B. randi (MI)
0.200
0.070
B. randi (PEIA)
0.047
E. viridis (KI)
E. viridis (HI)
3.119
0.410
E. similis (PEIA)
0.706
0.091
E. kuscheli (PEI)
3.0

The molecular phylogenies obtained with the different inference methods were topologically similar and recovered two main evolutionary lineages (denoted I and II in Fig. 1) for the *Ectemnorhinus* group of genera. Pairwise uncorrected p-distance comparisons of each monophyletic lineage / species within these lineages revealed mean inter-specific sequence divergence values of between 1.8 and 13.1%, and mean intra-specific diversity values ranging from 0.1 to 1.2 % (Table 2). Lineage I (85% bootstrap support from ME and 100% from MP) which contains all of the *Ectemnorhinus* species characterised in this study is basal to the lineage II (99% and 100% bootstrap support from ME and MP, respectively) containing representatives of the genera *Palirhoeus* and *Bothrometopus*. Of the three *Ectemnorhinus* species characterised, *E. viridis* is basal to *E. similis* and *E. kuscheli* and intra-specific divergence for this species is low despite the fact that the *E. viridis* individuals are from different (Heard and Kerguelen) islands. According to the age estimates in Fig. 2, *E. viridis* last shared a common ancestor with the *Ectemnorhinus* species from the Prince Edward Archipelago approximately 3.12 million years ago (m.y.a.). *Ectemnorhinus. kuscheli* from Prince Edward Island is basal to *E. similis* that occurs on both Marion Island and Prince Edward Island, and they shared their last common ancestor approximately 0.71 m.y.a. (Fig. 2).

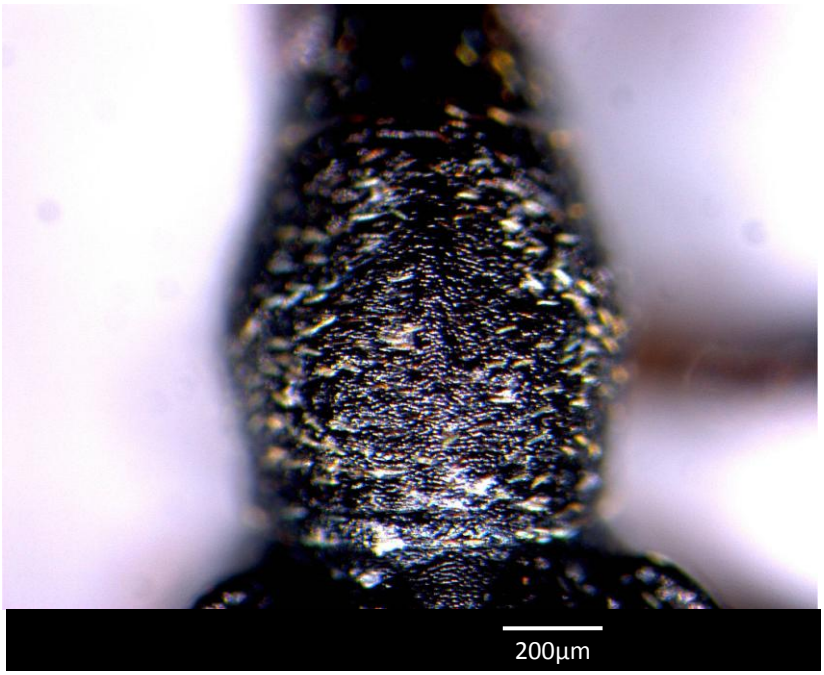
Lineage II comprises five monophyletic lineages (labelled a-e in Fig. 1) that coalesced approximately 4.22 m.y.a. These clades contain all nine *Bothrometopus* species characterised in this study as well as *Palirhoeus eatoni* (Fig. 1, clade d) suggesting that the monotypic genus *Palirhoeus* should be synonymised with *Bothrometopus* pending confirmation from nuclear gene analyses. Within the *Palirhoeus* lineage, which is estimated to have arisen approximately 0.696 m.y.a., the *P. eatoni* specimen from eastern Heard Island, is basal to the western Prince Edward Islands' specimens. *Bothrometopus gracilipes*, *B. angusticollis* and *B. sulcatus* group together in a monophyletic clade (Fig. 1, clade a) with 75-91% nodal support.

The Heard Island *B. gracilipes* lineage is estimated to have diverged from the remaining species approximately 1.69 m.a.y. The sister taxa *B. angusticollis* and *B. sulcatus*, represented by specimens from Ile Kergeulen, diverged approximately 0.356 m.y.a. with the phylogeny. Note that *B. gracilipes* and *B angusticollis* fall into the *gracilipes*-group of *Bothrometopus* species (Kuschel & Chown 1995) while *B. sulcatus* falls in the *fasciatus*-group of *Bothrometopus* species (Kuschel & Chown 1995). This suggests that separation of species on the basis of absence or presence of dorsal wall vaginal spicules, into the *fasciatus*- and *gracilipes*-groups of *Bothrometopus* species (Kuschel & Chown 1995), respectively, may not be valid.

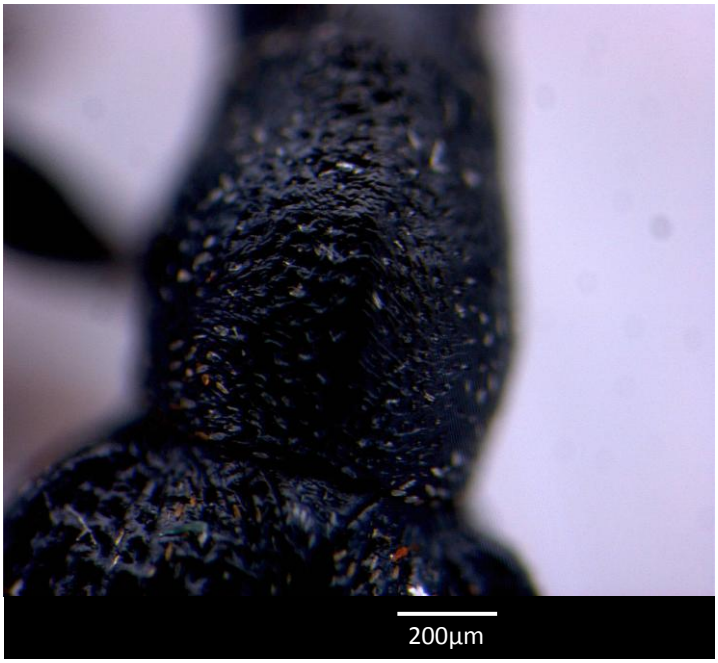
Bothrometopus fasciatus from Possession Island, groups with, and is basal to, *B. elongatus* from the Prince Edward Islands (Fig. 1, clade c). The estimated time to *B. fasciatus* and *B. elongatus* lineage coalescence is approximately 2.2 m.y.a. Individuals of *B. elongatus* from Prince Edward Island are distinct from those from Marion Island, diverging approximately 0.56 m.y.a. Additional *B. elongatus* specimens would need to be examined to determine the extent of gene flow between Prince Edward and Marion Islands.

When examining the remaining two clades (Fig. 1b & e) it became clear that both clades contain individuals from the Prince Edward Islands archipelago, identified morphologically as *B. parvulus*, but which are not sister taxa. One of these clades is sister to *B. randi* from the Prince Edward Islands, having diverged from this sister taxon approximately 3.3 m.y.a., whilst the other morphologically similar counterpart, groups with *B. brevis* from the Kerguelen and Heard Islands, constituting a lineage which is estimated to have arisen approximately 2.0 m.y.a. (Fig. 2).

Detailed external morphological examination of these two species, and comparison with images of the holotype of *B. parvulus* held by the Natural History Museum, London, revealed considerable similarity, with the exception of the microsculpture of the pronotum, which provides a reliable means of distinguishing between them (and also between some species on Possession Island, see Chown & Kuschel 1994). In the case of the holotype of *B. parvulus*, and indeed all material henceforth assigned to that species, the pronotal microscuplture appears pointillistic under a light microscope with granular microsculpture (Figs 3a & c), and alutaceous when examined using scanning electron microscopy (Fig. 4). By contrast, the other species, which we describe formally below, has a smoother appearance under both light (Fig. 3b) and electron microscopy (Fig. 4), with distinct large punctations. No other completely reliable means exist to distinguish morphologically between these two species, but the characters are 100% reliable, as assessed via two independent approaches. First, morphology-based in which one of us (SLC) with no advance knowledge of specimen identity, visually matched all specimens to the sequence data determinations with 100 % congruence. Second, based on morphology, additional material from Prince Edward Island was identified by one of us (AMT) and then provided to another author (GCG) who sequenced the material without prior knowledge of morphological assignment. The assignment match was 100 %. We also noted that the individuals that correspond to *B. parvulus* appear to be restricted to coastal regions whereas the new species is distributed island-wide. The new cryptic species, *Bothrometopus huntleyi*, initially identified as *B. parvulus* based on morphology, is formally described below and compared to *B. parvulus*.



a)



b)

c)

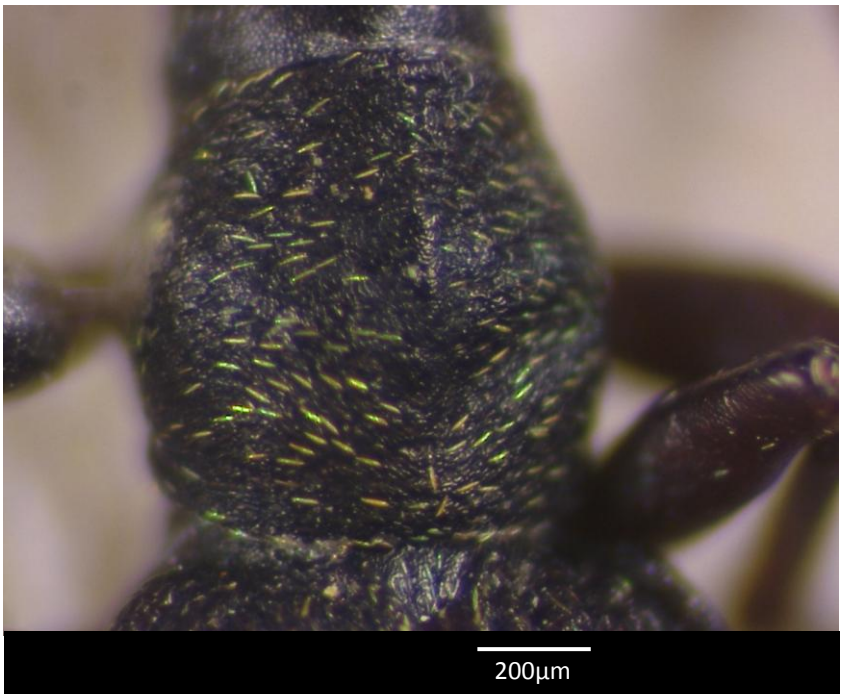


Fig 3. Light micrographs of the pronota of (a) *B. parvulus*: (b) *B. huntleyi*. and c) *B. parvulus* type specimen from the National History Museum, London. Both the type specimen and *B. parvulus* show granular micro sculpture on the pronotum. The pronotum of *B. huntleyi* is smoother in appearance.

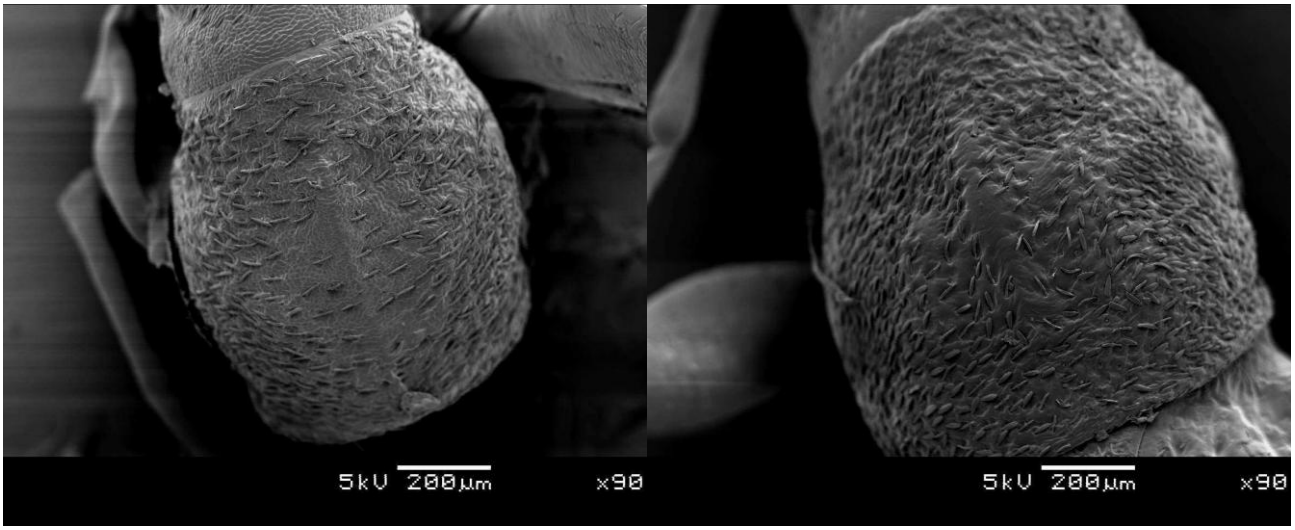


Fig. 4 Scanning electron microscopic comparison of the thorax of *B. parvulus* (LHS) and that of *B. huntleyi* (RHS) using scanning electron microscopy (SEM). No distinct setal patterning can be discerned, however the *B. parvulus* specimen appears to have a more granular surface and fewer scales than *B. huntleyi*. This feature can be observed with a standard, light microscope and can be used to readily distinguish *B. parvulus* from *B. huntleyi*.

***Bothrometopus huntleyi* n. sp.**

Fig. 5 Dorsal habitus of *B. huntleyi* n. sp. Male (length from anterior of eyes to posterior of elytra = 4.7 mm).

Description:

Length: (anterior of eyes to posterior of elytra): Overall: 3.1 – 5.5 mm; males: mean ± S.E. = 4.1 ± 0.03 mm (n = 156); females: 4.4 ± 0.04 mm (n = 136). Body dark brown to black with a variable covering of green to blue scales on the dorsal surface; the ventral surface is black (Fig. 5). The density of scales is highest on the elytra, most variable on the prothorax and sparse on the head and femora. The tibiae and tarsi lack scales, with the former having stiff, spine-like setae. On the elytra the scales occasionally form an anchor-shaped pattern, or two spots, one on each of the elytra. Where the scale density is high the scales are not imbricate. Occasionally, on the lateral margins of the elytra, small, fine and transparent to golden-brown to green erect hair-like scales may be present. These do not resemble the stiff, marked erect spines found on the elytra of species in the genus *Ectemnorhinus*. Antennae with light-brown to reddish-brown scape, reddish-brown funicle and dark-brown to almost black club. The first three funicle segments typically have the ratio 0.94:1:0.61 (Fs1:Fs2:Fs3) (n = 10). Epistome symmetric, sometimes with pronounced lobes, but also with a straight margin. Mandibles reddish-brown, each one asymmetric, with the dorsal tooth more pronounced than the ventral tooth, except after substantial wear. Labial palps three-segmented. Ommatidia coarse. Prothorax with an indistinct to distinct dorsal carina which can occasionally be entirely absent; where present it tends not to run the full length of the prothorax. Dorsal surface of the prothorax with pronounced punctations with an otherwise smooth surface between them. No granular microsculpturing is present. Elytra obovate each with a humeral carina which is moderately to well developed. Striations are pronounced as a consequence of deep punctations that are virtually contiguous. Legs reddish-brown to black with lighter colouration towards the base of the femora. Third tasal segment with a ventral surface of densely packed white setae forming a brush. Tarsal claw segment shorter than the other three segments combined. Aedeagus as in Fig. 6a with a unique basal sclerite. Female genitalia as in Fig. 6b.

