PHYLOGENY OF AMERONOTHROIDEA IN THE SOUTH POLAR REGION AND THE PHYLOGEOGRAPHY OF SELECTED SPECIES ON SUB-ANTARCTIC MARION ISLAND

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VERKLARING

Ek, die ondergetekende, verklaar hiermee dat die werk in hierdie proefskrif vervat, my eie oorspronklike werk is en dat ek dit nie vantevore in die geheel of gedeeltelik by enige universiteit ter verkryging van 'n graad voorgelê het nie.

DECLARATION

I, the undersigned, hereby declare that the work contained in this dissertation is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

Signature: .....

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ABSTRACT

Sub-Antarctic islands represent the only mid to high latitude terrestrial biomes in the Southern Hemisphere. These islands have various geological origins and histories, well-preserved terrestrial ecosystems and high levels of species endemism. In an attempt to understand the evolution and biogeography of terrestrial taxa in the South Polar Region, the first broad-scale molecular phylogeny was constructed for the unique terrestrial group, the ameronothroid mites (genus *Halozetes* (Oribatida)), collected from sub-Antarctic and Maritime Antarctic localities. Phylogenetic analyses based on a combined mitochondrial (cytochrome oxidase subunit I (COI)) and nuclear (histone-3 (H3)) sequence dataset indicated that the evolution of these mites were habitat specific (i.e. intertidal, supralittoral and terrestrial). Notwithstanding criticisms levelled against a molecular clock, the mites were evolutionary young (<10myo), contrary to their status as an ancient group predating Gondwana fragmentation. Biogeographic analyses indicated a complex pattern mainly sculpted by multiple independent dispersal events across the Antarctic Polar Frontal Zone similar to previous findings for other marine and terrestrial taxa. Also, the molecular phylogeny displayed considerable discourse with contemporary taxonomy suggesting the need for taxonomic revisions and reassessment of morphological characters. Sub-Antarctic Marion Island, the larger of the two islands comprising the Prince Edward Island archipelago (PEI), has experienced extensive glaciation and volcanism. To assess the impact of historical events (volcanism (including recent eruptions) and glaciation) and contemporary mechanisms (gene flow) on the genetic spatial distribution of species from Marion Island, two mite species namely *Eupodes minutus* (Prostigmata) and *Halozetes fulvus* (Oribatida) as well as a single plant species, *Azorella selago* (Apiaceae), were selected as model organisms. For independent phylogeographic analyses, mitochondrial sequence data (COI) were obtained for both mite species, while chloroplast sequence (*trn*H-psbA) and amplified fragment length polymorphism (AFLP) data were generated for the cushion plant, *A. selago*. Since *A. selago* is typified by two growth forms namely discrete cushions and continuous mats, it was essential to examine the growth dynamics prior to phylogeographic analyses. The sequence and fragment data indicated that both mite and plant species were significantly substructured across Marion Island. Manual comparisons indicated unique populations on the western (Kaalkoppie for *H. fulvus*, La Grange Kop for *E. minutus* and Mixed Pickle for *A. selago*), eastern (Bullard Beach for *H. fulvus* and Kildalkey Bay for *E. minutus*), northern (Middelman and Long Ridge for *H. fulvus*) and southern side (Grey Headed for *H. fulvus* and Watertunnel for *A. selago*) of the island. Importantly, the western side had unique localities for all species. Interestingly, based on the *H. fulvus* data, the western populations were relatively young, characterized by high migration rates, small effective (female) population sizes with no isolation-by-distance. The opposite scenario was found for the eastern populations. This spatial genetic structure described for species on Marion Island can be ascribed to both historical events and environmental conditions. These areas with their unique genetic composition are of special conservational concern; consequently this research will contribute to an active management plan for PEI, South Africa's only Special Nature Reserve.

OPSOMMING

Sub-Antarktiese eilande verteenwoordig die enigste terrestriële bioom in die middel tot hoër breedtegrades van die Suidelike Halfrond. Hierdie eilande besit 'n verskeidenheid van geologiese oorspronge en geskiedenisse, goed-bewaarde terrestriële ekosisteme en hoë vlakke van endemisme. In 'n poging om die evolusie en biogeografie van terrestriële taksa in die Suid Pool Area te verstaan, is die eerste grootskaalse molekulêre filogenie saamgestel vir 'n unieke terrestriële groep, die ameronothoïed miete (genus *Halozetes* (Oribatida: Ameronothroidea)), vanaf menigte sub-Antarktiese en Maritime Antarktiese lokaliteite. Filogenetiese analises gebaseer op die saamgestelde mitochondriale (sitokroom oksidase subeenheid I (COI)) en nukluêre (histoon-3 (H3)) basispaarvolgordes het aangedui dat die evolusie van hierdie miete habitat spesifiek is (m.a.w inter-gety, supralitoraal en terrestrieël). Ongeag die kritiek teenoor 'n molekulêre klok, is hierdie miete evolusionêr jonk (<10mjo), wat teenstrydig is met hulle status as 'n antieke groep wat terugdateer voor Gondwana fragmentasie. Biogeografiese analises het 'n komplekse patroon aangedui wat grotendeels gekarakteriseer word deur menigte onafhanklike verspreidingsgebeurtenisse bo-oor die Antarktiese Polêre Frontale Zone, wat ooreenstemmend is met vorige bevindinge vir ander mariene en terrestriële taksa. Die molekulêre filogenie het ook aansienlik verskil van die tradisionele taksonomie, dus is taksonomiese aanpassings en herklassifisering van morfologiese karakters noodsaaklik. Sub-Antarktiese Marion Eiland, die groter eiland van die Prins Edward eilandgroep (PEI), het uitermate glasiasie en vulkanisme ondervind. Om die impak van historiese gebeurtenisse (vulkanisme (insluitend onlangse uitbarstings) en glasiasie) en kontemporêre meganismes (geenvloei) op die genetiesgespasieërde verspreiding van spesies vanaf Marion Eiland te bepaal, was twee mietspesies naamlik *Eupodes minutus* (Prostigmata) en *Halozetes fulvus* (Oribatida) asook 'n enkele plantspesie, *Azorella selago* (Apiaceae), gekies as model organismes. Vir onafhanklike filogeografiese analises, was die mitochondriale basispaarvolgorde (COI) vir beide mietspesies bepaal, terwyl chloroplast basispaarvolgorde (*trn*H-psbA) asook geamplifiseerde fragmentlengte polimorfisme (AFLP) data gegenereer was vir die kussingplant, *A. selago*. Aangesien *A. selago* gekenmerk word deur twee groeivorme, naamlik diskrete kussings en aaneenlopende matte, was dit noodsaaklik om eers die groeidinamika van die plant te ondersoek alvorens 'n filogeografiese studie kon geskied. Die basispaarvolgordebepalings en fragmentdata het aangedui dat beide mietspesies sowel as die plantspesie betekenisvolle substruktuur vertoon regoor Marion Eiland. Informele vergelykings het unieke populasies aangedui op die westelike (Kaalkoppie vir *H. fulvus*, La Grange Kop vir *E. minutus* en Mixed Pickle vir *A. selago*), oostelike (Bullardstrand vir *H. fulvus* en Kildalkeybaai vir *E. minutus*), noordelike (Middelman en Long Ridge vir *H. fulvus*) en suidelike kant (Grey Headed vir *H. fulvus* en Watertunnel vir *A. selago*) van die eiland. Die westelike kant besit dus unieke lokaliteite vir al die spesies. Interressantheidhalwe het die *H. fulvus* data getoon dat die westelike populasies relatief jonk is en gekarakteriseer word deur hoë migrasiesyfers en klein effektiewe (vroulike) populasiegroottes met geen isolasie-oor-afstand nie. Die resultate vir die populasies aan die oostelike kant van die Marion Eiland was presies teenoorgesteld. Dié beskryfde substruktuur vir die spesies op Marion Eiland is afkomstig van beide historiese gebeurtenisse asook omgewingstoestande. Hierdie areas met hul unieke genetiese

samestelling, is belangrik vir natuurbewaring. Hierdie navorsing sal bydra tot die bestuursriglyne van PEI, Suid Afrika se enigste Spesiale Natuurreservaat.

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Thank you for all

your love and support

during this degree.

“Nothing in life is to be feared. It is only to be understood”.

-Marie Curie (1867-1934), polish-born physical chemist

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LIST OF ABBREVIATIONS

AFLP	Amplified fragment length polymorphism
AIC	Akaike Information Criterion
AMOVA	Analysis of Molecular Variance
APFZ	Antarctic Polar Frontal Zone
BI	Bayesian Inference
CI	Consistency Index
COI	Cytochrome oxidase subunit I gene
g	g-value (statistical inference)
H3	Histone-3 gene
Is	Island
M	Migration rate
ML	Maximum Likelihood
MP	Parsimony searches (Maximum Parsimony)
mtDNA	Mitochondrial DNA
myo	Million years old
mya	Million years ago
NCA	Nested Clade Analysis
p	*p*-value (statistical inference)
PCA	Principle component analysis
PEI	Prince Edward Island archipelago
rg	Raggedness statistic
RI	Retention Index
RAPD	Random Amplified Polymorphic DNA
SAMOVA	Spatial Analysis of Molecular Variance
SCAR	Scientific Committee on Antarctic Research
SAP	South Atlantic Province
SIP	South Indian Province
SPP	South Pacific Province
T	Divergence time
TMRCA	Time to Most Recent Common Ancestor
UK	United Kingdom
USA	United States of America
ybp	Years before present

LIST OF AREAS

South Polar Region	Border from 40° southern latitude
Sub-Antarctic Region	Borders between 46° and 55° southern latitude
Antarctic Region	Border from 60° southern latitude

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CHAPTER 1

LITERATURE SURVEY

INTRODUCTION

The oceanic masses that dominate the Southern Hemisphere have profoundly sculpted cladogenic events in terrestrial biological communities. In contrast, the Northern Hemisphere is dominated by continental landmass with limited oceanic masses. Northern hemispherical continents are all in close proximity to the Arctic polar cap, suggesting that recent glaciation / deglaciation (Pliocene / Pleistocene) cycles have potentially impacted on the distribution of species to a greater extend compared to the Southern hemispherical continental areas (Skottsberg 1960). In the Southern Hemisphere, the vast oceanic expanse and large scale distance between continents and the Antarctic, implies a limited impact on the distribution of taxa which suggest potentially older cladogenic events (Miocene) at least for continental areas. For example, the phylogeographic study conducted on springtails species in the South Polar Region showed that their diversification mostly occurred during the Miocene (~23-5 million years ago (mya)) and Stevens *et al.* (2006) suggested that the Continental Antarctic species were isolated from the sub-Antarctic and surroundings once the sea-ice was sufficient to restrict their oceanic dispersal. Also, comparative phylogeographic studies elucidating the effects of glaciation are limited in the Southern Hemisphere (specifically the South Polar Region) compared to the Northern Hemisphere. The only mid to high latitude southern terrestrial biomes are represented by groups of sub-Antarctic islands. These islands have well-preserved terrestrial ecosystems, relatively unharmed biotas (Chown *et al.* 1998; Chown *et al.* 2001), remarkably high levels of species endemism (Pugh 1993), suffered limited anthropomorphic disturbance (Smith & Lewis Smith 1987; Young 1995) and are ideal areas for the study of evolutionary biology (Chown *et al.* 1998 and references within). Nevertheless, despite representing a unique biota, the evolutionary history, colonization and biogeographical interrelationships among most groups remain ill-explored.

From an evolutionary perspective, the islands in the South Polar Region present some unique and interesting questions. This region contains landmasses of various ages that range from ancient continental islands to more recent subarial islands formed by volcanism. Some of these islands have experienced extreme glaciation events, while others appear to have been unglaciated (Bergstrom & Chown 1999; Chown *et al.* 1998). In addition to these variables, it is assumed that rising oceanic levels or directional shifts in ocean currents as a direct consequence of deglaciation of polar caps had a tremendous impact on the islands' biotas. The isolation of the Antarctic islands could have induced diversification and the formation of neo-endemics (Gillespie & Roderick 2002). Hence, intricate colonization patterns probably exist for the terrestrial species present on these islands. Whether oceanic dispersal or vicariance is responsible for the cladogenesis of the area's biota in Antarctica and surrounding islands, is still heavily debated (Greve *et al.* 2005; Myers & Giller 1988; Peck *et al.* 2006). Furthermore, biogeographic studies in this region are incomplete with limited research focussing on both marine and terrestrial environments. This presents an ideal template for novel research opportunities.

The usefulness of genetic data to answer ecological, evolutionary as well as conservation questions recently sparked an increase in molecular work on / around the South Polar Region (see for example Allegrucci *et al.*

2006; Bargelloni *et al.* 2000; Stevens & Hogg 2006; Verde *et al.* 2007). However, a major limitation that precludes detailed work is the isolated nature of the sub-Antarctic islands which give rise to sampling difficulties. In addition, only a limited number of biogeographic molecular studies have been undertaken in the region (mostly marine species), with most research focussing on the population structure of species (for example plants (reviewed in Skotnicki *et al.* 2000) and arthropods (Frati *et al.* 2001; Stevens & Hogg 2003)) in certain areas of the Antarctic or sub-Antarctic. Thus, a need exists for more evolutionary studies to obtain an accurate picture of the evolutionary history of various species in the South Polar Region. From a conservation point of view, it is also important to determine the impact of past environmental change on biodiversity and ecosystems of the South Polar Region, in particular when considering the need to document current biodiversity as well as the effect of predicted climate change on these areas.

The following literature will mainly focus on the South Polar Region especially on biogeographic and colonization patterns of selected arthropod species as well as the findings from previous phylogenetic studies. The focus will then shift to an intra-island scenario, with sub-Antarctic Marion Island forming the focal point, where species diversity, abundance and patterns will be discussed in a historical context. The two main objectives of this study will be dealt with separately in the form of research questions listed below the relevant literature sections.

South Polar Region

The South Polar Region can be divided into different zones based on botanical and biogeographic criteria. The three botanical zones comprise firstly of the South Atlantic Province (SAP) including Falkland Island, the Scotia Arc (South Georgia, South Orkney, South Shetland and South Sandwich Islands as well as the Antarctic Peninsula) and Bouvetøya Island; secondly of the South Indian Province (SIP) consisting of Crozet, Kerguelen, Heard and Prince Edward Islands and lastly, the South Pacific Province (SPP) comprising of Auckland, Campbell and Macquarie Islands together with New Zealand (Lewis-Smith 1984; Pugh & Convey 2000; Wardle 1991). Biogeographically, the Antarctic region comprise of Continental Antarctica (most of mainland Antarctica), Maritime Antarctica (South Orkney, South Shetland and South Sandwich Islands as well as the Antarctic Peninsula) and the sub-Antarctic region (Crozet, Heard, Kerguelen, Macquarie, Prince Edward and South Georgia Islands) (Gressit 1970; Holdgate 1964; Marshall & Pugh 1996; Wallwork 1969).

The break-up of the southern supercontinent Gondwana started when East Gondwana (Antarctica, Madagascar, India and Australia) separated from West Gondwana (South America and Africa) during Early Jurassic (~178mya) (Crame 1999). South America separated from Africa during the Early Cretaceous (~130mya), forming the South Atlantic Ocean. During the East Gondwana break-up, India separated during the Early Cretaceous (~120mya), while Madagascar separated from India during the Cretaceous - Tertiary boundary (~65.5mya). Australia and New Zealand separated during the Late Cretaceous (~80mya) (see Cattermole 2000). Gondwana break-up often feature in biogeographic studies as an explanation for the distribution of taxa that are currently found in

discontinuous regions that were previously part of Gondwana. An example includes the Proteaceae plant family that is only found in Chile, South Africa and Australia (Weston & Crisp 1996).

When only considering the history of Antarctica (when it was part of Pangaea), it was glaciated during the Carboniferous period (354-286mya). During the Jurassic period (213-144mya) it became deglaciated as a consequence of world climate changes and latitudinal migration caused by continental drift (Gould 1993; Parrish 1990). Throughout the Eocene (58–36mya), it was characterized by rich and temperate fauna and flora until fragmentation occurred during the Miocene (24–5mya) (Chaloner & Creber 1989; Creber 1990; Elliot 1985). The date when the (current) ice cap formed and expanded is contentious and ranged from 34mya to less than 3mya (see for example DeConto & Pollard 2003; Marshall & Pugh 1996). Presently, Antarctica is an isolated and frozen continent.

During prior and past fragmentation of Gondwana, multiple islands have formed in the vicinity of Antarctica. These Maritime and sub-Antarctic islands have different geological origins and histories. In general, oceanic islands can be divided into “Darwinian” or “fragment” islands. Darwinian islands are created *de novo*, while the other island type forms due to the fragmentation of continents (reviewed by Gillespie & Roderick 2002). Only a few sub-Antarctic islands have a continental origin, for example Auckland, Bounty, Campbell and Falklands Islands (reviewed by Bergstrom & Chown 1999). The ages of these islands range from 2 500mya (Falkland Islands) to 16mya (Campbell Island). However, most of the sub-Antarctic Islands are Darwinian islands since they originally developed as underwater volcanoes; examples included the Crozet, McDonald, Prince Edward, Kerguelen Islands (Chown *et al.* 1998; Hänel & Chown 1998; Quilty & Wheller 2000). Macquarie Island on the other hand is the only island composed of oceanic crust and rocks from deep within the earth's mantle (Varne *et al.* 1969). Ages of the islands range from 29-40mya (Kerguelen Island) to 0.08mya (McDonald Island) (for more detail see Chown *et al.* 1998).

The extent of glaciation also varies between the sub-Antarctic islands, for example Kerguelen and Heard Islands have experienced multiple glaciation events (9 and 11, respectively (Chown *et al.* 1998) during the past glacial maximum (16 000 years ago), while Macquarie Island has experienced none (Bergstrom & Chown 1999; Hall 1990; Selkirk *et al.* 1990). Evidence suggests that the sub-Antarctic islands located south of the Antarctic Polar Frontal Zone (APFZ), specifically those that comprise larger landmasses with higher elevations, are more glaciated than those situated north of the APFZ that are smaller and with lower elevations. Thus the island’s position relevant to the APFZ and the movement of the APFZ plays an important role in the climatic history of these islands (reviewed by Bergstrom & Chown 1999).

The interplay of various ages, climatic oscillations as well as the geological histories of these islands renders these groups ideal for studying processes and patterns that influence the genetic diversity of species. Arthropod

communities that are widespread throughout the South Polar Region represent ideal organisms to test the development of evolutionary biota on these islands.

Arthropods

The ability of arthropods to survive extreme environmental conditions coupled with their relatively high generation turnover (Hogg & Stevens 2002 and references within) makes them one of the most abundant and successful terrestrial groups world-wide. It is therefore not surprising that sub-Antarctic islands are largely dominated by arthropod taxa (Pugh 1993). Among these arthropods, Acari or Acarina (mites) are highly abundant and diverse and play a significant ecological role on sub-Antarctic islands (Burger 1985; Chown *et al.* 2001).

Typically, contemporary microarthropod faunas in the South Polar Region consist of acari (approximately 400 species), arachnids (60 species), myriapods (12 species) and hexapods (500 species) (Pugh 1993, 1997). When considering the zoogeography of mites in the South Polar Region, the Antarctic species are limited, ancient and highly endemic. This is probably due to isolation in combination with physiographic, geological, glaciological and climatic factors (Wallwork 1973). The sub-Antarctic is species-rich with a high degree of endemism. This high degree of endemism indicates that the sub-Antarctic could be seen as a separate zoogeographical province. In addition, the zoogeography of the sub-Antarctic is complicated by the closeness of Australasia in the east and South America in the west as well as the fact that some islands have a Darwinian or fragmented origin. Maritime Antarctic is also species-rich and the species are thought to be derived mostly from the sub-Antarctic (Pugh & Convey 2000; Wallwork 1966, 1967, 1969, 1973).

From an evolutionary, ecological and biogeographical perspective, the mite superfamily Ameronothroidea (Oribatida) is of particular interest. These mites often feature in biogeographical studies due to their limited dispersal capabilities and their status as an ancient group (Starý & Block 1998; Wallwork 1973). They are also abundant and well-represented in the South Polar Region (Pugh 1993; Starý & Block 1998; Wallwork 1973) and are capable of occupying different habitats, ranging from intertidal to terrestrial ecosystems (Marshall & Convey 2004; Pugh 1993).

For the mite suborder Oribatida, 78 species have been documented in the sub-Antarctic zone (Pugh 1993, Starý & Block 1998). Unfortunately the taxonomy of the superfamily Ameronothroidea (family Podacaridae) is dubious with various proposed classification schemes due to character plasticity (D. J. Marshall and L. Coetzee, personal communication). In general, the most accepted classification suggests that the superfamily Ameronothroidea comprises of 40 peri-Antarctic species partitioned into three genera namely *Podacarus* Grandjean, 1955; *Alaskozetes* Hammer, 1966 and *Halozetes* Berlese, 1917. Unfortunately the classification of the species and subspecies within these genera remains dubious. For example, some species like *Halozetes edwardensis* have been described from a single specimen, while other species like terrestrial *H. fulvus* and *H. crozetensis* or

intertidal *H. marionensis* and *H. intermedius* have no distinct morphological characters to differentiate between adult individuals (D. J. Marshall and L. Coetzee, personal communication). Species like *H. belgicae* and *A. antarcticus* have a circum-sub-Antarctic pattern and are represented by distinct subspecies in the western and eastern sub-Antarctic. These subspecies specific variation could confirm the hypothesis that there are two sub-Antarctic faunal provinces (Wallwork 1973). See Table 1.1 for details on the distribution of South Polar ameronothroid mites.

Table 1.1 Ameronothroid mite species (Podacaridae) with their respective distribution in the South Polar Region. Distinction is made between terrestrial (T), supralittoral (S), intertidal (I) or unknown (?) zones. The following abbreviations were used for the localities: Amsterdam and St. Paul Islands (AS), Antarctica (A), Antarctic Peninsula (AP), Bouvetøya Island (B), Campbell Island (CB), Crozet Islands (C), Falkland Islands (F), Gough Islands (G), Heard Island (H), Kerguelen Islands (K), Macquarie Island (M), New Zealand (NZ), Prince Edward/Marion Islands (PM), South Georgia Island (SG), South Orkney Islands (SO), South Sandwich Islands (SH), South Shetland Islands (SD) and South Africa (SA). Table adopted from Marshall and Convey (2004) as well as Pugh (1993).

Species	Habitat	Localities
Podacarus auberti Grandjean, 1955	T	SG, PM, C, K, H, M
Podacarus auberti occidentalis Wallwork, 1966	T	SG
Alaskozetes antarcticus (Michael, 1903)	S/T	F, SG, SO, SD, AP, PM, C, K, H, M
Alaskozetes antarcticus intermedius Wallwork, 1967	S/T	A, AP, SO, SH, SG, K
Alaskozetes antarcticus grandjeani Dalenius, 1958	S/T	H, M
Alaskozetes bovetoyaensis van Pletzen and Kok, 1971	S/T	PE, B
Halozetes belgicae (Michael, 1903)	S/T	SG, SO, SD, AP, PM, C, K, H, M, CB, AS
Halozetes belgicae brevipilis Wallwork, 1963	S/T	M
Halozetes belgicae longiseta Wallwork, 1967	S/T	SH
Halozetes marinus (Lohmann, 1907)	I	F, SG, SO, PM, C, K, H, M, CB, AS
Halozetes marinus devilliersi Engelbrecht, 1974	S	PM
Halozetes marinus minor Wallwork, 1966	?	CB
Halozetes marionensis Engelbrecht, 1974	I	PM, G
Halozetes intermedius Wallwork, 1963	I	H, K, M
Halozetes impeditus Niedbala, 1986	?	SD
Halozetes littoralis Wallwork, 1970	S/T	SG
Halozetes negrophagus Wallwork, 1967	S/?	AP
Halozetes plumosus Wallwork, 1966	S/?	CB
Halozetes bathamae Luxton, 1985	I	NZ
Halozetes otagoensis Hammer, 1966	S	NZ
Halozetes macquariensis (Dalenius and Wilson, 1958)	T	M, CB
Halozetes crozetensis (Richters, 1907)	T	F, C, K, H, M, CB, AS
Halozetes edwardensis van Pletzen and Kok, 1971	?	PM
Halozetes fulvus Engelbrecht, 1975	T	PM
Halozetes capensis Coetzee and Marshall, 2003	I	SA

Biogeography

The historical biogeography of the South Polar Region is contentious. The sub-Antarctic islands can be seen either as a single biogeograhical province (i. e. the islands share similar histories as well as certain parts of their biota) (Chown *et al.* 1998; Holdgate 1960; Vijver & Beyens 1999), or as a multi-regional province (i. e. the biotas and histories of the islands are too dissimilar to be included in a single province) (Cox 2001; Gressit 1970). Three theories exist to explain the geological distribution of terrestrial arthropods in the Antarctic and sub-Antarctic region, namely vicariance (fragmentation due to geological processes), dispersal (wind, water, rafting, zoohoria etc.) or a combination of vicariance and dispersal (see for example Brundin 1966; Hogg & Stevens 2002; Pugh & Convey 2000; Strong 1967; Wallwork 1973).

Before the origin of sub-Antarctic islands was known, the similarities between the colonists across the South Polar Region prompted the idea of vicariance. It is well known that the biotas within the SIP are very similar which can indicate a shared origin (for example Chown 1994; Lewis-Smith 1984; McInnes & Pugh 1998; Starý *et al.* 1997). At first it was suggested that the islands were part of the Sudamedie continent proposing an African origin for certain taxa (insects) (Jeannel 1964). However, it became clear that a vicariant origin for the taxa in the South Polar Region as a whole is doubtful (Wallace *et al.* 2002) but might apply to certain smaller geographic regions (Chown 1994; Craig *et al.* 2003; Greve *et al.* 2005). Since most of the sub-Antarctic islands formed *de novo* (LeMausier & Thomson 1990), dispersal seems to be the obvious mechanism. Indeed, a nestedness analysis conducted for Southern Ocean island biotas demonstrated that the distribution pattern of less mobile taxa (such as flightless insects) is most likely influenced by the proximity of continents and large islands (Greve *et al.* 2005).

Historically, vicariance has been the preferred choice of explaining species ranges (terrestrial and freshwater) that were separated by oceans. However, recent molecular dating from phylogenetic studies suggests that dispersal play an important role in creating regional biogeography (de Queiroz 2005). Recent molecular evidence from dispersal patterns on oceanic islands, indicates the importance of dispersal in terms of the evolution of the biodiversity of the island (Cowie & Holland 2006). Dispersal across the ocean accounts for the high level of endemism in the South Polar Region (post-Pleistocene colonization) (Gressit 1970). The type of dispersal usually varies between different species from the region. For example, Craig and colleagues (2003) suggested that the dispersal method for the Diptera species, *Crozetia* Davies, 1965 to the Crozet archipelago was likely either through wind or zoohoria (birds from Africa). Powered flight has also been suggested as a good dispersal attribute for pterygote insects (for example Coleoptera and Diptera) (Kushel 1990). Wind dispersal was suggested for soil organisms specifically nematodes in the Dry Valleys of Antarctica (Nkem *et al.* 2006). For amoronothroid mites, zoohoria and air currents were ruled out due to their size and life history phases (Marshall & Pugh 1996). Evidence obtained from “aerial plankton” also suggested that long distance air dispersal of mites was unlikely, since they were unable to survive the subsequent low temperature and high pressure (Pugh 2003; Pugh & Convey 2000). However, *Halozetes* and *Antarcticus* has been found to survive in seawater for extensive

time periods (LT_{50}=20+ days), which may favour ocean current dispersal (Pugh 1995; Strong 1967). It has been suggested that the presence of certain mite species can be explained by recent anthropogenic introductions (Pugh 2003).

The controversy behind the complex biogeography of the South Polar Region may also, in part, be attributed to species distributional data of taxonomically difficult groups (for example Gressitt, 1970; Greve *et al.* 2005; McInnes & Pugh 1998; Morrone 1998; Muñoz *et al.* 2004). But now with the powerful tool of genetic techniques, the complexity of the South Polar Region can be reassessed. A marked increase in molecular studies from this region has occurred, especially from the marine environment. Using various molecular markers and techniques, studies focussed mostly on population structure, however, an increase in phylogenetic relationships referring to dispersal and speciation patterns has also been noted. Some examples from the marine environment include notothenioid fishes (for example Bargelloni *et al.* 2000; Patarnello *et al.* 2003; Ritchie *et al.* 1996; Sanchez *et al.* 2007; Verde *et al.* 2007; Zane *et al.* 2006), benthos (Brandt *et al*. 2007), bivalves (Page & Linse 2002), sea urchins (Lee *et al.* 2004), isopods (Held 2000), krill (Jarman 2001; Zane *et al.* 1998) and allogromiids (Pawlowski *et al.* 2005). Terrestrial studies have mostly focused on the population structure of arthropods (for example Fanciulli *et al.* 2001; Frati *et al.* 2001; Stevens & Hogg 2003) and plants (reviewed in Skotnicki *et al.* 2000). A few phylogenetic studies have been conducted on midges (Allegrucci *et al.* 2006), springtails (Stevens & Hogg 2006) and moss (Skotnicki *et al.* 2004). However, more detailed studies are needed to obtain an overall pattern of the terrestrial environment of the South Polar Region, since most of the biogeographical studies are limited in terms of sampling and molecular markers. Many studies implement molecular clocks based on substitution rates due to the lack of fossil data (for example Allegrucci *et al*. 2006; Stevens & Hogg 2006). A way to improve these clock estimates can be to include additional genetic markers, specifically nuclear markers and to implement a multigene relaxed Bayesian molecular clock using relative rates. In this study, both mitochondrial (cytochrome oxidase subunit I) and nuclear (histone-3) markers were included in the relaxed Bayesian clock, in an attempt to calculate more accurate divergence dates for mite species from the South Polar Region.

Evolution

Some of the microarthropods in Antarctica potentially signify isolated microarthropod populations following the fragmentation of the southern supercontinent. These relict microarthropod populations have evolved independently, generating the high levels of endemism now characteristic of the Continental, sub-Antarctic and Maritime zones. The presence of certain microarthropods can also be explained by recent northern radiations (Marshall & Coetzee 2000; Marshall & Pugh 1996; Pugh & Convey 2000; Wallwork 1973). This theory is supported by the presence of certain ameronothroid mites (Podacaridae) in both South America and New Zealand (Hammer 1958, 1961, 1962, 1966, 1967, 1968) and recently, the first *Halozetes* (*Halozetes capensis* n.sp) has been described from South Africa (Coetzee & Marshall 2003). This is the most northern occurrence of *Halozetes* and this finding together with observations for the existing ocean current systems may support the

theory that the genus originated in the South Polar Region. It is also believed that the Podacaridae group represents an ancient group (Gondwanan origin), since it extends over two or three zones (Continental, Maritime and sub-Antarctic) with several subspecies being recognised (Wallwork 1973).

In addition to the hypothesis that the ameronothroid mites (specifically the Podacaridae family) evolved in the South Polar Region, they are also unique among arthropods, due to their occupation of various habitats (i. e. terrestrial, supralittoral and intertidal). Earlier work based on morphology and zoography, indicated that ancestral species within this group could be the intertidal taxa, specifically *H. marinus* and *H. littoralis,* and not the terrestrial species. *Halozetes marinus* and *H. littoralis* still occurs in the marine and intertidal regions, respectively, and may have endured glaciation to form the basis of radiation into terrestrial environments. The radiation may also have transpired independently in the western and eastern sub-Antarctic. The intertidal mites (specifically *H. marinus*) are therefore considered to be basal (Wallwork 1973). Supportive evidence was found for this hypothesis on sub-Antarctic Marion Island based on the relative ages of the different vegetation types and habitat specificity of various mite species. The authors reasoned that these older intertidal habitats acted as refugia for species during glaciation periods when terrestrial environments were largely covered by ice (Barendse *et al.* 2002). In addition, Mercer and colleagues (2002) also suggested that the specificity of species to the shore (epilithic biotope) is most likely due to the substantial age of this biotope compared to the younger vascular vegetation (post glacial) on Marion Island.