Etymology:

This new species is named in honour of the youngest biologist on the first biological and geological expedition (1965/1966) to the Prince Edward Islands: Brian John Huntley.

Remarks:

Bothrometopus huntleyi is a medium-sized *Bothrometopus* species – the genus varies from *c.* 2 mm to 10 mm in length (Chown & Kuschel 1994; Kuschel & Chown 1995). It is morphologically very similar to *B. parvulus* (C.O. Waterhouse) from the Prince Edward Islands and *B. brevis* (C.O. Waterhouse) from Kerguelen and Heard Islands. Adults of *B. huntleyi* can be separated from *B. parvulus* based on the former species' deep punctations and lack of granular microsculpture on the prothorax, dorsal carina which does not stretch from end to end of the prothorax, and typically lighter funicle segments of the antennae by comparison with the general body colouration. The most reliable distinguishing feature is the difference in microsculpture on the prothorax of the two species (as described above and shown in Figs 3 and 4). No characters have yet been found to distinguish the larvae.

Distribution:

Island-wide (coastal rocks and inland areas, see Chown 1989, 1992) on both Marion Island and Prince Edward Island. This contrasts with *B. parvulus*, which thus far has only been found on coastal rocks at both Marion Island and Prince Edward Island. The phylogeography of this new species is discussed in detail in Grobler *et al.* (2011).

a)

b)

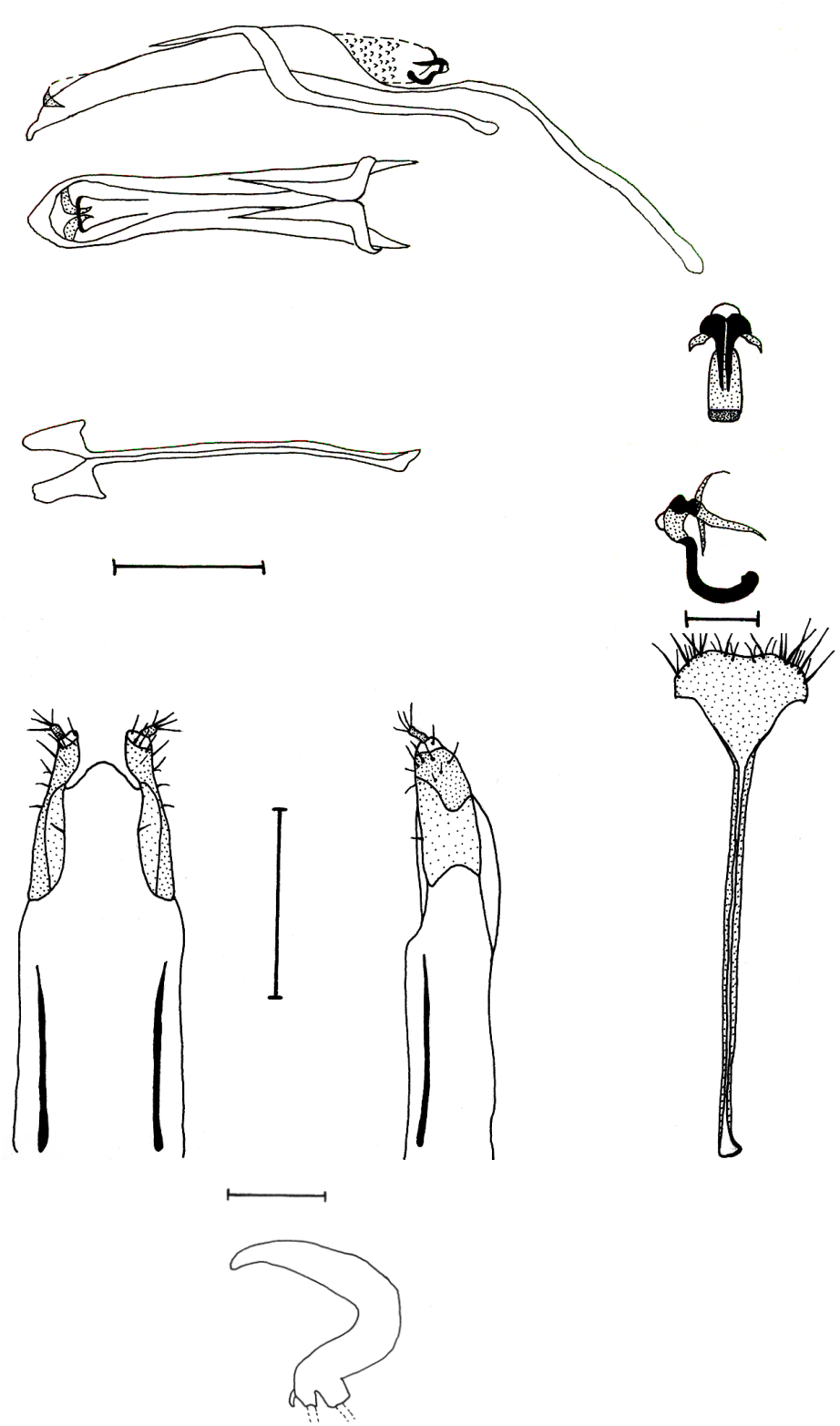


Fig. 6 *Bothrometopus huntleyi n. sp*. (a) Male genitalia with the aedeagus in lateral and dorsal views (scale bar = 0.5 mm) with the genital armature to the right (scale bar = 0.1 mm).
(b) Female genitalia in dorsal and lateral view (scale bar = 0.5 mm). The spermatheca is shown below (scale bar = 0.2 mm).

Material examined

Holotype:

♂, South Africa, Marion Island, 400 m above sea level, First Red Hill, S 46º53.412' E 37º48.21', Genbank no. GQ131999, voucher no. 180- 15, date collected April 2001, collector G.C. Grobler. Deposited in the Iziko South African Museum, Cape Town, South Africa.

Paratypes:

♀, South Africa, Marion Island, 0 m above sea level, Ship's Cove, S 46º51'41'' E 37º50'66'', Genbank no. GQ132012, voucher no. 16- 22, date collected April 2001, collector G.C. Grobler. Deposited in the Iziko South African Museum, Cape Town, South Africa.

♂, South Africa, Marion Island, 200 m above sea level, Junior's Kop, S 46º52.794' E 37º50.083', Genbank no. GQ131946, voucher no. 151- 18, date collected April 2001, collector G.C. Grobler. Deposited in the Iziko South African Museum, Cape Town, South Africa.

♀, South Africa, Marion Island, 600 m above sea level, Feldmark Plateau, S 46º56'35'' E 37º46'10'', Genbank no. GQ131952, voucher no. 339-1, date collected April 2002, collector G.C. Grobler. Deposited in the Iziko South African Museum, Cape Town, South Africa.

♂, South Africa, Marion Island, 400 m above sea level, First Red Hill, S 46º53.412' E 37º48.21', Genbank no. GQ131967, voucher no. 180-3, date collected April 2001, collector G.C. Grobler. Deposited in the Natural History Museum, London, United Kingdom.

♀, South Africa, Marion Island, 0 m above sea level, Ship's Cove, S 46º51'41'' E 37º50'66'', Genbank no. GQ131943, voucher no. 16-29, date collected April 2001, collector G.C. Grobler. Deposited in the Natural History Museum, London, United Kingdom.

♂, South Africa, Prince Edward Island, 0 m above sea level, Cave Bay, S 46º38.752' E 37º59.780', Genbank no. GQ131954, voucher no. 303-19, date collected April 2003, collector G.C. Grobler. Deposited in the Iziko South African Museum, Cape Town, South Africa.

♀, South Africa, Prince Edward Island, 672 m above sea level, Top of van Zinnerin Bakker, S 46º37.590' E 37º55.891', Genbank no. GQ131961, voucher no. 318-10, date collected April 2003, collector G.C. Grobler. Deposited in the Iziko South African Museum, Cape Town, South Africa.

♂, South Africa, Prince Edward Island, 0 m above sea level, Cave Bay, S 46º38.752' E 37º59.780', Genbank no. GQ131955, voucher no. 303-20, date collected April 2003, collector G.C. Grobler. Deposited in the Iziko South African Museum, Cape Town, South Africa.

♀, South Africa, Prince Edward Island, 400 m above sea level, S 46º38.211' E 37º57.482', Genbank no. GQ132006, voucher no. 307-9, date collected April 2003, collector G.C. Grobler. Deposited in the Iziko South African Museum, Cape Town, South Africa.
♂, South Africa, Prince Edward Island, 400 m above sea level, S 46º38.211' E 37º57.482', Genbank no. GQ132004, voucher no. 322-8, date collected April 2003, collector G.C. Grobler. Deposited in the Natural History Museum, London, United Kingdom.
♀, South Africa, Prince Edward Island, 0 m above sea level, Cave Bay, S 46º38.752' E 37º59.780', Genbank no. GQ131997, voucher no. 303-7, date collected April 2003, collector G.C. Grobler. Deposited in the Natural History Museum, London, United Kingdom.

Additional material was examined for the morphometric analysis on which the length measurements used in the description are based (A. Treasure and S.L. Chown, unpublished data).

Molecular comment

DNA barcoding, its recognised flaws notwithstanding (Rubinoff 2006), was considered here as a complementary tool for the unequivocal differentiation of *B. parvulus* from *B. huntleyi* The 43 nucleotide sites that are conserved within species, and consistently different between the two morphologically indistinct species occurring on PEIA are summarised in Table 3. When comparing the partial amino acid COI gene sequences of the thirteen species of the *Ectemnorhinus*-group of genera generated in this study, 17 non-synonymous amino acid substitutions were observed in the 84 ingroup taxon dataset. These non-synonymous amino acid substitutions revealed several consistent and therefore possibly diagnostic differences between species and include the following positions in our dataset: Codon 7 (I in *B. elongatus* and M in all other species, except for two *B. sulcatus* specimens which have a V at this position); Codon 19 (V in *E. viridis* and I in all other species); Codon 41 (all species within the genus *Ectemnorinus* have an I at this position, whereas a V is present in all species of the genera *Bothrometopus* and *Palirhoeus*); Codon 161 (T in *B. parvulus* and N in all other species); Codon 183 (V in *B. parvulus* and I in all other species); Codon 241 (M in *B. gracilipes*, and either a V or an L in all other species). As some of the species in this study are only represented by two specimens, additional data will need to be generated to determine the consistency and species-exclusivity of some of these characters.

TABLE 3 Summary of the 43 nucleotide sites in the COI gene region characterised in this study, that are consistently different between *B. parvulus* and *B. huntleyi*.

Nucleotide site	Base position	*B. parvulus*	*B. huntleyi*
9	3rd	T	A
33	3rd	T	C
39	3rd	T	A
42	3rd	A	G
63	3rd	T	A
64	1st	C	T
82	1st	C	T
84	3rd	T	G
87	3rd	A	T
141	3rd	T	C
153	3rd	T	A
195	3rd	A	T
285	3rd	T	A
288	3rd	T	C
321	3rd	A	C
333	3rd	C	T
348	3rd	T	C
360	3rd	A	G
366	3rd	T	C
444	3rd	A	T
468	3rd	T	A
482	2nd	C	A
486	3rd	A	T
492	3rd	C	T
493	1st	C	T
507	3rd	T	A
543	3rd	C	A
547	1st	G	A
585	3rd	T	C
615	3rd	C	T
618	3rd	T	C
648	3rd	A	T
651	3rd	A	T
669	3rd	T	C
672	3rd	C	A
699	3rd	C	T
706	1st	T	C
712	1st	G	A
714	3rd	C	T
750	3rd	G	A
765	3rd	T	C
780	3rd	C	T
876	3rd	C	T

Discussion

The phylogenetic analyses revealed three major points. First, the monotypic genus *Palirhoeus* is not readily distinguishable, on a mtCOI sequence basis, from the genus *Bothrometopus*, thus questioning the retention of the species *P. eatoni* in a separate genus, *Palirhoeus*, created by Kuschel (1971), and its position in Kuschel & Chown's (1995) phylogeny as basal to the genera *Bothrometopus* and *Ectemnorhinus*. Nonetheless, limited taxon and gene sampling means that we refrain from proposing formal generic synonymy. Second, the two species groups in the genus *Bothrometopus* (*fasciatus* group and *gracilipes* group) identified on the basis of absence or presence of dorsal wall vaginal spicules, by Kuschel & Chown (1995) are not supported by the COI gene phylogeny. *Bothrometopus gracilipes* and *B. angusticollis* fall into the *gracilipes* group of *Bothrometopus* species (Kuschel & Chown 1995) while *B. sulcatus* falls in the *fasciatus* group of *Bothrometopus* species (Kuschel & Chown 1995). The sister taxon relationship of *B. elongates*, which is assigned to the *gracilipes* group, with *B. fasciatus* from the *fasciatus* group of *Bothrometopus* species (Kuschel & Chown 1995) in the COI gene tree also raises questions regarding the phylogenetic utility of these two major groups. Third, what was previously considered a single species on the Prince Edward Islands, *B. parvulus* Jeannel, is clearly two species that are certainly not sister taxa, but rather share relationships with different species from our sample taxa. Identification of this cryptic species increases the number of species within the *Ectemnorhinus*-group of genera from 36 to 37.

Despite being a partial analysis of this group of weevils endemic to the South Indian Ocean Province Islands, the current study has important implications for interpretation of biogeographic and evolutionary dynamics in the region more generally. Perhaps the most significant point to emerge is that colonization of the Prince Edward Islands is likely to have taken place repeatedly from other islands in the South Indian Ocean Province. Thus, although *B. parvulus* and *B. randi* are sister species in the current tree (Fig. 1), the molecular clock based on a 2.3% nucleotide sequence divergence per million years estimate obtained from an arthropod mtDNA survey of Brower (1994), which has proven useful for studies of this group (see Grobler *et al.* 2006) indicates that divergence must have taken place approximately c. 3.3 m.y.a. (Fig. 2). This could not have happened on the Prince Edward Islands because the oldest date for the islands is approximately 0.5 m.y.a., and there is no geological evidence to suggest that they are much older than this (Boelhouwers *et al.* 2008). The date of the divergence between *B. huntleyi* and *B. brevis*, approximately 2.0 m.y.a. also suggests that an early colonization of the Prince Edward Islands is unlikely. Instead, the dated phylogeny suggests

that dispersal to the Prince Edward Islands must have occurred from elsewhere, sometime after the islands emerged, and on at least two separate occasions. Because we were unable to sample all taxa in the genus *Bothrometopus* (see Chown & Kuschel 1994, Kuschel & Chown 1995 for review) it seems likely that the colonization has been from species on the Crozet archipelago. *Bothrometopus randi* (the sister species of *B. parvulus*, based on this analysis) is known from Possession Island and other *Bothrometopus* species are widespread across the Crozet islands (Chown & Kuschel 1994). Such an hypothesis of colonization against the prevailing west wind drift is not new, and was in fact proposed by Dreux and Voisin in a series of works on the group (e.g. Dreux & Voisin 1987, 1989). Thus, unlikely as their hypotheses may have seemed initially, they cannot, on present evidence, be rejected. Indeed, it also appears that *P. eatoni* colonized the Prince Edward Islands relatively recently (Figs. 1, 2) and that dispersal between Marion Island and Prince Edward Island has been quite common since their emergence.

Several independent lines of evidence support this proposal of repeated colonization across the region. Using a molecular phylogenetic approach, Stevens *et al.* (2006) demonstrated that repeated colonizations across the sub-Antarctic islands probably took place from the late Miocene (c. 7 m.y.a.) to approximately 0.3 m.y.a. Likewise, recent investigations of the ameronothroid mite genera *Halozetes* and *Alaskozetes* have shown colonization of the islands by species in these genera over the last ten million years (Mortimer *et al.* 2010). These dates also correspond closely with those for dispersals among populations of the springtail *Cryptopygus antarcticus* in the Scotia Arc and Antarctic Peninsula region (McGaughran *et al.* 2010b), and trans-Drake Passage dispersal of the nudibranch *Doris kerguelenensis* (Wilson *et al.* 2009). However, the divergence times differ substantially for those estimated for the bull kelp *Durvillaea antarctica*, which apparently recolonized the South Indian Ocean Province Islands after its removal during the last glacial maximum, *c.* 16 000 years ago (Fraser *et al.* 2009).

These dispersal dates indicate that for the terrestrial species much of the diversification considerably preceded the last glacial maximum and many events date to either the Pliocene/early Pleistocene, or as soon as a particular island group (such as the Prince Edward Islands) emerged. Thus, it appears likely that the groups survived several glacial cycles in refugia on the islands, and are certainly not post-glacial colonists. Such proposals have been made previously for various groups (see discussions in Chown 1990a, Van der Putten *et al.* 2010). Indeed for the *Ectemnorhinus*-group of genera, Chown (1989, 1994) suggested that the species typical of the epilithic biotope, (i.e. those in the genera

Bothrometopus, *Palirhoeus* and *Disker*) probably radiated since the end of the Pliocene in the epilthic biotopes that must have come to predominate as a consequence of cooling (for revised climatic histories see Turner *et al.* 2009). The divergence times calculated on the basis of an arthropod mtDNA survey of Brower (1994) certainly support such a proposal. Whether the groups more typical of vegetated areas will show an equally deep history is not clear. However, the deep divergence time, approximately 6.46 m.y.a., found here between *Ectemnorhinus* (a genus in which species are typical of vegetated areas – Chown 1989, 1994) and *Bothrometopus* (restricted to eplithic biotopes) and the fairly substantial divergence dates among species within this genus (see also Grobler *et al.* 2006), suggests that they may well do so. That recent studies have supported the persistence of vascular plants on the South Indian Ocean Province Islands through several glacial periods (e.g. Scott 1985, van der Putten *et al.* 2010) also suggests that survival during these periods is likely. In consequence, the proposal that the genus *Ectemnorhinus* diversified following the last glacial maximum (Chown 1994) must be rejected. Similar hypotheses of recolonization of terrestrial areas from refugia, such as marine refugia in the case of the ameronothroid mites have also been rejected on the grounds of new molecular evidence (Mortimer *et al.* 2010). However, within particular species it remains clear that volcanic and glacial cycles and refugia on particular islands have played important roles in population structuring. Such structure has thus far been identified for indigenous springtails, mites, and weevils (Grobler *et al.* 2006, Mortimer & Jansen van Vuuren 2007, Myburgh *et al.* 2007, Grobler *et al.* 2011), and seems also to apply to a vascular plant species and to other insects (Mortimer *et al.* 2008, Groenewald, Chown & Jansen van Vuuren, unpublished data). Significantly, though, in a sub-Antarctic context such details are available only for the Prince Edward Islands, and to a lesser extent for Macquarie and Heard Islands (Skotnicki *et al.* 2004).

These results clearly indicate the need for further comprehensive molecular phylogenetic analyses of the biogeography of the region including a range of taxa. Only in this way will clearer reconstructions of the history and evolutionary relationships of the endemic and frequently enigmatic taxa in the region be established, and the hypotheses concerning the origins of the group (e.g. Jeannel 1964) assessed on sounder basis. Moreover, they suggest that hypotheses concerning the historical biogeography of the region based solely on distributional data are perhaps no longer as useful as they once were. The distributional data must be accompanied by modern phylogenetic analyses for two reasons. First, the phylogenetic approach can reveal divergence times and relationships more straightforwardly than other approaches (acknowledging that a match with earth history must

still be sought), thus helping to resolve biogeographic interpretation. Second, molecular evidence has been instrumental in revealing the presence of cryptic species, the existence of which can change interpretation substantially (Stevens *et al.* 2006, Torricelli *et al.* 2010). Given enhanced scientific cooperation across the Antarctic within a variety of scientific programmes, the development of comprehensive molecular phylogenies is likely to be achieved readily, and will almost certainly change current perspectives on the biogeography and biodiversity of the region, as this initial study has demonstrated.

Acknowledgments

Two referees provided useful comments on a previous version of the manuscript. We thank the South African National Antarctic Programme and the Australian Antarctic Division for supporting our work at the Prince Edward Islands and Heard Island, respectively. Lewis Davies provided access to material from Possession Island via the donation of several specimens to SLC, whilst material from Kerguelen Island was collected by Jacques Deere during a Scientific Committee on Antarctic Research (SCAR) exchange activity supported by the French Polar Institute (IPEV) and specifically Yves Frenot. We are indebted to Chris Lyal of the Natural History Museum, London for providing light micrographs of the *B. parvulus* holotype and to Helene Brettschneider for sample sorting and SEM photography of specimens. This work forms part of the SCAR Evolution and Biodiversity in Antarctica programme.

References

ALI, J.R. & AITCHISON, J.C. 2009. Kerguelen Plateau and the Late Cretaceous southern-continent bioconnection hypothesis: tales from a topographic ocean. *Journal of Biogeography*, **36**, 1778-1784.

ALLEGRUCCI, G., CARCHINI, G., TODISCO, V., CONVEY, P. & SBORDONI, V. 2006. A molecular phylogeny of Antarctic Chironomidae and its implications for biogeographical history. *Polar Biology*, **29**, 320-326.

ALONSO-ZARAZAGA, M.A. & LYAL, C.H.C. 1999. *A World Catalogue of families and genera of Curculionoidea (Insecta: Coleoptera) excluding (Scolytidae and Platypodidae).* Entomopraxis: Barcelona, 315 pp.

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.

BROWER, A.V.Z. 1994. Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. *Proceedings of the National Academy of Sciences of the U.S.A.*, **91**, 6491-6495.

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

CHOWN, S. L. 1990a. Possible effects of Quaternary climatic change on the composition of insect communities of the South Indian Ocean Province Islands. *South African Journal of Science*, **86**, 386-391.

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

CHOWN, S.L. 1992. A preliminary analysis of weevil assemblages in the sub-Antarctic: local and regional patterns. *Journal of Biogeography*, **19**, 87-98.

CHOWN, S.L. 1994. Historical ecology of sub-Antarctic weevils: patterns and processes on isolated islands. *Journal of Natural History*, **28**, 411-433.

CHOWN, S.L. & CONVEY, P. 2007. Spatial and temporal variability across life's hierarchies in the terrestrial Antarctic. *Philosophical Transactions of the Royal Society of London B*, **362**, 2307-2331.

CHOWN, S.L., & KUSCHEL, G. 1994. New *Bothrometopus* species from Possession Island, Crozet Archipelago with nomenclatural amendments and a key to its weevil fauna (Coleoptera: Curculionidae: Brachycerinae). *African Entomology*, **2**, 149-154.

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.

CHOWN, S.L., GREMMEN, N.J.M. & GASTON, K.J. 1998. Ecological biogeography of southern ocean islands: species-area relationships, human impacts, and conservation. *American Naturalist*, **152**, 562-575.

CRAIG, D.A., CURRIE, D.C. & VERNON, P. 2003. *Crozetia* Davies (Diptera: Silmuliidae): redescription of *Cr. Crozetensis*, *Cr. Seguyi*, number of larval instars, phylogenetic relationships and historical biogeography. *Zootaxa*, **259**, 1-39.

DE QUEIROZ, A. 2005. The resurrection of oceanic dispersal in historical biogeography. *Trends in Ecology and Evolution*, **20**, 68-73.

DE WEVER, A., LELIAERT, F., VERLEYEN, E., VANORMELINGEN, P., VAN DER GUCHT, K., HODGSON, D.A., SABBE, K. & VYVERMAN, W. 2009. Hidden levels of phylodiversity in Antarctic green algae: further evidence of glacial refugia. *Proceedings of the Royal Society of London B*, **276**, 3591-3599.

DREUX, P. & VOISIN, J.F. 1987. Notes sur le genre *Antarctonesiotes* Jeannel et sur le genre *Disker* nov., et description d'*Antarctonesiotes villiersi* n. sp. (Coleoptera, Curculionidae). *Nouvelle Revue d'Entomologie (N.S.)*, **4**, 313-321.

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.

FARRIS, J.S. 1969. A successive approximations approach to character weighting. *Systematic Biology*, **18**, 374-385.

FRASER, C.I., NIKULA, R., SPENCER, H.G. & WATERS, J.M. 2009. Kelp genes reveal effects of subAntarctic sea ice during the Last Glacial Maximum. *Proceedings of the National Academy of Sciences of the U.S.A.*, **106**, 3249-3253.

FRENOT, Y., GLOAGUEN, J.C., MASSE, L. & LEBOUVIER, M. 2001. Human activities, ecosystem disturbance and plant invasions in subantarctic Crozet, Kerguelen and Amsterdam Islands. *Biological Conservation*, **101**, 33-50.

GRESSITT, J.L. 1970. Subantarctic entomology and biogeography. *Pacific Insects Monograph*, **23**, 295-374.

GREVE, M., GREMMEN, N.J.M., GASTON, K.J. & CHOWN, S.L. 2005. Nestedness of South Ocean island biotas: ecological perspectives on a biographical conundrum. *Journal of Biogeography*, **32**, 155-168.

GROBLER, G.C., JANSE VAN RENSBURG, L., BASTOS, A.D.S., CHIMIMBA, C.T. & CHOWN, S.L. 2006. Molecular and morphometric assessment of the taxonomic status of *Ectemnorhinus* weevil species (Coleoptera: Curculionidae, Entiminae) from the sub-Antarctic Prince

Edward Islands. *Journal of Zoological Systematics and Evolutionary Research*, **44**, 200-211.

GROBLER G.C., BASTOS, A.D.S., CHIMIMBA, C.T. & CHOWN, S.L. 2011. Inter-island dispersal of flightless *Bothrometopus huntleyi* (Coleoptera: Curculionidae) from the sub-Antarctic Prince Edward Island Archipelago. *Antarctic Science*, **23**: 225-234.

GUINDON, S. & GASCUEL, O. 2003. A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. *Systematic Biology*, **52**, 696-704.

HALL, K. 2002. Review of present and Quaternary periglacial processes and landforms of the maritime and sub-Antarctic region. *South African Journal of Science*, **98**, 71-81.

JEANNEL, R. 1964. Biogéographie des terres australes de l'océan Indien. *Revue Francaise d'Entomologie*, **31**, 319-417.

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, 355-359.

KUSCHEL, G. & CHOWN, S.L. 1995. Phylogeny and systematics of the *Ectemnorhinus*-group of genera (Insecta: Coleoptera). *Invertebrate Taxonomy*, **9**, 841-863.

LIBRADO, P. & ROZAS, J. 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* doi: 10.1093/bioinformatics/btp187.

MCGAUGHRAN, A., CONVEY, P., STEVENS, M.I. & CHOWN, S.L. 2010a. Metabolic rate, genetic and microclimate variation among springtail populations from sub-Antarctic Marion Island. *Polar Biology*, in press.

MCGAUGHRAN, A., TORRICELLI, G., CARAPELLI, A., FRATI, F., STEVENS, M.I., CONVEY, P. & HOGG, I.D. 2010b. Contrasting phylogeographical patterns for springtails reflect different evolutionary histories between Antarctic Peninsula and continental Antarctica. *Journal of Biogeography*, **37**, 103-119.

MICHAUX, B. & LESCHEN, R.A.B. 2005. East meets west: biogeology of the Campbell Plateau. *Biological Journal of the Linnean Society*, **86**, 95-115.

MORTIMER, E. & JANSEN VAN VUUREN, B. 2007. Phylogeography of *Eupodes minutus* (Acari: Prostigmata) on sub-Antarctic Marion Island reflects the impact of historical events. *Polar Biology*, **30**, 471-476.

MORTIMER, E., MCGEOCH, M.A., DANIELS, S.R. & JANSEN VAN VUUREN, B. 2008. Growth form and population genetic structure of *Azorella selago* on Marion island. *Antarctic Science*, **20**, 381-390.

MORTIMER, E. & JANSEN VAN VUUREN, B., LEE, J.E., MARSHALL, D.J., CONVEY, P. & CHOWN, S.L. 2010. Mite dispersal among the Southern Ocean Islands and Antarctica before the last glacial maximum. *Proceedings of the Royal Society of London B*, in press.

MYBURGH, M., CHOWN, S.L., DANIELS, S.R. & JANSEN VAN VUUREN, B. 2007. Population structure, propagule pressure and conservation biogeography: lessons from indigenous and invasive springtails. *Diversity and Distributions*, **13**, 143-154.

PAPADOPOULOU, A., ANASTASIOU, I. & VOGLER, A.P. 2010. Revisiting the molecular clock: the mid-Aegean trench calibration. *Molecular Biology and Evolution*, (*In press*). (doi:10.1093/molbev/msq051)

POSADA, D. 2008. jModelTest: Phylogenetic Model Averaging. *Molecular Biology and Evolution*, **25**, 1253-1256.

PUGH, P.J.A. 2004. Biogeography of spiders (Araneae: Arachnida) on the islands of the Southern Ocean. *Journal of Natural History*, **38**, 1461-1487.

PUGH, P.J.A. & CONVEY, P. 2008. Surviving out in the cold: Antarctic endemic invertebrates and their refugia. *Journal of Biogeography*, **35**, 2176-2186.

RONQUIST, F. & HUELSENBECK, J.P. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19**, 1572-1574.

RUBINOFF, D. 2006. Utility of mitochondrial DNA barcodes in species conservation. *Conservation Biology*, **20**, 1026-1033.

SANMARTÍN, I., WANNTORP, L. & WINKWORTH, R.C. 2007. West Wind Drift revisited: testing for directional dispersal in the Southern Hemisphere using event-based tree fitting. *Journal of Biogeography*, **34**, 398-416.

SCOTT, L. 1985. Palynological indications of the Quaternary vegetation history of Marion Island (sub-Antarctic). *Journal of Biogeography*, **12**, 413-431.

SKOTNICKI, M.L., MACKENZIE, A.M., NINHAM, J.A. & SELKIRK, P.M. 2004. High levels of genetic variability in the moss *Ceratodon purpureus* from continental Antarctica, subantarctic Heard and Macquarie Islands, and Australasia. *Polar Biology*, **27**, 687-698.

STEVENS, M.I., GREENSLADE, P., HOGG, I.D. & SUNNUCKS, P. 2006. Southern Hemisphere springtails: could any have survived glaciation of Antarctica? *Molecular Biology and Evolution*, **23**, 874-882.

SWOFFORD, D. L., 2003: PAUP*. *Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4.10b.* Sinauer Associates, Sunderland, Massachusetts.

TAMURA, K., DUDLEY, J., NEI, M. & KUMAR, S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution*, **24**, 1596-1599.

THORNHILL, D.J., MAHON, A.R., NORENBURG, J.L. & HALANYCH, K.M. 2008. Open-ocean barriers to dispersal: a test case with the Antarctic Polar Front and the ribbon worm *Parborlasia corrugatus* (Nermetea: Lineidae). *Molecular Ecology*, **17**, 5104-5117.

TORRICELLI, G., CARAPELLI, A., CONVEY, P., NARDI, F., BOORE, J.L. & FRATI, F. 2010. High divergence across the whole mitochondrial genome in the pan-Antarctic springtail *Friesea grisea*: evidence for cryptic species? *Gene*, **449**, 30-40.

TURNER, J., BINDSCHADLER, R., CONVEY, P., DI PRISCO, G., FAHRBACH, E., GUTT, J., HODGSON, D., MAYEWSKI, P. & SUMMERHAYES, C. *eds*. 2009. *Antarctic Climate Change and the Environment.* Scientific Committee on Antarctic Research, Cambridge.