The opposite scenario was suggested for ameronothroid mites in the Northern Hemisphere. It is believed that the ameronothroid mite, *Ameronothrus* Berlese, 1896, used terrestrial areas as refugia during glaciation. In this case terrestrial species are considered to be ancestral (Schulte & Weigmann 1977). Both scenarios, intertidal to terrestrial or terrestrial to intertidal transition, can be tested in a phylogenetic framework. With such a phylogeny, more insight could also be gained for larger issues such as the origin of all oribatid mites. Clues could be obtained as to whether their ancestor was terrestrial (Procheş & Marshall 2002) or marine (Bernini *et al.* 2000).

Research questions:

1. What is the biogeographical pattern of terrestrial ameronothroid mites (specifically the genus *Halozetes*) in the South Polar Region?
2. When conducting manual comparisons, how do terrestrial biogeographical patterns found in this study and in the literature compare to previously described marine biogeographic patterns in the South Polar Region?
3. What are the relative diversification times for the ameronothroid species? Do their divergence times predate Gondwana fragmentation?
4. Are intertidal ameronothroid mites less derived (and older) than those inhabiting the terrestrial zone (Wallwork 1973) or does the opposite hypothesis apply (Shulte and Weigmann 1977)?

5. How does the dubious contemporary taxonomy of *Halozetes* compare to its molecular phylogeny?
6. What is the intra-specific variation within different *Halozetes* species across sub-Antarctic islands?

Intra-island scenario

Thus far the focus of the literature was on evolution in the South Polar Region. To enhance our understanding of intra-island population structure of different species in the South Polar Region, sub-Antarctic Marion Island was selected, because the geological history and the paleoclimatic conditions on the island are well understood. Also, the taxonomy of multiple species residing on the island is well documented. In addition, Marion Island is managed by the South African government with annual relief voyages to the island that provide ample opportunities for research.

Marion Island

The Prince Edward Island archipelago (PEI) consists of Marion Island (PEI; 46º54'S, 37º45'E) and Prince Edward Island (PEI; 46˚38'S, 37˚57'E), separated by 19km of ocean. PEI is situated in the Southern Ocean, approximately 2300km south-east of Cape Town, South Africa (see position in Chapter 2, Figure 2.1). The nearest landmass is the Crozet archipelago positioned approximately 950km east of Marion Island (Hänel & Chown 1998).

PEI formed *de novo* (i. e. Darwinian island *sensu* Gillespie & Roderick 2002) and constitutes the tips of an oceanic intraplate volcano (Hall 1990, 2002; Hänel & Chown 1998). Marion Island, the larger of the two islands (290 km^2), has a typical shield volcanic shape with the centre being dominated by high mountains containing an ice plateau at an altitude above 1km. This island is estimated to be between 1 million years and 0.5 million years old (myo) (Hänel & Chown 1998), however, recent K-Ar dating suggests Marion Island is 0.45myo (McDougall *et al.* 2001).

Marion Island is situated in the middle of the APFZ, and as such was noticeably influenced by climate change due to the oscillations in the APFZ. After its formation, it has experienced at least eight volcanic periods and five glacial stages (McDougall *et al.* 2001). Based on K-Ar age determinations, McDougall and colleagues suggested that the volcanic activity was episodic with most activity occurring about 450, 350, 240, 170, 110, 85, 50 and less than 10 000 years ago (ka) across the island. The most recent documented volcanic eruption occurred in 1980, in the vicinity of Kaalkoppie (western side of island). Two lava types are present on Marion Island, the older grey basalt lava dating back to the Pleistocene (for example Long Ridge, Cold Ridge, Feldmark Plateau) and the younger black basalt lava dating back to postglacial Holocene (McDougall *et al.* 2001). The black lava flows formed over a hundred scoriae cones that dominate the island's terrain (Hänel & Chown 1998).

Most of the glacial events on Marion Island (stage 2 (~10-35ka), 4 (~65-79ka), 6 (~132-198ka), 8 (~252-302ka), 12 (~428-480ka); see Figure 9 of McDougall *et al.* 2001) were intercalated with volcanic activity; however evidence exists that earlier volcanic eruptions overlapped with glacial events. The two most recent stages were interglacial (McDougall *et al.* 2001). It is believed that Marion Island has been covered by huge fields of permanent ice and snow during periods of extensive glaciation, probably due to temperatures that were 4 to 7°C lower than what they are now. The grey lava ridges on Marion support signs of extensive glaciations as recently as 12 000 to 16 000 years ago (Hobs *et al.* 1998). In the past, the east coast of Marion Island was covered by huge glaciers (Hänel & Chown 1998; Schulze 1971; Smith & Steenkamp 1990). Contrary to Marion Island, Prince Edward Island bears no signs of glaciation. The presence of ice sheets was never detected probably due to the lower altitude of the Prince Edward Island. Another explanation for the lack of glacial evidence could be the presence of erosion on the western side of the island (Hänel & Chown 1998).

The current climate and weather on Marion Island are greatly influenced by the Southern Ocean atmospheric system. The present weather on the island includes average temperatures of ~5°C, very high precipitation of ~2500 mm/year (mainly rain), high humidity (~83%) and a high degree of cloudiness with an annual sunshine duration of 30%. The north westerly winds predominate (70 - 200km/h). In addition, the island is also located in the so-called Roaring Forties (westerly winds). These westerly winds are coupled with cloudy conditions while the southerly winds from the Antarctic bring cold and clear conditions. Unfortunately this island is keeping pace with global warming. Over the last 30 years, records have shown that the annual average temperature has increased by 1.2°C while precipitation declined by 600mm (Hänel & Chown 2000; Schulze 1971; Smith & Steenkamp 1990; Smith & Gremmen 2004). The effect of the warmer and drier conditions is noticeable in the shrinking and disappearance of the ice plateau in centre of the island (Smith 2002; Summer *et al.* 2004; K. I. Meiklejohn, personal communication). It is also visible by the rapid transformation of the island by alien plant species (Gremmen *et al.* 1998). Multiple alien species have been introduced to Marion Island during the past and these species have significantly impacted on the structure and functioning of the ecosystem (Chown & Smith 1993; Gremmen & Smith 1999; Hänel & Chown 1998). This positive link between climate change and the establishment and distribution of alien species poses a big problem, especially in the sensitive sub-Antarctic ecosystem (Chown & Gaston 2000).

Marion Island has a semi-closed ecosystem that is dependant on the surrounding ocean. The island is considered nutrient-poor with the mineral content arising from direct salt-sea spray, indirect seabird and seal excreta as well as other organic deposits. Mineral content, moisture and climate also influence the vegetation of the island, which differs with altitude (Bergstrom & Chown 1999; Gremmen 1981; Smith & Gremmen 2004). Seven community complexes have been described: a) salt-spray, found near shorelines (*Crassula moschata*), b) biotic, found near animal activity along the shoreline (*Callitriche antarctica–Poa cookii*), c) fernbrake, found along drained slopes on the lowland (*Blechnum penna-marina*), d) *Acaena magellanica–Brachythecium,* found near

mires and slopes, e) *Juncus scheuchzerioides–Blepharidophyllum densifolium,* found near wet peat, f) polar desert, found at high altitudes and g) fellfield, found at exposed rocky areas (*Andreaea–Racomitrium crispulum*) (Smith & Mucina 2006). Fellfield is considered to be one of the oldest community complexes on sub-Antarctic islands (Scott 1985), with the flowering vascular cushion plant, *Azorella selago* Hook. f., 1847 (Apiaceae), dominating this habitat (Smith & Gremmen 2004). This widespread and long-lived pioneer species is well-adapted for the harsh sub-Antarctic environment; it has short internodes, a compact surface and its buds are protected on the soil level, where temperatures are more constant than the air (see Armesto 1980 and references within). On Marion Island, *A. selago* acts as keystone species since it forms nutrient rich environments for invertebrates and epiphytic plants (more detail below) (Barendse & Chown 2001; Huntley 1972). In addition, it shapes the landscape by affecting geomorphological processes (Boelhouwers *et al*. 2000).

Since PEI is a Darwinian island group, it must have been colonized from a potential source after its formation. In general, if a Darwinian island stays isolated from its source, evolutionary processes such as speciation and development of neo-endemics will occur (Gillespie & Roderick 2002). It is therefore not surprising that the isolated nature of Marion Island contributed to the presence of multiple endemic species (Chown *et al.* 2002). PEI has approximately 187 different plant species. These species include indigenous vascular plants (22 species) and multiple lichens, mosses and liverwort species (165 species). Only nine of these species are endemic, however, PEI also contains 34 other species that are endemic to SIP. Most alien plant species present on Marion Island are grasses like *Agrostis stolonifera* L., 1753 and *A. gigantea* Roth, 1788 (Hänel & Chown 2000; Smith & Gremmen 2004).

The fauna on Marion Island includes multiple species of seals, birds and penguins; however, it is mostly dominated by invertebrates. The terrestrial invertebrates (indigenous as well as introduced) include beetles, flies, moths, wasps, butterflies, aphids, spiders, mites, slugs and snails as well as earthworms and roundworms (Hänel & Chown 1998). The invertebrates play an essential role in the island's ecosystem, since they are the only herbivores and detrivores present. Invertebrates are responsible for the recycling of nutrients in the soil, for plant fertilization and for decomposition (Chown *et al.* 2002).

My research project mainly focussed on free-living terrestrial mite species. Marshall *et al.* (1999) identified 60 species from PEI, including Mesostigmata (8), Progstigmata (20), Oribatida (23) and Astigmata (9). Of these mites, all Mesostigmata, 58% of Prostigmata, 13% of Oribatida and 33% of Astigmata are endemic. On Marion, the habitats of mite species varies from the intertidal and supralittoral zone to rocky faces in lowland environments, fellfield regions, vegetated regions (mosses and flowering plants) as well as freshwater ponds and streams (Hänel & Chown 1998).

There is an important distinction between terrestrial and shoreline faunas. The terrestrial species from sub-Antarctic islands have common habitat requirements while the shoreline species have more specific habitat requirements (Marshall *et al.* 1999). The distribution patterns of several invertebrate species have been investigated on Marion Island (Barendse & Chown 2001; Chown 1990; Hugo *et al.* 2004). A significantly higher species richness exists in the older epilithic biotope (shoreline) compared to the younger vegetated biotope (Mercer *et al.* 2000). When investigating invertebrate communities in the mid-altitude fellfield (*Azorella selago* and rocky areas), most indigenous species were found with a relatively high species richness. In *A. selago*, *Eupodes minutus* Strandtmann, 1967 was by far the most abundant mite species (16,000 individuals m^{-2}), while *Halozetes fulvus* was most abundant in rocky areas between *Azorella* cushions (700 individuals m^{-2}) (Barendse & Chown 2001). Hugo and colleagues (2004) also found fine-scale structure in terms of distribution and abundance of mite and springtail species in *A. selago*. Their abundance was higher on the southern sides of the plants that were less exposed to wind and that had colder and drier conditions.

Phylogeography

Phylogeography is based on the genealogical histories of individual genes sampled from different populations where the spatial distribution of these differences can offer valuable insights into evolutionary processes (Maholtra *et al.* 1996). The phylogeographic structure of any species can indicate patterns of historical fragmentation as well as restricted gene flow, selection, mutation, drift and species-specific dispersal capabilities (for example Avise *et al.* 1979; Avise 1994; Knowles & Maddison 2002).

Evidence from Antarctica has shown that glaciation had a significant impact on the population structure of certain invertebrate species (mites and springtails) (for example Fanciulli *et al.* 2001; Frati *et al.* 2001; Stevens & Hogg 2003). In addition, when considering sub-tropical islands like the Hawaiian Islands or the Canary Islands; these islands have experienced extreme geological events (volcanism) which have also shaped the diversification of numerous indigenous invertebrate species (Emerson 2002; Roderick & Gillespie 1998). Even vertebrates can exhibit complex patterns on islands due to mountains acting as barriers to gene flow, for example the house mouse (*Mus musculus domesticus* Schwarz & Schwarz, 1943) on Madeira Island (Britton-Davidian *et al.* 2000). Thus, evidence from sub-tropical islands indicates that geological events have an impact on the population structure of species. This may also be the case for Marion Island, which has been characterized by numerous glaciation and volcanic events.

In general, alterations in species' habitats could cause them to go extinct in certain parts of their distribution range or they can radiate to more favourable environments. Another alternative is to survive in small refugia from where they spread out again in the presence of more favourable climatic conditions (Hewitt 2000). In the case of Marion Island, glaciation could have confined species into isolated refugia, while lava flows could have caused the extinction of multiple fauna and flora populations directly in their path. The multiple ridges and valleys on

Marion Island formed by volcanic eruptions during the past may potentially present long term extrinsic barriers to gene flow. Myburgh *et al.* (2007) examined the population structure of selected springtail species (indigenous and invasive) by means of COI on Marion Island (refer to Chapter 3, 4 or 5 for a map of Marion Island). Substantial genetic substructure was present in the two indigenous species (*Cryptopygus antarcticus travei* Déharveng, 1981 and *Tullbergia bisetosa* (Börner, 1903)) which correlated well with geological or glaciation events on the island. In addition, a refuge population was identified at the higher elevated Katedraalkrans. This locality could have remained ice-free during past glaciations (K. I. Meiklejohn, personal communication). Interestingly, Kildalkey Bay was distinct from the other localities based on its genetic profile. Possible explanations for this could be that this bay is the natural entry point for new invertebrates due to the prevailing winds and sea currents. In addition, this bay has frequently been used by sealers. This study provided valuable insight into the population structure of springtails across the island, however, the large scale pattern across the island remains unknown, which provides the ideal opportunity to conduct more comparative phylogeographic studies focussing on other species.

Research questions:

1. What is the independent phylogeographic population structure of the indigenous mite species, *Halozetes fulvus* and *Eupodes minutus,* across Marion Island? Can congruent patterns be manually identified for both species on the island?
2. When conducting manual comparisons, how does the observed phylogeographic pattern compare between the indigenous mite species (*H. fulvus* and *E. minutus*) and indigenous springtail species (*C. antarcticus* and *T. bisetosa* (Myburgh *et al*. 2007)) across Marion Island?
3. Is there a possible correlation between the two species' (*H. fulvus* and *E. minutus*) microhabitat and microclimate preferences and their genetic variability on the island?
4. What is the growth dynamics of *Azorella selago* on Marion Island since it has two distinct growth forms (i. e. discrete cushions and continuous mats)? (This needs to be assessed prior to phylogeography, since phylogeographic investigations assume sample independence.)
5. What is the phylogeographic population structure of *A. selago* across Marion Island? Is substructure present in this host plant and does its pattern coincides with patterns found for inhabiting microarthropods (like *E. minutus*) on Marion Island when compared manually?
6. What do all these phylogeographic patterns of species on the island mean in terms of conservation?

REFERENCES

Allegrucci, G., G. Carchini, V. Todisco, P. Convey and V. Sbordoni (2006). A molecular phylogeny of Antarctic chironomidae and its implications for biogeographical history. Polar Biology 29: 320-326.

Armesto, J., M. Arroyo and C. Villagrán (1980). Altitudinal distribution, cover and size structure of umbelliferous cushion plants in the high Andes of Central Chile. Acta Oecologica 1: 327-332.

Avise, J., C. Giblin-Davidson, J. Laerm, J. Patton and R. Lansman (1979). Mitochondrial DNA clones and matriarchal phylogeny within and among geographic populations of the pocket gopher, *Geomys pinetis*. Proceedings of the National Academy of Sciences of the United States of America 76: 6694-6698.

Avise, J. (1994). Molecular Markers, Natural History and Evolution. London and New York, Chapman and Hall.

Barendse, J. and S. Chown (2001). Abundance and seasonality of mid-altitude fellfield arthropods from Marion Island. Polar Biology 24: 73-82.

Barendse, J., R. Mercer, D. Marshall and S. Chown (2002). Habitat specificity of mites on sub-Antarctic Marion Island. Environmental Entomology 31: 612-625.

Bargelloni, L., S. Marcato, L. Zane and T. Patarnello (2000). Mitochondrial phylogeny of notothenioids: a molecular approach to Antarctic fish evolution and biogeography. Systematic Biology 49: 114-129.

Bergstrom, D. and S. Chown (1999). Life at the front: history, ecology and change on southern ocean islands. Trends in Ecology and Evolution 14: 472-477.

Berlese, M. (1917). Acariens. Deuxième Expèdition Antarctique Française Zoologie (1908-1910) 1-2.

Bernini, F., G. Carnevale, G. Bagnoli and S. Stouge (2000). An Early Ordovician oribatid mite from the Island of Oeland, Sweden. 4th Symposium of the Europea Association of Acarologists. Sienna.

Boelhouwers, J., S. Holness and P. Sumner (2000). Geomorphological characteristics of small debris flows on Junior's Kop, Marion Island, maritime sub-Antarctic. Earth Surface Processes and Landforms 25: 341-352.

Brandt, A., A. Gooday, S. Brandão, S. Brix, W. Brökeland, T. Cedhagen, M. Choudhury, N. Cornelius, B. Danis, I. De Mesel, R. Diaz, D. Gillan, B. Ebbe, J. Howe, D. Janussen, S. Kaiser, K. Linse, M. Malyutina, J. Pawlowski, M. Raupach and A. Vanreusel (2007). First insights into the biodiversity and biogeography of the Southern Ocean deep sea. Nature 447: 307-311.

Britton-Davidian, J., J. Catalan, M. da Graça Ramalhinho, G. Ganem, J. Auffray, R. Capela, M. Biscoito, J. Searle and M. da Luz Mathias (2000). Rapid chromosomal evolution in island mice. Nature 403: 158.

Brundin, L. (1966). Transantarctic relationships and their significance as evidenced by chironomid midges. Svenska Vteneskapsakademie Handlunge 11: 1-472.

Burger, A. (1985). Terrestrial food webs in the sub-Antarctic: island effects. In: Antarctic nutrient cycles and food webs (eds. W. Siegfried, P. Condy and R. Laws), pp. 582-291. Berlin, Springer.

Cattermole, P. (2000). Building Planet Earth: Five Billion Years of Earth History. Cambridge, Cambridge University Press.

Chaloner, W. and G. Creber (1989). The phenomenon of forest growth in Antarctica: a review. In: Origins and evolution of the Antarctic biota (ed. J. Crame), 47 pp. 85-88. London, Geological Society Special Publication.

Chown, S. (1990). 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. and V. Smith (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. (1994). Historical ecology of sub-Antarctic weevils (Coleoptera: Curculionidae): patterns and processes on isolated islands. Journal of Natural History 28: 411-433.

Chown, S., N. Gremmen and K. Gaston (1998). Ecological Biogeography of Southern Ocean Islands: Species-area relationships, human impacts and conservation. The American Naturalist 152: 562-575.

Chown, S. and K. Gaston (2000). Island-hopping invaders hitch a ride with tourists in the Southern Ocean. Nature 408: 637.

Chown, S., A. Rodrigues, N. Gremmen and K. Gaston (2001). World Heritage status and the conservation of Southern Ocean islands. Conservation Biology 15: 550-557.

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

Coetzee, L. and D. Marshall (2003). A new *Halozetes* species (Acari: Oribatida: Ameronothridia) from the marine littoral of southern Africa. African Zoology 38: 327-331.

Cowie, R. and B. Holland (2006). Dispersal is fundamental to biogeography and the evolution of biodiversity on oceanic islands. Journal of Biogeography 33: 193-198.

Cox, C. (2001). The biogeographic regions reconsidered. Journal of Biogeography 28: 511-523.

Craig, D., D. Currie and P. Vernon (2003). *Crozetia* Davies (Diptera: Simuliidae): rediscription of *Cr. crozetensis*, *Cr. seguyi*, number of larval instars, phylogenetic relationships and historical biogeography. Zootaxa 259: 1-39.

Crame, J. A. (1999). An evolutionary perspective on marine faunal connections between southernmost South America and Antarctica. Science Marine 63 [Suppl. 1]: 1-14.

Creber, G. (1990). The south polar forest ecosystem. In: Antarctic palaeobiology: its role in the construction of Gondwana (ed. T. Taylor), pp. 37-41. New York, Springer Verlag.

Dalenius, P. and O. Wilson (1958). On the soil fauna of the Antarctic and of the sub-Antarctic islands. The Oribatidae (Acari). Arkiv für Zoologi 11: 193-425.

DeConto, R. and D. Pollard (2003). Rapid Cenozoic glaciation of Antarctica induced by declining atmospheric CO2. Nature 421: 245-249.

de Queiroz, A. (2005). The resurrection of oceanic dispersal in historical biogeography. Trends in Ecology and Evolution 20: 68-73.

Elliot, D. (1985). Physical geography-geological evolution. In: Key environments – Antarctica (ed. D. Bonner), pp. 39-61. Oxford, Pergamon Press.

Emerson, B. (2002). Evolution on oceanic islands: molecular phylogenetic approaches to understanding pattern and process. Molecular Ecology 11: 951–966.

Engelbrecht, C. (1974). The genus *Halozetes* (Oribatei: Acari) on Marion Island. Navorsinge van die Nasionale Museum Bloemfontein 3: 1-25.

Engelbrecht, C. (1975). New ameronothroid (Oribatei, Acari) taxa from the republic of South Africa and the Islands of Gough and Marion. Navorsinge van die Nasionale Museum Bloemfontein 3: 53-88.

Fanciulli, P., D. Summa, R. Dallai and F. Frati (2001). High levels of genetic variability and population differentiation in *Gressittacantha terranova* (Collembola, Hexapoda) from Victoria Land, Antarctica. Antarctic Science 13: 246-254.

Frati, F., G. Spinsanti and R. Dallai (2001). Genetic variation of mtCOII gene sequences in the collembolan *Isotoma klovstadi* from Victoria Land, Antarctica: evidence for population differentiation. Polar Biology 24: 934-940.

Gillespie, R. and G. Roderick (2002). Arthropods on islands: colonization, speciation and conservation. Annual Revue of Entomology 47: 595-632.

Gould, S. (1993). The book of life. London, Edbury Hutchinson.

Grandjean, F. (1955). Sur un acarien des iles Kerguélen, *Podacarus auberti* (Oribate). Mémoires du Muséum nationale d'histoire naturelle, Paris, Serie A, Zoology 8: 109-150.

Gremmen, N. (1981). The vegetation of the sub-Antarctic Islands Marion and Prince Edward. The Hague, Dr W. Junck BV Publishers.

Gremmen, N., S. Chown and D. Marshall (1998). Impact of the introduced grass *Agrostis stolonifera* on vegetation and soil fauna communities at Marion Island, sub-Antarctic. Biological Conservation 85: 223-231.

Gremmen, N. and V. Smith (1999). New records of alien vascular plants from Marion and Prince Edward Islands, Sub-Antarctic. Polar Biology 21: 401-409.

Gressit, J. (1970). SubAntarctic Entomology: Particularly of South Georgia and Heard Island. Pacific insects monographs 23: 295-374.

Greve, M., N. Gremmen, K. Gaston and S. Chown (2005). Nestedness of Southern Ocean island biotas: ecological perspectives on a biogeographical conundrum. Journal of Biogeography 32: 155-168.

Hall, K. (1990). Quaternary glaciations in the southern ocean: sector 0° long-180° long. Quarternary Science Reviews 9: 217-228.

Hall, K. (2002). Review of present and Quaternary peri-glacial processes and landforms of the maritime and sub-Antarctic region. South African Journal of Science 98: 71-81.

Hammer, M. (1958). Investigations on the oribatid fauna of the Andes Mountains I. The Argentine and Bolivia. Biologiske Skrifter 10: 129.

Hammer, M. (1961). Investigations on the oribatid fauna of the Andes Mountains II. Peru. M. Biologiske Skrifter 13: 157.

Hammer, M. (1962). Investigations on the oribatid fauna of the Andes Mountains III. Chile. Biologiske Skrifter 13: 96.

Hammer, M. (1966). Investigations on the oribatid fauna of the Andes Mountains I. The Argentine and Bolivia. Kongelige Dansk Videnskabernes Selskabs, Biologiske Skrifter 10: 1-129.

Hammer, M. (1967). Investigations on the oribatid fauna of New Zealand II. Biologiske Skrifter 15: 64.

Hammer, M. (1968). Investigations on the oribatid fauna of New Zealand III. Biologiske Skrifter 16: 96.

Hänel, C. and S. Chown (1998). An introductory guide to the Marion and Prince Edward Island Special Nature Reserves, 50 years after annexation. Pretoria, Department of Environmental Affairs and Tourism.

Held, C. (2000). Phylogeny and biogeography of serolid isopods (Crustacea, Isopoda, Serolidae) and the use of ribosomal expansion segments in molecular systematics. Molecular Phylogenetics and Evolution 15: 165-178.

Hewitt, G. (2000). The genetic legacy of the Quaternary ice ages. Nature 405: 907-913.

Hobs, J., J. Lindesay and H. Bridgeman (1998). Climates of the Southern Continents. Chichester, Wiley.

Hogg, I. and M. Stevens (2002). Soil Fauna of Antarctic Coastal Landscapes. In: Geoecology of Antarctic Ice-Free Coastal Landscapes. Ecological Studies (eds. L. Beyer and M. Bölter), 154 pp. 265-282. Berlin, Springer-Verlag.

Holdgate, M. (1960). Fauna of the mid-Atlantic islands. Proceedings of the Royal Society of London B 152: 550-567.

Holdgate, M. (1964). Terrestrial ecology in the Maritime Antarctic. In: Biologie Antarctique, Antarctic Biology (eds. R. Carrick, M. Holdgate and J. Prévost), pp. 181-194. Paris, Hermann.

Hugo, E., M. McGeoch, D. Marshall and S. Chown (2004). Fine scale variation in microarthropod communities inhabiting the keystone species *Azorella selago* on Marion Island. Polar Biology 27: 466-473.

Huntley, B. (1972). Notes on the ecology of *Azorella selago* Hook.f. South African Journal of Botany 38: 103-113.

Jarman, S. (2001). The evolutionary history of krill inferred from nuclear large subunit rDNA sequence analysis. Biological Journal of the Linnean Society 73: 199-212.

Jeannel, R. (1964). Biogeographie des terres Australes de l'Ocean Indien. Revue Francaise d'Entomologie 31: 319-417.

Knowles, L. and W. Maddison (2002). Statistical phylogeography. Molecular Ecology 11: 2623-2635.

Kushel, G. (1990). Biogeographical aspects of the subantarctic islands. Roma Accademiae Internazionale de Lincei 85: 575-591.

Lee, Y., M. Song, S. Lee, R. Leon, S. Godoy and I. Canete (2004). Molecular phylogeny and divergence time of the Antarctic sea urchin (*Sterechinus neumayeri*) in relation to the South American sea urchins. Antarctic Science 16: 29-36

LeMausier, W. and J. Thomson (1990). Volcanoes of the Antarctic Plate and Southern Ocean. Washington, American Geophysical Union.

Lewis-Smith, R. (1984). Terrestrial plant biology of sub-Antarctic and Antarctic. In: Antarctic ecology (ed. R. Laws), pp. 161-162. London, Academic Press.

Lohmann, H. (1907). Die Meeresmilben der Deutschen Südpolar-Expedition. Deutshe Südpolar-Expedition 9: 361-413.

Luxton, M. (1990). The marine littoral mites of the New Zealand region. Journal of the Royal Society of New Zealand 20: 367-418.

Maholtra, A., R. Thorpe, H. Black, J. Daltry and W. Wüster (1996). Relating geographic patterns to phylogenetic processes. New Uses of New Phylogenies. Oxford, Oxford University Press.

Marshall, D. and P. Pugh (1996). Origin of the inland Acari of Continental Antarctica, with particular reference to Dronning Maud Land. Zoological Journal of the Linnean Society 118: 101-118.

Marshall, D., N. Gremmen, L. Coetzee, B. O'Connor, P. Pugh, P. Theron and E. Ueckermann (1999). New records of Acari from the sub-Antarctic Prince Edwards Islands. Polar Biology 21: 84-89.

Marshall, D. and L. Coetzee (2000). Historical biogeography and ecology of a Continental Antarctic mite genus, *Maudhemia* (Acari, Oribatida): Evidence for a Gondwanan origin and Pliocene-Pleistocene speciation. Zoological Journal of the Linnean Society 129: 11-128.

Marshall, D. and P. Convey (2004). Latitudinal variation in habitat specificity of ameronothrid mites (Oribatida). Experimental and Applied Acarology 34: 21-35.

McDougall, I., W. Verwoerd and L. Chevallier (2001). K-Ar goechronology of Marion Island, Southern Ocean. Geological Magazine 138: 1-17.

McInnes, S. and P. Pugh (1998). Biogeography of limno-terrestrial Tardigrada, with particular reference to the Antarctic fauna. Journal of Natural History 25: 31-36.

Mercer, R., S. Chown and D. Marshall (2000). Mite and insect zonation on a Marion Island rocky shore: a quantitative approach. Polar Biology 23: 775-784.

Michael, A. (1903). Acarida (Oribatidae). In: Expédition Antarctique Belge. Résultats du voyage S. Y Belgica en 1897-1898-1899. Rapports cientifiques, Zoologie, Acariens Libres 10: 1-6.

Morrone, J. (1998). On Udvardy's Insulantarctica province: a test from the weevils (Coleoptera: Curculionoidea). Journal of Biogeography 25: 947-955.

Muñoz, J., A. Felicísimo, F. Cabezas, A. Burgaz and I. Martínez (2004). Wind as a long-distance dispersal vehicle in the Southern Hemisphere. Science 304: 1144-1147.

Myburgh, M., S. Chown, S. Daniels and B. Jansen van Vuuren (2007). Population structure, propagule pressure and conservation biogeography in the sub-Antarctic: lessons from indigenous and invasive springtails. Diversity and Distributions 13: 143-154.

Myers, A. and P. Giller (1988). Analytical biogeography: an integrated approach to the study of animal and plant distributions. London, Chapman and Hall.

Niedbala, W. (1986). Several species of moss-mites (Acari: Oribatida) from the Antarctic coastal zone. Polish Polar Research 7: 119-126.

Nkem, J., D. Wall, R. Virginia, J. Barrett, E. Broos, D. Porazinska and B. Adams (2006). Wind dispersal of soil invertebrates in the McMurdo Dry Valleys, Antarctica. Polar Biology 29: 346-352.

Page, T. and K. Linse (2002). More evidence of speciation and dispersal across the Antarctic Polar Front through molecular systematics of Southern Ocean *Limatula* (Bivalvia: Limidae). Polar Biology 25: 818-826.

Parrish, J. (1990). Gondwanan palaeogeography and palaeoclimatology. In: Antarctic palaeobiology: its role in the construction of Gondwana (ed. E. Taylor), pp. 15-26. New York, Springer Verlag.

Patarnello, T., S. Marcato, L. Zane, V. Varotto and L. Bargelloni (2003). Phylogeography of the *Chionodraco* genus (Perciformes, Channichthydae) in the Southern Ocean. Molecular Phylogenetics and Evolution 28: 420-429.