UDVARDY, M.D.F. 1987. The biogeographical realm Antarctica: a proposal. *Journal of the Royal Society of New Zealand*, **17**, 187-194.

VAN DE VIJVER, B., GREMMEN, N.J.M. & BEYENS, L. 2005. The genus *Stauroneis* (Bacillariophyceae) in the Antarctic region. *Journal of Biogeography*, **32**, 1791-1798.

VAN DE WOUW, M., VAN DIJK, P. & HUISKES, A. 2007. Regional genetic diversity patterns in Antarctic hairgrass (*Deschampsia antarctica* Desv.). *Journal of Biogeography*, **35**, 365-376.

VAN DER PUTTEN, N., VERBRUGGEN, C., OCHYRA, R., VERLEYEN, E. & FRENOT, Y. 2010. Subantarctic flowering plants: pre-glacial survivors or post-glacial immigrants? *Journal of Biogeography*, **37**, 582-592.

VINK, C.J. & PHILLIPS, C.B. 2007. First record of *Sitona discoideus* Gyllenhal 1834 (Coleoptera: Curculionidae) on Norfolk Island. *New Zealand Journal of Zoology*, **34**, 283-287.

WILSON, N.G., SCHRÖDL, M. & HALANYCH, K.M. 2009. Ocean barriers and glaciations: evidence for explosive radiation of mitochondrial lineages in the Antarctic sea slug *Doris kerguelenensis* (Mollusca, Nudibranchia). *Molecular Ecology*, **18**, 965-984.

CHAPTER 5

Inter-island dispersal of flightless *Bothrometopus huntleyi* (Coleoptera: Curculionidae) from the sub-Antarctic Prince Edward Island Archipelago

G.C. Grobler[1*], A.D.S. Bastos[1,2], C.T. Chimimba[1,3] and S.L. Chown[3]

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

[2]*Department of Biological Sciences, University of Cyprus, Nicosia, Cyprus*

[3]*Centre for Invasion Biology, Department of Botany and Zoology, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa*

The results presented here have been published in *Antarctic Science* **23,** 225-234 (2011).

Abstract: *Bothrometopus huntleyi* is a flightless weevil endemic to the volcanically-formed sub-Antarctic Prince Edward Islands archipelago that arose approximately 0.5 million years ago (m.y.a.). Since emergence, a series of volcanic and glaciation events have occurred on Marion Island, whilst Prince Edward Island, the second island constituting the archipelago, has remained largely unaffected by glaciation. Cytochrome oxidase I gene analyses indicate that major historical dispersal events in this species are linked to the geologically discrete histories of these islands and underlie the high haplotype diversity (0.995) recovered for the Prince Edward Islands archipelago. The estimated time to haplotype coalescence of ~0.723 m.y.a. is in keeping with estimated dates of island emergence, and the majority of individuals appear to have descended from a relict, high-altitude population that is still present on Marion Island. The first major inter-island dispersal event occurred ~0.507 m.y.a., coinciding with the oldest dated rocks on Marion Island. Apart from this early inter-island colonisation, only one other between-island dispersal event was detected. The genetically discrete *B. huntleyi* complexes on each of the islands of the Prince Edward Islands archipelago together with the low levels of inter-island gene flow reaffirm the need to control alien invasive mice, which are restricted to Marion Island, and which prey on this weevil species.

Key words: mtDNA, COI gene, conservation biogeography, dispersal, invasion biology, phylogeography

Introduction

Bothrometopus huntleyi (Grobler et al. 2011) (Curculionidae: Coleoptera: Hexapoda) belongs to the *Ectemnorhinus* group of genera (Kuschel & Chown 1995), also known as the tribe Ectemnorhinini (Alonso-Zarazaga & Lyal 1999), which is restricted to islands of the sub-Antarctic South Indian Ocean Province (SIP). This group of weevils comprises eight genera and 37 flightless species (Kuschel & Chown 1995, Grobler *et al*. 2006, Grobler *et al.* 2011), of which seven occur on the Prince Edward Islands. Of these seven species, five, including *B. huntleyi*, are endemic to the archipelago, with *Bothrometopus randi* (Jeannel 1953) also being found on the Crozet archipelago to the east, and *Palirhoeus eatoni* (C.O. Waterhouse 1876) occurring on all SIP islands (Chown & Klok 2001, Kuschel & Chown 1995). Recent molecular studies both of this group of weevils (Grobler *et al*. 2006) and of other taxa in the region (e.g. Stevens *et al*. 2006, Myburgh *et al*. 2007) have suggested that relationships among species, within and among islands and archipelagos (including the Prince Edward Island archipelago) may be much more complex than suggested by morphological analyses. In consequence, it may be argued that the status of *B. huntleyi* on the Prince Edward Island archipelago has not been fully resolved, and that it might be expected to show substantial phylogeographic structure associated with the different histories of the two islands which lie only 19 km apart. Perhaps most significantly, Marion Island, the larger of the two Prince Edward Islands, was extensively glaciated whilst the smaller Prince Edward Island shows no evidence of glaciation (Boelhouwers *et al*. 2008). McDougall *et al*. (2001) suggested that Marion Island had five distinct cold periods and eight volcanic ages, with two of these accompanying major glaciations (Fig. 1). Although the exact sequence and spatial extent of volcanic and glacial events is currently the subject of revision, this overall scenario appears to be robust (Boelhouwers *et al*. 2008).

In consequence, substantial differences among populations of *B. huntleyi* on the two islands might be expected. Such differences would not only be of biogeographic significance (see e.g. Chown 1992), but would also have substantial conservation implications given the differences in invasive alien species on the islands, especially the absence of house mice on Prince Edward Island, which have a dietary preference for weevils on Marion Island (Chown & Smith 1993). Here we set out to examine the evolutionary dynamics shaping the population structure of *B. huntleyi*, which occurs on both islands, is widely distributed across Marion Island, and has until recently been confounded with *B. parvulus* (Grobler *et al*. 2011).

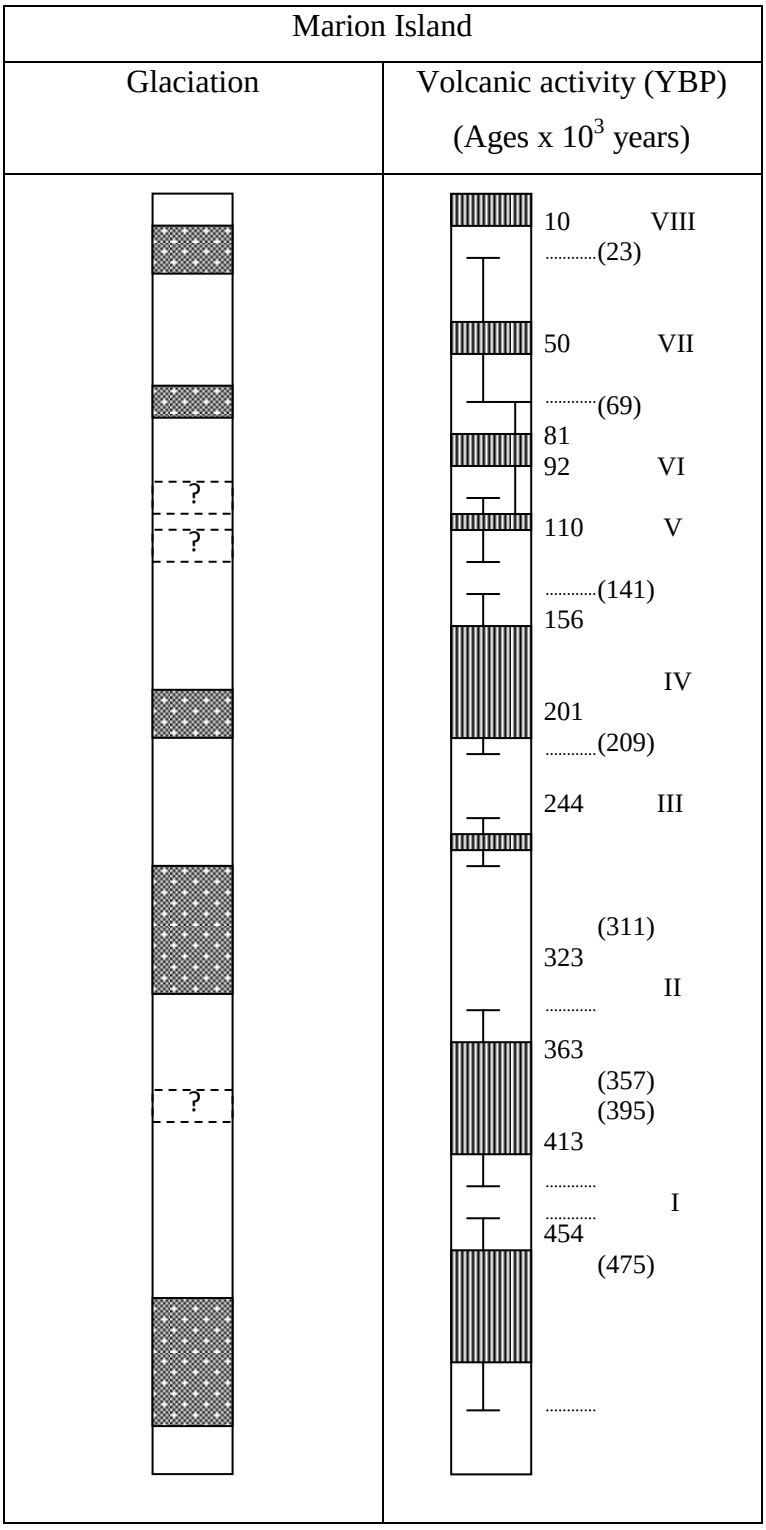


Fig. 1 Correlation of volcanic activity and glaciation on Marion Island. The shaded bars represent periods of glaciation or volcanic activity with the older events at the bottom of the figure and the most recent events at the top. This diagram is presented with the permission of the Geological Magazine and Prof. McDougal (McDougal *et al*. 2001).

Materials and Methods

Systematics and biology

Although the genus *Bothrometopus* has previously been separated into two distinct groups, *viz*. the *fasciatus*- and *gracilipes*-groups, which are distinguished by, respectively, the absence and presence of vaginal spicules (see Dreux & Voisin 1984, 1986, 1987, Kuschel & Chown 1995 for earlier work on the genus and its now synonymous allies), Grobler *et al.* (2011) have called for the re-evaluation of these groups.

Because *B. huntleyi* was previously not distinguished from the very similar *B. parvulus*, and because it is now clear that *B. parvulus* is restricted to coastal areas of both islands (Grobler *et al.* 2011), it can be reasonably assumed, from the previous literature on habitat use in the group (e.g. Chown 1989), that *B. huntleyi* predominantly occurs in epilithic moss cushions from coastal rock faces to high altitude fellfield and polar desert. The larvae and adults feed on algae, lichens, and bryophytes (Chown 1989, 1992). Individuals may occur occasionally on *Azorella selago*, but feed on epiphytic algae and bryophytes, rather than on the plant itself (Chown 1989, 1992). On both Marion Island and Prince Edward Island, *B. huntleyi* shows distinct variation in size, associated both with elevation and habitat type, although differences among the two islands are not pronounced (Chown 1992, Chown & Smith 1993, Chown & Klok 2003).

Study sites and sampling

Specimens of *B. huntleyi* were collected during three consecutive years (April 2001 – April 2003) from 14 localities from the eastern side of Marion Island (Fig. 2). Due to restricted access to Prince Edward Island, weevils were only sampled from 5 localities on one occasion in April 2003 (Fig. 2). For each island, the specimens were collected from a range of altitudes, and individuals selected for genetic characterisation were chosen to ensure the broadest possible size and colour variation representation, for each sampling locality. The geographic coordinates, altitude and weevil size range recorded for each sampling locality are summarized in Table 1. All specimens were collected by hand and preserved in absolute ethanol. *Bothrometopus brevis* (C.O. Waterhouse) from Heard Island was selected as an outgroup, since it is the closest known sister taxon of *B. huntleyi* (Grobler *et al.* 2011: Table 1).

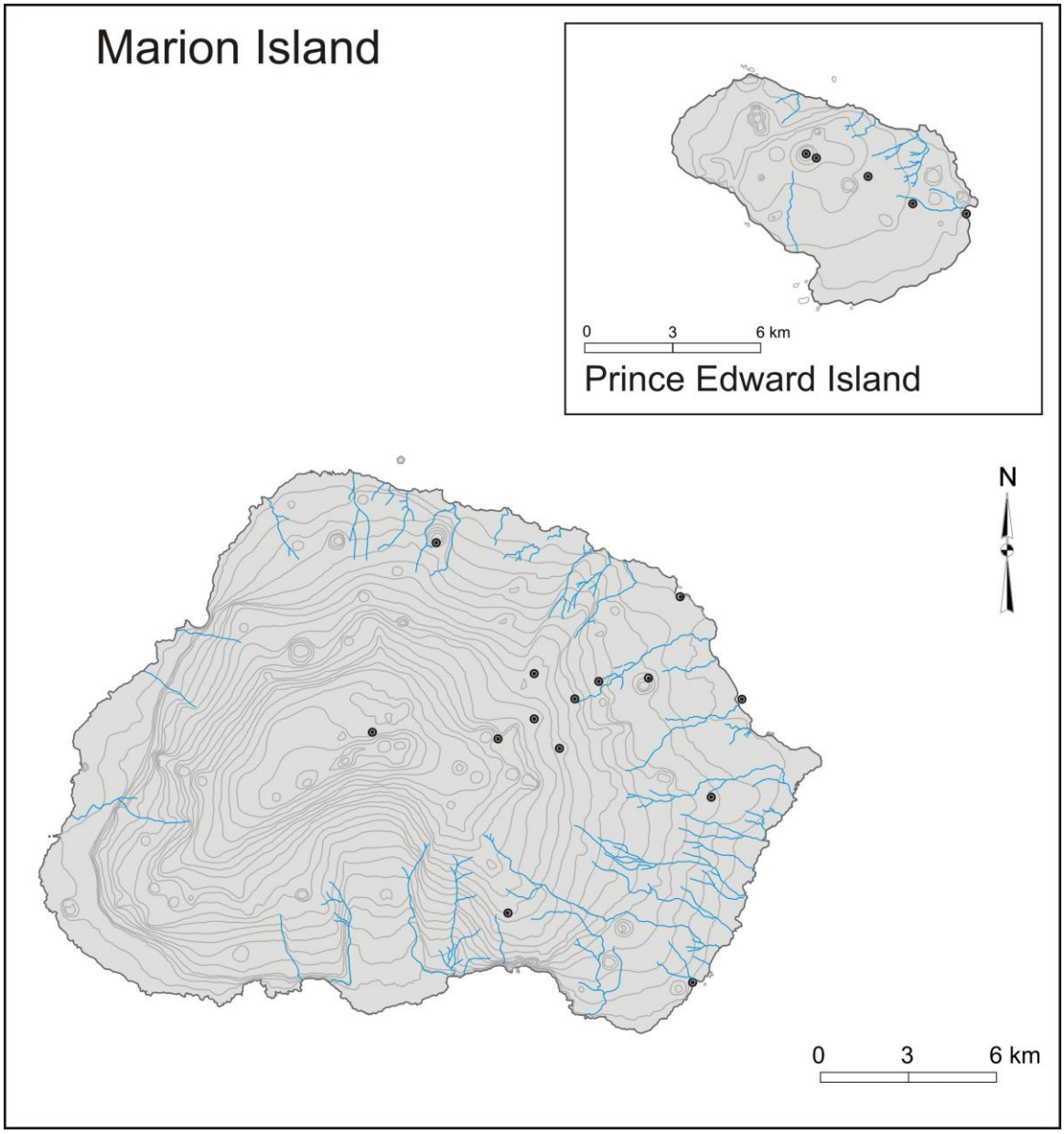


Fig. 2 Map indicating flightless weevil (*Bothrometopus huntleyi*) sampling localities on the Prince Edward Island Archipelago and that correspond to the geographic coordinates summarised in Table 1.

TABLE 1 Summary of the 19 sampling localities on Marion (MI) and Prince Edward Islands (PEI) from which the genetically characterised *Bothrometopus huntleyi* specimens included in this study were collected.

Island	Sampling Locality (a.s.l)	Geographic coordinates	Number of specimens per locality (size range in mm)
MI	Kildalkey Bay (0 m)	S 46º57'38.3'' E 37º51'22.2''	1 (3.92)
MI	Ships Cove (0 m)	S 46º51'41'' E 37º50'66''	8 (3.31 - 5.00)
MI	Trypot Beach (0 m)	S 46º53'05.2'' E 37º52'06''	1 (3.30)
MI	Stony Ridge (150 m)	S 46º54'88.1'' E 37º51'48.4''	1 (4.61)
MI	Junior's Kop (200 m)	S 46º52.794' E 37º50.083'	4 (3.15 - 5.00)
MI	Tafelberg (250 m)	S 46º53'03.5'' E 37º48'20.1''	1 (3.69)
MI	Repetto's Hill (300 m)	S 46º50'35.7'' E 37º44'21.8''	4 (3.36 - 4.77)
MI	First Red Hill (400 m)	S 46º53.412' E 37º48.21'	5 (2.80 - 4.08)
MI	Long Ridge South (450 m)	S 46º52'45'' E 37º47'00''	2 (3.08 - 5.08)
MI	First Red Hill (600 m)	S 46º53.647' E 37º47.208'	4 (2.96 - 4.72)
MI	Halfway Kop (600 m)	S 46º54'06'' E 37º47'83''	6 (3.08 - 5.31)
MI	Feldmark Plateau (600 m)	S 46º56'35'' E 37º46'10''	2 (3.39 - 5.15)
MI	Katedraalkrans (800 m)	S 46º53.896' E 37º46.482'	4 (3.60 - 4.85)
MI	Ice Plateau (1000 m)	S 46º54.29' E 37º45.375'	8 (3.92 - 5.69)
PEI	Cave Bay (0 m)	S 46º38.752' E 37º59.780'	4 (2.46 - 4.62)
PEI	(200 m)	S 46º38.457' E 37º58.396'	5 (3.31 - 4.39)
PEI	(400 m)	S 46º38.211' E 37º57.482'	5 (3.31 - 4.31)
PEI	(600 m)	S 46º37.533' E 37º55.985'	4 (3.54 - 5.00)
PEI	TvZB (672 m)	S 46º37.590' E 37º55.891'	4 (3.54 - 4.46)

a.s.l.: above sea level; TvZB: Samples collected at the top of Van Zinderen Bakker.

Genetic characterization

DNA from each individual was extracted from a single leg which, following removal from ethanol was washed and rehydrated in distilled water for 10 minutes, prior to being frozen in liquid nitrogen and ground using an Eppendorf micro pestle (Merck, South Africa). DNA was extracted using the High Pure PCR Template Preparation Kit (Roche Applied Science) using the supplier's procedure for the isolation of nucleic acids from mammalian tissue with modification to the proteinase K tissue lysis incubation step, which was performed for 48 hrs instead of the recommended 1 hr for mammalian tissue.

COI primers, GF-1858 and GR1-2938 which are specific for weevils from Marion Island (Grobler *et al*. 2006) were used to amplify a 1059 bp PCR product under previously described reaction conditions (Grobler *et al*. 2006) using a thermal cycling profile that consisted of an initial denaturation step at 94ºC for 90 s, followed by 40 cycles of 94ºC for 22 s, 46ºC for 30 s and 72ºC for 1 min and concluding with a final extension step of 1 min at 72ºC. PCR products of the correct size were purified directly from the tube using a Roche High Pure PCR Product Purification Kit (Roche Applied Science). Automated cycle sequencing with the ABI PRISM Big Dye^{TM} Terminator version 3.0 (Applied Biosystems) and two internal primers, GF5-1940 and GR5-2935 (Grobler *et al*. 2006), in separate reactions, was performed at an annealing temperature of 46ºC. Sequences were viewed, edited and aligned in MEGA4 (Tamura *et al.* 2007), resulting in a homologous dataset 885 bp in length.

Phylogenetic analyses

Neighbor-Joining (NJ) and Minimum Evolution (ME) algorithms were used to construct distance trees in MEGA4 (Tamura *et al*. 2007) and maximum likelihood analyses were performed in PhyML (Guindon & Gascuel 2003) using the model of sequence evolution identified as the best-fit model under the Akaike Information Criterion (AIC) in ModelTest version 3.06 (Posada & Crandall 1998). Nodal support was assessed by 100,000 and 5,000 bootstrap replications, respectively. Maximum parsimony (MP) analyses were performed in PAUP* v4.0b10 (Swofford 2003). Characters had equal weights in the initial analysis and were subsequently reweighted using the rescaled consistency index (RCI). Starting trees were obtained by random stepwise addition of sequences and branch swapping was performed

using the tree-bisection-reconnection (TBR) algorithm. Bayesian Inference (BI) using MrBayes version 3.1 (Huelsenbeck & Ronquist 2001) was performed using priors guided by the best-fit model and parameters in ModelTest (Posada & Crandall 1998), namely the HKY85 model (Hasegawa et al. 1985) with the proportion of invariable sites (I) and gamma distribution shape parameter (Γ) being 0.791 and 1.6671, respectively. The analysis was initiated with random starting trees and run for 10,000,000 generations with Markov chains sampled every 2,000 generations. Of the 5000 trees obtained, 1250 were discarded as "burn-in". Tracer plots were visually inspected and tracer diagnostics (standard deviation of split frequencies, effective sample size), as implemented in MrBayes and Tracer v1.4 (Drummond & Rambaut 2007) were inspected to ensure that stationarity had been reached.

The equality of evolutionary rates between lineages was evaluated using the relative rate test (Li & Bousquet 1992) in PHYLTEST version 2.0 (Kumar 1996). The likelihood ratio test (Felsenstein 1981, Felsenstein 1988) was also performed by calculating and comparing the log likelihood scores with and without the molecular clock enforced. Divergence times were estimated using the 2.3% nucleotide sequence divergence per million years, estimated from an arthropod mtDNA survey (Brower 1994) and the appropriate parameter-rich model of evolution (Papadopoulou et al. 2010) selected under the AIC in ModelTest (Posada and Crandall 1998). 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, ML and BI analyses were constrained to be monophyletic and the HKY+I+Γ model was enforced using a strict molecular clock model. The results of two independent runs were merged and analyzed with Tracer v1.4 and TreeAnnotator v1.4.7 (Drummond & Rambaut 2007).

The program TCS version 1.21 (Clement *et al*. 2000) was used to generate a haplotype cladogram displaying the number of base pair differences between haplotypes. TCS version 1.21 (Clement *et al*. 2000) incorporates the cladogram estimation algorithm described by Templeton *et al*. (1992) and provides 95% parsimoniously plausible branch connections between the different haplotypes.

Mismatch analyses, population expansion and diversity estimates

Pairwise mismatch distributions, where the observed pairwise mismatch distributions were fitted to a stepwise expansion model by a generalized least square procedure following Schneider & Excoffier (1999), as implemented in Arlequin version 2.0 (Schneider *et al*.

2000), were generated. A constant size population is expected to show a ragged, multimodal distribution, while an expanding population is consistent with a smooth unimodal distribution.

In each case, the raggedness index assesses the match of the real data to the model while the overall validity of the estimated expansion model was tested by comparing the distribution of the test statistic, SSD (sum of squared differences) between the observed and the estimated mismatch distribution using a bootstrap approach. Evidence for departure from the estimated expansion model is given by significant SSD values (Excoffier & Schneider 1999).

The validity of a stepwise expansion model for the data was tested by Monte Carlo Markov chain simulations (1000 steps) in Arlequin version 2.0 (Schneider *et al*. 2000). The time of the main expansion in generations (*t*) was estimated from the equation $\tau = 2\mu t$ using the moment estimator of time to the expansion (τ) computed in Arlequin version 2.0 (Schneider *et al*. 2000) and a mutation rate (μ) of 2.3% nucleotide sequence divergence per million years for arthropod mtDNA (Brower 1994). As the weevils complete one generation in one-year (Chown & Scholtz 1989), *t* could also be used as the time of the main expansion in years. Arlequin version 2.0 (Schneider *et al*. 2000) was utilized to estimate the essential population parameter θ using Watterson's (1975) estimate, which is based on the number of segregating sites among the sequences, and Tajima's (1983) estimate, which is based on the calculation of the mean number of pairwise differences of the sequences. Tajima's estimate of θ puts more weight on ancient mutations and therefore reflects ancient population events (Fu 1997).

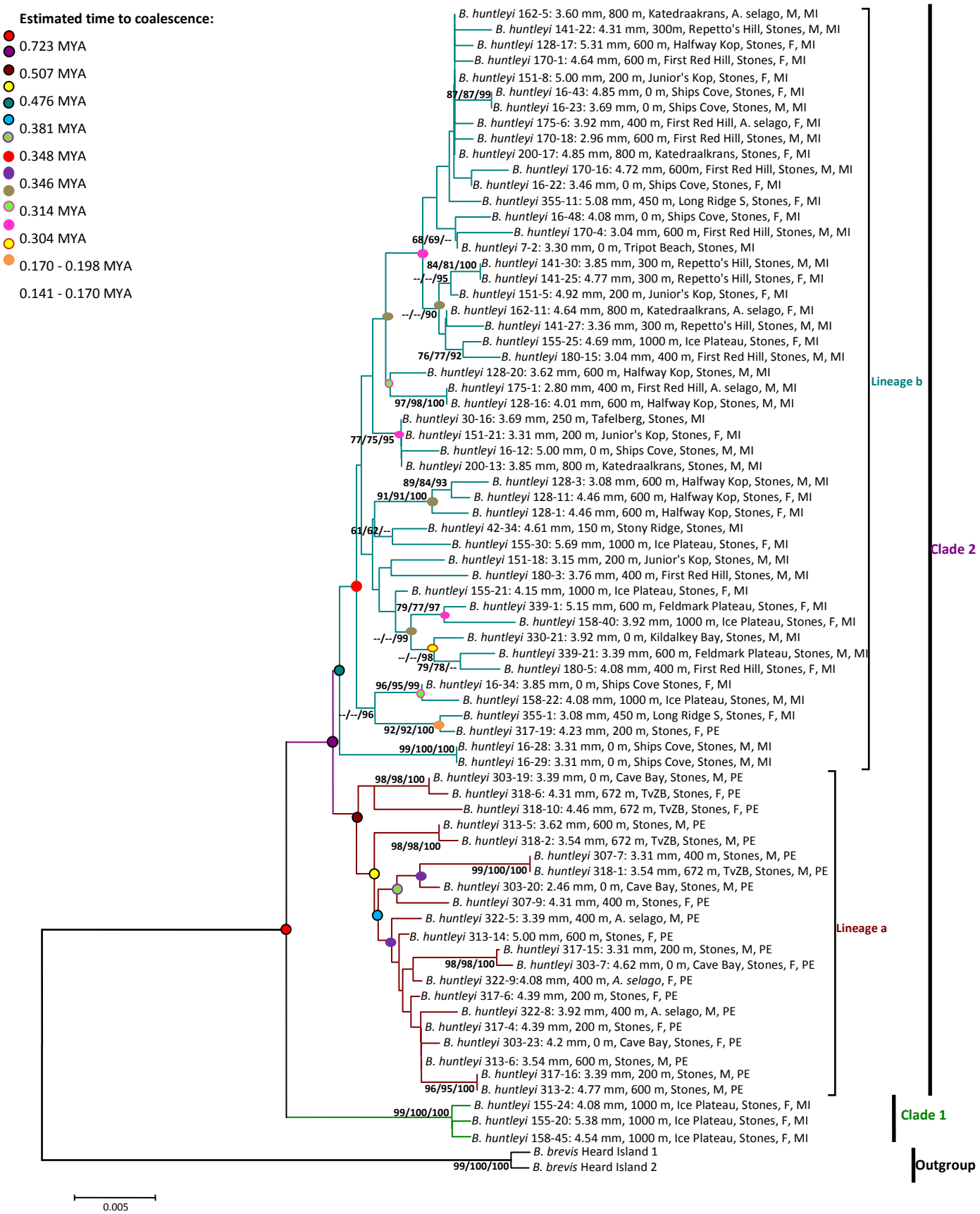
Fu's (1997) F_S test of neutrality, as estimated in Arlequin version 2.0 (Schneider *et al*. 2000), and the R_2 test of neutrality (Ramos-Onsins & Rozas 2002), as estimated in DNASP version 5 (Librado & Rozas 2009), were shown to be powerful tests for detecting recent population expansions under assumptions of neutrality with R_2 showing better results for small sample sizes and F_S for large sample sizes. The R_2 statistic is based on the difference between the number of singleton mutations and the average number of nucleotide differences among sequences within a population sample (Ramos-Onsins & Rozas 2002). The haplotype diversity (h) and nucleotide diversity (π) were estimated in DNASP version 5 (Librado & Rozas 2009).

Results

A homologous region of 885 bp corresponding to the 5' end of the COI gene was generated for 73 *B. huntleyi* individuals and two *B. brevis* outgroup specimens. All sequences have been deposited in the Genbank database under accession numbers GQ131940-GQ132014. Of the 885 sites characterised, 768 were conserved across all 75 sequences and 82 of the 117 variable sites were parsimony informative. Under the HKY+I+Γ model of sequence evolution, a transition (ti)/transversion (tv) ratio of 14.5 and base frequencies of A = 0.3069, C = 0.1590, G = 0.1497 and T = 0.3844 (% AT = 70.2%) were recovered. Third base position substitutions accounted for 88.03% of the variation with 11.11% and 0.85% being due to first and second base substitutions, respectively. Mutations at the nucleotide level gave rise to five non-synonymous amino acid substitutions at codons 4, 191, 219, 238, and 289. Of the 73 *B. huntleyi* individuals sequenced, 62 had unique haplotypes corresponding to a haplotype diversity (h) of 0.995 and a nucleotide diversity (π) of 0.01278 for the Prince Edward Islands archipelago. When considering each island individually, 43 unique haplotypes were recovered from the 51 Marion Island specimens sequenced (h = 0.992 and π = 0.01130), whilst for Prince Edward Island, 19 unique haplotypes were identified from 22 individuals (h = 0.987 and π = 0.00890).

MP analysis recovered 76125 equally parsimonious trees with homoplasy indices of: CI=0.520; RI=0.827 and RCI=0.430 when characters were assigned equal weights. Successive weighting with the RCI recovered the same number of equally parsimonious trees and homoplasy indices of CI=0.699, RI=0.889 and RCI=0.621. All methods of phylogenetic inference (Fig. 3) revealed the presence of two distinct clades.