Pawlowski, J., J. Fahrni, J. Guiard, K. Conlan, J. Hardecker, A. Habura and S. Bowser (2005). Allogromiid foraminifera and gromiids from under the Ross Ice Shelf: morphological and molecular diversity. Polar Biology 28: 514-522.

Peck, L., P. Convey and D. Barnes (2006). Environmental constraints on life histories in Antarctic ecosystems: tempos, timings and predictability. Biological Reviews 81: 75-109.

Procheş, S. and D. Marshall (2002). Diversity and biogeography of southern African intertidal Acari. Journal of Biogeography 29: 1201-1215.

Pugh, P. (1993). A synonymic catalogue of the Acari from Antarctica, the sub-Antarctic Islands and the Southern Ocean. Journal of Natural History 27: 323-421.

Pugh, P. (1995). Littoral Mites of South Georgia. Journal of Zoology 236: 649-660.

Pugh, P. (1997). Acarine colonisation of Antarctica and the islands of the Southern Ocean: the role of zoohoria. Polar Record 33: 113-122.

Pugh, P. and P. Convey (2000). Scotia Arc Acari: antiquity and origin. Zoological Journal of the Linnean Society 130: 309-328.

Pugh, P. (2003). Have mites (Acarina: Arachnida) colonised Antarctica and the islands of the Southern Ocean via air currents? Polar Record 39: 239-244.

Quilty, P. and G. Wheller (2000). Heard Island and the McDonald Islands: a window into the Kerguelen Plateau. Papers and proceedings of the Royal Society of Tasmania 133: 1-12.

Ritchie, P., L. Bargelloni, A. Meyer, J. Taylor, J. McDonald and D. Lambert (1996). Mitochondrial Phylogeny of Trematomid Fishes (Nototheniidae, Perciformes) and the Evolution of Antarctic Fish. Molecular Phylogenetics and Evolution 5: 383-390.

Roderick, G. and R. Gillespie (1998). Speciation and phylogeography of Hawaiian terrestrial arthropods. Molecular Ecology 7: 519-531.

Sanchez, S., A. Dettaï, C. Bonillo, C. Ozouf-Costaz and G. Lecointre (2007). Molecular and morphological phylogenies of the Antarctic teleostean family Nototheniidae, with emphasis on the *Trematominae*. Polar Biology 30: 155-166.

Schulte, G. and G. Weigmann (1977). The evolotion of the family Ameronothridae (Acari: Oribatei). Ecological Aspects. Acarologia 19: 167-173.

Schulze, B. (1971). The climate of Marion Island. In: Marion and Prince Edward Islands (eds. E. Van Zinderen Bakker, J. Winterbottom and R. Dryer), pp. 16-31. Cape Town, AA Balkema.

Scott, L. (1985). Palynological indications of the Quaternary vegetation history of Marion Island (sub-Antarctic). Journal of Biogeography 12: 413-432.

Selkirk, P., R. Seppelt and D. Selkirk (1990). Subantarctic Macquarie Island: environment and biology. Cambridge, Cambridge University Press.

Skotnicki, M., J. Ninham and P. Selkirk (2000). Genetic diversity, mutagenesis and dispersal of Antarctic mosses – a review of progress with molecular studies. Antarctic Science 12: 363-373.

Skotnicki, M., A. Mackenzie, J. Ninham and P. Selkirk (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.

Skottsberg, C. (1960). Remarks on the Plant Geography of the Southern Cold Temperate Zone. Proceedings of the Royal Society of London. Series B, Biological Sciences 152: 447-457.

Smith, V. and R. Lewis Smith (1987). The biota and conservation status of sub-Antarctic islands. Environment International 13: 95-104.

Smith, V. and M. Steenkamp (1990). Climatic change and its ecological implications at a sub-Antarctic Island. Oecologia 85: 14-24.

Smith, V. (2002). Climatic change in the Sub-Antarctic: An illustration from Marion Island. Climate Change 52: 345-357.

Smith, V. and N. Gremmen (2004). The Flora of Marion and Prince Edward Island. The Netherlands.

Smith, V. and L. Mucina (2006). Vegetation of Subantarctic Marion and Prince Edward Islands. In: The Vegetation of South Africa, Lesotho and Swaziland (eds. L. Mucina and C. Rutherford), pp. 701–723. Pretoria, South African National Biodiversity Institute.

Starý, J., W. Block and P. Greenslade (1997). Oribatid mites (Acari: Oribatida) of sub-Antarctic Heard Island. Journal of Natural History 31: 545-553.

Starý, J. and W. Block (1998). Distribution and biogeography of oribatid mites (Acari: Oribatida) in Antarctica, the sub-Antarctic islands and nearby land areas. Journal of Natural History 32: 861-894.

Stevens, M. and I. Hogg (2003). Long-term isolation and recent range expansion from glacial refugia revealed for the endemic springtail *Gomphiocephalus hodgsoni* from Victoria Land, Antarctica. Molecular Ecology 12: 2357-2369.

Stevens, M. and I. Hogg (2006). Contrasting levels of mitochondrial DNA variability between mites (Penthalodidae) and springtails (Hypogastruridae) from the Trans-Antarctic Mountains suggest long-term effects of glaciation and life history on substitution rates, and speciation. Soil Biology and Biochemistry 38: 3171-3180.

Stevens, M., P. Greenslade, I. Hogg and P. Sunnucks (2006). Southern Hemisphere springtails: could any have survived glaciation of Antarctica? Molecular Biology and Evolution 23: 874-882.

Strong, J. (1967). Ecology of terrestrial arthropods at Palmer station, Antarctic Peninsula. Antarctic Research Series 10: 357-371.

Summer, P., K. Meicklejohn, J. Boelhouwers and D. Hedding (2004). Climate change melts Marion Island's snow and ice. South African Journal of Science 100: 395-398.

Varne, R., R. Gee and P. Quilty (1969). Macquarie Island and the cause of oceanic linear magnetic anomalies. Science 166: 230-232.

Verde, C., G. Lecointre and G. di Prisco (2007). The phylogeny of polar fishes and the structure, function and molecular evolution of hemoglobin. Polar Biology 30: 523-539.

Vijver, B and L. Beyens (1999). Biogeography and ecology of freshwater diatoms in Subantarctica: a review. Journal of Biogeography 26: 993-1000.

van Pletzen, R. and D. Kok (1971). Oribatei. In: Marion and Prince Edward Islands (eds. E. van Zinderen Bakker, J. Winterbottom and R. Dryer), pp. 16-31. Cape Town, AA Balkema.

Wallace, P., F. Frey, D. Weis and M. Coffin (2002). Origin and evolution of the Kerguelen Plateau, Broken Ridge, and Kerguelen Archipelago: Editorial. Journal of Petrology 43: 1105-1108.

Wallwork, J. (1963). The Oribatei (Acari) of Macquarie Island. Pacific Insects 5: 721-769.

Wallwork, J. (1966). Some Cryptostigmata (Acari) from South Georgia. British Antarctic Survey Bulletin 9: 1-20.

Wallwork, J. (1967). Cryptostigmata (Oribatid Mites). Antarctic Research Series 10: 105-122.

Wallwork, J. (1969). The zoogeography of Antarctic Cryptostigmata. In: Proceedings of the 2nd International Congress of Acarology, Budapest (ed. G. Evans), pp. 17-20. Budapest, Akadémiai Kiado.

Wallwork, J. (1970). Acarina (Cryptostigmata) of South Georgia. Pacific Insects Monograph 23: 168-178.

Wallwork, J. (1973). Zoogeography of some terrestrial micro-arthropoda in Antarctica. Biological Reviews 48: 233-259.

Wardle, P. (1991). Vegetation of New Zealand. Cambridge, Cambridge University Press.

Weston, P. and M. Crisp (1996). Trans-Pacific biogeographic patterns in the Proteaceae. In: The origin and evolution of Pacific Island Biotas, New Guinea to eastern Polynesia: Patterns and processes (eds. A. Keast and S. Miller), pp. 215–232. Amsterdam, SPB Academic Publishing.

Young, E. (1995). Conservation values, research and New Zealand's responsibilities for the Southern Ocean Island and Antarctica. Pacific Conservation Biology 2: 99-112.

Zane, L., L. Ostellari, L. Maccatrozzo, L. Bargelloni, B. Battaglia and T. Patarnello (1998). Molecular evidence for genetic subdivision of Antarctic krill (*Euphausia serperba* Dana) populations. Proceedings of the Royal Society of London B Biological Sciences 265: 2387-2391.

Zane, L., S. Marcato, L. Bargelloni, E. Bortolotto, C. Papetti, M. Simonato, V. Varotto and T. Patarnello (2006). Demographic history and population structure of the Antarctic silverfish *Pleuragramma antarcticum.* Molecular Ecology 15: 4499-4511.

CHAPTER TWO TO FIVE

This thesis is written in the form of scientific articles that were either published or will be submitted to an international peer-reviewed journal. The references of each chapter correspond to the prescribed format of the specific journal.

CHAPTER 2

MOLECULAR PHYLOGENY OF ANTARCTIC AMERONOTHROIDS: BIOGEOGRAPHIC COMPLEXITY UNVEILED

(will be submitted to *Molecular Phylogenetics and Evolution*)

ABSTRACT

Reported herein is the first broad-scale molecular phylogeny for a group of terrestrial ameronothroid mites, focussing on the genus *Halozetes* (Oribatida: Ameronothroidea), as a basis for understanding the evolution and biogeography of terrestrial taxa in the South Polar Region. Both mitochondrial (cytochrome oxidase subunit I (COI)) and nuclear (histone-3 (H3)) markers were used. A total of 10 species (114 samples) from 9 localities (Heard, Kerguelen, Marion, Macquarie, Possession, South Georgia and South Sandwich Islands as well as the Antarctic Peninsula and South Africa) were included. Parsimony searches (MP), maximum likelihood (ML) and Bayesian Inference (BI) based on the combined COI and H3 sequence dataset rendered similar tree topologies. Evidence suggested that the evolution of these mites (which have largely been restricted to the different habitat zones i.e. intertidal, supralittoral and terrestrial) had been driven largely by habitat specificity with niche availability being the key factor. Molecular data also suggested that this group was relatively young (<10myo) and that the biogeography was largely sculpted by multiple independent dispersal events. My molecular phylogeny also displayed considerable discourse with contemporary taxonomy suggesting the need for taxonomic revisions and reassessment of morphological characters. In short, the biogeographic history of the Antarctic in combination with sub-Antarctic islands is significantly more complex than originally anticipated and detailed studies of a similar nature to the one presented here are needed to further elucidate evolution and biogeography in the area.

INTRODUCTION

The biogeography of the South Polar Region is both complex and contentious. Its complexity derives from the compound history of the continent and its surrounding islands. Antarctica itself is an amalgam of a large, eastern Antarctic block and a western complex of accreted terranes (Clarke, 2003; Vaughan and Storey, 2000). Likewise, its surrounding islands have a variety of geological origins and histories, ranging from relatively young volcanic islands like the Prince Edwards (McDougall et al., 2001), to more complex older islands and archipelagos such as South Georgia (~120 million years old (myo)) and the Kerguelen islands (~29myo) (Chown et al., 1998; Peck et al., 2006; Wallace et al., 2002). The contention has come largely from a debate about the origins of terrestrial Antarctic biotas (Allegrucci et al., 2006; Greve et al., 2005; Marshall and Pugh, 1996; Morrone, 1998; Udvardy, 1987) and whether they are a consequence of dispersal or of vicariance.

Low species endemism in some groups, particularly the mosses (Bednarek-Ochyra, 2000; Ochyra et al., in press), combined with substantially more extensive glaciation of the Antarctic, and many of its surrounding islands, during the last glacial maximum than at present (Hall, 2002; Peck et al., 2006), has encouraged the view that the majority of terrestrial Antarctic species are relatively recent arrivals, with perhaps a few microbial or protozoan taxa being substantially older. However, this idea is controversial (e.g. Brundin, 1966, 1998; Craig et al., 2003; Darlington, 1970; Kuschel and Chown, 1995; Pugh, 2004; Wallwork, 1973), and several studies have shown that at least some parts of the Continental Antarctic biota have a vicariant origin and are likely pre-Pleistocene endemics (Allegrucci et al., 2006; Marshall and Coetzee, 2000). Consequently, the extent to which dispersal and vicariance has contributed to current biogeographic patterns in the terrestrial biota, and the relationship between the faunas of Antarctica and its surrounding islands (e.g. Barrat and Mougin, 1974; McInnes and Pugh, 1998; Wilkinson, 1990) remains vigorously debated (Chown and Convey, 2007; reviewed in Greve et al., 2005; Peck et al., 2006).

Perhaps one of the main reasons for the controversy is that most analyses have been based on species-level distributional data for taxonomically problematic groups (e.g. Gressitt, 1970; Greve et al., 2005; McInnes and Pugh, 1998; Morrone, 1998; Muñoz et al., 2004; Pugh, 2004). Although such approaches have provided valuable initial insights into the biogeography of the region, they are confounded by several problems that can be better resolved with phylogenetic information for taxa in the areas under consideration (for comprehensive discussion see Brooks and McLennan, 2001; Brooks and van Veller, 2003; Morrone and Crisci, 1995). The development of knowledge concerning the biogeography and evolution of notothenioid fish in the Antarctic readily illustrates this point. Early work, based on morphological phylogenies and species distributions, suggested a progressive adaptation of more derived groups to cold, high-Antarctic environments, and subsequent radiation in the latter to form a species flock (reviewed in Clarke and Johnston, 1996). It also suggested that most sub-Antarctic taxa predate the isolation of Antarctica. Recent molecular phylogenetic work challenged this perspective. For the notothenioids, several sub-Antarctic taxa emerged after a variety of Antarctic species. Also, these fishes were

characterized by a recurrent pattern of dispersal across the Antarctic Polar Frontal Zone, and divergence within it (Bargelloni et al., 2000). Similar patterns of dispersal and speciation across the Antarctic Polar Frontal Zone have been recorded in other marine taxa following molecular phylogenetic assessments (e.g. Page and Linse, 2002). Recently, Brandt and colleagues (2007) assessed the marine biodiversity of the Weddell Sea. They recovered a vast amount of previously undescribed taxa from this region (for example >70% ostacods and 88% isopods caught were new to science) emphasizing the high level of biodiversity that exists, contrary to assumption that diversity is deprived around the Antarctic. This high level of diversity could be attributed to shelf-ice retreat and advance which forced species into deeper water, facilitating multiple migrations between the continental shelf and abyssal plains. Additional support included high levels of genetic similarity found between species (for example *Epistominella exigua* (Brady, 1884) and *Oridorsalis umbonatus* (Reuss, 1851)) from the continental shelf and the Deep Sea. When considering benthos, the APFZ does not limit their distribution (as for pelagic species); rather it forms a connection between the Southern Ocean and other ocean basins.

In short, molecular work has revealed a level of marine biogeographic complexity in the region that was previously undetected. Although several terrestrial studies have noted the variability in relationships between different Antarctic areas and the surrounding islands (McInnes and Pugh, 1998; Morrone, 1998; Pugh et al., 2002; Pugh, 2004), none to date have resolved the reasons for this complexity, nor explicitly examined the question of whether the biogeography of the terrestrial biota shows similar patterns to that found in marine systems (but see Greve et al., 2005 for discussion). Although the marine and terrestrial environments differ considerably (Peck et al., 2006), there is no *a priori* reason why some form of congruence in the biogeographic patterns of their biotas could not exist, especially given the fact that both environments would have experienced substantial regional changes in climate through time (see Clarke, 2003), and that dispersal to the Antarctic and to its surrounding islands has been well documented (Greenslade et al., 1999; Marshall and Chalmers, 1997).

Undoubtedly the main reason for the current situation is the absence of molecular phylogenetic work for most terrestrial species. To date, most molecular work examined the population structure of specific species within given areas of the Antarctic or sub-Antarctic, especially in plants (reviewed in Skotnicki et al., 2000) and arthropods (Frati et al., 2000; Stevens and Hogg, 2003). Exceptions are one recent investigation of the moss *Ceratodon purpureus* (Hedwig) Bridel, 1826 (Skotnicki et al., 2004), the springtail *Cryptopygus* Willem, 1901 (Stevens et al., 2006) and several studies of seabirds (e.g. Burg and Croxall, 2001, 2004; Nunn, 1996), which can, however, be considered an essentially pelagic group (Chown et al., 1998). This situation is understandable given the large size of Antarctica and the difficulty of reaching its isolated fragments of ice-free land, as well as the wide distribution and isolation of the Southern Ocean islands. Indeed, many of these areas are only now being investigated (Bargagli et al., 2004; Convey et al., 2000ab; Convey and McInnes, 2005; Marshall and Chown, 2002; Stevens and Hogg, 2002).

Nonetheless, recent collaborative efforts within the Scientific Committee on Antarctic Research (http://www.scar.org/researchgroups/lifescience/eba/) have enabled cross-regional sampling to be undertaken. Therefore, I present here the first broad-scale molecular phylogenetic study of a group of terrestrial organisms, the ameronothroid mites, as a basis for understanding the evolution and biogeography of terrestrial taxa in the Antarctic region. I focussed on these mites since they often feature in biogeographical studies due to their limited dispersal capabilities, their abundance in the South Polar Region and their assumed status as an ancient group (e.g. Starý and Block, 1998; Wallwork, 1973). They are also abundant and well represented in the South Polar Region (Pugh, 1993; Starý and Block, 1998; Wallwork, 1973). Lastly, they are unique considering their ability to occupy a diverse array of habitats (intertidal, supralittoral and terrestrial) (Marshall and Convey, 2004; Pugh, 1993) which allowed me to test several biogeographical and evolutionary hypotheses. Such as, do congruent biogeographic patterns exist for both marine and terrestrial taxa in the South Polar Region? What are the relative divergence estimates for ameronothroids? Are the ameronothroids occupying the intertidal zone, older due to glaciation, than those in the terrestrial zone as previously suggested (for example Barendse et al., 2002; Wallwork, 1973)?

MATERIALS AND METHODS

Sample collection

The taxonomy of the superfamily Ameronothroidea (Oribatida) is uncertain with various proposed classifications. In general, it is thought that the superfamily comprises approximately 40 peri-Antarctic species partitioned into three genera namely *Podacarus* Grandjean, 1955; *Alaskozetes* Hammer, 1955 and *Halozetes* Berlese, 1917 (family Podacaridae). Although the focus of this study is on *Halozetes,* representative specimens of both *Alaskozetes* and *Podacarus* were also included in the analyses (refer to Table 2.1). Of the fifteen *Halozetes* species, I included eight *Halozetes* species (*H. fulvus, H. crozetensis, H. belgicae, H. marinus, H. marionensis, H. intermedius, H. macquariensis* and *H. capensis*) incorporating four subspecies (*H. m. devilliersi, H. m. marinus, H. b. micki* and *H. b. brevipilis*). At first, the closely related *Podacarus auberti* and *Alaskozetes antarcticus* (including *A. a. intermedius*) was selected as outgroup material. However, preliminary results indicated that both groups cluster within *Halozetes*. Therefore, the more distantly related oribatid taxa such as *Aquanothrus montanus* (Ameronothroidea) (believed to be an ancient species (Bayley, 1998)), *Magellozetes antarcticus* (Ceratozetoidea), *Macquarioppia striata* (Liacaroidea) and *Platynothrus skottsbergi* (Camisiidae), were included. The inclusion of successive outgroups can increase the accuracy of phylogenetic reconstruction (Graybeal, 1998). The islands included are Heard, Kerguelen, Macquarie, Marion, Possession, South Georgia and South Sandwich. Localities from the Antarctic Peninsula and South Africa (*H. capensis* and *A. montanus*) were also included (Figure 2.1). Where possible, two mite specimens (per species) from two geographically diverse locations per island / landmass were included (see Figure 2.1 and Table 2.1). This was done to provide some assessment of intra-island variation.

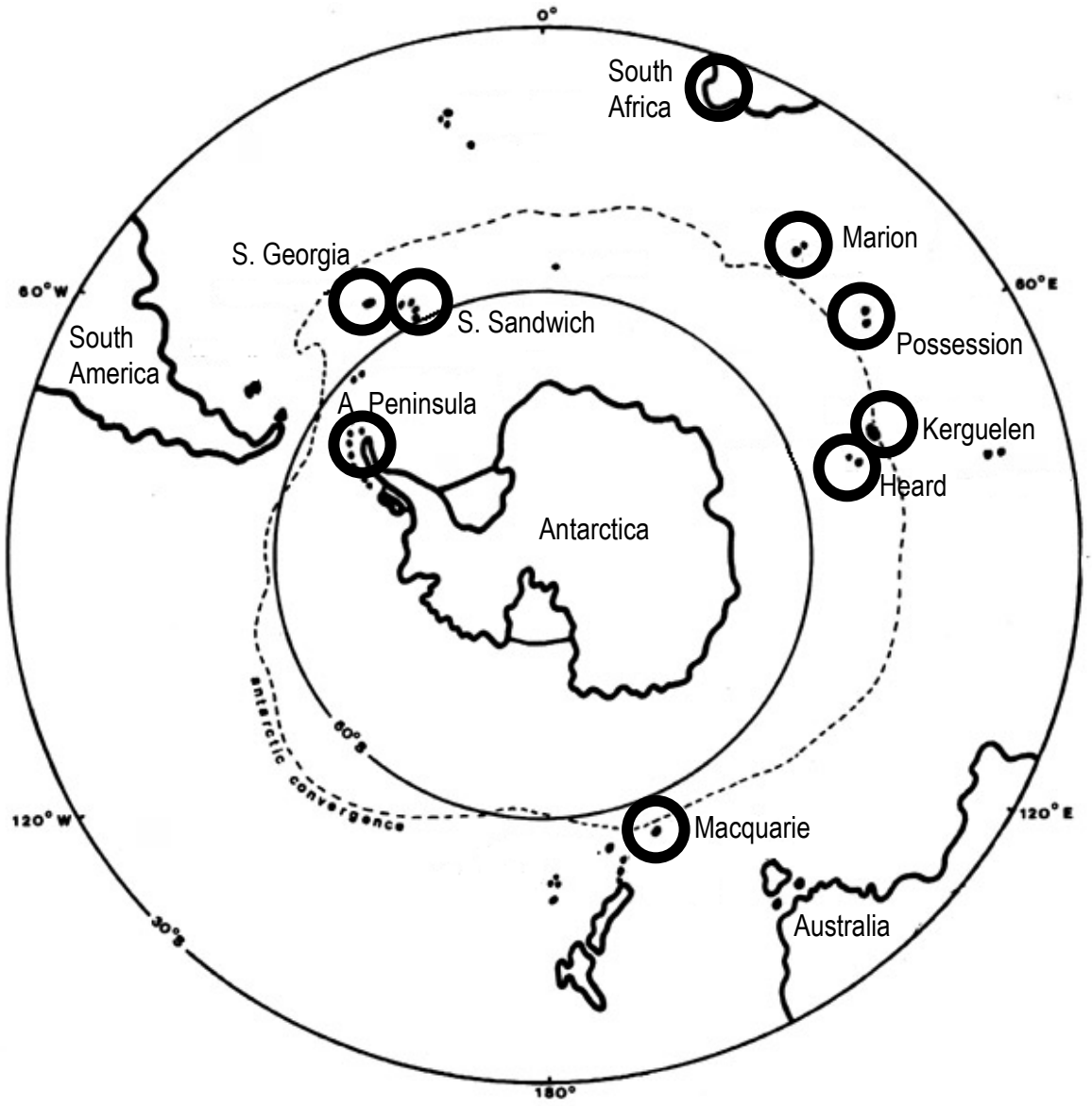


Figure 2.1 A map of the South Polar Region including sampling sites. More detail on the species and the localities on the specific islands can be obtained from Table 2.1. This figure was adapted from Smith and Gremmen (2004).

Table 2.1 Species are indicated in bold, followed by their respective authorities, the number of specimens as well as their respective localities. Whether the species inhabit terrestrial (T), supralittoral (S) or intertidal (I) zones are also indicated (Marshall and Convey, 2004; Pugh, 1993).

Species	T, S, I	Locality
Aquanothrus montanus Engelbrecht, 1975 (n=2)	T	Augrabies (AG), South Africa (n=2)
Magellozetes antarcticus (Michael, 1895) (n=4)	T/S	Schlieper Bay (SB), South Georgia (n=4)
Macquarioppia striata (Wallwork, 1963) (n=2)	T/S	Third Sister (TS), Marion Island (n=2)
Platynothrus skottsbergi Trägårdh, 1931 (n=2)	T	Hound Bay (H), South Georgia (n=2)
Podacarus auberti Grandjean, 1955 (n=10)	T	Goodhope Bay (GB), Marion Is (n=2) Archway Bay (AB), Marion Is (n=2) Atlas Cove (AC), Heard Is (n=4) Bird Island (BI), South Georgia (n=5) Schlieper Bay (SB), South Georgia (n=2)
Alaskozetes antarcticus (Michael, 1903) (n=27)	T/S	Goodhope Bay (GB), Marion Is (n=4) Base area (B), Kergeulen Is (n=2) Brothers Point (BP), Macquarie Is (n=2) Terrada Point (T), Brabant I (n=2) Leonie I (L), Marguerite Bay (n=2) Anchorage I (A), Marguerite Bay (n=2) Goudier I (G), Port Lockroy (n=2) Sven Foyn Harbour (SF), Lifeboat Is (n=2) Paradise Harbour (PH), Antarctic Peninsula (n=2)
Halozetes fulvus Engelbrecht, 1975 (n=4)	T	Trypot Beach (TB) (n=2) Bullard Beach (BL) (n=2)
Halozetes capensis Coetzee and Marshall, 2003 (n=1)	I	Kommetjie (K), South Africa
Halozetes crozetensis (Richters, 1907) (n=6)	T	Atlas Cove (AC), Heard Is (n=2) Val Studor (VS), Kerguelen Is (n=4)
Halozetes macquariensis (Dalenius and Wilson, 1958) (n=4)	T	Brother's Point (BP), Macquarie Is (n=4)
Halozetes belgicae (Michael, 1903) (n=26)	T/S	Macaroni Bay (MB), Marion Is (n=4) Base area (B), Possession Is (n=2) Atlas Cove (AC), Heard Is (n=2) Isle Australia (IA), Kerguelen Is (n=2) Base area (B), Kerguelen Is (n=2) Green Gorge (GG), Macquarie Is (n=2) Anchorage I (A), Marguerite Bay (n=2) Leonie I (L), Marguerite Bay (n=2) Cook I (C), South Sandwich (n=3) Schlieper Bay (SB), South Georgia (n=6)
Halozetes intermedius Wallwork, 1963 (n=8)	I	Isle Australia (IA), Kergeulen Is (n=2) Base area (B), Kergeulen Is (n=2) Green Gorge (GG), Macquarie Is (n=2) Brothers Point (BP), Macquarie Is (n=2)
Halozetes marinus (Lohmannn, 1907) (n=10)	I	Transvaal Cove (TC), Marion Is (n=4) Atlas Cove (AC), Heard Is (n=2) Buckles Bay (BB), Macquarie Is (n=2) Hasselborough Bay (HB), Macquarie Is (n=2)
Halozetes marionensis Engelbrecht, 1974 (n=4)	I	Macaroni Bay (MB), Marion Is (n=4)

DNA extraction

Total genomic DNA was isolated using a phenol / chloroform extraction method described by Maniatis et al., (1982) and used in Mortimer and Jansen van Vuuren (2007). Briefly, a single mite was placed in 550µl of DNA lysis buffer (1M NaCl, 1M Tris, 0.5M EDTA, 10% SDS solution) and 8µl Proteinase K (10mg/ml). The extraction was incubated at 55°C for 5 hours, phenol-chloroform extracted, ethanol precipitated and eluted in 20µl distilled water (Hillis et al., 1996). Conventional DNA extraction procedures sometimes failed to yield DNA of useable quality. In these cases, DNA was extracted with the DNeasy Tissue Kit (QIAGEN, Hilden, Germany) following the manufacturer's recommendations.

PCR and sequencing

The majority of studies investigating arthropod relationships are based on the mitochondrial cytochrome oxidase subunit I gene (COI) (e.g. Navajas et al., 1996; Simon et al., 1994; Söller et al., 2001). A drawback in the exclusive use of mtDNA characters is that the data reach saturation and become homoplasic at which point they suffer from the same problems as are often encountered when dealing with morphological data. To enhance phylogenetic signal at the deeper nodes, the nuclear histone-3 gene (H3) was included. In general, slow-evolving nuclear genes have proven to be useful at taxonomic evaluation among invertebrates (Matsuo and Yamazaki, 1998; Matsuo, 2000).

The universal primers designed by Folmer et al., (1994) were used for amplification of COI resulting in a fragment of 494bp. The H3 primers H3AF and H3AR (Colgar et al., 1998) had limited success with the amplification of *Halozetes* DNA. Hence, species-specific primers were designed from the sequence alignment of the few successfully amplified specimens. The sense primer (HIST3F: CGTAAGTCGGCGCCCAGC) and the antisense primer (HIST3R: GACCCGTTTGGCGTGAATTGC) successfully amplified a 320bp fragment. This fragment corresponded to the 2nd exon (partial), 2nd intron and the 3rd exon (partial) of the H3 gene of *Drosophila hydei* (Akhmanova et al., 1995).

Mitochondrial and nuclear PCR reactions were carried out in 30µl reaction volumes and included 2µl of genomic DNA, 3µl 10X reaction buffer, 3µl of 25mM $MgCl_2$, 3µl of 1mM dNTP solution, 30pmol of each primer and 1 unit of Taq polymerase (Super-therm, Southern Cross Biotechnology, Cape Town, South Africa). The final volume was adjusted with deionised distilled water. All PCR reactions were done under similar cycling parameters which included an initial denaturation step at 94°C for 1 minute followed by 35 cycles of 94°C for 30 seconds, 40°C (COI) or 50°C (H3) for 30 seconds, 72°C for 45 seconds. A final extension step at 72°C for 5 minutes completed the reactions.

Amplicons were electrophoresed in 1% agarose gels. Bands were excised and purified with the Wizard SV Gel and PCR clean-up system (Promega, Madison, USA) according to the manufacturer's recommendations.

Nucleotide sequencing was carried out using BigDye® Terminator 3.1 mix (Applied Biosystems, Warington, UK). Sequencing cocktails were cleaned using Centrisep spin columns (Applied Biosystems, Warington, UK) and the products were analyzed on a 3100 automated sequencer (Applied Biosystems, Warington, UK). Electropherograms of the raw data were manually checked and edited with Sequence Editor™ software v1.0.3a (Applied Biosystems, Warington, UK).

Sequence analysis

The gene sequences (COI and H3) were aligned with Clustal X 1.81 (Thompson et al., 1997) using the multiple alignment mode. All alignments were checked for alignment errors and for stop codons. Uncorrected nucleotide *p*-distances as well as the AT/GC-content were calculated in MEGA 3.1 (Kumar et al., 2000). The transition to transversion ratio was determined using Treepuzzle 5.2 (Schmidt et al., 1999).

Phylogenetic analysis

Phylogenetic analysis included parsimony searches (MP), maximum likelihood (ML) and Bayesian Inference (BI). All analyses were performed on (a) the mitochondrial COI gene singly, (b) the single H3 gene and (c) the combined COI and H3 datasets after the partition homogeneity test indicated no conflicting signal in the data (p=1.000). Bootstrap values exceeding 75% (MP and ML) and posterior probabilities higher than 0.95 (BI) were considered to be statistically well supported (refer to Hillis and Bull, 1993; Hillis and Huelsenbeck, 1995). These different phylogenetic estimation methods (MP, ML and BI) were applied to check that the trees obtained were not an artefact of the method used.

MP, employing the heuristic search criterion with TBR branch swapping, was implemented in PAUP* 4.0b10 (Swofford, 2000). Nodal support was assessed through 1 000 bootstrap replicates. Bremer support values, indicating the number of unambiguous changes along branches (Bremer, 1994), were calculated in TreeRot 2c f (Sorensen, 1999) in conjunction with PAUP*. According to Wahlberg and Nylin (2003), values between five and eight were considered as good support, while values larger than eight were considered as strong support. Both the consistency index (CI) and retention index (RI) were calculated in PAUP*.

Modeltest 3.06 (Posada and Crandall, 1998) was used to determine the model of evolution that best fitted the data. The Akaike Information Criterion (AIC) was preferentially used because it minimizes those parameters that do not contribute phylogenetic information (Bernham and Anderson, 2002; Nylander et al., 2004). ML searches were performed in PAUP*. Given computational time limitations, I used PhyML 2.4.4 (Guindon and Gascuel, 2003) to assess the robustness of nodes through 1000 bootstrap replicates. Following this, a strict consensus tree was constructed using Consense in Phylip 3.5c (Felsenstein, 1993). The Shimodaira and Hasegawa (1999) test was conducted on the combined ML tree to assess the robustness of contemporary taxonomic boundaries to alternative hypotheses.

Posterior probabilities for the BI analysis were determined by running one cold and three heated chains for 5 000 000 generations in MrBayes 3.1.1 (Ronquist and Huelsenbeck, 2003). My combined analysis, which comprised two gene fragments, was analyzed in a partitioned manner to allow the selection of different optimal models for each partition (Ronquist and Huelsenbeck, 2003). The optimal model, selected under AIC criteria (see above), was specified as prior for each partition. Each analysis was repeated and trees were sampled every 100 generations. Stability was determined using the sump command in MrBayes and the first 35 000 generations were excluded as burn-in. Posterior probabilities for nodes were calculated from the remaining topologies.

Molecular clock

Two methods were implemented to estimate the divergence time of the ameronothroid mites, namely a relaxed Bayesian clock and a conventional molecular clock approach. The relaxed Bayesian clock method is designed for multigene data and uses relative rates. Two software packages are included namely PAML 3.15 and Multidivtime 3.15 (Thorne and Kishino, 2002). In the analysis, I used a simplified version of the combined tree as the reference tree and included both gene datasets. In short, the Markov chain was selected every 10 000 generations of every 100th cycle. The burn-in was set to 10 000. Default parameters were used except for a few specified priors where "rttm" (expected time between root and the tip) was set to 20mya (SD=20mya), "rtrate" (rate of evolution at the ingroup node) was set to 0.003 (SD=0.003) and the "bigtime" (highest number of time units between the root and the tip) was set to 150mya (approximate time of the Gondwana break-up). Both the prior and posterior were estimated to determine if the clock results were based on the data or the priors. The unconstrained tree was used to interpret the different rates (or ages) for each species. All the rates were divided by the "bigtime" prior. Due to the lack of fossil data to calibrate the dates, the rate obtained for *H. fulvus* was standardized to an upper and lower boundary of 1.0mya and 0.5mya. These dates were selected given that *H. fulvus* is endemic to Marion Island and these are believed to be the approximate ages of the island.

In terms of the conventional molecular clock, it is generally thought that the COI gene in arthropods evolves at an average rate of 2% to 2.3% per million years (Brower, 1994; DeSalle et al., 1987). Salomone et al. (2002) used the average of this rate (2.15% per million years) to determine the divergence times for oribatid mites of the family S*teganacaridae* on the Canary Islands. Thus, a rate of 2.15% per million years was applied to the COI data to estimate divergence times within the clades. It is important to note that all these dates should be seen only as estimates, especially because the standard errors associated with them are large.

Biogeographic analysis

Two approaches were used to investigate the biogeographic history of ameronothroid mites in the Southern Oceans: The first (DiVa 1.1; Ronquist, 1997) is an event-based method that considers vicariance, dispersals and extinctions (but see Brooks and McLennan, 2001; Yuan et al., 2005). The second (LaGrange 1.0.1; Ree et al., 2005) is a likelihood approach that incorporates a parametric inference which takes both anagenetic and

cladogenetic changes into account. For DiVa, I defined two geographic units namely sub-Antarctic (Marion, Heard, Kerguelen, Macquarie, South Georgia and Possession Islands) and Maritime Antarctic (Antarctic Peninsula and South Sandwich Islands), because I was interested in dispersal across the Antarctic Polar Frontal Zone (APFZ). Analyses were based on a simplified version of the combined (COI and H3) tree (all duplicate taxa were trimmed from the tree and I retained a single representative specimen per species). The current distribution of each species was coded as present or absent in each of these areas. For the DiVa analyses, I set the "maxareas" option of the "optimize" command to two. For LaGrange, I set the "root age" to 10 million years ago (mya) and 20mya, respectively, because of the uncertainty around the age of this node. Similar to the DiVa analyses, I used the simplified version of the combined tree and specified two geographical units (sub-Antarctic and Maritime Antarctic).

RESULTS

Largely congruent results were found irrespective methods of analyses or whether genes were analyzed singly or in combination. I therefore presented my main description of the phylogeny only for the combined analyses. I did, however, highlight important phylogenetic aspects pertinent to the single gene analyses.

Gene analysis

Combined analysis

The combined data set comprised 708bp (H3: 214bp; COI: 494bp) (see single genes for details; sequences will be submitted to GenBank prior to manuscript submission and voucher specimens will be submitted to the National Museum in Bloemfontein, South Africa) for 111 specimens. The optimal model that provided the best fit for the combined data was TVM+I+G (I=0.5345; G=0.5870; base frequencies A=0.2926, C=0.1773, G=0.1430 and T=0.3871; and the rate matrix R(a) [A-C]=0.5023, R(b) [A-G]=6.2954, R(c) [A-T]=0.6405, R(d) [C-G]=0.9836, R(e) [C-T]=6.2954 and R(f) [G-T]=1.000), and this model was used in ML searches. BI analyses were run with the optimal models specified for each of the two character partitions (COI and H3; see below for optimal model selected). MP analysis resulted in 1598 equally most parsimonious trees (tree length=1502, CI=0.3182, RI=0.8539).

Congruent topologies were retrieved irrespective of the method of analyses (MP, ML and BI). The ML topology is shown in Figure 2.2. When considering the outgroups, the combined analyses (as well as the single gene analyses) showed minor discrepancies in the exact placement of the outgroup taxa. For all the analyses (singly and combined), the relative position of the distantly related outgroups, *M. antarcticus* and *M. striata,* was unclear. The combined analyses do, however, show that *M. antarcticus* was paraphyletic. The two closely related outgroups *Alaskozetes antarcticus* (MP=96, ML=99, BI=98, Bremer=16) and *Podacarus auberti* (MP=100, ML=100, BI=100, Bremer=11) both clustered within the ingroup *Halozetes*. In addition, not all the species included in my analyses proved to be monophyletic. *Halozetes belgicae* was paraphyletic, with specimens from

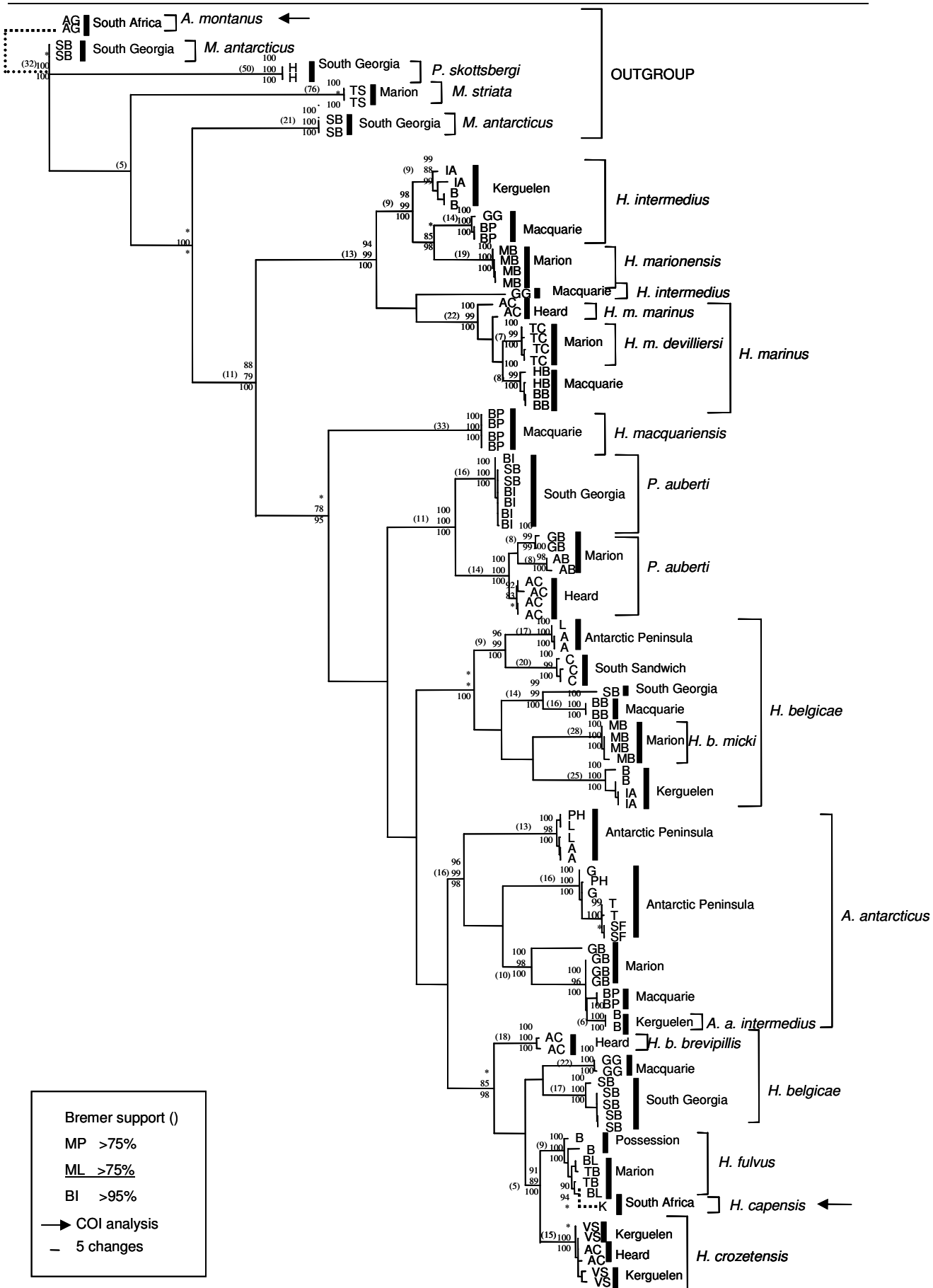


Figure 2.2 See legend on following page

Figure 2.2 Maximum Likelihood topology (-ln*L*=7295.55) as constructed for the 708bp combined COI and H3 gene segments. The numbers above the branches indicate bootstrap support for Parsimony searches (1000 replicates) and Maximum likelihood (1000 replicates), respectively, while the posterior probabilities of Bayensian Inference (5 million generations) are indicated underneath the branch. Bremer support values are indicated in brackets (only values >5). Specimens for which only COI sequences were available are indicated with arrows. The specimen names refer to the sampling localities and are presented Table 2.1.

Heard, Macquarie and South Georgia islands forming part of a more derived clade, and those from Marion, Heard, Macquarie and the South Sandwich islands, and the Antarctic Peninsula forming part of a more basal clade. *H. intermedius* was also paraphyletic. The remainder of the species were monophyletic.

Two main groups based on habitat preference were discernable in all topologies. The one group comprised the interidal mite species (*H. marinus*, *H. intermedius* and *H. marionensis*) (MP=94, ML=99, BI=100, Bremer=13), while the supralittoral / terrestrial mite species (*H. macquariensis, P. auberti, A. antarcticus, H. belgicae*, *H. crozetensis* and *H. fulvus*) (ML=78, BI=95) formed a clade.

H3

In the nuclear H3 gene the exons were conserved across all taxa. The intron region of both the ingroup and outgroup species was highly variable and could not be aligned. Because of this, the intron region was removed from further analyses. Phylogenetic analyses of the H3 gene were therefore based on 214bp of the H3 gene (2nd and 3rd exon and a small portion of the 2nd intron that could be unambiguously aligned). When the outgroup taxa were included, the 214bp fragment had 65 variable sites of which all were parsimony informative. When considering only the ingroup, 35 characters were variable and all were parsimony informative. The H3 gene had a mean GC-content of 50.3%. The transition to transversion ratio (Ts:Tv) was 0.99:1.

ML and BI recovered topologies that were largely congruent with the MP topologies. The substitution model that provided the best fit for the nuclear data was K80+G (G=0.2739 and the base frequencies were equal). MP analyses resulted in 24 equally most parsimonious trees (tree length=94, CI=0.8085, RI=0.9584).

The trees based on the nuclear data were better resolved at the deeper nodes with less resolution for more recent clusterings (data not shown). Of the distantly related oribatid outgroups, I was unsuccessful with the amplification of the H3 gene of A. *montanus*. Thus, only *M. antarcticus* and *M. striata* were included. Also, the H3 gene of *H. capensis* failed to amplify. Similar to the combined analyses, the closely related outgroups (*A. antarcticus* and *P. auberti*) grouped within *Halozetes* and *H. belgicae* proved to be paraphyletic.

Uncorrected divergence values separating species ranged between 0.1% (*H. marionensis* vs *H. intermedius*) to 24.8% (*M. striata* vs *H. macquariensis* and *M. striata* vs *P. auberti*). Divergence values within species were low with several species, such as *H. crozetensis*, *H. marionensis*, *H. macquariensis* and *M. striata,* having no intra-specific variation. The largest within species divergence was 2.2% within *M. antarcticus* and 1% within *H. belgicae, A. antarcticus* and *H. marinus.*

COI

A 494bp fragment of the COI gene was amplified and sequenced for 114 specimens. This fragment contained no stop codons. When including the outgroup taxa, 244 characters were variable and 230 parsimony informative. When considering only the ingroup, these values decreased only slightly to 208 variable and 197 parsimony informative characters. The Ts:Tv was estimated to be 2.2:1.

Phylogenetic trees were largely congruent irrespective of method of analyses (MP, ML and BI). When considering the ML and BI analyses, the model of substitution that provided the best fit for the data was TVM+I (invariance= 0.4580)+G (gamma=0.6339); base frequencies A=0.2859, C=0.1672, G=0.1252 and T=0.4218; and the rate matrix R(a) [A-C] = 0.4370, R(b) [A-G]= 6.5954, R(c) [A-T]=0.5348, R(d) [C-G]=0.7116, R(e) [C-T]=6.5954 and R(f) [G-T]=1.000). The MP search resulted in 2400 equally most parsimonious trees (tree length=1680, CI=0.2708, RI=0.8293).

In contrast to the H3 gene, the tips of the trees were well resolved whereas deeper nodes collapsed following rigorous statistical testing (data not shown). Minor discrepancies were evident in the exact placement of the outgroup taxa, however, as expected *Aquanothrus montanus* always grouped basal to all the other species (ML=100, BI=100, Bremer=32). Interestingly, the single *H. capensis* specimen from South Africa (Kommetjie) grouped within *H. fulvus* (MP=100, ML=99, Bremer=11). The remainder of the phylogenetic findings were similar to the combined analyses.

The mean AT-content of the COI gene was 63.5% increasing to 76.3% when only the 3rd codon position was considered. When the tree topology was ignored and only the contemporary taxonomy considered, the uncorrected nucleotide divergences between species ranged from 2.6% (*H. capensis* vs *H. fulvus*) to 25.1% (*A. montanus vs H. capensis*). Intra-specific variation ranged from 0% (four *H. macquariensi*s specimens were characterized by a single haplotype) to 14.8% (*H. belgicae* collected from Marion, Kerguelen, Possession, Macquarie, South Georgia, South Sandwich Islands as well as the Antarctic Peninsula).

SH test

To verify whether *H. belgicae* and *H. intermedius* represented monophyletic species or whether they were indeed paraphyletic as the phylogenetic analyses suggested, a SH test (Shimodaira and Hasegawa, 1999) was

implemented. Enforcing the monophyly of *H. intermedius*, the constraint tree was not statistically worse (–ln*L* 7295.47, *p*=0.299) than the best unconstrained tree. On the contrary, enforcing the monophyly of *H. belgicae*, the constraint tree was statistically worse (-ln*L* 7333.69, *p*=0.004), thus supporting the paraphyly of *H. belgicae*.

Molecular Clock

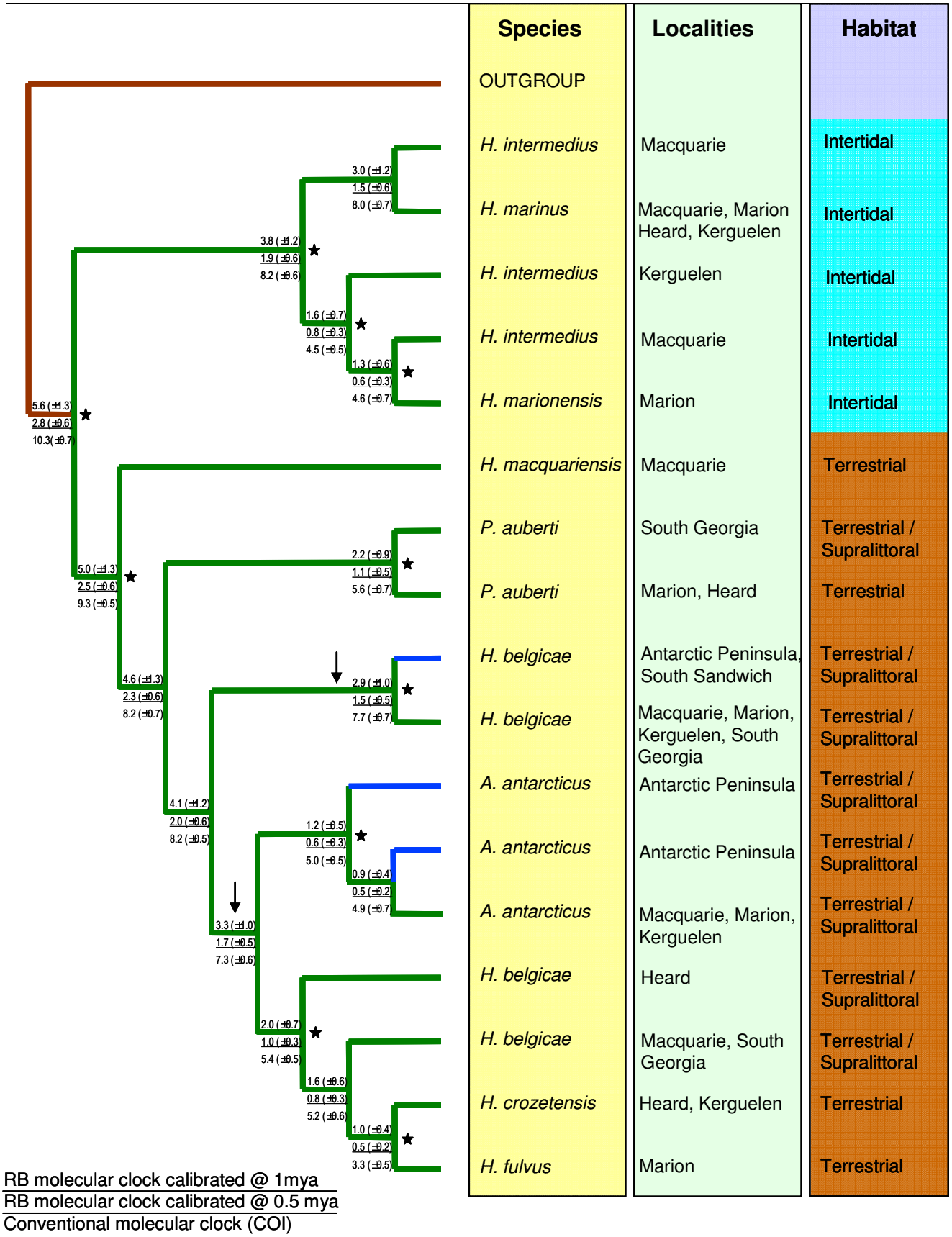
In general, the conventional molecular clock estimates were slightly higher than the relaxed Bayesian clock estimates (Figure 2.3). However, both analyses indicated that the *Halozetes* group was relatively young. Applying the relaxed Bayesian clock, and keeping in mind that these dates have large errors associated with them, the divergence time between intertidal species (*H. marinus*, *H. intermedius* and *H. marionensis*) and the terrestrial / supralittoral species (*H. macquariensis, P. auberti, A. antarcticus, H. belgicae*, *H. crozetensis* and *H. fulvus*) was estimated to be 2.8(±0.6) to 5.6(±1.3)mya. The divergence estimates of *Halozetes* species (including *Antarcticus* and *Podacarus*) ranged from 0.6(±0.3)-1.3(±0.6) (*H. marionensis* vs *H. intermedius*) to 2.5(±0.6)-5.0(±1.3)mya (*H. macquariensis* vs *P. auberti*, *A. antarcticus*, *H. belgicae*, *H. crozetensis* and *H. fulvus*) (see Figure 2.3 for more details).

Biogeography

Even though DiVa has been criticised (for example Brooks and McLennan, 2001; Yuan et al., 2005), both DiVa and LaGrange indicated multiple dispersal events between the sub-Antarctic and Maritime Antarctic (Scotia Arc excluding South Georgia Island) region. The analyses conducted for the species' entire distribution range compared to the specific localities included in this study, rendered similar results. According to the DiVa analyses, two independent dispersal events occurred (see Figure 2.3). Since DiVa is parsimony based, I could not discern whether the two dispersals represent two independent dispersals to Maritime Antarctic or one dispersal to Maritime Antarctic and one dispersal back to the sub-Antarctic. For La Grange, the log-likelihood estimates indicated three major dispersal events between sub-Antarctic and Maritime Antarctic (see Figure 2.3). The highest likelihood of La Grange indicated that three independent dispersals occurred to Maritime Antarctic. The different "root ages" (i.e. 10mya and 20mya) rendered similar results.

DISCUSSION

My results suggest that dispersal biogeography has been an important mechanism for shaping the biotic communities in the South Polar Region. My results further suggest that the evolution of these mites was habitat specific with niche occupation being a possible driver for diversification. Molecular data show this group to be relatively recent and certainly post date large scaled continental drift. Current taxonomic designations based on morphology are in conflict with my molecular topology, suggesting taxonomic revisions and reassessment of alpha taxonomic characters.



★Bootstrap values >75% and / or posterior probabilities >95%

Figure 2.3 See legend on next page.

Figure 2.3 The simplified topology of the combined (COI and H3) ML tree with the conventional COI nucleotide clock estimates as well as the relaxed molecular Bayesian estimates (RB) plotted on each node (see Figure 2.2 for original ML tree). The localities and habitats are indicated next to each species. The blue lines represent major dispersal events that were found using LaGrange (Ree et al., 2005) and the arrows indicate the DiVa results (Ronquist, 1997).

Biogeography

When considering my tree topology, it becomes evident that sub-Antarctic and Maritime Antarctic taxa are intermingled with little congruence between the distributional data and phylogenetic clustering. For example, *H. intermedius* and *H. macquariensis*, both species with restricted distributions (<3 sub-Antarctic islands) appeared to be older than wide-spread species such as *A. antarcticus* and *H. crozetensis* (Figure 2.3, see Marshall and Convey, 2004; Pugh, 1993 for distributional data). Biogeograpical analyses (DiVa and LaGrange analyses) indicated multiple independent dispersals of amoronothroid mites between Maritime Antarctic (Scotia Arc excluding South Georgia Island) and sub-Antarctic (i. e. across the APFZ), a finding that was also clear when considering sampling localities on my phylogenetic tree (Figure 2.3). Even though my findings were based on a single specimen and single gene fragment (COI), it is clear that *H. capensis* was extremely closely related to *H. fulvus* (Marion Island). Notwithstanding current taxonomy (*H. capensis* may be a synonym of *H. fulvus*); the presence of this specimen on Marion Island constitutes a dispersal either to or from the African continent.

When comparing my results to a previous study conducted on the moss species, *Ceratodon purpureus,* a similar scenario was evident. The authors used nuclear ITS-1 and RAPD data to infer genetic variation within and between populations collected from various localities in the Antarctic, sub-Antarctic (Heard, Macquarie Island and New Zealand) and Australia. Again, sub-Antarctic and Antarctic sequences were intermingled. The ITS-1 sequences of Australia grouped sister to the remainder of the localities. The sequences from Heard Island and Europe shared similarities, while Macquarie Island sequences were more related to the Antarctic sequences. Similar to the mites, this may imply multiple independent dispersals in the Antarctic region (Skotnicki et al., 2004).

Even more fascinating is that the pattern retrieved among in terrestrial taxa (moss and mites) is also found in the marine environment, specifically in Antarctic notothenioid fish and bivalve fauna. Bargelloni and colleagues (2000) sequenced the 16S and 12S ribosomal DNA for the notothenioid fishes. They also found that a complex pattern existed in these fishes and that the Antarctic species could not be separated from sub-Antarctic species. For example the Harpagiferidae, which is mostly restricted to the sub-Antarctic waters, grouped sister to the Artedidraconidae, which are found in the waters close to Antarctica. The authors suggested a recurring pattern of dispersal across the APFZ with subsequent diversification for some of these fishes. Similar results were obtained for the bivalve, *Limatula* Searles-Wood, 1839 (Page and Linse, 2002). The phylogenetic tree (18S, 16S and ITS-

1) showed the mixed groupings of sub-Antarctic and Antarctic specimens. Again it was suggested that dispersal across the APFZ coupled with speciation occurred.

Habitat specificity

It is generally accepted that life on earth originated in the sea. The ancestral aquatic arthropods gradually progressed from the fringes of coastal areas onto the land where they steadily evolved into the magnitude of terrestrial insects and other hexapods known today (McGavin, 2001; Vermeij and Dudley, 2000, and references within). Specifically referring to oribatid mites, Procheş and Marshall (2001) argued that the ancestor to this group evolved on land although contemporary species are largely associated with marine and intertidal environments. Bernini and colleagues (2000) took the opposite view and suggested that oribatid mites evolved in the sea. Wallwork (1973) was the first to propose, based on zoography, that the more ancestral species within the Antarctic Ameronothroidea (which includes *Halozetes*) were the intertidal taxa (not fully aquatic but similarly not completely terrestrial), specifically *H. marinus* and *H. littoralis*, and not the terrestrial species. Since then studies like Barendse and colleagues (2002) confirmed this for Marion Island based on the relative ages of the different vegetation types and habitat specificity of mites. They argued that these older intertidal habitats acted as refugia for species during glaciation periods when terrestrial environments were largely covered by ice. The pattern in the Northern Hemisphere appeared somewhat different with Schulte and Weigmann (1977) arguing that the Ameroidea, specifically *Ameronothrus* Berlese, 1896, used terrestrial areas as refugia during glaciation and that these terrestrial species are therefore ancestral.

I speculate that the evolution of the ameronothroid mites (which are restricted to the different habitat zones i.e. intertidal, supralittoral and terrestrial) has been driven largely by habitat specificity with niche availability. This was supported by the tree topology which indicated two well-supported clades corresponding to habitat preference (intertidal and terrestrial / supralittoral) (see Figure 2.3). The availability of suitable outgroups hampered robust conclusions regarding to ancestral state of the outgroups confined to terrestrial habitats and to intertidal zones. The other member of the family Podacaridae, *Antarcticola* Wallwork, 1967, occurs in both the intertidal and supralittoral environments (Pugh, 1993). However, given the uncertainty regarding the taxonomy of this group (the other outgroup taxa such as *Alaskozetes* and *Podacarus* grouped within *Halozetes*) and the fact that *Antarcticola* was not included here, complicated evolutionary inferences. Thus, at this stage it is impossible to determine which group (intertidal or terrestrial / supralittoral) is more primitive or derived since the intertidal and terrestrial / supralittoral clades formed sister groups.

Molecular clock

A conventional molecular clock as well as a relaxed Bayesian clock was applied to the data to determine divergence dates. Molecular clocks are dependent on the genes used; therefore these dates should only be seen as estimates, especially since no fossil data are available for calibration. The estimates of both clocks indicated

that this ameronothroid mite group was young (<10myo). This is contrary to the belief that it is an ancient group. The distribution of the ameronothroid mites in the South Polar Region lead Wallwork (1973) to believe that the existence of these mites predates the break-up of the southern continents. The estimates are also consistent with molecular clock calculations for *Limatula* (for 16S: 1.36-8.03myo; for 18S: 6.81-19.12myo and for ITS-1: 0.24-2.87myo) and most of the notothenoid fishes (5-12myo). Thus, the molecular data indicated that ameronothoid mites together with several other groups, diverged after the APFZ was formed since the Drake Passage opened ~30-35mya (Livermore et al., 2005; Livermore et al., 2007). According to my tree topology, *H. belgicae* was the first species to cross the APFZ during the Pliocene, followed by *A. antarcticus,* probably on two separate occasions during the Pliocene-Pleistocene boundary.

According to my divergence estimates, episodic and rapid evolution in the ameronothroid mites may have occurred during the Miocene (23.5–5.3mya), Pliocene (5.3–1.8mya) as well as at the beginning of the Pleistocene (1.8–0.10mya) epochs. During this time, the earth experienced major global temperature cooling partly due to plate tectonics as well as paleogeographic and paleoceanographic events. The South Polar Region was enclosed by a circum-polar ice sheet and the circum Antarctic current also intensified. Glaciation in the Antarctic peaked during the Middle Miocene and together with polar cooling, had mayor affects on the Indian Ocean basin (Banakar and Hein, 2000; Williams and Handwerger, 2005; Zachos et al., 1992). In addition, most of the sub-Antarctic islands that had already surfaced by this time were geologically dynamic as evidenced by the Kerguelen Plateau (Bohaty et al., 2003) and the origin of the stratovolcano on Possession Island (Bellair, 1964; Lameyre and Nougier, 1982). During this dynamic period, it is very likely that a niche for sub-Antarctic marine mites could have developed (S. Proches, personal communication). The sub-Antarctic islands' position relative to the APFZ that originated ~30-35mya, as well as the movement of the APFZ further influenced the climate (and also speciation) on different islands (Hall, 1990).

Taxonomy

For the ameronothroid mites, which are distributed across the Maritime and sub-Antarctic region, discrepancies existed between molecular and morphological data. A similar scenario was apparent in the South Polar marine system, referring to the notothenioid fishes, discrepancies also existed between molecular and morphological data. Using mitochondrial markers (16S and 12S), the authors emphasized the lack of monophyly that exist for most notothenioid species that are distributed in both Antarctic and sub-Antarctic waters (Bargelloni et al., 2000). My data showed clear inconsistencies between the contemporary taxonomy and molecular evidence. What is proposed is a full taxonomic revision for the genus *Halozetes*, and indeed the larger ameronothroid group where detailed morphological investigations may help to resolve "unexpected" relationships when taking traditional classifications into account. The most obvious and surprising inconsistencies in terms of taxonomy were *Alaskozetes antarcticus* and *Podacarus auberti*, my original closely-related outgroup taxa, that nested within *Halozetes*. I propose according to my genetic data, that the generic status of *Podacarus* and *Alaskozetes* should

be reassessed since these do not constitute robust genera. This implies that the current morphological characters being used for taxonomic identification are insufficient.