Fig. 3 Neighbour-Joining (NJ) tree of *Bothrometopus huntleyi* from Marion (MI) and Prince Edward (PEI) Islands and *B. brevis* outgroup from Heard Island , based on 885 nucleotides of the mitochondrial cytochrome oxidase I (COI) gene inferred using the HKY85 model of sequence evolution. The taxon name contains the species designation, sample number, body length measurement, altitude and/or sampling locality, sex and island of origin. Nodal support values ≥ 60 obtained from NJ and ML (100,000 and 5,000 bootstrap replications, respectively) and ≥ 90 from Bayesian Inference (BI) and expressed as a percentage, are indicated NJ/ML/BI. '--' denotes bootstrap support values below 60 (NJ and ML) and below 90 (BI). (On page 201)


Estimated time to coalescence:
0.723 MYA
0.507 MYA
0.476 MYA
0.381 MYA
0.348 MYA
0.346 MYA
0.314 MYA
0.304 MYA
0.170 - 0.198 MYA
0.141 - 0.170 MYA
B. huntleyi 162-5: 3.60 mm, 800 m, Katedraakrans, A. selago, M, MI
B. huntleyi 141-22: 4.31 mm, 300m, Repetto's Hill, Stones, M, MI
B. huntleyi 128-17: 5.31 mm, 600 m, Halfway Kop, Stones, F, MI
B. huntleyi 170-1: 4.64 mm, 600 m, First Red Hill, Stones, F, MI
B. huntleyi 151-8: 5.00 mm, 200 m, Junior's Kop, Stones, F, MI
87/87/99
B. huntleyi 16-43: 4.85 mm, 0 m, Ships Cove, Stones, F, MI
B. huntleyi 16-23: 3.69 mm, 0 m, Ships Cove, Stones, M, MI
B. huntleyi 175-6: 3.92 mm, 400 m, First Red Hill, A. selago, F, MI
B. huntleyi 170-18: 2.96 mm, 600 m, First Red Hill, Stones, M, MI
B. huntleyi 200-17: 4.85 mm, 800 m, Katedraalkrans, Stones, F, MI
B. huntleyi 170-16: 4.72 mm, 600m, First Red Hill, Stones, M, MI
B. huntleyi 16-22: 3.46 mm, 0 m, Ships Cove, Stones, F, MI
B. huntleyi 355-11: 5.08 mm, 450 m, Long Ridge S, Stones, F, MI
B. huntleyi 16-48: 4.08 mm, 0 m, Ships Cove, Stones, F, MI
B. huntleyi 170-4: 3.04 mm, 600 m, First Red Hill, Stones, M, MI
68/69/--
B. huntleyi 7-2: 3.30 mm, 0 m, Tripot Beach, Stones, MI
84/81/100
B. huntleyi 141-30: 3.85 mm, 300 m, Repetto's Hill, Stones, M, MI
--/--/95
B. huntleyi 141-25: 4.77 mm, 300 m, Repetto's Hill, Stones, F, MI
B. huntleyi 151-5: 4.92 mm, 200 m, Junior's Kop, Stones, F, MI
--/--/90
B. huntleyi 162-11: 4.64 mm, 800 m, Katedraalkrans, A. selago, F, MI
B. huntleyi 141-27: 3.36 mm, 300 m, Repetto's Hill, Stones, M, MI
B. huntleyi 155-25: 4.69 mm, 1000 m, Ice Plateau, Stones, F, MI
76/77/92
B. huntleyi 180-15: 3.04 mm, 400 m, First Red Hill, Stones, M, MI
B. huntleyi 128-20: 3.62 mm, 600 m, Halfway Kop, Stones, M, MI
B. huntleyi 175-1: 2.80 mm, 400 m, First Red Hill, A. selago, M, MI
97/98/100
B. huntleyi 128-16: 4.01 mm, 600 m, Halfway Kop, Stones, M, MI
B. huntleyi 30-16: 3.69 mm, 250 m, Tafelberg, Stones, MI
77/75/95
B. huntleyi 151-21: 3.31 mm, 200 m, Junior's Kop, Stones, F, MI
B. huntleyi 16-12: 5.00 mm, 0 m, Ships Cove, Stones, M, MI
B. huntleyi 200-13: 3.85 mm, 800 m, Katedraalkrans, Stones, M, MI
89/84/93
B. huntleyi 128-3: 3.08 mm, 600 m, Halfway Kop, Stones, M, MI
91/91/100
B. huntleyi 128-11: 4.46 mm, 600 m, Halfway Kop, Stones, F, MI
B. huntleyi 128-1: 4.46 mm, 600 m, Halfway Kop, Stones, F, MI
61/62/--
B. huntleyi 42-34: 4.61 mm, 150 m, Stony Ridge, Stones, MI
B. huntleyi 155-30: 5.69 mm, 1000 m, Ice Plateau, Stones, F, MI
B. huntleyi 151-18: 3.15 mm, 200 m, Junior's Kop, Stones, M, MI
B. huntleyi 180-3: 3.76 mm, 400 m, First Red Hill, Stones, M, MI
B. huntleyi 155-21: 4.15 mm, 1000 m, Ice Plateau, Stones, F, MI
79/77/97
B. huntleyi 339-1: 5.15 mm, 600 m, Feldmark Plateau, Stones, F, MI
B. huntleyi 158-40: 3.92 mm, 1000 m, Ice Plateau, Stones, F, MI
--/--/99
B. huntleyi 330-21: 3.92 mm, 0 m, Kildalkey Bay, Stones, M, MI
--/--/98
B. huntleyi 339-21: 3.39 mm, 600 m, Feldmark Plateau, Stones, M, MI
79/78/--
B. huntleyi 180-5: 4.08 mm, 400 m, First Red Hill, Stones, F, MI
96/95/99
B. huntleyi 16-34: 3.85 mm, 0 m, Ships Cove Stones, F, MI
B. huntleyi 158-22: 4.08 mm, 1000 m, Ice Plateau, Stones, M, MI
--/--/96
B. huntleyi 355-1: 3.08 mm, 450 m, Long Ridge S, Stones, F, MI
92/92/100
B. huntleyi 317-19: 4.23 mm, 200 m, Stones, F, PE
99/100/100
B. huntleyi 16-28: 3.31 mm, 0 m, Ships Cove, Stones, M, MI
B. huntleyi 16-29: 3.31 mm, 0 m, Ships Cove, Stones, M, MI
98/98/100
B. huntleyi 303-19: 3.39 mm, 0 m, Cave Bay, Stones, M, PE
B. huntleyi 318-6: 4.31 mm, 672 m, TvZB, Stones, F, PE
B. huntleyi 318-10: 4.46 mm, 672 m, TvZB, Stones, F, PE
B. huntleyi 313-5: 3.62 mm, 600 m, Stones, M, PE
98/98/100
B. huntleyi 318-2: 3.54 mm, 672 m, TvZB, Stones, M, PE
B. huntleyi 307-7: 3.31 mm, 400 m, Stones, M, PE
99/100/100
B. huntleyi 318-1: 3.54 mm, 672 m, TvZB, Stones, M, PE
B. huntleyi 303-20: 2.46 mm, 0 m, Cave Bay, Stones, M, PE
B. huntleyi 307-9: 4.31 mm, 400 m, Stones, F, PE
B. huntleyi 322-5: 3.39 mm, 400 m, A. selago, M, PE
B. huntleyi 313-14: 5.00 mm, 600 m, Stones, F, PE
B. huntleyi 317-15: 3.31 mm, 200 m, Stones, M, PE
98/98/100
B. huntleyi 303-7: 4.62 mm, 0 m, Cave Bay, Stones, F, PE
B. huntleyi 322-9:4.08 mm, 400 m, A. selago, F, PE
B. huntleyi 317-6: 4.39 mm, 200 m, Stones, F, PE
B. huntleyi 322-8: 3.92 mm, 400 m, A. selago, M, PE
B. huntleyi 317-4: 4.39 mm, 200 m, Stones, F, PE
B. huntleyi 303-23: 4.2 mm, 0 m, Cave Bay, Stones, F, PE
B. huntleyi 313-6: 3.54 mm, 600 m, Stones, M, PE
B. huntleyi 317-16: 3.39 mm, 200 m, Stones, M, PE
96/95/100
B. huntleyi 313-2: 4.77 mm, 600 m, Stones, M, PE
99/100/100
B. huntleyi 155-24: 4.08 mm, 1000 m, Ice Plateau, Stones, F, MI
B. huntleyi 155-20: 5.38 mm, 1000 m, Ice Plateau, Stones, F, MI
B. huntleyi 158-45: 4.54 mm, 1000 m, Ice Plateau, Stones, F, MI
B. brevis Heard Island 1
99/100/100
B. brevis Heard Island 2
Lineage b
Lineage a
Clade 2
Clade 1
Outgroup
0.005

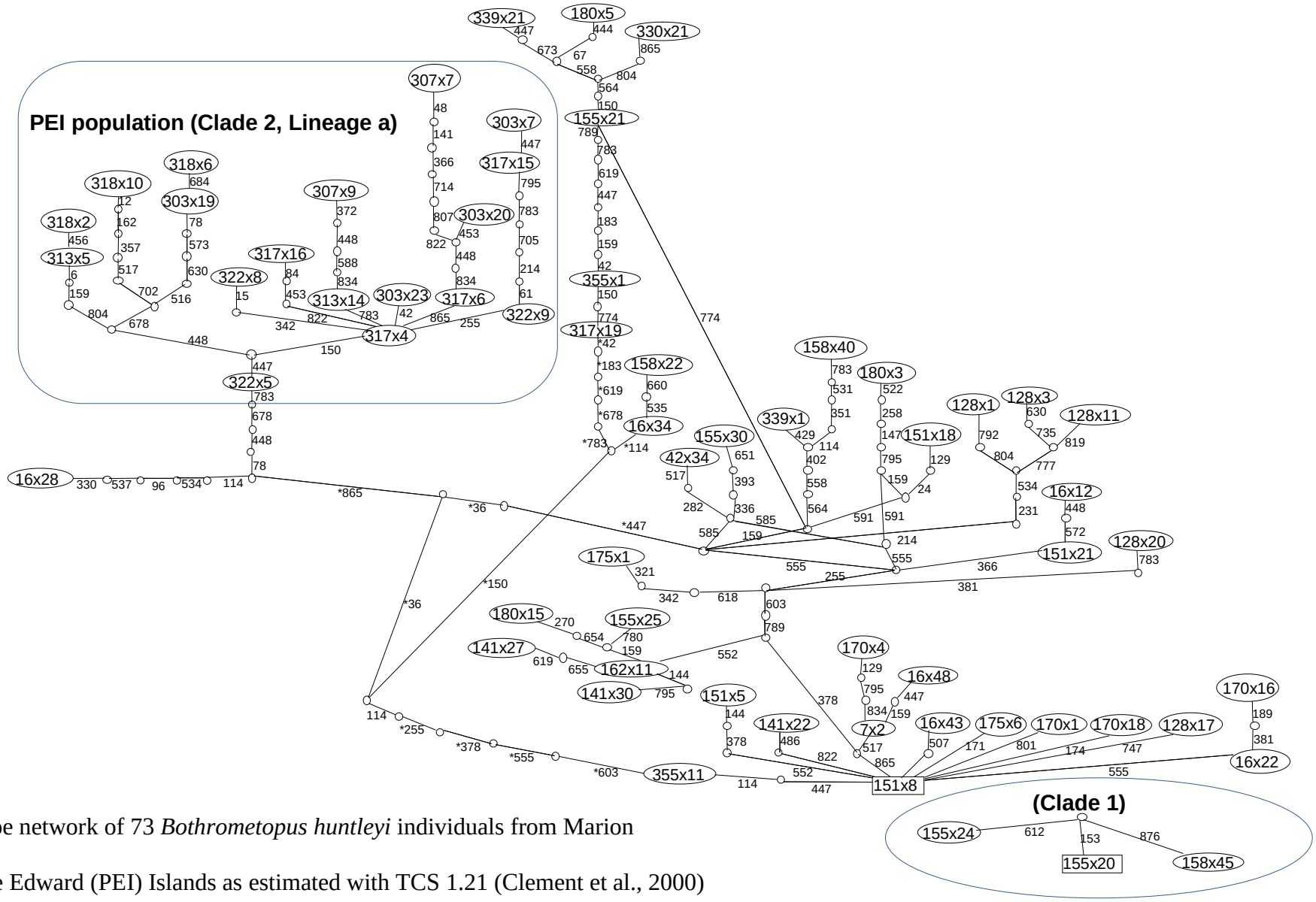


Fig. 4 Haplotype network of 73 *Bothrometopus huntleyi* individuals from Marion (MI) and Prince Edward (PEI) Islands as estimated with TCS 1.21 (Clement et al., 2000)

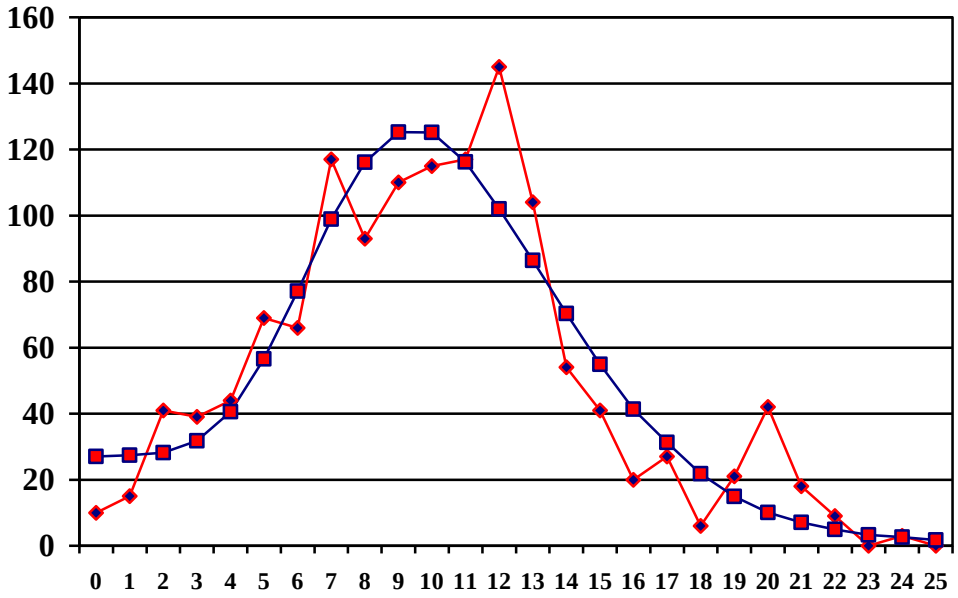
Clade 1 (99%-100% support) comprised three individuals from Marion Island collected from 1000 m a.s.l. that were basal to the second and larger clade comprising individuals from Marion Island and Prince Edward Island, suggesting that *B. huntleyi* originally colonised Marion Island at high altitude. For the remaining individuals that grouped within clade 2, two major lineages could be discerned albeit with low levels of support. The first lineage (labelled 'a' in Fig. 3) contains individuals collected from Prince Edward Island alone, whilst lineage 'b' consists solely of individuals collected from Marion Island, the only exception being individual 317-19. Clade 2 is characterised by low bootstrap support for the internal nodes and by shallow divergences, with high support values occurring primarily at the terminal nodes. The haplotype network (Fig.4) indicates that clade 1, the high altitude, relict population present on Marion Island, is separated from clade 2 by more than 12 steps. The two major lineages within clade 2, namely 'a' and 'b', which correspond to Prince Edward Island and Marion Island, respectively are separated from each other by more than seven steps.

Tests for rate heterogeneity revealed that *B. huntleyi* lineages from the Prince Edward Islands archipelago do not evolve at significantly different rates. The estimated time to coalescence of *B. huntleyi* is approximately 0.723 m.y.a., predating the oldest dated rocks, but possibly not island emergence. The first major inter-island dispersal event, is estimated to have occurred ca. 0.216 million years (m.y.) later, at which time the islands were fully formed, following which *B. huntleyi* dispersed to the remainder of Marion Island during predominantly glaciations-free periods. The results indicate that subsequent to this range expansion, the populations on the two islands have remained largely isolated with the only evidence of inter-island gene flow being detected for a single haplotype (317-19). This individual, sampled from Prince Edward Island shares recent common ancestry with individual 355-1 from Long Ridge South (Fig. 3) on Marion Island, and an estimated time to coalescence of approximately 0.02 m.y.a. (Fig. 3).