The topology indicated that both *H. belgicae* and *H. intermedius* were paraphyletic. When enforcing the monophyly of *H. intermedius*, the constraint tree was not statistically worse, therefore I cannot statistically support the paraphyly of this genus. Importantly, only a single specimen from Macquarie Island clustered outside of the main *H. intermedius* group, and it is likely that this aberrant placement may be the result of statistical analyses rather than true taxonomy. In contrast, the SH test unequivocally indicated a paraphyletic origin for *H. belgicae*. Taking into account that *H. belgicae* is the most widespread of all *Halozetes* species (Marshall and Convey, 2004; Pugh, 1993; Wallwork, 1973), the results are perhaps not that surprising. It has also been found that there are various morphological differences including individual differences present in this species (Marshall, personal observation). This implies that species boundaries are not well-defined with any robust morphological characters.

Other terrestrial studies focussing on groups such as *Cryptopygus antarcticus travei* Déharveng, 1981 (Collembola) that is similarly widespread in the South Polar Region, can also be described as complex. Stevens and colleagues (2006) have shown that several paraphyletic relationships exist for *Cryptopygus* species. These findings, when taken in concert with mine, indicate that analyses based on species distributional data of taxonomically difficult groups can be problematic. Adequate molecular and morphological taxonomic knowledge of the chosen taxa may be a prerequisite before attempting to explain the complex biogeography in the South Polar Region.

To conclude, the taxonomy of the ameronotroid mites is in need of revision and my molecular study may provide a guideline for the future morphological studies that will be conducted on *Halozetes.* A similar pattern of mixed groupings of sub-Antarctic and (Maritime) Antarctic taxa was observed in both terrestrial and marine environments. It was proposed that multiple independent dispersals of taxa occurred across the APFZ with diversification within it. The molecular dates indicated that the ameronothroid mites were a young group and diverged after the formation of the APFZ. I also found evidence that the evolution of the ameronothroid mites was driven by habitat specificity. Thus, the biogeographic history of the Antarctic in combination with sub-Antarctic islands is significantly more complex than originally anticipated and detailed studies of a similar nature to the one presented here are needed to elucidate evolution and biogeography in the area.

REFERENCES

Akhmanova, A. S., Bindels, P. C., Xu, J., Miedema, K., Kremer, H., Hennig, W., 1995. Structure and expression of histone H3.3 genes in *Drosophila melanogaster* and *Drosophila hydei*. Genome 38, 586-600.

Allegrucci, G., Carchini, G., Todisco, V., Convey, P., Sbordoni, V., 2006. A molecular phylogeny of Antarctic chironomidae and its implications for biogeographical history. Polar Biol. 29, 320-326.

Banakar, V. K., Hein, J. R., 2000. Growth response of a deep-water ferromanganese crust to evolution of the Neogene Indian Ocean. Mar. Geol. 162, 529–540.

Bargagli, R., Skotnicki, M. L., Marri, L., Pepi, M., Mackenzie, A., Agnorelli, C., 2004. New record of moss and thermophilic bacteria species and physico-chemical properties of geothermal soils on the northwest slope of Mt. Melbourne (Antarctica). Polar Biol. 27, 423-431.

Barendse, J., Mercer, R. D., Marshall, D. J., Chown, S. L., 2002. Habitat specificity of mites on sub-Antarctic Marion Island. Environ. Entomol. 31, 612-625

Bargelloni, L., Marcato, S., Zane, L., Patarnello, T., 2000. Mitochondrial phylogeny of notothenioids: a molecular approach to Antarctic fish evolution and biogeography. Syst. Biol. 49, 114-129.

Barrat, A., Mougin, J. L., 1974. Donneés numériques sur la zoogéographie de l'avifane Antarctique et Subantarctique. CNFRA 33, 1-18.

Bayley, I., 1998. Beetle-mites that take to the water. Invertebrata Tasmania's Invertebrate Newsletter. 12, 4.

Bednarek-Ochyra, H., Väňa, J., Ochyra, R., Lewis Smith, R. I., 2000. The liverwort flora of Antarctica. W. Szafer Institute of Botany, Polish Academy of Sciences, Cracow.

Bellair, P., 1964. Recent data on the geology of Isles Crozet. In: Adie, R. J., (Ed), Antarctic Geology. Proc 1st Internatl Symp Antarctic Geol. Elsevier, Amsterdam, pp 3-7.

Berlese, M., 1917. Acariens. Deuxième Expèdition Antarctique Française Zoologie (1908-1910) 1-2.

Bernham, K. P., Anderson, D. R., 2002. Model selection and multimodel inferences, a practical information-theoretic approach. Springer, New York.

Bernini, F., Carnevale, G., Bagnoli, G., Stouge, S., 2000. An Early Ordovician oribatide mite from the Island of Oeland, Sweden. 4th Symposium of the Europea Association of Acarologists, Sienna.

Bohaty, S. M., Wise, S. W., Duncan, R. A., Moore, C. L., Wallace, P. J., 2003. Neogene Diatom Biostratigraphy, Tephra Stratigraphy, and Chronology of ODP Hole 1138A, Kerguelen Plateau. In: Frey, F. A., Coffin, M. F., Wallace, P. J., Quilty, P. G. (Eds), Proceedings of the Ocean Drilling Program. Scientific Results 183, 1-53.

Brandt, A., Gooday, A. J., Brandão, S. N., Brix, S., Brökeland, W., Cedhagen, T., Choudhury, M., Cornelius, N., Danis, B., De Mesel, I., Diaz, R. J., Gillan, D. C., Ebbe, B., Howe, J. A., Janussen, D., Kaiser, S., Linse, K., Malyutina, M., Pawlowski, J., Raupach, M., Vanreusel A., 2007. First insights into the biodiversity and biogeography of the Southern Ocean deep sea. Nature 447, 307-311.

Bremer, K., 1994. Branch support and treestability. Cladistics 10, 295-304.

Brooks, D. R., McLennan, D. A., 2001. A comparison of a discovery-based and an event-based method of historical biogeography. J. Biogeogr. 28, 757-768.

Brooks, D. R., van Veller, M. G. P., 2003. Critique of parsimony analysis of endemicity as a method of historical biogeography. J. Biogeogr. 30, 819-825.

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, 313-320.

Brundin, L. Z., 1966. Transantarctic relationships and their significance as evidenced by chironomid midges. Svensk. Vet. Akad. Hand. 11, 1-472.

Brundin, L. Z., 1988. Phylogenetic biogeography. In: Myers, A. A., Giller, P. S., (Eds), Analytical biogeography: an integrated approach to the study of animal and plant distributions. Chapman and Hall, London, pp. 343–369.

Burg, T. M., Croxall, J. P., 2001. Global relationships amongst black-browed and grey-headed albatrosses: analysis of population structure using mitochondrial DNA and microsatellites. Mol. Ecol. 10, 2647-2660.

Burg, T. M., Croxall, J. P., 2004. Global population structure and taxonomy of the wandering albatross species complex. Mol. Ecol. 13, 2345-2355.

Chown, S. L., Gaston, K. J., Williams, P. H., 1998. Global patterns in species richness of pelagic seabirds: the Procellariiformes. Ecography 21, 342-350.

Chown, S. L., Convey, P., 2007. Spatial and temporal variability across life's hierarchies in the terrestrial Antarctic. Philos. Trans. R. Soc. Lond. B. Biol. Sci. (DOI 10.1098/rstb.2006.1949).

Clarke, A., Johnston, I. A., 1996. Evolution and adaptive radiation of Antarctic fishes. Trends Ecol. Evol. 212-218.

Clarke, A., 2003. Evolution, adaptation and diversity: global ecology in an Antarctic context. In: Huiskes, A. H. L. W., Gieskes, W. C., Rozema, J., Schorno, R. M. L., van der Vies, S. S., Wolff W. J. (Eds), Antarctic biology in a global context. Backhuys Publishers, Leiden, pp.3-17.

Coetzee, L., Marshall, D. J., 2003. A new *Halozetes* species (Acari: Oribatida: Ameronothridia) from the marine littoral of southern Africa. Afr. Zool. 38, 327-331.

Colgar, D. J., McLauchlan, A., Wilson, G. D. F., Livingston, S. P., Edgecombe, G. D., Macaranas, J., Cassis, G., Gray, M. R., 1998. Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. Aust. J. Zool. 46, 419-437.

Convey, P., Smith, R. I. L., Peat, H. J., Pugh, P. J. A., 2000a. The terrestrial biota of Charcot Island eastern Bellingshausen Sea, Antarctica an example of extreme isolation. Antarct. Sci. 12, 406-413.

Convey, P., Greenslade, P., Pugh, P. J. A., 2000b. Terrestrial fauna of the South Sandwich Islands. J. Nat. Hist. 34, 597-609.

Convey, P., McInnes, S. J., 2005. Exceptional tardigrade-dominated ecosystems in Ellsworth Land, Antarctica. Ecology 86, 519-527.

Craig, D. A., Currie, D. C., Vernon, P., 2003. *Crozetia* Davies (Diptera: Simuliidae): redescription of *Cr. crozetensis*, *Cr. sequyi*, number of larval instars, phylogenetic relationships and historical biogeography. Zootaxa 259, 1-39.

Dalenius, P., Wilson, O., 1958. On the soil fauna of the Antarctic and of the sub-Antarctic islands. The Oribatidae (Acari). Arkiv für Zoologi. 11, 193-425.

Darlington, P. J., 1970. A practical criticism of Hennig-Brundin 'phylogenetic systematics' and Antarctic biogeography. Syst. Zool. 19, 1–18.

DeSalle, R., Freedman, T., Prager, E. M., Wilson, A. C., 1987. Tempo and mode of sequence evolution in mitochondrial DNA of Hawaiian *Drosophila*. Mol. Phylogenet. Evol. 13, 157-164.

Engelbrecht, C., 1974. The genus *Halozetes* (Oribatei: Acari) on Marion Island. Navors. Nas. Mus.Bloemfontein 3, 1-25.

Engelbrecht, C., 1975. New ameronothroid (Oribatei, Acari) taxa from the republic of South Africa and the Islands of Gough and Marion. Navors. Nas. Mus. Bloemfontein 3, 53-88.

Felsenstein, J., 1993. PHYLIP (Phylogeny Inference Package) version 3.5c. Distributed by the author. Department of Genetics, University of Washington, Seattle.

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3, 294-299.

Frati, F., Spinsanti, G., Dallai, R., 2001. Genetic variation of mtCOII gene sequences in the collembolan *Isotoma klovstadi* from Victoria Land, Antarctica: evidence for population differentiation. Polar Biol. 24, 934-940.

Grandjean, F., 1955. Sur un acarien des iles Kerguélen, *Podacarus auberti* (Oribate). Mémoires du Muséum nationale d'histoire naturelle, Paris, Serie A. Zoology 8, 109-150.

Graybeal, A., 1998. Is it better to add taxa or characters to a difficult phylogenetic problem? Syst. Biol. 47, 9-17.

Greenslade, P., Farrow, R. A., Smith, J. M. B., 1999. Long distance migration of insects to a subantarctic island. J. Biogeogr. 26, 1161-1167.

Gressitt, J. L., 1970. SubAntarctic entomology and biogeography. In: Gressitt, J. L. (Ed), SubAntarctic Entomology Particularly of South Georgia and Heard Island. Pacif. Insects Monogr. 23, 295-374.

Greve, M., Gremmen, N. J. M., Gaston, K. J., Chown, S. L., 2005. Nestedness of Southern Ocean island biotas: ecological perspectives on a biogeographical conundrum. J. Biogeogr. 32, 155-168.

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

Hammer, M., 1955. Alaskan oribatids. Acta Arctica 7, 36.

Hall, K. J., 1990. Quaternary glaciations in the Southern Ocean: sector 0º long -180º long. Quat. Sci. Rev. 9, 217-228.

Hall, K., 2002. Review of Present and Quaternary periglacial processes and landforms of the maritime and sub-Antarctic region. S. Afr. J. Sci. 98, 71-81.

Hillis, D. M., Bull, J. J., 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Syst. Biol. 42, 182-192.

Hillis, D. M., Huelsenbeck, J. P., 1995. Assessing molecular phylogenies. Science 267, 255-256.

Hillis, D. M., Moritz, C., Mable, B., 1996. Molecular Systematics. Sinauer Associates, Sunderland.

Hurst, L., 2002. The Ka/Ks ratios: diagnosing the form of sequence evolution. Trends Genet. 18, 486.

Kumar, S., Tamura, K., Jakobsen, I., Nei, M., 2000. MEGA: Molecular evolutionary genetics analyses. Version 3.1. University Park: Pennsylvania State University.

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

Lameyre, J., Nougier, J., 1982. Geology of Ile de l'Est Crozet archipelago (TAAF). In: Craddock, C. (Ed), Antarctic Geoscience. Univ Wisconsin Press, Madison, pp 767-770.

Livermore, R., Nankivell, A., Eagles, G., Morris, P., 2005. Paleogene opening of Drake Passage. Earth Planet. Sci. Lett. 236, 459– 470.

Livermore, R., Hillenbrand, C. D., Meredith, M., Eagles, G., 2007. Drake Passage and Cenozoic climate: An open and shut case? Geochem. Geophys. Geosyst. 8, Q01005, doi:10.1029/2005GC001224.

Lohmann, H., 1907. Die Meeresmilben der Deutschen Südpolar-Expedition. Dt. Südpol.-Exped. 9, 361-413.

Maniatis, T., Fritsch, E., Sambrook, J., 1982. Molecular cloning. Cold Spring Harbor, New York.

Marshall, D. J., Pugh, P. J. A., 1996. Origin of the inland Acari of Continental Antarctica, with particular reference to Dronning Maud Land. Zool. J. Linn. Soc. 118, 101-118.

Marshall, W. A., Chalmers, M. O., 1997. Airborne dispersal of antarctic terrestrial algae and cyanobacteria. Ecography 20, 585-594.

Marshall, D. J., Coetzee, L., 2000. Historical biogeography and ecology of a Continental Antarctic mite genus, *Maudheimia* (Acari, Oribatida): evidence for a Gondwanan origin and Pliocene-Pleistocene speciation. Zool. J. Linn. Soc. 129, 111-128.

Marshall, D. J., Chown, S. L., 2002 .The acarine fauna of Heard Island. Polar Biol. 25, 688-695.

Marshall, D. J., Convey, P., 2004. Latitudinal variation in habitat specificity of ameronothrid mites (Oribatida). Exp. Appl. Acarol. 34, 21-35.

Matsuo, Y., Yamazaki, T., 1989. Nucleotide variation and divergence in the histone multigene family in *Drosophila melanogaster*. Genetics 122: 87-97.

Matsuo, Y., 2000. Molecular evolution of the histone 3 multigene family in the *Drosophila melanogaster* species subgroup. Mol. Phylogen. Evol. 16, 339-343.

McDougall, I., Verwoerd, W., Chevallier, L., 2001. K-Ar goechronology of Marion Island, Southern Ocean. Geol. Mag. 138, 1-17.

McGavin, G. C., 2001. Essential Entomology: An Order-by-Order Introduction. Oxford University Press, US.

McInnes, S. J., Pugh, P. J. A., 1998. Biogeography of limno-terrestrial Tardigrada, with particular reference to the Antarctic fauna. J. Biogeogr. 25, 31-36.

Michael, A., (1895).

Michael, A., (1903). Acarida (Oribatidae). In: Expédition Antarctique Belge. Résultats du voyage S. Y Belgica en 1897-1898-1899. Rapports cientifiques, Zoologie, Acariens Libres 10, 1-6.

Morrone, J. J., Crisci, J. V., 1995. Historical biogeography: introduction to methods. Annu. Rev. Ecol. Syst. 26, 373-401.

Morrone, J. J., 1998. On Udvardy's Insulantarctica province: a test from the weevils (Coleoptera: Curculionoidea). J. Biogeogr. 25, 947-955.

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 Biol. 30, 471-476.

Muñoz, J., Felicísimo, A. M., Cabezas, F., Burgaz, A. R., Martínez, I., 2004. Wind as a long-distance dispersal vehicle in the Southern Hemisphere. Science 304, 1144-1147.

Navajas, M., Fournier, D., Lagnel, D., Boursot, P., 1996. Mitochondrial COI sequences in mites: evidence for variation in base composition. Insect Mol. Biol. 5, 1-5.

Nei, M., Kumar, S., 2000. Molecular Evolution and Phylogenetics. Oxford University press.

Nunn, G. B., Cooper, J., Jouventin, P., Robertson, C. J. R., Robertson, G. G., 1996. Evolutionary relationships among extant albatrosses (Procellariiformes: Diomedeidae) established from complete cytochrome-ß gene sequences. The Auk 113, 784-801.

Nylander, J. A. A. A., Ronquist, F., Huelsenbeck, J. P., Nieves-Aldrey, J. L., 2004. Bayesian phylogenetic analysis of combined data. Syst. Biol. 53, 47-67.

Ochyra, R., Bednarek-Ochyra, H., Lewis Smith, R. I., In press. The moss flora of Antarctica. Cambridge University Press.

Page, T. J., Linse, K., 2002. More evidence of speciation and dispersal across the Antarctic Polar Front through molecular systematics of Southern Ocean *Limatula* (Bivalvia: Limidae). Polar Biol. 25, 818-826.

Peck, L. S., Convey, P., Barnes, D. K. A., 2006. Environmental constraints on life histories in Antarctic ecosystems: tempos, timings and predictability. Biol. Rev. 81: 75-109.

Posada, D., Crandall, K., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817-818.

Procheş, S., Marshall, D. J., 2001. Back to the sea: secondary marine organisms from a biogeographical perspective. Biol. J. Linn. Soc. 73, 1-7.

Pugh, P. J. A., 1993. A synonymic catalogue of the Acari from Antarctica, the sub-Antarctic Islands and the Southern Ocean. J. Nat. Hist. 27, 323-421.

Pugh, P. J. A., Dartnall, H. J. G., McInnes, S. J., 2002. The non-marine Crustacea of Antarctica and the islands of the Southern Ocean: biodiversity and biogeography. J. Nat. Hist. 36, 1047-1103.

Pugh, P. J. A., 2004. Biogeography of spiders (Araneae: Arachnida) on the islands of the Southern Ocean. J. Nat. Hist. 38, 1461-1487.

Ree, R. H., Moore, B. R., Webb, C. O., Donoghue, M. J., 2005. A Likelihood framework for inferring the evolution of geographic range on phylogenetic trees. Evolution 59, 2299–2311

Richters, F., 1907. Die Fauna der Moosrasen des Gauss-Bergs und einiger südlicher Inseln. Dt. Südpol.-Exped. 9, 261-302.

Ronquist, F., 1997. Dispersal-vicariance analysis: A new approach to the quantification of historical biogeography. Syst. Biol. 46, 195-203.

Ronquist, F., Huelsenbeck, J., 2003. MrBayes3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572-1574.

Salomone, N., Emerson, B., Hewitt, G., Bernini, F., 2002. Phylogenetic relationships among the Canary Island Steganacaridae (Acari, Oribatida) inferred from mitochondrial DNA sequence data. Mol. Ecol. 11, 79–89.

Schmidt, H. A., Strimmer, K., Vingron, M., von Haeseler, A., 1999. Computional Molecular Biology, Germany.

Schulte, G., Weigmann, G., 1977. The evolution of the family Ameronothridae (Acari: Oribatei). II Ecological Aspects. Acarologia, 19, 167-173.

Shimodaira, H., Hasegawa, M., 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inferences. Mol. Biol. Evol. 16, 114-116.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Lui, H., Flook, P., 1994. Evolution, Weighing and Phylogenetic Utility of Mitochondrial Gene Sequences and a Compilation of Conserved Polymerase Chain Reaction Primers. Ann. Entomol. Soc. Am. 87, 651- 701.

Skotnicki, M. L., Ninham, J. A., Selkirk, P. M., 2000. Genetic diversity, mutagenesis and dispersal of Antarctic mosses – a review of progress with molecular studies. Antarct. Sci. 12, 363-373.

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 Biol. 27, 687-698.

Smith, V. R., Gremmen, N. J. M., 2004. The flora of Marion and Prince Edward Island. Data Analysis Ecology, The Netherlands.

Söller, R., Woltmann, A., White, H., Blohm, D., 2001. Phylogenetic relationships within terrestrail mites (Acari:Prostigmata:Parasitengona) inferred from comparative DNA sequence analysis of the mitochondrial cytochrome oxidase subunit I gene. Mol. Phylogen. Evol. 18, 47-53

Sorensen, M. D., 1999. TreeRot, version 2. Boston University, Boston, MA.

Starý, J., Block, W., 1998. Distribution and biogeography of oribatid mites (Acari: Oribatida) in Antarctica, the sub-Antarctic islands and the nearby land areas. J. Nat. Hist. 32, 861-894.

Stevens, M. I., Hogg, I. D., 2002. Expanded distributional records of Collembola and Acari in southern Victoria land, Antarctica. Pedobiologia 46, 485-495.

Stevens, M. I., Hogg, I. D., 2003. Long-term isolation and recent range expansion from glacial refugia revealed for the endemic springtail *Gomphiocephalus hodgsoni* from Victoria Land, Antarctica. Mol. Ecol. 12, 2357-2369.

Stevens, M. I., Greenslade, P., Hogg, I. D., Sunnucks, P., 2006. Southern hemisphere springtails: Could any have survived glaciation of Antarctica? Mol. Biol. Evol. 23, 874-882.

Swofford, D., 2000. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other methods). Version 4. Sinauer, Sunderland.

Trägårdh, I., 1931. Acarina from the Juan Fernandez Islands. In: Skottsberg, C. (Ed), The Natural Hisotry of Juan Fernandez and Easter Island III. Almquist and Wiksells, Boktrykerie, pp 553-628.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., Higgins, D. G., 1997. The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 24, 4876-4882.

Thorne, J. L., Kishino, H., 2002. Divergence time and evolutionary rate estimation with multilocus data. Syst. Biol. 51, 689-702.

Udvardy, M. D. F., 1987. The biogeographical realm Antarctica: a proposal. J. R. Soc. N. Z. 17, 187-194.

Vaughan, A. P. M., Storey, B. C., 2000. The eastern palmer Land shear zone: a new terrane accretion model for the Mesozoic development of the Antarctic Peninsula. J. Geol. Soc. Lond. 157, 1243-1256.

Vermeij, G. J., Dudley, R., 2000. Why are there so few evolutionary transitions between aquatic and terrestrial ecosystems. Biol. J. Linn. Soc. 70, 541-554.

Wahlberg, N., Nylin, S., 2003. Morphology versus molecules resolution of the positions of *Nymphalis*, *Polygonia* and related genera (Lepidoptera: Nymphalidae). Cladistics 19, 213–223.

Wallace, P. J., Frey, F. A., Weis, D., Coffin, M. F., 2002. Origin and evolution of the Kerguelen Plateau, Broken Ridge and Kerguelen Archipelago: editorial. J. Petrology 43, 1105-1108.

Wallwork, J. A., 1963. The Oribatei (Acari) of Macquarie Island. Pac. Insects 5, 721-769.

Wallwork, J. A., 1973. Zoogeography of some terrestrial micro-arthropoda in Antarctica. Biol. Rev. 48, 233-259.

Wilkinson, D. M., 1990. Multivariate analysis of the biogeography of the protozoan genus *Nebela* in southern temperate and antartctic zones. Eur. J. Protistol. 26, 117-121.

Williams, T., Handwerger, D., 2005. A high-resolution record of early Miocene Antarctic glacial history from ODP Site 1165, Prydz Bay. Paleoceanography 20, 2017.

Yuan, Y., Wohlhauser, S., Moeller, M., Klackenberg, J., Callmander, M. W., Kuepfer, P., 2005. Phylogeny and Biogeography of *Exacum* (Gentianaceae): A Disjunctive Distribution in the Indian Ocean Basin Resulted from Long Distance Dispersal and Extensive Radiation. Syst. Biol. 54, 21-34.

Zachos, J. C., Rea, D. K., Seto, K., Nomura, R., Nitsuma, N., 1992. Paleogene and early Neogene deep-water paleoceanography of the Indian Ocean as determined from benthic foraminer stablecarbon and oxygen isotope records. In: Duncan, R. A., Rea, D. K., Kidd, R. B., von Rad, U., Weissel, J. K. (Eds), Scientific Drilling in the Indian Ocean. Geophys. Mono. Paleoceanography 20, 2017.

CHAPTER 3

PHYLOGEOGRAPHY OF *EUPODES MINUTUS* (ACARI: PROSTIGMATA) ON SUB-ANTARCTIC MARION ISLAND REFLECTS THE IMPACT OF HISTORICAL EVENTS

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ABSTRACT

Marion Island, situated ~2300km south-east of Cape Town, South Africa, has experienced multiple volcanic and glaciation events during its history. To better understand the impact of these events on species' genetic structure, we determined the phylogeographic population structure of the mite, *Eupodes minutus*. We included 57 individuals sampled from 11 localities across the island. Our analyses based on the mitochondrial COI gene suggest a population expansion as would typically be expected when species recover after being confined to refugia. Standard *Phi* (Φ) -statistics and a Spatial Analysis of Molecular Variance (SAMOVA) identified unique populations on the south-western and south-eastern sides of the island. We argue that multiple volcanic events on the southern side of Marion, in combination with glaciations, effectively isolated these populations from each other.

INTRODUCTION

Marion Island (PEI; 46º54'S, 37º45'E), together with Prince Edward Island (PEI; 46˚38'S, 37˚57'E), forms part of the Prince Edward Island archipelago (PEI) situated in the Southern Ocean. Marion Island is generally believed to be between 500 000 and 1 million years old (McDougall 1971; Smith 1987; Hänel and Chown 1998; McDougall et al. 2001). During this time, several glacial cycles (~five) and volcanic events (~eight) have characterized the island's history (McDougall et al. 2001) and it can safely be assumed that these events have contributed significantly to shaping the current spatial distribution of genetic variation for indigenous fauna and flora. Specifically, glaciation events would have confined fauna and flora to isolated refugia. Volcanism causes the extinction of populations directly in the path of lava flows; in addition, these lava flows also present long term extrinsic barriers to gene flow.

Mite and Collembola species dominate the terrestrial fauna in the South Polar Region (Wallwork 1973). These invertebrates play an essential role in nutrient recycling on sub-Antarctic islands since they are the only herbivores and detrivores present (Burger 1985; Tréhen et al. 1985; Chown et al. 2002). Sixty mite species have been recorded on PEI (Marshall et al. 1999). The fungivorous prostigmatid mite, *Eupodes minutus* Strandtmann, 1967 is the most abundant species on Marion Island and is found most commonly in the cushion plant *Azorella selago* Hook. f., 1847 (Barendse et al. 2002). Hugo (2006) reports that even though *E. minutus* is found across the island, it is most abundant on the calmer eastern side, has no altitude preference, and prefers warmer and drier conditions.

Very little is known about the genetic variation of the fauna and flora that inhabit Southern Ocean islands. Only a few phylogeographic studies have been conducted on Antarctica (see for example Fanciulli et al. 2001; Frati et al. 2001; Hogg and Stevens 2002; Stevens and Hogg 2003) and New Zealand (Garrick 2002; Garrick et al. 2004) focusing primarily on springtails (Collembola). However, similar studies are lacking for other sub-Antarctic Islands. To extend our limited knowledge, we present our preliminary findings detailing the phylogeographic population structure of *E. minutus* across Marion Island based on analyzed data for the mitochondrial cytochrome oxidase subunit I (mtCOI) gene (for usefulness of this gene see Salomone et al. 1996; Frati et al. 2000; Garrick 2002; Hogg and Stevens 2002; Garrick et al. 2004; Stevens and Hogg 2003). This represents, to our knowledge, the first documentation of intra-specific genetic variation for invertebrates on Marion Island and one of only a few studies pertaining to Southern Ocean islands. This study forms part of a larger project aiming to document the spatial distribution of both genetic and ecological variation for a large group of species from Marion Island; data that will allow us to search for congruent patterns and processes underlying the evolutionary history of invertebrates on this Southern Ocean island.

MATERIALS AND METHODS

Sample collection

We collected 57 *E. minutus* specimens from 11 localities across Marion Island during the 2005 relief voyage (Figure 3.1). Mites were sampled primarily from the cushion plant, *A. selago*. Specimens were preserved in absolute ethanol by means of Tullgren extractions (Southwood 1978). Due to their small size, *E. minutus* was identified using light microscopy (50x magnification) prior to preservation in absolute ethanol.

DNA extraction

Individual mites were pipetted into separate 1.5ml-microcentrifuge tubes and incubated for 30 minutes at 50°C to evaporate the excess ethanol. Prior to DNA extraction, the mites were softened by two freeze / thaw cycles at (minus) -70°C and 50°C respectively (Rainer Söller, personal communication). Total genomic DNA was then extracted from individual mites using the QIAamp DNA micro kit (QIAGEN, Hilden, Germany) according to the manufacturer's recommendations.

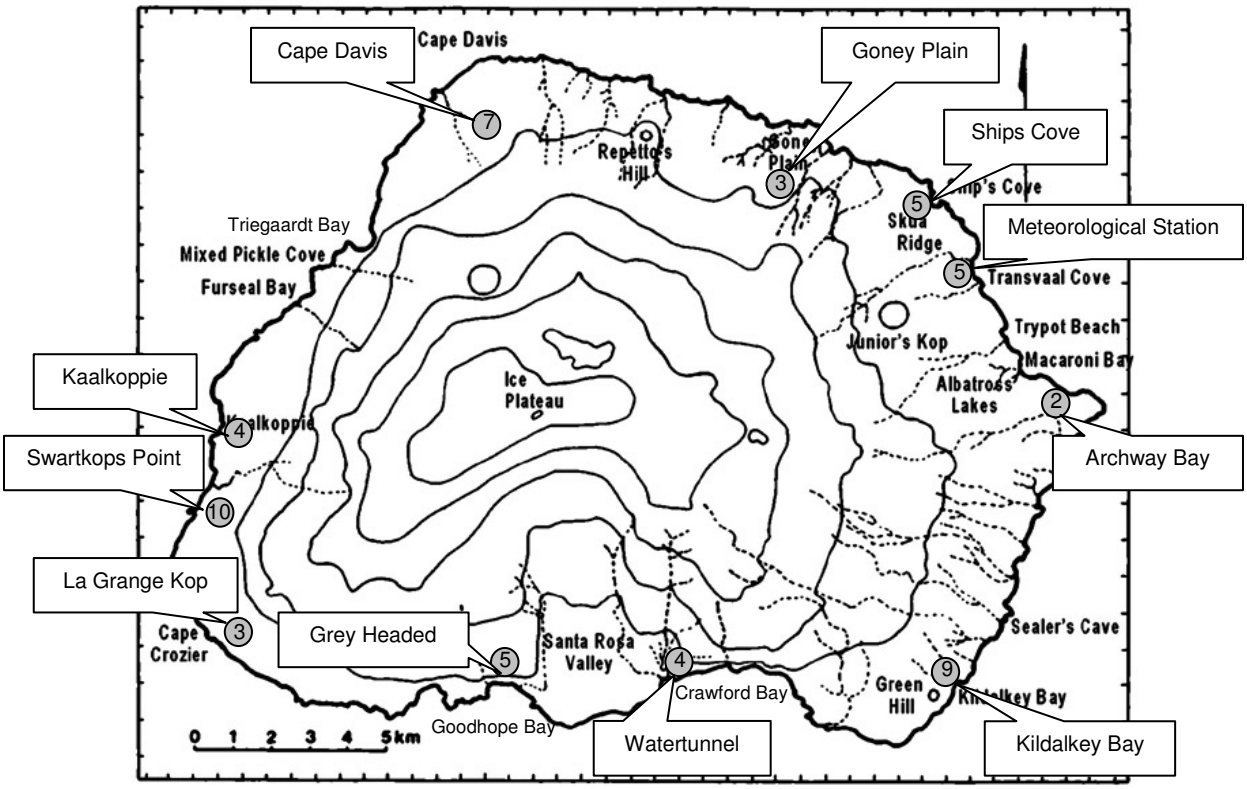

Figure 3.1 A map indicating the sampling localities of *E. minutus* across Marion Island (adapted from Smith and Gremmen (2004)). The number of specimens included per locality is indicated in each case.

PCR and sequencing

COI sequences were amplified from five to ten pooled *E. minutus* individuals using primers described by Folmer et al. (1994). These sequences were used as a template to design species-specific primers with PerlPrimer 1.1.5 (Marshall 2003). A nested PCR had to be performed due to the small quantity of DNA extracted from each individual. The first primer set amplified a ~500bp stretch followed by the two nested PCRs with two overlapping fragments within this stretch. The total fragment, comprised of the two overlapping nested fragments, was 413bp in length. In the initial PCR, the species-specific forward primer EMCOIF1 (5'-GAAATCCAGAAATAATAGGTC-3') was used in combination with the reverse primer (HCO 2198, Folmer et al. 1994). The two sets of nested PCR primer pairs consisted of EMCOIF3 (5'-CTATAATGTATTAGTAACAAGAC-3') and EMCOIR2 (5'-TGAAGTGAGAAAATGGT-3'), and EMCOIF4 (5'-CAGGGTGAACAGTATATC-3') and EMCOIR3 (5'-TGTGTTGTAATTTCGATCT-3').

All the PCR reactions were performed using 10ng of DNA under the following cycling parameters: 94°C for 1 minute, (94°C for 30 seconds, 50°C for 30 seconds and 72°C for 45 seconds) for 35 cycles with a final extension step at 72°C for 5 minutes. PCR products were gel purified with the Wizard SV Gel and PCR clean-up system (Promega, Madison, USA) according to the manufacturer's recommendations. Nucleotide sequencing was carried out using the forward primers (i.e. EMCOIF3 and EMCOIF4) with half-reactions of BigDye® Terminator 3.1 mix (Applied Biosystems, Warrington, UK). Purified sequencing products were run on an ABI 3100 automated sequencer (Applied Biosystems, Warrington, UK). Electropherograms of the raw data were edited with BioEdit 7.0.5 (Hall 2005). All *E. minutus* sequences were deposited in GenBank (accession numbers DQ675083-DQ675139).

Sequence analysis

Sequences were aligned with Clustal X 1.81 (Thompson et al. 1997) using the multiple alignment mode with default parameters. PAUP* 4.0b10 (Swofford 2000) was used to assess the nucleotide composition and the number of parsimony-informative sites. Sequence divergences were determined in MEGA 3.1 (Kumar et al. 2000). Haplotypes were identified using Collapse 1.2 (Posada 2004). To depict the evolutionary relationships (number of mutational steps) among the haplotypes, we constructed a median-joining network using NETWORK 4.1.1.2. (Polzin and Daneshmand 2004).

A demographic method was implemented to investigate the spatial distribution of mitochondrial variation (SAMOVA, Dupanloup et al. 2002). SAMOVA 1.0 provides a way to identify spatially differentiated groups allowing for the identification of (possible) barriers to gene flow. A hierarchical analysis of molecular variance (AMOVA as implemented in Arlequin 2.0, Schneider et al. 2000) was used to estimate traditional *Phi* (Φ) – statistics reporting the degree of variation between populations as well as between individuals within these. In

both instances, tests of significance for departure from the null distribution were performed using permutational procedures (1000 randomisations) in Arlequin 2.0.

To investigate whether haplotype frequencies deviated from equilibrium, Fu's (1997) *F*-statistic was calculated using Arlequin 2.0. To further investigate if the deviation from equilibrium might be due to past demographic changes, we plotted the number of mutational changes separating haplotypes against their respective frequencies (Rogers and Harpending 1992). A χ^2 goodness-of-fit test was used to compare observed and expected distributions. The raggedness statistic (*rg*) (Harpending et al. 1993; Harpending 1994), an indication of the smoothness of the mismatch distribution (population growth = lower *rg* vales), was calculated in Arlequin 2.0. We also employed coalescence-based methodology to calculate exponential growth rates (*g*-values in Fluctuate 1.30 (part of LAMARC software package), Kuhner et al. 1998) for *E. minutus*.

RESULTS AND DISCUSSION

A total of 413bp (137 amino acids) were obtained for the COI gene. Of these, 22 sites were polymorphic and 14 parsimony informative among the 57 individual mites that were sequenced. Twenty-four haplotypes characterized the 57 mite specimens. The most common haplotype (EM2) represented 33% of all our specimens (see Figure 3.2 and Table 3.1). The sequences had an average AT-content of 69.3%, increasing to 87.2% when only 3rd codon positions were considered. Eight of the substitutions resulted in amino acid changes (data available on request). The mean transition: transversion ratio was 1.8: 1. The highest uncorrected sequence divergence separating specimens was 2.2% between mites sampled at the localities of Swartkops Point and Kildalkey Bay (see Figure 3.1).

The median-joining network (Figure 3.2) displayed a star-like pattern; this type of pattern is strongly suggestive of a recent population expansion (e.g. Avise 1994; Conroy and Cook 2000; Jolly et al. 2005). The most common haplotype (EM2) formed the core, connecting multiple haplotypes separated by one or three mutational steps. Fu's *F*-statistic (-25.72938, $p<0.001$) was highly significant indicating a deviation from equilibrium. An analysis of mismatch distributions confirmed the observed change in population demography (data not shown). When combining all sampled populations into a single unit (sample sizes for single populations were not sufficient to allow for meaningful statistical analyses), the null model of sudden population expansion could not be rejected ($p=0.2$). Similarly, the raggedness statistic ($rg=0.05$, $p=0.12$) supported a population expansion.

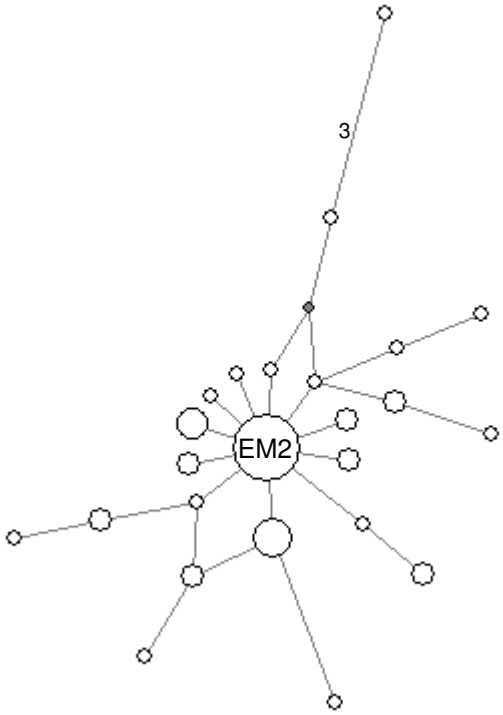

Figure 3.2 The median-joining network, depicting the number of mutational steps separating the 24 haplotypes detected for 57 *E. minutus* specimens using NETWORK 4.1.1.2. The most common haplotype (EM2) is indicated. Missing haplotypes are shown in grey. Lines separating haplotypes represent one mutational change unless otherwise indicated. See Table 3.1 for haplotype distribution.

Table 3.1 A summary of the haplotype diversity, nucleotide diversity and the haplotype distribution at each of the 11 sample sites across Marion Island.

Populations	Haplotype diversity	Nucleotide diversity	Haplotypes
Cape Davis (n=7)	0.810±0.130	0.005±0.004	EM2, EM4, EM11, EM13, EM17, EM22
Goney Plain (n=3)	1.000±0.272	0.005±0.005	EM2, EM6, EM21
Ships Cove (n=5)	0.900±0.161	0.005±0.004	EM2, EM3, EM15, EM24
Meteorological Station (n=5)	0.800±0.164	0.004±0.003	EM2, EM6, EM14
Archway Bay (n=2)	1.000±0.500	0.007±0.008	EM2, EM23
Kildalkey Bay (n=9)	0.889±0.091	0.006±0.004	EM1, EM2, EM7, EM9, EM10, EM16
Watertunnel (n=4)	0.833±0.222	0.005±0.004	EM2, EM9, EM12
Grey Headed (n=5)	0.900±0.161	0.003±0.003	EM2, EM7, EM15, EM17
La Grange Kop (n=3)	1.000±0.272	0.006±0.006	EM5, EM8
Swartkops Point (n=10)	0.889±0.075	0.007±0.004	EM2, EM7, EM15, EM18, EM19, EM20
Kaalkoppie (n=4)	0.500±0.265	0.001±0.001	EM1, EM2

Hence, all analyses are consistent with a sudden expansion event. Importantly, the current *g*-value was strongly positive (*g*=+1357.3). This would indicate that some, if not all of the population(s) of *E. minutus* on Marion Island is still expanding despite past and recent climate changes. As mentioned earlier, this mite species favours warm and dry conditions (Hugo 2006). We argue that the increase in temperature (1.2°C) and the concomitant decrease in moisture (600mm) observed on Marion Island over the past ~30 years (Smith 2002; Smith and Gremmen 2004) favours this species and may, at least in part, account for its population growth.

The spatial distribution of genetic variation across populations is shaped by many factors both historical as well as contemporary. Specifically relating to Marion Island, it is well established that several glacial and volcanic episodes have occurred during the history of the island (see McDougall et al. 2001). More recently, environmental conditions influence gene flow among populations which impacts directly on the level of differentiation among populations. Perhaps surprising given the demographic history of this Southern Ocean island, AMOVA indicated that almost all of the variation was within populations (99%) rather than between populations (1%) (Φ_{ST}= 0.00890, *p*=0.36) as would be typical of a large, panmictic population with high levels of gene flow. Important to note though that our sample sizes for most of the populations was small (<5 mites per locality) which greatly reduces statistical power.

When regarding sampling localities as populations, population pair-wise Φ_{ST} analyses indicated that the localities of La Grange Kop (south-western side of Marion Island) and Kildalkey Bay (south-eastern side of Marion Island) were significantly differentiated from each other (Φ_{ST}=0.07053, *p*=0.04). Significant differences among certain populations were indeed confirmed by a SAMOVA. Φ_{CT} was maximized at five groups (Φ_{CT}=0.108, *p*<0.001) which mainly corresponded to localities on the eastern (Ships Cove and Archway Bay), south-eastern (Kildalkey Bay), south-western (Swartkops Point) and northern (Cape Davis) side with the rest of the localities (mainly southern, western and north-eastern side of the island) grouping together. When the number of groups were increased (>5 groups), Swartkops Point consistently grouped separately; it never grouped with the nearby localities of Kaalkoppie and La Grange Kop. Although this may appear counter intuitive, the failure of this population to group with geographically close populations may be explained through an examination of the haplotype distribution. Three of the ten specimens included from Swartkops Point had unique haplotypes (haplotype diversity = 0.889±0.075) with only a single haplotype shared with the nearby populations at La Grange Kop and Kaalkoppie, respectively (see Table 3.1). Additionally, the relatively low sample sizes for Kaalkoppie (n=4) and La Grange Kop (n=3) may not adequately have captured the genetic variation present in these populations (a similar scenario could explain the clustering of populations on the eastern side of the island where the population sampled at Meteorological Station do no group with the localities of Ship's Cove to the north and Archway Bay to the south). Notwithstanding the relatively high intra-population nucleotide diversities (*cf*. inter-population values) and small sample sizes, it would appear from our preliminary data that unique populations are

present on the south-western (vicinity of La Grange Kop and Swartkops Point) and south-eastern side (Kildalkey Bay) of the island.

Climatic and environmental conditions vary extensively across Marion Island. The western side of the island typically experiences cold winds of high speed that blow from the Antarctic over the island (Smith 1987). Young as well as older lava, interspersed with sparse fellfield vegetation, covers large areas of the western side of the island resulting in a very inhospitable environment compared to the eastern side which is characterized mainly by older grey lava covered by vegetation (van Zinderen Bakker et al. 1971; Smith and Gremmen 2004). The significant influence that these differences have on the fauna and flora across the island has recently been demonstrated (Melodie McGeoch, personal communication). This may similarly hold for the localities of La Grange Kop (together with Swartkops Point situated on the south-western side of Marion Island) and Kildalkey Bay (situated on the south-eastern side of Marion Island). Specifically, extensive glaciation has been documented from Triegaardt Bay in the north-west to Goodhope Bay in the south, as well as at Crawford Bay in the south (McDougall et al. 2001). A similar barrier to gene flow is evidenced by the Santa Rosa Valley in the south, an area of extensive lava (both old and recent) with sparse vegetation cover.

To conclude, we show that *E. minutus* has undergone a recent population expansion with subsequent growth in population size. Notwithstanding that only 1% of the genetic variation is accounted for by variation among populations, we show that certain populations are indeed unique (Kildalkey Bay and La Grange Kop); a situation that is probably a true reflection of the influence of past biotic and abiotic factors on invertebrate populations across Marion Island. However, to fully understand and substantiate our preliminary findings, the population structure of more species with more individuals per sampling site needs to be investigated which will help increase the power of the statistical inferences.

REFERENCES

Avise JC (1994) Molecular Markers, Natural History and Evolution. Chapman and Hall, London, New York.

Barendse J, Mercer RD, Marshall DJ, Chown SL (2002) Habitat specificity of mites on sub-Antarctic Marion Island. Environ Entomol 31: 612-625

Burger AE (1985) Terrestrial food webs in the sub-Antarctic: island effects. In: Siegfried WR, Condy PR, Laws RM (eds) Antarctic nutrient cycles and food webs. Springer, Berlin, Heidelberg, New York, pp 582-591

Chown SL, McGeogch MA, Marshall DJ (2002) Diversity and conservation of invertebrates on the sub-Antarctic Prince Edward Islands. Afr Entomol 10: 67-82

Conroy CJ, Cook JA (2000) Phylogeography of a post-glacial colonizer: *Microtus longicaudatus* (Rodentia: Muridae). Mol Ecol 9: 165-175

Dupanloup I, Schneider S, Excoffier L (2002) A simulated annealing approach to structure of populations. Mol Ecol 11: 2751-2581

Fanciulli PP, Summa D, Dallai R, Frati F (2001) High levels of genetic variability and population differentiation in *Gressittacantha terranova* (Collembola, Hexapoda) from Victoria Land, Antarctica. Antarctic Sci 13: 246-254

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotechnol 3: 294-299

Frati F, Dell'Ampio E, Casasanta S, Carapelli A, Fanciulli PP (2000) Large amounts of genetic divergence among Italian species of the genus *Orchesella* (Insecta, Collembola) and the relationships of two new species. Mol Phylog Evol 17: 456-461

Frati F, Spinsanti G, Dallai R (2001) Genetic variation of mtCOII gene sequences in the collembolan Isotoma klovstadi from Victoria Land, Antarctica: evidence for population differentiation. Polar Biol 24:934-940

Fu YX (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147: 915-925

Garrick R (2002) Phylogeography of a new species of Collembola (Neanuridea) from Tallaganda State Forest, New South Wales. MSc dissertation, Australian National University

Garrick R, Sands CJ, Rowell DM, Tait NN, Greenslade P, Sunnucks P (2004) Phylogeography recapitulates topography: very fine-scale local endemism of a saproxylic "giant" springtail at Tallaganda in the Great Dividing Range of south-east Australia. Mol Ecol 13: 3329-3344

Hall T (2005) BioEdit v7.0.5. Ibis Therapeutics, a division of Isis Pharmaceuticals, Carlsbad

Hänel C, Chown SL (1998) An introductory guide to the Marion and Prince Edward Island Special Nature Reserves, 50 years after annexation. Department of Environmental Affairs and Tourism, Pretoria.

Harpending HC, Sherry ST, Rogers AR, Stoneking M (1993) The genetic structure of ancient human populations. Curr Anthropol 34: 483-496

Harpending H (1994) Signature of ancient population growth in a low resolution mitochondrial DNA mismatch distribution. Hum Biol 66: 591–600

Hogg ID, Stevens MI (2002) Soil Fauna of Antarctic Coastal Landscapes. In: Beyer L, Bölter M (eds) Geoecology of Antarctic Ice-Free Coastal Landscapes. Ecological Studies 154: 265-282

Hugo EA (2006) Spatial patterns in the microarthropod community associated with *Azorella selago* (Apiaceae) on the sub-Antarctic Prince Edward Islands. PhD dissertation, Stellenbosch University

Jolly MT, Jollivet D, Gentil F, Thiebaut E, Viard F (2005) Sharp genetic break between Atlantic and English Channel populations of the polychaete *Pectinaria koreni*, along the north coast of France. Heredity 94: 23-32

Kuhner MK, Yamato J, Felsenstein, J (1998) Maximum likelihood estimation of population growth rates based on the coalescent. Genetics 149: 429-434

Kumar S, Tamura K, Jakobsen I, Nei M (2000) MEGA: Molecular evolutionary genetics analyses. Version 3.1. University Park, Pennsylvania State University

Marshall DJ, Gremmen NJM, Coetzee L, O'Connor BM, Pugh PJA, Theron PD, Ueckermann EA (1999) New records of Acari from the sub-Antarctic Prince Edwards Islands. Polar Biol 21: 84-89

Marshall O (2003) PerlPrimer v1.1.5: An application to design primers for PCR, Bisulphite PCR, Real-time PCR and Sequencing. http://perlprimer.sourceforge.net

McDougall I (1971) Geochronology. In: Van Zinderen Bakker EM, Winterbottom JM, Dryer RA (eds) Marion and Prince Edward Islands. Balkema, Cape Town, pp 72-77

McDougall I, Verwoerd W, Chevallier L (2001) K-Ar goechronology of Marion Island, Southern Ocean. Geol Mag 138: 1-17

Polzin T, Daneshmand SV (2004) NETWORK 4.1.1.2. Fluxus Technology Ltd. Steiner (MP) algorithm

Posada 2004. COLLAPSE 1.2 Free Software Foundation Inc, Boston, Massachusetts

Rogers AR, Harpending H (1992) Population growth makes waves in the distribution of pairwise genetic differences. Mol Biol Evol 9: 552-569

Salomone N, Frati F, Bernini F (1996) Investigation on the taxonomic status of *Steganacarus magnus* and *Steganacarus anomalus* (Acari: Oribatida) using mitochondrial DNA sequences. Exp Appl Acarol 20: 607-615

Schneider S, Roessli D, Excoffier L (2000) Arlequin: a software for population genetics data analysis. Version 2.000. Genetics and Biometry Laboratory, Department of Anthropology, University of Geneva

Smith VR (1987) The environment and biota of Marion Island. Suid-Afrikaanse Tydskrif vir Wetenskap 83: 211-220

Smith VR (2002) Climate change in the sub-Antarctic: an illustration from Marion Island. Clim Change 52: 345-357

Smith VR, Gremmen NJM (2004) The Flora of Marion and Prince Edward Island. Data Analysis Ecology, The Netherlands

Southwood TRE (1978) Ecological methods with particular reference to the study of insect populations. Chapman & Hall, London

Strandtmann RW (1967) Terrestrial Prostigmata (Trombidiform mites). Ant Res Ser 10: 51-80

Stevens MI, Hogg ID (2003) Long-term isolation and recent range expansion from glacial refugia revealed for the endemic springtail *Gomphiocephalus hodgsoni* from Victoria Land, Antarctica. Mol Ecol 12: 2357-2369

Swofford DL (2000) PAUP*. Phylogenetic analyses using parsimony (*and other methods). Version 4.0b2a. Sinauer, Sunderland.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 24: 4876-4882

Tréhen P, Bouché M, Vernon P, Frenot Y (1985) Organization and dynamics of *Oligochaeta* and *Diptera* on Possession Island. In: Siegfried WR, Condy PR, Laws RM (eds) Antarctic nutrient cycles and food webs. Springer, Berlin, Heidelberg, New York, pp 606-613

van Zinderen Bakker EM, Winterbottom JM, Dyer RA (1971) Marion and Prince Edward Islands. Report on the South African biological and geological expedition 1965-1666. A.A. Balkema, Cape Town

Wallwork J (1973) Zoogeography of some terrestrial micro-arthropoda in Antarctica. Biol Rev 48: 233-259

CHAPTER 4

CLIMATE CHANGE, HISTORICAL VICARIANCE AND CONTEMPORARY GENE FLOW: DISENTANGLING EFFECTS ON THE POPULATION STRUCTURE OF THE ORIBATID MITE *HALOZETES FULVUS* ON SUB-ANTARCTIC MARION ISLAND

(will be submitted to *Molecular Ecology*)

ABSTRACT

Sub-tropical islands have been utilized extensively to assess phylogeographic patterns exhibited by indigenous taxa. Complex population patterns were found as a direct consequence of volcanic evolution of these islands. In comparison, volcanic Southern Ocean islands also experienced extreme glaciation, which may further influence population structure; however, these islands are virtually devoid of population-level studies, with the exception of sub-Antarctic Marion Island, where population substructure was detected for some indigenous mites and springtails. However, these particular phylogeographic studies had low sample sizes which may have limited statistical inferences. Thus, I conducted a large-scale phylogeographic study on the mite species, *Halozetes fulvus*, to assess the impact of historical, environmental and demographic factors on its population structure across Marion Island. A total of 291 samples from 30 localities were included. Based on the mitochondrial COI gene, a high level of haplotype diversity was detected illustrating the genetic complexity on the island. The PCA findings indicated that the localities from the northern and eastern side of the island grouped together while the southern and western side formed a group. This result is consistent with topographic and climatic conditions on the island. My analyses also indicated the presence of unique populations on the north-eastern, eastern, southern and south-western side of Marion Island. Long Ridge situated on the north-eastern side, constituted a barrier to gene flow. I found that the western populations were relatively young, characterized by high migration rates, small effective female population sizes with no isolation-by-distance. The exact opposite was true for populations on the eastern side of Marion Island, where populations were older with lower migration rates and substantially larger effective female population sizes with isolation-by-distance. The western side of Marion Island has experienced several recent volcanic and glaciation events which, in concert, cause local extinctions of populations. In contrast, the eastern side of Marion Island provides a stable environment, however unique populations may be explained by past sealing activities in conjunction with the notion that the south-eastern tip of Marion Island may constitute a natural entry point for microarthropods. Thus, I propose that the population structure of *H. fulvus* on Marion Island has been sculpted by historical events (volcanism and glaciation), environmental factors (like strong winds) and contemporary factors (like gene flow).

INTRODUCTION

Sub-tropical archipelagos or island chains (such as the Hawaiian Islands, Galapagos Islands or Canary Islands) have long been a focal point for evolutionary studies ever since Darwin's voyage of the Beagle to the Galapagos Islands during the 19th century (Darwin 1859). These studies have generally demonstrated that most organisms on these islands arrived from adjacent continental areas through dispersal and rafting. Once established on these islands, their population genetic structures are sculpted by local historical or environmental factors which gave rise to complex phylogeographic patterns and may even produce cladogenic events driven largely by niche availability (see reviews of Emerson 2002; Gillespie & Roderick 2002).

When considering well-studied sub-tropical islands such as the Hawaiian or the Canary archipelagos, the pattern that generally emerged between taxa (on species and population level) is a 'step-like progression' from the oldest to the youngest island in the island chains (reviewed by Roderick & Gillespie 1998; Juan *et al.* 2000). In the Hawaiian archipelago, speciation data for multiple arthropod genera (~28) that constitute 75% of the endemic biota (Roderick & Gillespie 1998), revealed complex phylogeographic patterns driven by processes such as dispersal, gene flow, historical barriers as well as habitat availability (Jordan *et al.* 2005). On the Canary Islands, studies conducted on for example beetles (*Calathus* Stephens, 1827, *Pimelia* Rafinesque, 1815 and *Eutrichopus* Tschitscherin, 1897), mites (*Steganacarus* Ewing, 1917), spiders (*Dysdera* Latreille, 1804) and cockroaches (*Loboptera* Brunner von Wattenwyl, 1865), all showed vacariant sister lineages on the larger Tenerife Island (reviewed by Juan *et al.* 2000). For example, the COI gene of the oribatid mite, *Steganacarus carlosi* Niedbala, 1984, from the Tenerife Island indicated three phylogroups attributable to volcanism (Salomone *et al.* 2002). For the flightless darkling beetle, *Pimelia,* it was found that high levels of genetic variation and geographical structure were present in populations from the older Tenerife Island, while the populations on the younger western islands had limited phylogeoraphic structure (Moya *et al.* 2006). One would expect that the volcanic nature of these islands would influence small arthropods with limited dispersal abilities, however this pattern even holds true for vertebrate species such as the house mouse (*Mus musculus domesticus* Schwarz & Schwarz, 1943) on Madeira Island (Britton-Davidian *et al.* 2000; Gündüz *et al.* 2001).

These complex phylogeographic patterns present on sub-tropical islands gave rise to the question whether similar patterns also exist for species on less-explored islands like the Southern Ocean islands. In contrast to sub-tropical islands, Southern Ocean islands have also experienced glaciations which could further add to their complexity. Glaciations at the poles caused sea-levels to lower on sub-tropical islands, thereby effectively increasing the land area (see Jordan *et al*. 2005). On the contrary, glaciations on Southern Ocean islands caused much of these islands to be covered in ice and snow, thereby confining species to refugia. To date, limited phylogeographic studies have been conducted on Southern Ocean islands which have dynamic histories and a diverse array of habitats. These limitations can be ascribe to these islands' strict conservation regulations (many have World Heritage Status (Chown *et al.* 1998)) and limited travel accessibility.

Southern Ocean islands are diverse in terms of their origins (either continental fragments or volcanic) and geological ages (~0.01 million years old (myo) to 2 500myo; see Chown *et al*. 1998; Bergstrom & Chown 1999). The majority of these islands are geographically isolated, with the distance to the nearest continent (excluding Antarctica) ranging between 530km to 5 000km (data taken from Chown *et al*. 1998). Given these attributes, biologists of various disciplines have been fascinated by the species diversity present on these islands. Some authors (e.g. Sanmartin & Ronquist 2004; Stevens *et al.* 2006) have argued that species richness mirrors the distribution patterns predating the break-up of Gondwanaland. This argument has largely been proposed to account for diversity on the Antarctic continent and cannot readily be extrapolated to the majority of Southern Ocean islands since these are volcanic in origin. Stevens and colleagues (2006) convincingly argued that glaciation and isolation may, at least in part, be responsible for the diversification of the Collembola genus *Cryptopygus* which has a circumpolar distribution (see also Chown 1990 for data on Marion Island; Stevens & Hogg 2003). However, various other factors (such as recurrent dispersal and colonization or even the physical attributes of the islands themselves) may likewise have influenced the patterns observed today. Specifically for invertebrates, Chown *et al*. (1998) found a direct correlation between species richness on Southern Ocean islands and vascular plant richness (this is not surprising since most insects on the islands are herbivores) as well as the islands' distance to the nearest continent (hinting at the role of colonization and dispersal).

Marion Island (46°54'S; 37°45'E) together with Prince Edward Island (46°38'S; 37°57'E), forms the Prince Edward Island archipelago (PEI) situated in the middle of the Antarctic Polar Frontal Zone (APFZ). PEI together with Funk Seamont, is situated on a long-lasting hotspot that can be traced back to the south-eastern side (Volcan de l'Androy) of Madagascar (~88 million years ago (mya)) (Duncan 1981; Storey *et al.* 1995). Recent evidence also suggested that this hotspot played a major part in the break-up of Greater India and Madagascar during the same time period (Storey *et al.* 1995). Thus, PEI formed *de novo* and constitutes the tips of an oceanic intraplate volcano (Hall 1990; Hänel & Chown 1998; Hall 2002). Marion Island, the larger of the two islands, is believed to be between 1 and 0.5 million years old (myo) (Hänel & Chown 1998); however according to recent K-Ar dating, Marion Island is 0.45myo (McDougall *et al.* 2001). The influence of past climatic changes are well documented for this southern oceanic island (see McDougall *et al*. 2001) with evidence for at least eight volcanic periods and five glacial stages. The climatic history of the sub-Antarctic was greatly influenced by oscillations in the APFZ (Hall 1990); evidence suggested that islands located south of the APFZ, specifically those that are bigger with higher elevations, were more glaciated than those situated north of the APFZ (Bergstrom & Chown 1999).

The few phylogeographic studies focusing on indigenous microarthropods (mites (Mortimer & Jansen van Vuuren 2007) and springtails (Myburgh *et al*. 2007)) on Marion Island, had reduced statistical power due to small sample sizes and these authors could not determine whether factors other than isolation influenced the current population structure of microarthropods on Marion Island. Since both these studies had limited sample sizes, my

aim for this study was to conduct a large-scale phylogeographic study on the abundant oribatid mite, *Halozetes fulvus* Engelbrecht, 1975, to assess its population structure on Marion Island including the relative influences of isolation and migration. In addition, my aim was to compare the influence of geological (historical) events on the population structure of *H. fulvus* on Marion Island (glaciation and volcanism) to genetic patterns observed for species on sub-tropical islands. The genus *Halozetes* is interesting because these mites inhabit terrestrial, supralittoral and intertidal zones and have a wide distribution range in the South Polar Region (Pugh 1993; Marshall & Convey 2004).

MATERIALS AND METHODS

Sample collection

I collected 291 *H. fulvus* specimens from 30 localities across Marion Island during the 2004 and 2005 relief voyages (see Figure 4.1 for details). Mites were sampled from plant material such as mosses (*Ditrichum strictum* (Hook. f. et Wils.) Hampe, 1867)), tussock grass (*Poa cookii* Hook. f., 1879) and flowering plants (*Azorella selago* Hook. f., 1847, *Cotula plumosa* Hook. f., 1864 and *Crassula moschata* G. Forst., 1787). Specimens were extracted into propylene glycol by means of Tullgren extraction funnels (Southwood 1978) and subsequently preserved in absolute ethanol.

DNA extraction

Total genomic DNA was isolated using a phenol / chloroform extraction method described by Maniatis and co-workers (1982). Briefly, a single mite was placed in 550µl of DNA lysis buffer (1M NaCl, 1M Tris, 0.5M EDTA and 10% SDS solution) and 8µl Proteinase K (10mg/ml). The extraction was incubated at 55°C for 5 hours, phenol-chloroform extracted, ethanol precipitated and resuspended in 20µl deionised distilled water (Hillis *et al.* 1996).

PCR and sequencing

PCR reactions were carried out in 30µl reaction volumes and included 2µl of genomic DNA, 3µl of a 10X reaction buffer, 3µl of 2mM $MgCl_2$, 3µl of 1mM dNTP solution, 3pmol of each primer (LCO1490 and HCO2198 (Folmer *et al*. 1994)) and 1 unit of Taq polymerase (Super-Therm, Southern Cross Biotechnology, Cape Town, South Africa). The final volume was adjusted with deionised distilled water. The cycling parameters included an initial denaturation step at 94°C for 1 minute followed by 35 cycles of 94°C for 30 seconds, 40°C for 30 seconds and 72°C for 45 seconds. A final extension step at 72°C for 5 minutes completed the reactions. PCR products were purified with the Wizard SV Gel and PCR clean-up system (Promega, Madison, USA) according to the manufacturer's recommendations.