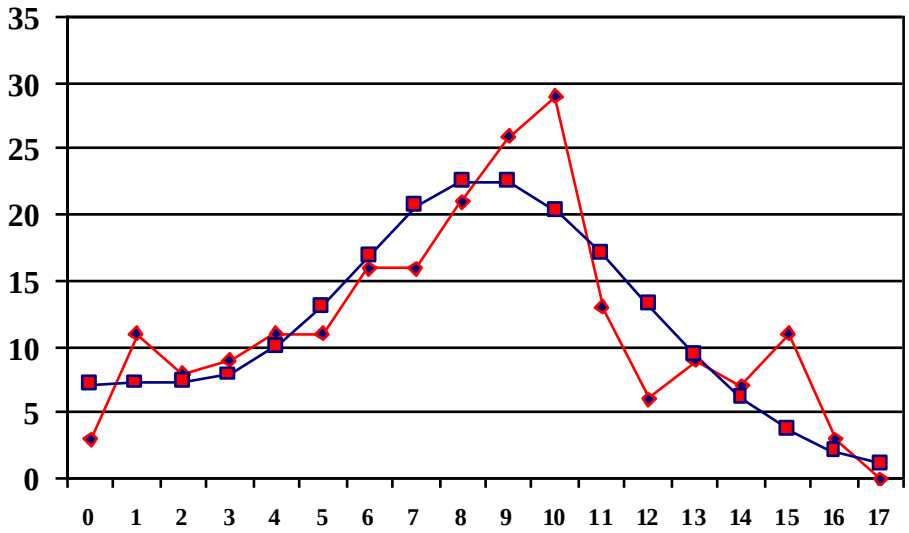
The mismatch distributions for individuals collected on each island are presented in Fig. 5. Neither the SSD nor the raggedness index values (Table 2) showed any significant deviation from the expansion model, indicating a population expansion for both Marion Island and Prince Edward Island. The time of the main expansion events calculated using $\tau = 2\mu t$ (Table 2) correspond to ca. 0.2 m.y. before present (BP).

Fu's *Fs* statistic (Table 3) showed statistically significant and large negative values for both islands that are indicative of a population expansion (Fu 1997). The R_2 statistic (Table 3)

of Ramos-Onsins & Rozas (2002) also indicates population expansion. The different estimations for θ are presented in Table 3.



a)



b)

Fig. 5. Mismatch distribution for *Bothrometopus huntleyi* individuals from: a) Marion (MI) and b) Prince Edward Islands (PEI). Circles represent the simulated stepwise expansion model and the squares represent the observed data.

TABLE 2 Mismatch parameters for *Bothrometopus huntleyi* from Marion (MI) and Prince Edward (PEI) Islands: τ is the expansion parameter or date of the growth or decline measured in units of mutational time with $\tau = 2\mu t$ where μ is the mutation rate, ant t is the time in generations. θ_0 and θ_1 are the substitution rates before and after expansion. SSD is the test of the validity of a stepwise expansion model based on the sum of the square deviations between the observed and the expected mismatch. The raggedness index quantifies the smoothness of the observed pairwise differences distribution. The statistical significance of both SSD and the raggedness index are estimated with a parametric bootstrap approach (probability values: * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$). The timing of the most important expansion in each group ($t_{divergence}$) was calculated on the basis of the equation $\tau = 2\mu t$.

Population	Mean number of differences	Mismatch observed variance	τ (alpha = 0.100 confidence values)	θ_0	θ_1	SSD	P(Sim. Ssd >= Obs. Ssd)	Raggedness index	P(Sim. Rag. >= Obs. Rag.)	$t_{divergence}$(year)
PEI	8.076	15.123	9.491(5.764-12.577)	0.059	28.268	0.006	0.753	0.013	0.840	206326(125394-273413)
MI	10.074	21.551	8.854(6.683-15.504)	2.245	48.137	0.004	0.497	0.007	0.574	192478(145282-337043)

TABLE 3 Summary statistics for *Bothrometopus huntleyi* from Marion (MI) and Prince Edward (PEI) Islands showing Fu's (1997) *Fs* values, Tajima's (1983) D as well as estimations for θ (probability values: * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$).

Population	Fu's (1997) F_s test	Ramos-Onsins and Rozas (2002) R_2 statistic	P(sim_*Fs* <=obs_*Fs*)	Watterson's (1975) estimate (θ(S))	Tajima's (1983) estimate (θ(π))
PEI	-7.686**	0.081	0.003	11.952	8.196
MI	-24.614***	0.053	<0.0001	18.368	10.233

Discussion

Time to *B. huntleyi* haplotype coalescence on the Prince Edward Islands archipelago is estimated to have occurred approximately 0.723 m.y.a. This date is older than that of the oldest dated rocks (0.45 m.y.a.), but remains within the estimated emergence time of the islands of less than 1 m.y.a. (McDougall *et al*. 2001; Boelhouwers *et al.* 2008). It is probable that the founder population was restricted to high elevation areas of Marion Island for a period of approximately 0.216 m.y. High elevation refugia in the form of nunataks have been identified for Marion Island both on geomorphological and molecular grounds (Boelhouwers *et al*. 2008; Mortimer *et al.* 2010). Moreover, recent studies from Antarctica are also providing evidence of refugia for invertebrate taxa that initially appear to be at odds with the geological evidence (Convey *et al*. 2008).

Bothrometopus huntleyi is estimated to have then dispersed to the remainder of Marion Island and on to Prince Edward Island, approximately 0.507 m.y.a. This occurrence coincides with the oldest dated rocks on the island (Boelhouwers *et al*. 2008). Individual 317-19 from Prince Edward Island groups with individuals (92 % bootstrap support; Fig. 3) in the Marion Island-associated lineage b in clade 2, and represents a lineage that became established on Prince Edward Island much later at *c*. 0.02 m.y.a. When comparing the dates estimated in Fig. 3 with the estimated times of glaciations and volcanic activity on Marion Island (Fig. 1) it can be seen that, while numerous divergence events coincide with times of volcanic activity, very few divergence events occurred during the first and third glaciations periods. Thus, it appears that genetic divergence of *B. huntleyi* is linked to volcanic activity on Marion Island, especially at those times when the island was not glaciated. Quite what mechanism of transport was involved is difficult to determine, but complex associations between volcanism and population structure are well known for animals from other island systems (e.g. Paulay 1985, Vandergast *et al*. 2004, Emerson *et al*. 2006, Moya *et al*. 2007).

It should be noted that for *B. huntleyi* only one instance of migration from Marion Island to Prince Edward Island occurred after Prince Edward Island was originally colonised (Fig. 1). This contrasts markedly with the multiple migration events noted for *Ectemnorhinus* weevils from Marion Island to Prince Edward Island (Grobler *et al*. 2006). The difference between *B. huntleyi* and *Ectemnorhinus* weevils could be explained by habitat preference. *Ectemnorhinus* weevils occur mainly on vegetation (Chown 1989, 1992), and are thus more likely to be transported by birds than *B. huntleyi* which mainly occurs on rock surfaces and

hides in crevices in the rocks. *Bothrometopus huntleyi* individuals also have adhesive brushes on the tarsomeres 1-3 (Kuschel & Chown 1995) that prevent the weevils from being easily lifted by the frequent, strong south-westerly winds.

The results of the mismatch distribution analyses are consistent with that of the phylogeny as they indicate that the major expansion event occurred on Marion Island approximately 0.192 m.y.a., with lower and upper bounds of ± 0.145 to ± 0.337 m.y.a., respectively. Most divergence times calculated for Marion Island (Fig. 3) fall within the upper bound estimates of the mismatch analyses. Similarly, most Prince Edward Island divergence estimates (Fig. 3) also fall within the lower and upper bounds of ± 0.125 to ± 0.273 m.y.a., respectively as estimated by the mismatch distribution analyses which revealed that a major expansion event occurred on Prince Edward Island approximately 0.206 m.y.a.

At present, the high elevation Marion Island founder population is intact (clade 1) and two island-discrete population complexes occur which are separated by an ocean barrier of 19 km. Historical inter-island migration has been minimal and uni-directional (from Marion Island to Prince Edward Island only) compared to other endemic *Ectemnorhinu*s weevils studied to date. The isolation of the population on Prince Edward Island from the population on Marion Island probably resulted from a combination of factors, including a lack of both volcanic activity and of bird and/or wind-assisted dispersal between the islands. The genetic data are thus consistent with the lack of morphological differences between the individuals found on the islands, and the assumption of a shared species. Whilst the phylogeny did not recover highly-supported island-specific clades, the haplotype network (Fig. 3) revealed that *B. huntleyi* individuals from Prince Edward Island are genetically more similar to each other than to individuals from Marion Island. It is therefore likely that these allopatric populations will continue to diverge as long as the natural inter-island isolation is maintained.

An important conservation biogeographic (see Whittaker *et al*. 2005) insight from our work is that anthropogenic gene flow between populations on Marion Island and Prince Edward Island should be prevented. Thus, the current practise of limiting visits to Prince Edward Island to a minimum, and the strict quarantine provisions for such visits (e.g. Anonymous 1996, Davies *et al*. 2007) should be maintained. Moreover, the genetic distinctiveness of the *B. huntleyi* population on Marion Island, which is under threat via increased size-selective predation by the house mouse, *Mus musculus domesticus* (Gleeson & van Rensburg 1982, Chown & Smith 1993, Smith *et al*. 2002; Jansen van Vuuren & Chown 2007), also underscores the need to control mice (see Angel *et al*. 2009) on Marion Island to

ensure that both the unique genetic variation on Marion Island, and the evolutionary dynamics of the archipelago are conserved.

Acknowledgements

The authors thank I. McDougall and the Geological Magazine for the permission to use Fig. 1, taken from McDougall *et al.* (2001), and the referees for their helpful comments on a previous version of the ms. The Directorate Antarctica and Islands of the South African Department of Environmental Affairs and Tourism provided logistic support at Marion Island via the South African National Antarctic Programme (SANAP). This work was funded by a SANAP NRF grant (GUN2068301).

References

ALONSO-ZARAZAGA, M.A. & LYAL, C.H.C. 1999. *A World Catalogue of families and genera of Curculionoidea (Insecta: Coleoptera) excluding (Scolytidae and Platypodidae).* Entomopraxis: Barcelona, 315pp.

ANGEL, A., WANLESS, R.M., & COOPER, J. 2009. Review of impacts of introduced house mouse on islands in the Southern Ocean: are mice equivalent to rats? *Biological Invasions*, **11,** 1743-1754.

ANONYMOUS. 1996. Prince Edward Islands Management Plan. Department of Environmental Affairs and Tourism, Pretoria, South Africa.

BOELHOUWERS, J.C., MEIKLEJOHN, K.I., HOLNESS, S.D. & HEDDING, D.W. 2008. Geology, geomorphology and change. *In* CHOWN, S.L. & FRONEMAN, P.W., *eds. The Prince Edward Islands: Land-sea interactions in a changing ecosystem*. Sun Press, Stellenbosch, 65-96.

BROWER, A.V.Z. 1994. Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. *Proceedings of the National Academy of Science of the United States of America*, **91**, 6491-6495.

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

CHOWN, S.L. 1992. A preliminary analysis of weevil assemblages in the sub-Antarctic: local and regional patterns. *Journal of Biogeography*, **19**, 87-98.

CHOWN, S.L. & KLOK, C.J. 2001. First record of *Palirhoeus eatoni* (Coleoptera: Curculionidae) from sub-Antarctic Heard Island. *African Entomology*, **9**, 193-194.

CHOWN, S.L. & KLOK, C.L. 2003 Altitudinal body size clines: Latitudinal effects associated with changing seasonality. *Ecography*, **26**, 445-455.

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.

CLEMENT, M., POSADA, D. & CRANDALL, K. 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, **9**, 1657-1660.

CONVEY, P., GIBSON, J.A.E., HILLENBRAND, C.D., HODGSON, D.A., PUGH, P.J.A., SMELLIE, J.L. & STEVENS, M.I. 2008. Antarctic terrestrial life – challenging the history of the frozen continent? *Biological Reviews*, **83**, 103-117.

DAVIES, S.J., CHOWN, S.L. & JOUBERT, L.J. 2007. Renewed management systems and provisions for South Africa's sub-Antarctic islands. *Papers and Proceedings of the Royal Society of Tasmania*, **141,** 115-120.

DREUX, PH. & VOISIN, J.F. 1984. Description de *Bothrometopus derelictorum*, n. sp. et notes sur le genre *Bothrometopus* Jeannel, 1940. [Coleoptera, Curculionidae, Ectemnorrhininae]. *Revue Francaise d'Entomologie (N.S.),* **6**, 33-38.

DREUX, PH. & VOISIN, J.F. 1986. Note sur les genres *Mesembriorrhinus* Jeannel et *Palirhoeus* Kuschel (Coleoptera, Curculionidae). *Nouvelle Revue d'Entomologie (N.S.)*, **3**, 257-261.

DREUX, PH. & VOISIN, J.F. 1987. Notes sur le genre *Antarctonesiotes* Jeannel et sur le genre *Disker* nov., et description d'*Antarctonesiotes villiersi* n. sp. (Coleoptera, Curculionidae). *Nouvelle Revue d'Entomologie (N.S.),* **4**, 313-321.

DRUMMOND, A.J. & RAMBAUT, A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, **7**, 214.

EMERSON, B.C., FORGIE, S., GOODACRE, S. & OROMI, P. 2006. Testing phylogeographic predictions on an active volcanic island: *Brachyderes rugatus* (Coleoptera: Curculionidae) on La Palma (Canary Islands). *Molecular Ecology*, **15**, 449-458.

EXCOFFIER, L. & SCHNEIDER, S. 1999. Why hunter-gatherer populations do not show signs of Pleistocene demographic expansions. *Proceedings of the National Academy of Sciences of the United States of America*, **96**, 10597-10602.

FELSENSTEIN, J. 1981. Evolutionary trees from DNA sequences: A maximum likelihood approach. *Journal of Molecular Evolution*, **17**, 368-376.

FELSENSTEIN, J. 1988. Phylogenies from molecular sequences: Inference and reliability. *Annual Reviews in Genetics*, **22**, 521-565.

FU, Y.X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection, *Genetics*, **143**, 557-570.

GLEESON, J.P. & VAN RENSBURG, P.J.J. 1982. Feeding ecology of the house mouse *Mus musculus* on Marion Island. *South African Journal of Antarctic Research*, **12**, 34-39.

GROBLER, G.C., JANSE VAN RENSBURG, L., BASTOS, A.D.S., CHIMIMBA, C.T. & CHOWN, S.L. 2006. Molecular and morphometric assessment of the taxonomic status of *Ectemnorhinus* weevil species (Coleoptera: Curculionidae, Entiminae) from the sub-Antarctic Prince Edward Islands. *Journal of Zoological Systematics and Evolutionary Research*, **44**, 200-211.

GROBLER, G.C., BASTOS, A.D.S., TREASURE, A. & CHOWN, S.L. 2011. Cryptic species, biogeographic complexity and evolutionary history of the *Ectemnorhinus*-group in the sub-Antarctic, including a description of *Bothrometopus huntleyi*, n. sp. *Antarctic Science*, 23: 211-224.

GUIDON, S. & GASCUEL, O. 2003. A simple, fast and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology*, **52**, 696-704.

HASEGAWA, M. KISHINO, H & YANO, T. 1985. Dating of human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution*, **22**, 160-174.

HUELSENBECK, J.P. & RONQUIST, F. 2001. MRBAYES: Bayesian inference of phylogeny. *Bioinformatics*, **17**, 754-755.

JANSEN VAN VUUREN, B.J. & CHOWN, S.L. 2007. Genetic evidence confirms the origin of the house mouse on sub-Antarctic Marion Island. *Polar Biology*, **30**, 327-332.

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

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.

KUSCHEL, G. & CHOWN, S.L. 1995. Phylogeny and systematics of the *Ectemnorhinus*-group of genera (Insecta: Coleoptera). *Invertebrate Taxonomy*, **9**, 841-863.

LI, P. & BOUSQUET, J. 1992. Relative-rate test for nucleotide substitutions between two lineages. *Molecular Biology and Evolution*, **9**, 1185–1189.