Amplicons were cycle sequenced using BigDye® Terminator 3.1 mix (Applied Biosystems, Warrington, UK). Unincorporated dye label was removed by sephadex columns before the samples were run on an ABI 3100

automated sequencer (Applied Biosystems, Warrington, UK). Electropherograms of the raw data were manually checked and edited with Sequence Editor™ software (Applied Biosystems, Warrington, UK). All *H. fulvus* sequences generated in this study were deposited in GenBank (DQ883230-DQ883330).

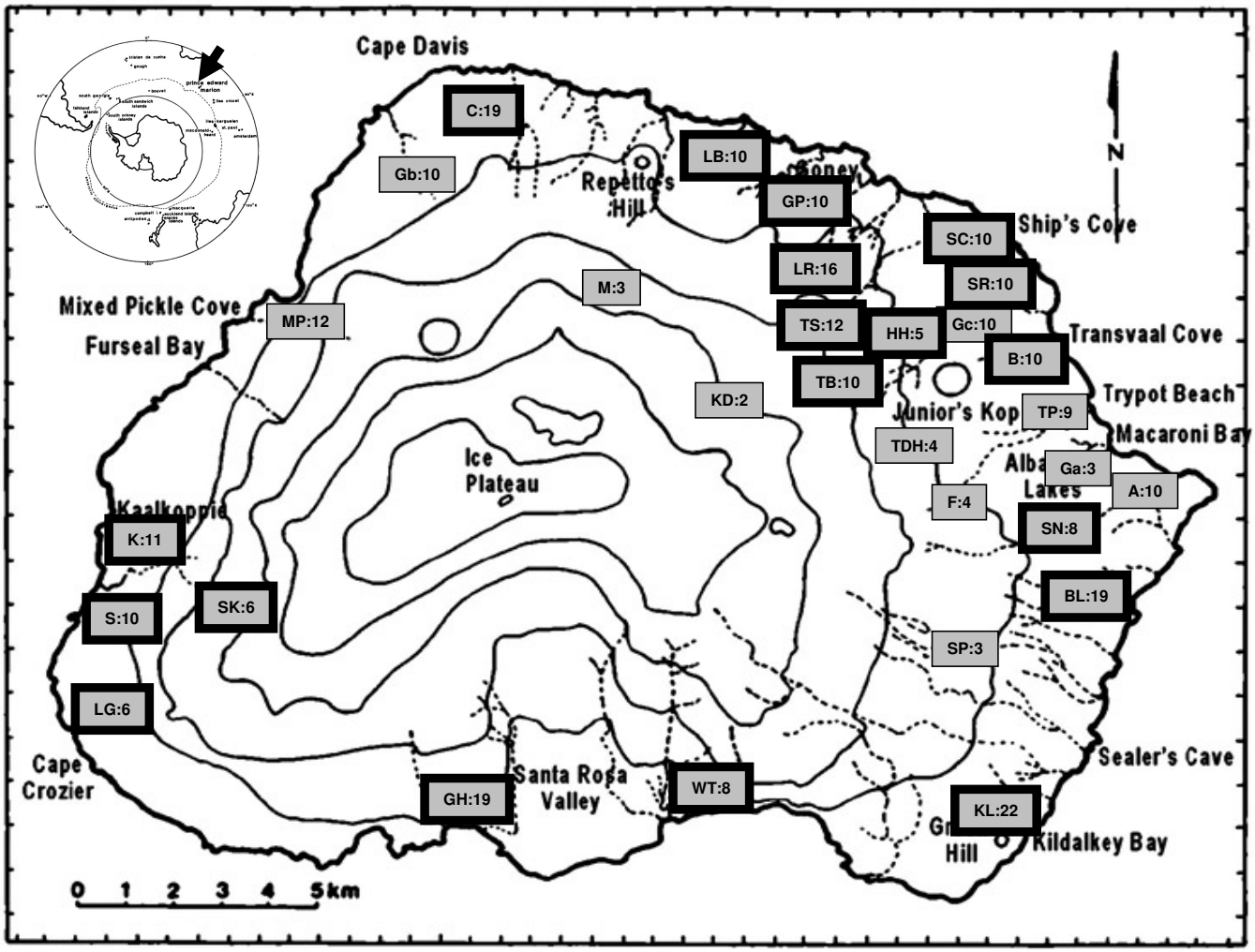

Figure 4.1 An illustration indicating the sampling localities of *H. fulvus* across Marion Island. The populations referred to in the text are indicated with the following symbols: Archway Bay - A, Meteorological Station - B, Bullard Beach - BL, Cape Davis - C, Fred's Hill - F, Grey Headed - GH, Goney Plain - GP, GPS coordinates (46°54'S; 37°53'E) - Ga, GPS coordinates (46°50'S; 37°41'E) - Gb, GPS coordinates (46°53'S; 37°52'E) - Gc, Hoppie's Hell - H, Kaalkoppie - K, Katedraalkrans - KD, Kildalkey Bay - KL, Log Beach - LB, La Grange Kop - LG, Long Ridge - LR, Middelman - M, Mixed Pickle - MP, Ships Cove - SC, Swartkops Point - S, Skuinskop - SK, Stoney Ridge - SN, Soft Plume - SP, Skua Ridge - SR, Tafelberg - T, Third Sister - TS, Tafelberg - T, Tom Dick & Harry - TDH, Trypot - TP and Watertunnel - W. The number of specimens included per locality is indicated. Localities highlighted in the discussion are shown in bold. Figure adapted from Smith and Gremmen (2004).

Sequence analysis

Sequences were aligned with Clustal X 1.81 (Thompson *et al.* 1997) using the multiple alignment mode. PAUP* 4.0b10 (Swofford 2000) was used to assess the nucleotide composition and the number of parsimony-informative sites. Nucleotide diversities were determined in MEGA 3.1 (Kumar *et al.* 2000) and corrected for ancestral polymorphism using the formula $Dxy=D-0.5(Dx+Dy)$ (Nei & Li 1979) where the corrected value (*Dxy*) is the average diversity (*D*) between populations after within-population diversity (*Dx* and *Dy*) has been subtracted. Haplotypes were identified using Collapse 1.2 (Posada 2004) and verified in Arlequin 3.0 (Excoffier *et al.* 2005). The evolutionary model that best fit my data was determined with Modeltest 3.06 (Posada & Crandall 1998). To determine whether I have sampled the actual number of haplotypes present in the population, the software EstimateS 7.51 was implemented (Colwell 2005). The non-parametric Chao 2 and Jacknife 2 estimators were used to construct an individual-based rarefaction curve (see Gotelli & Colwell 2001; Magurran 2004; Myburgh *et al.* 2007). Fifty randomisations (sampling without replacement) and 1000 randomisations (sampling with replacement) were performed to compare and to ensure that accurate results were obtained. To depict the evolutionary relationship (number of mutational steps) among the haplotypes I constructed a median-joining network using NETWORK 4.1.1.2 (Polzin & Daneshmand 2004). ParsProb 1.1 was used to determine the number of mutational steps under the 95% probability criterion (Templeton *et al*. 1992).

A demographic method was implemented to investigate the spatial distribution of mitochondrial variation using SAMOVA 1.0 (Dupanloup *et al.* 2002). This method provides a subjective way to identify spatially differentiated groups allowing for the identification of (possible) barriers to gene flow among them. An hierarchical analysis of molecular variance (AMOVA, Excoffier *et al.* 2005 as implemented in Arlequin 3.0) was used to estimate traditional *Phi* (Φ)–statistics reporting the degree of differentiation between populations as well as between individuals within these. Additionally, pair-wise Φ_{ST} was also estimated between all populations. In both instances, tests of significance (null distributions) were provided by permutational procedures (1000 randomisations).

To investigate whether haplotype frequencies deviated from neutrality, Fu's (1997) *F*-statistic was calculated in Arlequin 3.0. One possible cause for a deviation from equilibrium could be past demographic changes. To further investigate this, I plotted the number of mutational changes separating haplotypes against their respective frequencies as proposed by Rogers and Harpending (1992). A χ^2 goodness of fit test was used to compare observed and expected distributions. I estimated the time of demographic change using the equation $\tau=2\mu t$, where *tau* (τ) signifies the time of expansion in mutational units and μ represent the per locus mutation rate for COI (2.15% per million years following Salomone *et al*. 2002). A generation time of one year was assumed for *H. fulvus*. The raggedness statistic (*rg*) (Harpending *et al.* 1993, Harpending 1994), an indication of the smoothness of the mismatch distribution (population growth = lower *rg* values), was calculated in Arlequin 3.0. To confirm the

results, a coalescence-based methodology was also employed to calculate exponential growth rates (*g*-values in Fluctuate 1.30 (part of the LAMARC software package); Kuhner *et al*. 1998) for different populations across Marion Island.

To distinguish historical processes (e.g. glaciation) from recurrent processes (e.g. gene flow), I performed a nested clade analysis (NCA, GeoDis 2.4; Posada *et al.* 2000). The parsimony network was divided into a set of hierarchical nesting clades following Templeton *et al*. (1995). Null models of no haplotype-geographic association were generated through 1000 permutations. In instances where null hypotheses were rejected, the latest inference key (Templeton 2004) was used to determine possible factors that may be responsible for the significant haplotype–geographic associations. This kind of analysis has an additional benefit in that it would highlight problems with sampling design (both in terms of sampling numbers as well as geographic coverage).

Knowles and Maddison (2002) argued that NCA could sometimes deduce the wrong demographic and evolutionary processes to explain the observed spatial genetic structure. To reduce the risk of inferring incorrect evolutionary patterns, the coalescent-based MDIV program (Nielson & Wakeley 2001) was used to calculate theta ($\Theta=N_{ef}\mu$), directional migration or gene flow ($M=N_{ef}m$), divergence time ($T=t/N_{ef}$) and the time to the most recent common ancestor ($TMRCA=t\mu$) (N_{ef} represents the female effective population size, t represents the generation time, and μ represents the per locus mutation rate). Due to computational constraints, the analyses were only applied to localities that were especially noteworthy in terms of pair-wise Φ_{ST} values and to selected coastal localities. The 95% credibility intervals were calculated whenever possible. Because of computational and time constraints, I could not always calculate these values for the migration rate since an asymptote was never obtained. The pairwise simulations were run for 20 million generations with *Mmax* set at 200 (due to problem mentioned above) while *Tmax* was set at 10 as suggested by Nielson (2002). The analyses were conducted under the HKY (Hasegawa *et al*. 1985) finite sites model. Similar to my demographic population calculations above, a mutation rate of 2.15% per million years (or 1.17×10^{-5} substitutions per site per year) and a generation time of one year was assumed in all calculations. *T*, indicating the time of gene divergence, and *TMRCA*, an indication of population divergence, were multiplied by their respective Θ values to correct for different effective population sizes (for more information on methodology see Nielson & Wakeley 2001; Bowie *et al*. 2006; Bulgin *et al*. 2003; Griswold & Baker 2002).

Isolation-by-distance results when populations follow a typical stepping-stone model of evolution where gene flow occurs mainly between neighbouring populations. This pattern of evolution often typifies populations that are (relatively) stable over time. I used a Mantel test (as implemented in Arlequin 3.0) to compare geographical distances between sampling sites against genetic *p*-distances among populations (sampling sites were assumed to represent populations). Given that the high altitudinal interior of Marion Island has always been covered in snow with permafrost, I assumed that mites would not cross the vegetation line but rather circumvent these

higher elevations. Geographical distances were thus calculated along the most likely colonization routes rather than "as the crow flies".

In addition to the Mantel test, I also implemented principle component analysis (PCA) using the program Spatial Analysis in Macroecology (SAM 1.1) (Rangel *et al.* 2006). I strived to determine fine-scale spatial structure in my dataset. Localities together with their geographical coordinates, nucleotide diversity and haplotype diversity were included to determine whether there is a correlation between geographical distance and genetic distance.

RESULTS

I sequenced 545bp of the COI gene which translated into 181 functional amino acids; no stops codons or any insertions or deletions were found. Blast searches (GenBank) were also conducted which confirmed the authenticity of my sequences. Of the 545bp, 84 sites were polymorphic and 47 parsimony informative. A total of 101 haplotypes were found of which 73 were singletons. This was reflected in the relatively high haplotype diversity of 0.921±0.010. The non-parametric estimators (EstimateS) indicated that most of the haplotypes present in this species were sampled since the rarefaction curves plateaued once 250 of the 291 individuals were reached (data not shown). The average AT-content for my data set was 65.6%, increasing to 82.7% when only 3rd codon positions were considered. The mean transition: transversion ratio was 3.3: 1. The highest sequence divergence was 2.8% between specimens sampled at Kildalkey Bay and Bullard Beach. The highest pairwise nucleotide divergence (corrected for ancestral polymorphism) between populations was 0.91% between Middelman (n=3) and Soft Plume (n=3). On average, correcting for ancestral polymorphism reduced divergence values by ~0.2%.

A median-joining network, depicting the level of connectedness among haplotypes, is shown in Figure 4.2. None of my haplotypes were more divergent than the 95% confidence interval (10 steps), and could therefore be connected into a single network. The pattern that emerged is typical of a (relatively) recent population expansion (see for example Avise 1994; Conroy & Cook 2000; Jolly *et al*. 2005). The three most common haplotypes (HF37 comprising 21%, HF55 comprising 14% and HF5 comprising 11% of the specimens, respectively) formed cores with several haplotypes connecting to them in a star-like pattern. However, most haplotypes were connected by single mutational steps with some outliers being separated by up to four mutational changes. Missing haplotypes in a median-joining network may be the result of insufficient sampling. However, given my comprehensive sampling in combination with large sample sizes I argue that these more distantly related haplotypes may be remnants of the earlier structure prior to the most recent demographic events. More details on the number of haplotypes at each locality as well as the nucleotide and haplotype diversity can be found in Table 4.1.

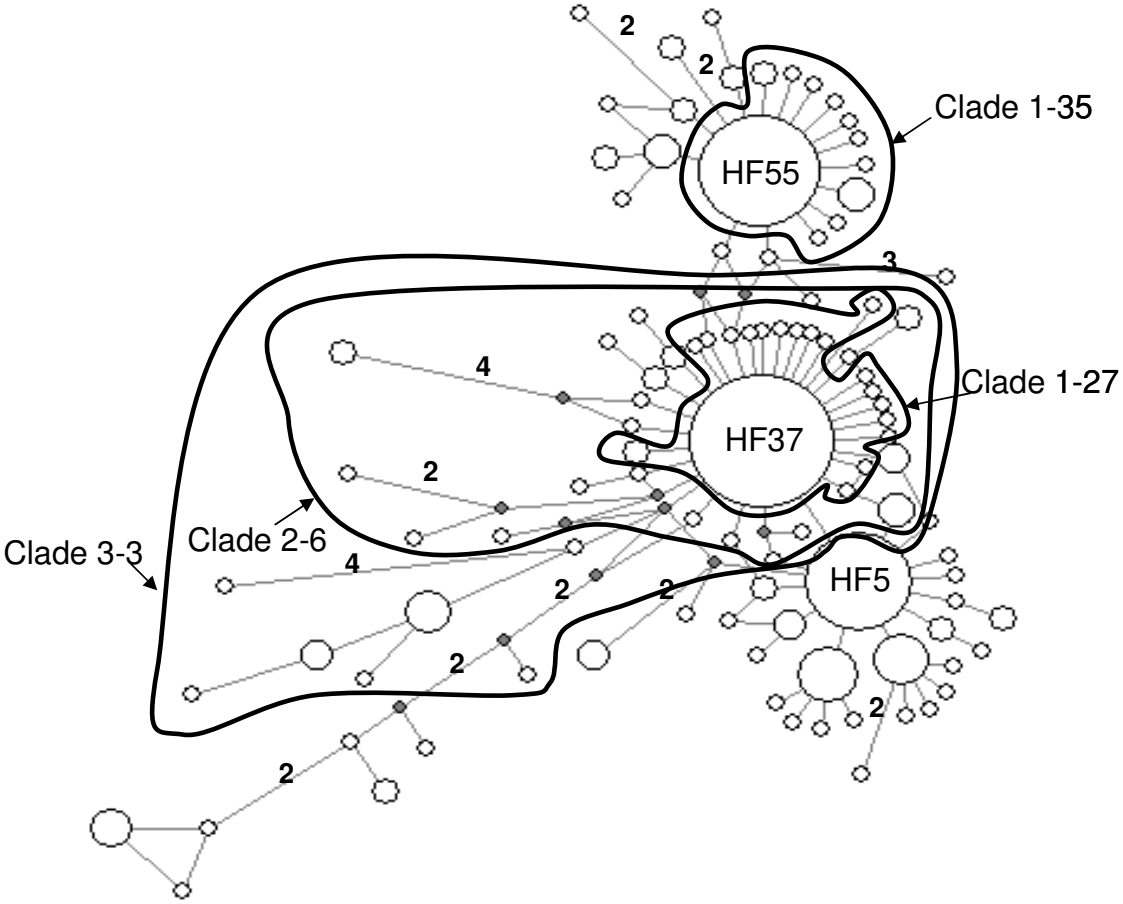

Figure 4.2 The median-joining network constructed for 291 *H. fulvus* specimens. The most common haplotypes are indicated. Missing haplotypes are shown in grey. Lines separating haplotypes represent one mutational change unless otherwise indicated.

Table 4.1 A summary of Arlequin 3.0 results indicating haplotype diversity, nucleotide diversity and the haplotype distribution at each of the 30 sample sites across Marion Island. See Figure 4.1 for GPS coordinates of Ga, Gb and Gc.

Populations	Haplotype diversity	Nucleotide diversity	Haplotypes
Cape Davis (n=19)	0.9298±0.0466	0.007726±0.004484	HF5, HF9, HF18, HF33, HF37, HF39, HF46, HF55, HF82, HF91, HF92, HF95, HF99
Middelman (n=3)	0.6667±0.3143	0.001223±0.001526	HF55, HF61
Log Beach (n=10)	0.9778±0.0540	0.005953±0.003787	HF4, HF5, HF9, HF18, HF27, HF30, HF31, HF55, HF88
Goney Plain (n=10)	0.9778±0.0540	0.005178±0.003369	HF1, HF3, HF5, HF9, HF37, HF53, HF57, HF78, HF84
Long Ridge (n=16)	0.8500±0.0772	0.005963±0.003632	HF5, HF26, HF37, HF38, HF43, HF64, HF85, HF87, HF99
Third Sister (n=12)	0.8485±0.1039	0.006950±0.004237	HF5, HF37, HF47, HF48, HF73, HF85, HF94, HF95
Katedraalkrans (n=2)	1.0000±0.5000	0.003670±0.004494	HF5, HF40
Tafelberg (n=10)	0.9333±0.0773	0.006565±0.004115	HF9, HF10, HF29, HF37, HF55, HF75, HF81, HF100
Hoppie's Hell (n=5)	0.7000±0.2184	0.001835±0.001708	HF1, HF5, HF37
Ships Cove (n=10)	0.9111±0.0773	0.005994±0.003808	HF18, HF20, HF34, HF37, HF65, HF71, HF79
Skua Ridge (n=10)	0.7778±0.1374	0.004444±0.002972	HF18, HF35, HF37, HF55, HF59, HF85
Meteorological Station (n=10)	0.9556±0.0594	0.007870±0.004813	HF1, HF5, HF11, HF12, HF23, HF37, HF54, HF55
Tom, Dick & Harry (n=4)	0.8333±0.2224	0.004587±0.003693	HF37, HF55, HF58
Trypot (n=9)	0.5833±0.1833	0.003568±0.002526	HF5, HF37, HF55, HF89
Fred's Hill (n=4)	0.8333±0.2224	0.014067±0.009931	HF55, HF83, HF95
Stoney Ridge (n=8)	0.9643±0.0772	0.008650±0.005392	HF2, HF5, HF7, HF36, HF44, HF55, HF67
Archway Bay (n=10)	0.9556±0.0594	0.009623±0.005747	HF18, HF21, HF37, HF41, HF42, HF55, HF66, HF80
Bullard Beach (n=19)	0.9415±0.0375	0.010258±0.005758	HF5, HF13, HF14, HF22, HF51, HF55, HF68, HF69, HF89, HF90, HF91, HF93, HF98
Soft Plume (n=3)	1.0000±0.2722	0.003670±0.003460	HF9, HF18, HF86
Kildalkey Bay (n=22)	0.8139±0.0593	0.007673±0.004422	HF5, HF8, HF9, HF17, HF35, HF37, HF55, HF95, HF96
Watertunnel (n=8)	0.8571±0.1083	0.006356±0.004126	HF5, HF24, HF45, HF55, HF60
Grey Headed (n=19)	0.9298±0.0466	0.008799±0.005025	HF5, HF16, HF18, HF37, HF49, HF50, HF55, HF63, HF65, HF68, HF70, HF74, HF76
La Grange Kop (n=6)	0.7333±0.1552	0.003425±0.002616	HF5, HF37, HF55
Skuinskop (n=6)	0.8000±0.1721	0.005627±0.003921	HF5, HF32, HF37, HF55
Swartkops Point (n=10)	0.9556±0.0594	0.006320±0.003984	HF5, HF9, HF18, HF37, HF45, HF55, HF80, HF101
Kaalkoppie (n=11)	0.7636±0.1066	0.001935±0.001562	HF5, HF18, HF25, HF37, HF80
Mixed Pickle (n=12)	0.9091±0.0795	0.006422±0.003960	HF3, HF7, HF18, HF37, HF52, HF55, HF56, HF65, HF77
Ga (n=3)	1.0000±0.2722	0.006116±0.005316	HF37, HF55, HF65
Gb (n=10)	0.9556±0.0594	0.009949±0.005921	HF5, HF6, HF15, HF19, HF28, HF62, HF66, HF72
Gc (n=10)	0.8000±0.1001	0.009704±0.005791	HF37, HF55, HF58, HF95, HF97

Interestingly, although the three common haplotypes are wide-spread across Marion Island, there appears to be some structure to their distribution as well as to the spread of singletons. When performing a SAMOVA, six groups were identified (Φ_{CT}=0.087; p<0.001) which corresponded to localities on the eastern side of Marion Island (group 1: Archway Bay; group 2: Bullard Beach), the north-eastern side (group 3: Cape Davis, Long Ridge, Hoppie's Hell, Tafelberg and Third Sister), the southern side (group 4: Grey Headed) and the south-western side (group 5: Kaalkoppie and Swartkops Point). The last group included the remainder of the localities which were spread across Marion Island. When regarding sampling localities as populations, AMOVA indicated that 92.9% of the variation was within populations with the remaining 7.1% between populations. Despite of this relatively low value, there was significant population substructure (Φ_{ST}=0.007, p<0.001). Population pair-wise Φ_{ST} values indicated that the localities of Bullard Beach (n=19), Long Ridge (n=16) (both on the eastern side), Middelman (northern side) (n=3), Grey Headed (southern side) (n=19) and Kaalkoppie (south-western side) (n=11) were significantly different to more than 40% of the populations. Other localities that also have noteworthy pair-wise Φ_{ST} values were Cape Davis (n=19), Log Beach (n=10), Third Sister (n=12) and Tom, Dick and Harry (n=4).

Fu's FS test returned a highly negative value (-25.27983; p<0.001), indicating a deviation from neutrality. The mismatch distribution indicated a correlation between my data and the expected values under a null model of sudden expansion (p=0.22). When sampling localities (n≥10) were considered separately, mismatch distributions indicated a change in population demography for most of the populations across Marion Island. These included Swartkops Point (p=0.2), Kaalkoppie (p=0.3), Mixed Pickle (p=0.4), Cape Davis (p=0.9), Log Beach (p=0.4), Goney Plain (p=0.2), Long Ridge (p=0.8), Tafelberg (p=0.9), Skua Ridge (p=0.9), 3rd sister (p=0.8), Hoppie's Hell (p=0.9), Meteorological Station (p=0.7), Archway Bay (p=0.7), Bullard Beach (p=0.4), Kildalkey Bay (p=0.3) as well as Grey Headed (p=0.9). The raggedness statistic (rg) was low (0.017, p=0.21) indicating population growth. It should be noted that some of these population demographic signatures may reflect ancient events rather than recent population trends. For example, when I calculated the time of population expansion for Bullard Beach, it would seem that the population expanded approximately 308 000 years ago. Similar trends were found for other localities such as Archway Bay (~240 000 years ago), Kildalkey Bay (~256 000 years ago) and Meteorological Station (~217 000 years ago). These results of population increase were corroborated by coalescent methods with the exponential growth factor (g), as calculated by Fluctuate, returning a relatively high positive g-value (g=438.77) for *H. fulvus* across Marion Island. Isolation-by-distance often results where populations are stable over time.

The inferred population history underlying the genetic structure of *H. fulvus* suggests that restricted gene flow with isolation-by-distance (isolation) as well as contiguous range expansions (migration) have played a role. A closer examination of the four different clades that showed a significant association between geography and genetics, revealed a contiguous range expansion for Clade 1-27 (inference key pathway 1-2-11-12-NO). This

clade included the wide-spread central haplotype HF37 as well as haplotypes connecting to it by single mutational differences. Restricted gene flow with isolation-by-distance was found for Clade 1-35 (inference key pathway 1-2-3-4-NO), Clade 2-6 (inference key pathway 1-2-3-4-NO) as well as the entire cladogram (inference key pathway 1-2-3-4-NO). Clade 3-3 (inference key pathway 1-2-11-17-NO) had an inconclusive outcome.

I used a coalescent approach to determine θ (an indicator of population size), gene flow and divergence times (as calculated in MDIV) (see Table 4.2 for results). Several noteworthy findings emerged. First, the effective (female) population sizes (N_{ef}) for localities on the western side of the island (Kaalkoppie, Swartkops Point, Mixed Pickle, Skuinskop and La Grange Kop) were considerably smaller compared to population sizes on the eastern side of Marion Island (Bullard Beach, Kildalkey Bay, Stoney Ridge and Archway Bay). Values given in Table 4.2 should be seen as an indication of relative size rather than as absolute numbers. Secondly, migration rates (gene flow) between populations on the western side (especially between Kaalkoppie and Mixed Pickle) were greater than migration rates between populations on the eastern side. Thirdly, the divergence times between populations on the western side of the island were zero, indicating that these populations have not yet diverged from one another. This finding is not surprising given the high migration rate between populations on the western side of Marion Island. The *TMRCA* calculated for populations on the western side of the island ranged from ~327 000 years before present (ybp) (Kaalkoppie and La Grange Kop) to ~771 000ybp (Kaalkoppie and Cape Davis). In contrast, on the eastern side where lower migration rates characterized populations, there was some evidence that the populations have either recently diverged or are in the process of diverging. Divergence rates varied from ~29 000ypb (between Bullard Beach and Watertunnel) to ~134 000ybp (between Bullard Beach and Stoney Ridge). As expected, *TMRCA* for populations on the eastern side (ranged from ~760 000ybp (Bullard Beach and Watertunnel) to ~856 000ybp (Bullard Beach and Kildalkey Bay)) was older than estimates for the western populations.

Markedly different evolutionary histories (effective (female) population sizes, migration rates, divergence times and *TMRCA* estimates; see above) were found for populations from Kaalkoppie and surroundings (western side) when compared to Bullard Beach and surroundings (eastern side). To further investigate patterns across Marion Island, the following sites were selected to give an overall representation of the island (northern, eastern, southern and western side): Cape Davis, Log Beach, Meteorological Station, Bullard Beach, Kildalkey Bay, Watertunnel, Grey Headed, La Grange, Kaalkoppie and Mixed Pickle. High levels of gene flow existed within and between the southern localities (Kildalkey Bay, Watertunnel and Grey Headed), western localities (La Grange, Kaalkoppie and Mixed Pickle) and northern localities (Cape Davis and Log Beach) of the island. A dramatic decrease in gene flow was observed on the eastern side (extending from Log Beach all to way down to Kildalkey Bay).

Table 4.2 MDIV results estimated from mitochondrial data for selected populations. The estimates of theta (and consequently N_{ef}), migration rates ($M = N_{ef}m$), divergence time (T) and the time to the most recent common ancestor (*TMRCA*) are indicated. To convert the estimates of *T* and *TMRCA* into years before present (ybp), a generation time of 1 year and a mutation rate of 1.17×10^{-5} substitutions per site per year was used. The 95% credibility intervals were calculated whenever possible and are indicated between brackets ([a]-the upper credibility interval could not be estimated since the parameter did not converge back to zero). A) MDIV results for Kaalkoppie and surrounding localities. B) MDIV results for Bullard Beach and surrounding localities.

A:

Population 1	*Population 2*	*theta (θ)*	N_{ef}	*M (Gene Flow)*	*TMRCA*	*T*
Swartkops Point	**Kaalkoppie**	2.43 (1.30-5.94)	199 701 (110 945-506 934)	9.2 (2->200[a])	537 119.69 ybp	0
La Grange Kop	**Kaalkoppie**	1.21 (0.58-3.85)	103 264 (49 499-328 568)	17.6 (1.2->200[a])	327 347.98 ybp	0
Skuinskop	**Kaalkoppie**	1.39 (0.68-5.95)	118 626 (58 033-507 788)	1.2 (1.2->200[a])	367 740.56 ybp	0
Grey Headed	**Kaalkoppie**	4.14 (1.91-8.34)	353 318 (163 004-711 756)	1.6 (1.2->200[a])	696 035.84 ybp	0
Mixed Pickle	**Kaalkoppie**	2.64 (1.44-6.17)	225 304 (122 893-526 563)	45.6 (1.6->200[a])	484 403.67 ybp	0
Cape Davis	**Kaalkoppie**	4.83 (2.87-9.31)	412 204 (244 933-794 538)	16.8 (2.4->200[a])	770 821.42 ybp	0

B:

Population 1	*Population 2*	*theta (θ)*	N_{ef}	*M (Gene flow)*	*TMRCA*	*T*
Archway Bay	**Bullard Beach**	7.63 (4.88-14.40)	651 163 (416 471-1 228 931)	2.1 (1->200[a])	853 023.26 ybp	104 186.05 ybp
Kildalkey	**Bullard Beach**	5.45 (3.44-9.68)	465 116 (293 578-826 115)	1.9 (0.8->200[a])	855 813.95 ybp	83 720.93 ybp
Stoney Ridge	**Bullard Beach**	7.89 (4.90-14.71)	673 352 (418 178-1 255 387)	3.2 (0.6->200[a])	834 956.26 ybp	134 670.36 ybp
Watertunnel	**Bullard Beach**	5.67 (2.75-11.45)	483 892 (234 692-977 171)	3.1 (0.5->200[a])	759 709.84 ybp	29 033.95 ybp
Meteorological Station	**Bullard Beach**	6.84 (4.06-12.45)	583 742 (346 490-1 062 490)	2.7 (0.6->200[a])	834 751.44 ybp	70 049.07 ybp

My Mantel test for the total population did not indicate isolation-by-distance (p=0.95, r=-0.14). When conducting a Mantel test on all the western and southern localities of the island (Watertunnel, Grey Headed, La Grange, Skuinskop, Swartkops Point, Kaalkoppie and Mixed Pickle), no isolation-by-distance was detected (p=0.21, r=0.20). However, to minimize my sampling bias towards the eastern (remainder) of the island, I conducted a Mantel test on randomly selected localities from the eastern side (Long Ridge, Tafelberg, Third Sister, Hoppie's Hell, Ships Cove, Skua Ridge, Meteorological Station, Trypot, Archway Bay, Stoney Ridge, Bullard Beach and Kildalkey Bay). These localities did indicate isolation-by-distance (p=0.05, r=0.32). Since my MDIV results indicated a substantial drop in gene flow between Log Beach and Meteorological Station, a Mantel test was conducted on localities in the vicinity, namely Meteorological Station, Ships Cove, Skua Ridge, Third Sister, Long Ridge and Hoppie's Hell. The Mantel test results indicated the presence of isolation-by-distance (p=0.054, r=0.447).