LIBRADO, P. & ROZAS, J. 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **doi**: 10.1093/bioinformatics/btp187.

MCDOUGALL, I., VERWOERD, W. & CHEVALLIER, L. 2001. K-Ar geochronology of Marion Island, Southern Ocean. *Geological Magazine*, **138**, 1-17.

MORTIMER, E. & JANSEN VAN VUUREN, B., LEE, J.E., MARSHALL, D.J., CONVEY, P. & CHOWN, S.L. 2010. Mite dispersal among the Southern Ocean Islands and Antarctica before the last glacial maximum. *Proceedings of the Royal Society of London B*, in press.

MOYA, O., CONTRERAS-DIAZ, H.G., OROMI, P. & JUAN, C. 2007. Phylogeography of a ground beetle species in La Gomera (Canary Islands): the effects of landscape topology and population history. *Heredity*, **99**, 322-330.

MYBURGH, M., CHOWN, S.L., DANIELS, S.R. & JANSEN VAN VUUREN, B. 2007. Population structure, propagule pressure and conservation biogeography: lessons from indigenous and invasive springtails. *Diversity and Distributions*, **13**, 143-154.

PAPADOPOULOU, A., ANASTASIOU, I. & VOGLER, A.P. 2010. Revisiting the molecular clock: the mid-Aegean trench calibration. *Molecular Biology and Evolution*, **27**, 1659-1672.

PAULAY, G. 1985. Adaptive radiation on an isolated oceanic island: the Cryptorhynchinae (Curculionidae) of Rapa revisited. *Biological Journal of the Linnean Society*, **26**, 95-187.

POSADA, D. & CRANDALL, K.A. 1998. MODELTEST: Testing the model of DNA substitution. *Bioinformatics*, **14**, 817-818.

RAMOS-ONSINS, S.E. & ROZAS, J. 2002. Statistical properties of new neutrality tests against population growth. *Molecular Biology and Evolution*, **19**, 2092-2100.

SCHNEIDER, S. & EXCOFFIER, L. 1999. Estimation of past demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites: application to human mitochondrial DNA. *Genetics*, **152**, 1079-1089.

SCHNEIDER, S., ROESSLI, D. & EXCOFFIER, L. 2000. Arlequin ver. 2.000: A software for population genetics data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

SMITH, V.R., AVENANT, N.L. & CHOWN, S.L. 2002. The diet and impact of house mice on a sub-Antarctic island. *Polar Biology*, **25**, 703-715.

STEVENS, M.I., GREENSLADE, P., HOGG, I.D. & SUNNUCKS, P. 2006. Southern Hemisphere springtails: could any have survived glaciation of Antarctica? *Molecular Biology and Evolution*, **23**, 874-882.

SWOFFORD, D. L., 2003: PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.

TAJIMA, F. 1983. Evolutionary relationship of DNA sequences in finite populations. *Genetics*, **105**, 437-460.

TAMURA, K., DUDLEY, J., NEI, M. & KUMAR, S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution*, **24**, 1596-1599.

TEMPLETON, A.R., CRANDALL, K.A. & SING, C.F. 1992. A caldistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping. III. Cladogram estimation. *Genetics*, **132**, 619-633.

VANDERGAST, A.G., GILLESPIE, R.G. & RODERICK, G.K. 2004. Influence of volcanic activity on the population genetic structure of Hawaiian *Tetragnatha* spiders: fragmentation, rapid population growth and the potential for accelerated evolution. *Molecular Ecology*, **13**, 1729-1743.

WATERHOUSE, C.O. 1876. Description of a new species of *Ectemnorhinus* from Kerguelen Island. *Entomologist's Monthly Magazine*, **13**, 51-52.

WATTERSON, G.A. 1975. On the number of segregation sites. *Theoretical Population Biology*, **7**, 256-276.

WHITTAKER, R.J., ARAÚJO, M.B., PAUL, J., LADLE, R.J., WATSON, J.E.M. & WILLIS, K.J. 2005. Conservation biogeography: assessment and prospect. *Diversity and Distributions*, **11**, 3-23.

CHAPTER 6

Concluding comments on the Phylogenetic relationships and phylogeograpic patterns as observed within the *Ectemnorhinus*-group of weevils (Coleoptera: Curculionidae) from the sub-Antarctic Prince Edward Island Archipelago

The Prince Edward Island Archipelago (PEIA) has been the subject of intensive research for more than 60 years since its annexation by South Africa in 1948, (See Hänel & Chown 1999 and Chown & Froneman 2008 for a more information). When considering the species of the *Ectemnorhinus* group of genera endemic to the PEIA (Kuschel & Chown, 1995) that was known prior to this study, namely *Ectemnorhinus similis* (Waterhouse, 1885), *Ectemnorhinus marioni* (Jeannel, 1940), *Bothrometopus parvulus* (Waterhouse, 1885), *Bothrometopus elongates* (Jeannel, 1953), *Bothrometopus randi* (Jeannel 1953) and *Palirhoeus eatoni* (Waterhouse 1876), it can be seen that the last time new species, within the *Ectemnorhinus* group of genera on the PEIA, were identified was 57 years ago in 1953 by Jeannel. In this study we identified and described two new species within the *Ectemnorhinus* group of genera from the PEIA. The first is *Ectemnorhinus kussheli* that occurs only on PEI and the second is *Bothrometopus huntleyi* from both MI and PEI.

Using a combined molecular and morphometric approach the current study showed that *E. similis* (Waterhouse, 1885) and *E. marioni* (Jeannel, 1940) should be synonymized as *E. similis* as *E. similis* has priority. We found no evidence that weevils cluster on the basis of body size or according to plant species from which they were collected when analyzed phylogenetically. In addition, multivariate analyses of the MI samples showed no separation of *Ectemnorhinus* individuals according to previous suggested morphologically- and ecologically-defined distinguishing characteristics (Crafford et al. 1986; Chown and Scholtz 1989; Chown 1990). We also confirm 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). While only one species of *Ectemnorhinus* weevils was found on MI, both the genetic and the morphometric analyses

indicated two size-distinct species on PEI with a sequence divergence value of 1.5 % 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). 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 have been designated *E. kuscheli*. One possible reason for the different status of *Ectemnorhinus* species between the two islands may 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. Results from this study indicate that the *Ectemnorhinus* weevils lineages coalesce at approximately 0.49 MYA on PEI, soon after the islands emerged, while coalescence on MI was dated to have occurred approximately 0.16 million years later.

When examining the population dynamics of *Ectemnorhinus* weevils on the PEIA, we see that PEI was colonized before MI. This is probably due to the fact that PEI was not glaciated like MI (Verwoerd 1971). The PEI population then acted as the source population for the colonization of MI by *Ectemnorhinus* weevils some time before the last glaciations where the weevils were able to colonize the whole of MI. The separation by distance of the PEI *Ectemnorhinus* weevils from those on MI then gave raise to two species by alopatric speciation, namely *E. kucheli* on PEI and *E. similis* on MI. During the last glaciation MI was extensively glaciated with only the southwestern corner of the island being free of ice. This extensive glaciation of MI would result in the eradication of all *E. similis* on MI except for those occurring on the ice-free southwestern corner of the island, of which a remnant population is still present on MI that are closer related to *E. kucheli* on MI than any other MI populations. We propose that at the end of the last glacial maximum, when the ice started to melt, the coastal areas of MI emerged first from beneath the ice and were available for the re-colonization of weevils. The movement of the weevils that were isolated in the south-western corner of MI, along the coastal areas of the island, was assisted by the strong, frequent south-western winds (Schulze, 1971). Subsequent Holocene post-glacial volcanism (Hall 1978, 1982, 2004) was then responsible for the fragmentation of the new migrants, to the rest of

MI, in small pockets surrounding by fresh, uninhabitable lava. The time it took for the different populations of isolated weevils to recover from the devastation of the lava as well as the time it took for the lava that isolated the weevils in small populations to become inhabitable for re-colonization of weevils, was sufficient for the weevils in each population to diverge from those in the other populations. When the Holocene black lavas became re-colonizeable the weevils from the different isolated populations could again migrate to the rest of the island. At present the members of the different genetically identified populations occur geographically in the same area and in some cases even on the same plant, but yet no noticeable gene flow was detected between them. We thus suggest that the time of isolation, before the Holocene post-glacial black lavas became hospitable, were long enough and that the populations were small enough that reproductive isolation could be induced to form a number of sub-species. We have recognised two sub-species of *E. kucheli* on PEI and seven sub-species of *E. similis* on MI. The fact that the living conditions and food available for all the different isolated populations were the same makes it not surprising that members of all the different sub-populations are morphologically indistinct.

When examining *B. huntleyi* we found that *Bothrometopus* weevils first colonised Marion Island at high altitude approximately 0.447 MYA where they were probably restricted to a small area through glaciation. Following a period of extended isolation, the data suggest that approximately 0.117 MY later, *B. huntleyi* dispersed to the remainder of Marion Island as well as over the 19 km stretch of sea to Prince Edward Island, at a time coinciding with glaciation-free volcanism. At present, the high elevation Marion Island founder population is intact and accompanied by two discrete population complexes separated by 19 km of sea on the two islands. Historical inter-island migration has been minimal and uni-directional (from Marion Island to Prince Edward Island only) and coincided with glaciation-free volcanic stages on Marion Island. It has also been revealed that *B. huntleyi* individuals from Prince Edward Island are genetically more similar to each other than to individuals from Marion Island. It is therefore likely that these allopatric populations will continue to diverge as long as the natural inter-island isolation is maintained.

We then examined 13 species from the genera *Palirhoeus* Kuschel, *Bothrometopus* Jeannel, and *Ectemnorhinus* G.R. Waterhouse from 22 populations from Marion Island, Prince Edward Island, Heard Island, Kergeulen Island and Possession Island. These genera are all within the *Ectemnorhinus* group of genera (Kuschel & Chown, 1995). Although this is only a partial analysis of this group of weevils, the study has cast significant light on the evolution and biogeography thereof, with important implications for interpretation of biogeographic

and evolutionary dynamics in the region more generally. Perhaps the most significant point to emerge is that colonization of the Prince Edward Islands is likely to have taken place repeatedly from other islands in the South Indian Ocean Province. We also suggest that the genus *Palirhoeus* is clearly not readily distinguishable on a mtCOI sequence basis from the genus *Bothrometopus*, thus questioning the retention of the species *P. eatoni* in a separate genus, and its position in Kuschel & Chown's (1995) phylogeny as basal to the genera *Bothrometopus* and *Ectemnorhinus*. The two species groups in the genus *Bothrometopus* (*fasciatus* group and *gracilipes* group) identified on morphological grounds by Kuschel & Chown (1995) also do not have support on an mtCOI basis.

Conservation recommendations

An important conservation biogeographic (see Whittaker *et al*. 2005) insight from our work is that anthropogenic gene flow between populations on Marion Island and Prince Edward Island should be prevented. Thus, the current practise of limiting visits to Prince Edward Island to a minimum, and the strict quarantine provisions for such visits (e.g. Anonymous 1996, Davies *et al*. 2007) should be maintained. Moreover, the genetic distinctiveness of the weevil populations on Marion Island, which is under threat via increased size-selective predation by the house mouse, *Mus musculus domesticus* (Gleeson & van Rensburg 1982, Chown & Smith 1993, Smith *et al*. 2002; Jansen van Vuuren & Chown 2007), also underscores the need to control mice (see Angel *et al*. 2009) on Marion Island to ensure that both the unique genetic variation on Marion Island, and the evolutionary dynamics of the archipelago are conserved.

Future research

There are numerous studies left to be done on the *Ectemnorhinus* group of genera. We are only highlighting a few:

We suggest further comprehensive molecular phylogenetic analyses of the biogeography of the region including all the species in the *Ectemnorhinus* group of genera as this would give the complete picture.

As the population study of the *Ectemnorhinus* weevils gave many new insights on biogeography and species dynamics, the same studieds from the other species on the PEIA might give us an even clearer picture of the biogeography of the islands.

More extensive sampling from all areas of PEI is needed in order to give a complete picture of the biogeography on PEI.

We suggest that breeding experiments between individuals from the different sub-species of *E. kucheli* on PEI and of *E. similis* on MI should be undertaken to determine whether they are unable to interbreed or whether there are some external factors that prevent gene flow to occur between them.

References

ANGEL, A., WANLESS, R.M., & COOPER, J. 2009. Review of impacts of introduced house mouse on islands in the Southern Ocean: are mice equivalent to rats? *Biological Invasions*, **11,** 1743-1754.

ANONYMOUS. 1996. Prince Edward Islands Management Plan. Department of Environmental Affairs and Tourism, Pretoria, South Africa.

BOELHOUWERS, J.C., MEIKLEJOHN, K.I., HOLNESS, S.D. & HEDDING, D.W. 2008. Geology, geomorphology and change. *In* CHOWN, S.L. & FRONEMAN, P.W., *eds. The Prince Edward Islands: Land-sea interactions in a changing ecosystem*. Sun Press, Stellenbosch, 65-96.

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

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. & FRONEMAN, P.W. 2008. *eds. The Prince Edward Islands. Land-Sea Interactions in a Changing Ecosystem*. African Sun Media, Stellenbosch.

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.; 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.

DAVIES, S.J., CHOWN, S.L. & JOUBERT, L.J. 2007. Renewed management systems and provisions for South Africa's sub-Antarctic islands. *Papers and Proceedings of the Royal Society of Tasmania*, **141,** 115-120.

GLEESON, J.P. & VAN RENSBURG, P.J.J. 1982. Feeding ecology of the house mouse *Mus musculus* on Marion Island. *South African Journal of Antarctic Research*, **12**, 34-39.

Hall, K.J. 1978. Evidence for Quaternary glaciation of Marion Island (Sub-Antarctic) and some implications. In Van Zinderen Bakker E.M. (ed.) *Antarctic Glacial History and World Palaeo-environments.* A.A. Balkema, Rotterdam, pp. 137-147.

Hall, K. 1982. Rapid deglaciation as an initiator of volcanic activity: an hypothesis. *Earth Surface Processes and Landforms* 7: 45-51.

Hall, K. 2004. Quaternary glaciation of the sub-Antarctic Islands. In Ehlers J. & Gibbard P.I. (Eds.). *Quaternary Glaciations - Extent and Chronology, Part III*. Elsevier, Amsterdam, pp. 339-345.

Hänel, C. & Chown, S.L. 1999. Fifty years at Marion and Prince Edward Islands: A bibliography of scientific and popular literature. *South African Journal of Science*, **95**, 87-112.

JANSEN VAN VUUREN, B.J. & CHOWN, S.L. 2007. Genetic evidence confirms the origin of the house mouse on sub-Antarctic Marion Island. *Polar Biology*, **30**, 327-332.

JEANNEL, R. 1940. Coléoptères. Croisière du Bougainville aux îles australes francaises. *Mémoires du Muséum National d'Histoire Naturelle (N.S.)*, **14**, 63-201.

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

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). *Invertebrate Taxonomy*, **9**, 841-863.

Schulze, B. R., 1971: The climate of Marion Island. 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. 16-31.

SMITH, V.R., AVENANT, N.L. & CHOWN, S.L. 2002. The diet and impact of house mice on a sub-Antarctic island. *Polar Biology*, **25**, 703-715.

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, C.O. 1885. Description of two new Curculionidae (*Ectemnorhinus*) from Marion Islands. *Annals and Magazine of Natural History, London*, **16**, 121-123.

WATERHOUSE, C.O. 1876. Description of a new species of *Ectemnorhinus* from Kerguelen Island. *Entomologist's Monthly Magazine*, **13**, 51-52.

WHITTAKER, R.J., ARAÚJO, M.B., PAUL, J., LADLE, R.J., WATSON, J.E.M. & WILLIS, K.J. 2005. Conservation biogeography: assessment and prospect. *Diversity and Distributions*, **11**, 3-23.