According to PCA, the first and second principal component axes accounted for most of the variation in my data set (p<0.05). Thus, the differences in localities (first principal component) were the main contributor to variation (49.9%), followed by nucleotide diversity (second principle component) (32.5%) and haplotype diversity (17.6%). From both scatterplots of the principal components, it did appear if the localities from the northern and eastern side of the island formed a group, while the localities from the southern and western side clustered together (Figure 4.3). It was also found that the nucleotide diversity decreased from the northern / eastern side of the island towards the southern / western side of the island.

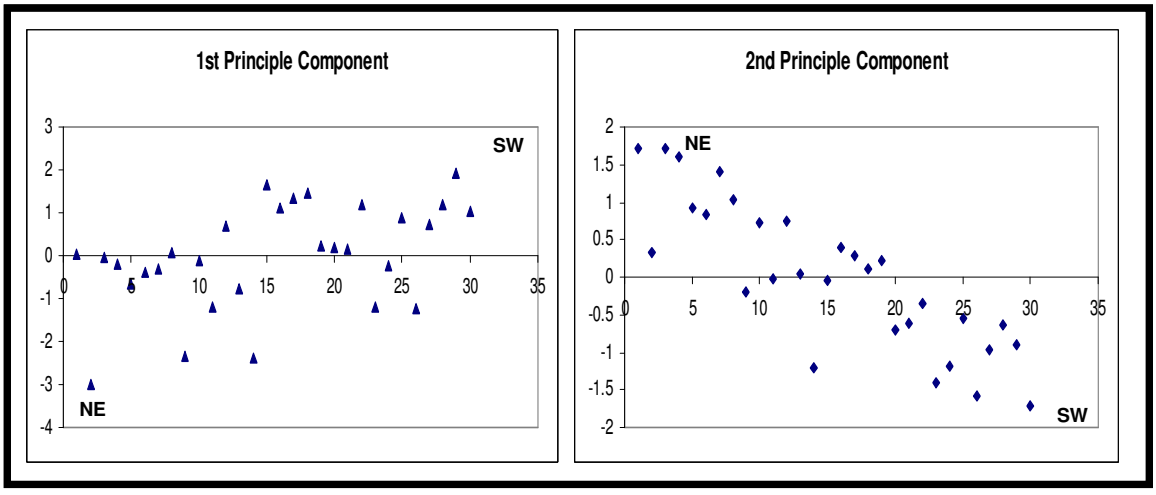

Figure 4.3 Scatterplot illustrating the first (localities) and second (nucleotide diversities) principal components of the principle component analysis (PCA). For both principle component graphs, the localities from the northern and eastern side (NE) group together (underneath line) and the localities from the southern and western side (SW) group together (above line).

DISCUSSION

To my knowledge, I compiled the largest phylogeographic study for a terrestrial mite species on any Southern Ocean island. Similar to sub-tropical islands, it is apparent from my findings together with previous results (Mortimer & Jansen van Vuuren 2007; Myburgh *et al.* 2007) that exceedingly complex population patterns are present on sub-Antarctic Marion Island. In addition, the population genetic structure within *H. fulvus* appears to be sculpted by an interplay of historic isolation events (most likely associated with climatic and geological events) as well as contemporary mechanisms (such as gene flow).

Intra-island complexity

Within *H. fulvus*, a large proportion of the genetic variance was accounted for by variation within populations (92.9%) with a much smaller (yet highly significant) component between populations (7.1%, $p<0.001$). A high number of haplotypes (n=101) were present in the total population of 291 individuals. My rarefaction curves (using both Chao 2 and Jacknife 2 estimators) indicated that most of the haplotype diversity was obtained within my current sample size. This high level of haplotype diversity was also detected for the prostigmatid mite, *E. minutus* (Mortimer & Jansen van Vuuren 2007), as well as Collembola (springtail) species, *Cryptopygus antarcticus travei* Déharveng, 1981 and *Tullbergia bisetosa* (Börner, 1903) (Myburgh *et al.* 2007) on Marion Island. In the case of springtails, the diversity was substantially higher on Marion Island than for springtail species (*Gomphiocephalus hodgsoni* Carpenter, 1908 and *Desoria klovstadi* (Carpenter, 1902)) from the Antarctic (Frati *et al*. 2001; Stevens & Hogg 2003). In conjunction, the findings for both mites and springtails may indicate that the high levels of genetic variation may be due to intra-island conditions specific to Marion.

When considering the oribatid mite species, *Steganacarus carlosi,* on the sub-tropical Canary archipelago (Tenerife Island), these mites had a well-defined population structure as a direct consequence of volcanic evolution of the island (Salomone *et al.* 2002). In comparison with *S. carlosi, H. fulvus* on Marion Island has a much more complex population structure, which is not surprising considering that volcanic Marion Island has been dissected by numerous glacial events (McDougall *et al.* 2001). My data suggested there is a correlation between genetic and geographic distance; localities from the northern and eastern side of the island group together, while the southern and western localities group together. This scenario fits well with the climatic conditions on the island. The northern and eastern sides of the island have calmer weather conditions compared to the harsher conditions on the western and southern sides of the island. When considering all my findings, unique populations were present in the eastern (Bullard Beach), north-eastern (Cape Davis, Log Beach, Third Sister, Long Ridge, Tafelberg and Hoppie's Hell), southern (Grey Headed) and south-western (Kaalkoppie and Swartkops Point) side of the Island. To simplify my results, I especially focused on Kaalkoppie and surroundings ("western side of the island") and Bullard Beach and surroundings ("eastern side of the island"), since very similar results were previously obtained for *E. minutus* (Mortimer & Jansen van Vuuren, 2007) and *C. antarcticus* (Myburgh *et al.* 2007). The unique populations on the north-eastern and southern side are discussed briefly.

The spatial distribution of the species on the western side of the island was dominated by high migration rates (and correspondingly, my Mantel test also indicated no isolation-by-distance) and small female effective population sizes. Interestingly, my data also suggested that gene flow on the western side of Marion Island was more prevalent along the coastal plateaus rather than crossing over the higher elevations to the central part of the island. The highest migration rate (rate value=45.6) in this study was between the coastal localities of Mixed Pickle and Kaalkoppie. Even when considering a broader geographic area, a migration rate of 16.8 was observed between the coastal localities of Cape Davis and Kaalkoppie (±8km apart). These migration estimates among geographically more distant populations were considerably higher than the values estimated for localities that were more closely situated but at elevation (for example the migration rate of 1.2 between Skuinskop and Kaalkoppie (±1km apart) and 1.6 between Grey Headed and Kaalkoppie (±7km apart)).

Given the relatively high migration rates estimated between localities, it was expected that these western populations have not yet fully diverged, with time to divergence (*T*) being zero (see Table 4.2). The *TMRCA* ranged between 327 000ybp to 771 000ybp. As one would expect, the western side consisted of expanding populations. The time of expansion estimated for Kaalkoppie occurred roughly 52 000 years ago and can be traced back to catastrophic events that occurred in the vicinity during the past. The event at Kaalkoppie may be due to both volcanic eruptions (VIII *sensu* McDougall *et al*. 2001) occurring at the higher altitudes across the island (20 000 to 70 000 years ago), as well as glaciation events (Stage 4) occurring along the west coast between Triegaardt Bay and Goodhope Bay (87 000±20 000 to 40 000±15 000). These findings were not surprising when considering that the western side of the island is known for its inhospitable environment due to multiple young (black) and old (grey) lava flows that are interspersed with sparse fellfield vegetation. This side is also bombarded by cold, high speed winds from the Antarctic (Smith & Lewis Smith 1987) which may assist in migration.

In contrast to the western side of the island, populations along the eastern seaboard of Marion Island are characterized by substantially larger effective female population sizes and generally low migration rates (see Table 4.2 for values) with isolation-by-distance. The highest migration rate estimated for any of the eastern localities was 3.2 between Bullard Beach and Stoney Ridge (<1km apart). These low migration rates would indicate a more stable panmictic population. This restricted gene flow observed between the eastern and western side of the island was also supported by NCA. The divergence times also indicated that the populations and the genes have diverged. The divergence times for populations along the east coast varied between 29 000ybp (between Watertunnel and Bullard Beach) to 134 000ybp (between Stoney Ridge and Bullard Beach). The *TMRCA* estimates were much higher for eastern populations (ranged from 760 000ybp (between Watertunnel and Bullard Beach) to 856 000ybp (between Kildalkey Bay and Bullard Beach)) in comparison with the western side. The population expansion at Bullard Beach was estimated at roughly 308 000 years ago. Bullard Beach is located on moraine dating back to the Pleistocene epoch and the only volcanic (II) and glaciation activity (Stage

12) around 350 000 years ago, were in the vicinity of Crawford Bay (south) (McDougall *et al*. 2001). I strongly doubt that this event directly influenced the species' populations at Bullard Beach. I rather propose that even though westerly winds predominate, it is believed that Kildalkey Bay (±5km from Bullard Beach) is a natural entry point for new species / arrivals onto the island (S.L. Chown; R. Mercer, personal observation). This is supported by the presence of new plant (woody plants) and insect (moths) species at Kildalkey Bay (S. Slabber, personal observation). Kildalkey Bay may also be an entry point due to ocean current directional changes during the Quaternary period (Graham *et al*. 2003) and that east-to-west winds have been observed on Macquarie Island (Watson 1967; Wallwork 1973). When considering Bullard Beach's close vicinity to Kildalkey Bay, I speculate that the entry point for new arrivals may extend to the south-eastern side (between Bullard Beach and Kildalkey Bay) rather than a single locality. Additionally, sealing activities during the eighteenth and nineteenth century may have inadvertently facilitated the introduction of invertebrate individuals to the eastern side of the island.

Again, the isolated stable populations on the eastern side fit well with the environmental conditions and the history of the eastern side. Contrary to the western side, the eastern side can be described as calmer, with mainly older grey lava covered by vegetation (van Zinderen Bakker *et al.* 1971; Smith and Gremmen 2004). Previous studies have indicated that these contrasting conditions between the western and eastern side have a significant impact on the fauna and flora across the island (M.A. McGeoch, personal communication).

SAMOVA as well as pairwise Φ_{ST} values indicated the presence of unique populations on the north-eastern side of the island. My MDIV analyses also revealed a strong decline in gene-flow at the north-eastern side of the island (Log Beach to Meteorological Station), and conversely an increase at the south-eastern side (Kildalkey Bay to Watertunnel). This decline in gene-flow was supported by the Mantel test. On the north-eastern side, one of Marion Island's older grey lava formations, namely Long Ridge (formed during Pleistocene) is present (McDougall *et al.* 2001). When conducting MDIV analyses on Long Ridge and its surrounding localities, very low levels of gene flow were detected between Long Ridge and Log Beach, Goney Plain, Ships Cove, Skua Ridge and Hoppie's Hell with the exception of Third Sister, with high levels of gene flow (data not shown). Isolation-by-distance was also found. Thus, I speculate that Long Ridge on the north-eastern side of the island, may effectively act as a barrier to gene flow to the surrounding localities.

High levels of gene flow were present on the southern side (between Kildalkey Bay, Watertunnel and Grey Headed) similar to the western side. As mentioned before, this was not surprising considering that conditions are very similar to the rough and environmentally unstable western side of the island. Extensive glaciation has been found from north-western side of the island (Triegaardt Bay near Mixed Pickle) towards the southern side of the island (Goodhope Bay near Grey Headed and Crawford Bay near Watertunnel) (McDougall *et al*. 2001). In addition, the Santa Rosa Valley with extensive lava flows (both old (pre-Pleistocene) and recent (Holocene)

(McDougall *et al*. 2001)) and sparse vegetation cover is present in the vicinity of Grey Headed, which may be responsible for the unique population.

Conservation

Congruent results obtained for invertebrates (mites and springtails), highlight certain areas on Marion Island that are of special conservation concern. The vicinities surrounding Bullard Beach and Kildalkey Bay on the eastern side as well as Kaalkoppie, Swartkops Point and La Grange Kop on the south-western side, are especially important for conservation of indigenous invertebrate populations. Invertebrates play an important role in the ecosystem of sub-Antarctic islands and to preserve the natural composition of invertebrate is thus of great importance. I propose that strict regulations should be implemented to conserve these important localities. With the steady increase of annual temperatures (1.2°C) and the associated decrease in moisture (600mm) (Smith 2002, Smith & Gremmen 2004), there are concerns that alien invertebrates may out compete the indigenous species due their ability to grow and reproduce faster (Barendse 2000; Chown *et al*. 2002). My findings suggested that the *H. fulvus* population is growing on Marion Island, similar to previous findings obtained for *E. minutus* (Mortimer & Jansen van Vuuren 2007) even though *H. fulvus* is known to prefer warm and wet conditions compared to *E. minutus*'s preference for warm and dry conditions (Hugo 2006). One may speculate that the change in climate does not have a negative impact on these species' population growth.

To conclude, similar to findings for sub-tropical islands, very complex phylogeographic patterns exist for species on sub-Antarctic Marion Island. The population structure of *H. fulvus* on Marion Island was more complex than initially anticipated due to the presence of numerous haplotypes. I found two evolutionary processes present on Marion Island that were the driving force for the diversification of species. The western and southern side of the island were dominated by gene flow probably due to the environmental conditions and multiple catastrophic events (glaciation and volcanism) during the past and present, while the calmer eastern side was isolated with more stable populations. Since both historical and recurrent processes were responsible for diversification of species on Southern Ocean islands, I advise that scientists need to widen their scope when conjecturing scenarios to explain their molecular data in a phylogeographic analysis.

REFERENCES

Avise JC (1994) *Molecular Markers, Natural History and Evolution*. Chapman and Hall, London and New York.

Barendse J (2000) *Patterns in the distribution and abundance of terrestrial arthropods on sub-Antarctic Marion Island*. MSc Thesis, University of Pretoria.

Bergstrom DM, Chown SL (1999) Life at the front: history, ecology and change on southern ocean islands. *Trends in Ecology and Evolution,* **14**, 472-477.

Bowie RCK, Fjeldså J, Hackett SJ, Bates JM, Crowe TM (2006) Coalescent models reveal the relative roles of ancestral polymorphism, vicariance, and dispersal in shaping phylogeographical structure of African montane forest robin. *Molecular Phylogenetics and Evolution*, **38**, 171-188.

Britton-Davidian J, Catalan J, da Graça Ramalhinho M, Ganem G, Auffray JC, Capela R, Biscoito M, Searle JB, da Luz Mathias M (2000) Rapid chromosomal evolution in island mice. *Nature*, **403**, 158.

Bulgin NL, Gibbs HL, Vickery P, Baker AJ (2003) Ancestral polymorphisms in genetic markers obscure detection of evolutionarily distinct populations in the endangered Florida grasshopper sparrow (*Ammodramus savannarum floridanus*). *Molecular Ecology*, **12**, 831-844.

Chown SL (1990) 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 SL, Gremmen NJM, Gaston K (1998) Ecological Biogeography of Southern Ocean Islands: Species-area relationships, human impacts and conservation. *The American Naturalist,* **152**, 562-575.

Chown SL, McGeoch MA, Marshall DJ (2002) Diversity and conservation of invertebrates on the sub-Antarctic Prince Edward Islands. *African Entomology*, **10**, 67-82.

Colwell RK (2005) *Estimates: statistical estimation of species richness and shared species from samples, Version 7.5.* User's Guide and application published at: http://purl.oclc.org/estimates.

Conroy CJ, Cook JA (2000) Phylogeography of post-glacial colonizer: *Microtus longicaudatus* (Rodentia: Muridae). *Molecular Ecology,* **9**, 165-175.

Darwin C (1859) *On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life*. John Murray, London.

Duncan RA (1981) Hotspots in the southern oceans – an absolute frame of reference for motion of the Gondwana continents. *Tectonophysics*, **74**, 29-42.

Dupanloup I, Schneider S, Excoffier L (2002) A simulated annealing approach to structure of populations. *Molecular Ecology*, **11**, 2751-2581.

Emerson BC (2002) Evolution on oceanic islands: molecular phylogenetic approaches to understanding pattern and process. *Molecular Ecology*, **11**, 951-966.

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, **1**, 47-50.

Frati F, Spinsanti G, Dallai R (2001) Genetic variation of mtCOII gene sequences in the collembolan *Isotoma klovstadi* from Victoria Land, Antarctica: evidence for population differentiation. *Polar Biology*, **24**, 934-940.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology,* **3**, 294-299.

Fu YX (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics*, **147**, 915-925.

Gillespie R, Roderick G (2002) Arthropods on islands: colonization, speciation and conservation. *Annual Revue of Entomology*, **47**, 595-632.

Gotelli JN, Colwell RK (2001) Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. *Ecology Letters*, **4**, 379 - 391.

Graham MH, Dayton PK, Erlandson JM (2003) Ice ages and ecological transitions on temperate coasts. *Trends in Ecology and Evolution*, **18**, 33-40.

Griswold CK, Baker AJ (2002) Time to the most recent common ancestor and divergence times of populations of common chaffinches (*Fringilla coelebs*) in Europe and Northe Africa: Insights into Pleistocene refugia and current levels of migration. *Evolution*, **56**, 143-153.

Gündüz İ, Auffray JC, Britton-Davidian J, Catalan J, Ganem G, Ramalhinho MG, Mathias ML, Searle JB (2001) Molecular studies on the colonization of the Madeiran archipelago by house mice. *Molecular Ecology*, **10**, 2023-2029.

Hall KJ (1990) Quaternary glaciations in the southern ocean: sector 0° long - 180° long. *Quarternary Science Reviews*, **9**, 217-228.

Hall KJ (2002) Review of present and Quaternary peri-glacial processes and landforms of the maritime and sub-Antarctic region. *South African Journal of Science,* **98**, 71-81.

Hänel C, Chown SL (1998) *An introductory guide to the Marion and Prince Edward Island Special Nature Reserves, 50 years after annexation*. Department of Environmental Affairs and Tourism, Pretoria.

Harpending HC, Sherry ST, Rogers AR, Stoneking M (1993) The genetic structure of ancient human populations. *Current Anthropology,* **34**, 483-496.

Harpending H (1994) Signature of ancient population growth in a low resolution mitochondrial DNA mismatch distribution. *Human Biology,* **66**, 591-600.

Hasegawa M, Kishino H, Yano T (1985) Dating the human–ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution*, **22**, 160-174.

Hillis D, Moritz C, Mable B (1996) *Molecular Systematics*. Sinauer Associates, Sunderland.

Hugo EA (2006) *Spatial patterns in the microarthropod community associated with Azorella selago (Apiaceae) on the sub-Antarctic Prince Edward Islands*. PhD thesis, University of Stellenbosch.

Jolly MT, Jollivet D, Gentil F, Thiebaut E, Viard F (2005) Sharp genetic break between Atlantic and English Channel populations of the polychaete *Pectinaria koreni*, along the North coast of France. *Heredity,* **94**, 23-32.

Jordan S, Simon C, Foote D, Englund RA (2005) Phylogeographic patterns of Hawaiian *Megalagrion* damselflies (Odonata: Coenagrionidae) correlate with Pleistocene island boundaries. *Molecular Ecology*, **14**, 3457-3470.

Juan C, Emerson BC, Oromí P, Hewitt GM (2000) Colonization and diversification: towards a phylogeographic synthesis for the Canary Islands. *Trends in Ecology and Evolution,* **15**, 104-109.

Knowles LL, Maddison WP (2002) Statistical phylogeography. *Molecular Ecology*, **11**, 2623-2635.

Kuhner MK, Yamato J, Felsenstein J (1998) Maximum likelihood estimation of population growth rates based on the coalescent. *Genetics*, **149**, 429-434.

Kumar S, Tamura K, Jakobsen I, Nei M (2000) *MEGA: Molecular evolutionary genetics analyses. Version 3.1.* University Park, Pennsylvania State University.

Magurran AE (2004) *Measuring biological diversity*. Blackwell Publishing, Oxford.

Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning. Cold Spring Harbor, New York.

Marshall DJ, Convey P (2004) Latitudinal variation in habitat specificity of ameronothrid mites (Oribatida). *Experimental and Applied Acarology,* **34**, 21-35.

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

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.

Moya Ó, Contreras-Díaz HG, Oromí P, Juana C (2006) Using statistical phylogeography to infer population history: Case studies on *Pimelia* darkling beetles from the Canary Islands. *Journal of Arid Environments*, **66**, 477–497.

Myburgh M, Chown SL, Daniels SR, Jansen van Vuuren B (2007) Population structure, propagule pressure and conservation biogeography in the sub-Antarctic: lessons from indigenous and invasive springtails. *Diversity and Distributions*, **13**, 143-154.

Nei M, Li WH (1979) Mathematical model for studying genetic variation interms of restriction endonucleases. *Proceedings of the National Academy of Sciences, USA,* **76**, 5269-73.

Nielsen R, Wakeley J (2001) Distinguishing migration from isolation: a Markov chain Monte Carlo approach. *Genetics*, **158**, 885-896.

Nielson R (2002) *MDIV software*. Available from <http://www.biom.cornell.edu/Homepages?Rasmus_Nielson/files.html>.

Polzin T, Daneshmand SV (2004) *NETWORK 4.1.1.2. Fluxus Technology Ltd. Steiner (MP) algorithm.*

Posada D, Crandall K (1998) Modeltest: testing the model of DNA substitution. *Bioinformatics*, **14**, 817-818.

Posada D, Crandall KA, Templeton AR (2000) GEODIS: a program for the cladistic nested analysis of the geographical distribution of genetic haplotypes. *Molecular Ecology*, **9**, 487-488.

Posada (2004) *COLLAPSE 1.2 Free Software Foundation Inc*. Boston, USA.

Pugh PJA (1993) A synonymic catalogue of the Acari from Antarctica, the sub-Antarctic Islands and the Southern Ocean. *Journal of Natural History*, **27**, 323-421.

Rangel TFLVB, Diniz-Filho JAF, Bini LM (2006) Towards an integrated computational tool for spatial analysis in macroecology and biogeography. *Global Ecology and Biogeography,* **15**, 321-327.

Roderick GK, Gillespie RG (1998) Speciation and phylogeography of Hawaiian terrestrial arthropods. *Molecular Ecology*, **7**, 519-531.

Rogers AR, Harpending H (1992) Population growth makes waves in the distribution of pairwise genetic differences. *Molecular Biology and Evolution,* **9**, 552-569.

Salomone N, Emerson B, Hewitt G, Bernini F (2002) Phylogenetic relationships among the Canary Island Steganacaridae (Acari, Oribatida) inferred from mitochondrial DNA sequence data. *Molecular Ecology*, **11**, 79-89.

Sanmartin I, Ronquist F (2004) Southern Hemisphere biogeography inferred by event-based models: plant versus animal patterns. *Systematic Biology*, **53**, 216-243.

Smith VR, Lewis Smith R (1987) The biota and conservation status of sub-Antarctic islands. *Environment International,* **13**, 95-104.

Smith VR (2002) Climatic change in the Sub-Antarctic: An illustration from Marion Island. *Climate Change,* **52**, 345-357.

Smith VR, Gremmen NJM (2004) *The Flora of Marion and Prince Edward Island.* The Netherlands.

Southwood TRE (1978) *Ecological methods with particular reference to the study of insect populations*. Chapman and Hall, London.

Stevens MI, Hogg ID (2003) Long-term isolation and recent range expansion from glacial refugia revealed for the endemic springtail *Gomphiocephalus hodgsoni* from Victoria Land, Antarctica. *Molecular Ecology,* **12**, 2357-2369.

Stevens MI, Greenslade P, Hogg ID, Sunnucks P (2006) Southern Hemisphere springtails: could any have survived glaciation of Antarctica? *Molecular Biology and Evolution*, **23**, 874-882.

Storey M, Mahoney JJ, Saunders AD, Duncan RA, Kelley SP, Coffin MF (1995) Timing of hot spot-related volcanism and the breakup of Madagascar and India. *Science*, **267**, 852-855.

Swofford D (2000) *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other methods)*. Sinauer.

Templeton AR, Crandall KA, Sing CF (1992) A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics*, **132**, 619-633.

Templeton AR, Routman E, Phillips CA (1995) Separating population structure from population history: a cladistic analysis of the geographical dispersion of mitochondrial DNA haplotypes in the tiger salamander, *Ambystoma tigrinum*. *Genetics*, **140**, 767-782.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, **24**, 4876-4882.

van Zinderen Bakker EM, Winterbottom JM, Dyer RA (1971) *Marion and Prince Edward Islands. Report on the South African biological and geological expedition 1965–1966*. A.A. Balkema, Cape Town.

Wallwork JA (1973) Zoogeography of some terrestrial micro-arthropoda in Antarctica. Biological Reviews, **48**, 233-259.

Watson KC (1967) The terrestrial Arthropoda of Macquarie Island. *ANARE Scientific Reports B (Zool),* **99**, 90.

CHAPTER 5

GROWTH FORM AND POPULATION GENETIC STRUCTURE OF *AZORELLA SELAGO* ON SUB-ANTARCTIC MARION ISLAND

(E. Mortimer, M. A. McGeoch, S. R. Daniels, B. Jansen van Vuuren 2007. *Antarctic Science,* in press)

ABSTRACT

Seven community complexes have been described across sub-Antarctic Marion Island, amongst these fellfield that typically comprise low plant cover dominated by the flowering cushion plant, *Azorella selago*. *Azorella* is considered a keystone species since it forms nutrient rich environments for microarthropod communities and epiphytic plants. Two distinct growth forms typify *Azorella,* namely discrete cushions and continuous mats. Whether these continuous mats consist of a single large cushion individual, or whether several individual plants merge, interdigitating to form a continuous area, remains moot. As such, it is important to obtain some measure of *Azorella* growth dynamics before embarking on phylogeographic studies. Previous genetic studies indicated that several of these microarthropod species are significantly substructured across Marion Island, but it remains unclear whether similar subdivisions characterize *Azorella*. We used chloroplast sequence data (*trn*H-psbA) and amplified fragment length polymorphism (AFLP) to investigate these questions. No sequence variation characterized the *trn*H-psbA region in *Azorella* across Marion Island. In contrast, the AFLP results indicated that an *A. selago* mat comprises multiple individuals. We argue that mats can be formed through at least two processes namely fragmentation, where parts of the cushion plant die off creating open areas for the establishment of different individuals and / or to a high density of interdigitating individuals merging to form the mat. Fragment data further indicated significant substructure for *Azorella* across Marion Island (F_{ST}=0.101, p=0.01) and we attribute this to past vicariance.

INTRODUCTION

Sub-Antarctic islands are interesting evolutionary entities due to their various geological origins (continental or volcanic) and histories (glaciation and volcanism) (Chown *et al.* 1998). Moreover, these islands are isolated from continents, implying restricted gene flow which ultimately results in high levels of species endemism (Emerson 2002). Sub-Antarctic Marion Island (46º54'S, 37º45'E) is the larger of two islands comprising the Prince Edward Island group. Prince Edward (46º38'S, 37º57'E), the second island in this group, is separated from Marion Island by approximately 19km. Similar to most other Southern Ocean Islands, the Prince Edward Island archipelago has a volcanic origin (Hänel & Chown 1998; McDougall *et al.* 2001) and based on recent K-Ar dating, Marion Island is estimated to be ~0.45 million years old (myo) (McDougall *et al.* 2001). As such, the biota on this island is probably representative of recent (post-Pleistocene) colonization events (Verwoerd 1971; Chown 1994).

Vegetation or habitat types are greatly influenced by soil moisture and wind exposure on Marion Island. Seven community complexes have been described which include salt-spray (*Crassula moschata*; restricted to shorelines), biotic (*Callitriche antarctica–Poa cookii*; along the shoreline and inland near animal activity), fernbrake (*Blechnum penna-marina*; drained slopes on the lowland), *Acaena magellanica–Brachythecium* (near mires and slopes), *Juncus scheuchzerioides–Blepharidophyllum densifolium* (wet peat), polar desert (at high altitudes) and fellfield (*Andreaea–Racomitrium crispulum*; exposed rocky environments) (Smith & Mucina 2006). Fellfield, arguably one of the oldest community complexes on sub-Antarctic islands (Scott 1985), consists of low plant cover dominated by the flowering vascular cushion plant, *Azorella selago* Hook. f., 1847 (Apiaceae) (Frenot *et al.* 1998; Smith & Gremmen 2004). In addition, *A. selago* is a long-lived species that colonizes deglaciated areas (Frenot *et al.* 1993, 1998) and is also associated with the development of landforms such as vegetation-banked terraces and patterned ground (Boelhouwers *et al.* 2003). Invertebrate population densities inside plants are much higher than in the surrounding epilithic biotope; for example, 16,000 individuals m^{-2} have reported for the prostigmatid mite *Eupodes minutus* Strandtmann, 1967 and 6,000 individuals m^{-2} for the springtail *Cryptopygus dubius* Déharveng, 1981 (Barendse & Chown 2001). *Azorella selago* is considered a keystone species since it forms nutrient rich environments for microarthropod communities and epiphytic plants (Huntley 1972; Barendse & Chown 2001; Hugo *et al.* 2004; le Roux & McGeoch 2004), significantly increasing the level of biodiversity associated with fellfield habitat at high altitudes (McGeoch *et al.* in press).

Despite the clear functional significance of *A. selago* to sub-Antarctic ecosystems and geomorphology, very little is known about the reproductive biology and population dynamics of the species (Frenot & Gloaguen 1994; le Roux & McGeoch 2004). For example, attempts to germinate seeds of the species have been largely unsuccessful, and the seed embryo appears to require time (under currently unknown conditions) to mature after release (Frenot & Gloaguen 1994). In addition, plant size is not always an accurate estimator of plant age because of high between-site differences in plant growth rates (le Roux & McGeoch 2004). On Marion Island, the frequency of young *A. selago* plants in sampled plots was found to be extremely low (le Roux & McGeoch 2004)

and successful establishment events are thought to be patchily distributed in both time and space. In general, however, cushion plants (for example *Azorella* Lam., 1783, *Plantago* L. 1753, *Draba* L. 1753, *Werneria* Kunth, 1820 and *Arenaria* L. 1753) are known to have two distinct growth forms, i.e. discrete cushions and continuous mats (also referred to as cultivated beds by Heilbron 1925 and carpets by Huntley 1972).

In *A. selago*, discrete cushions take the form of generally low growing, compact, circular plants that become hemispherical, irregular or crescent-shaped as they age (McGeoch *et al.* in press). In fellfield habitats, these discrete plants are evenly to randomly positioned within an epilithic biotope (le Roux 2004). The second growth form, namely mats, are characterized by large (sometimes several tens of meters in length and/or breadth), contiguous areas completely covered by *A. selago* (Huntley 1972). Cushion plants in the family Apiaceae are known to commonly develop into multiple (genetically similar) plants from a process of fragmentation, where parts of the plant die off creating open areas and separate fragments (or clones, as the fragments are genetically identical individuals) of the plant survive independently (Heilbron 1925; Armesto 1980). This would mean that other individuals (plants with different genotypes) may establish between genetically similar cushions; however, this hypothesis has not been tested. Therefore, it is currently not known whether mats consist of a single very large cushion individual, or whether several individual plants merge, interdigitating to form a continuous mat. This same question was raised decades ago by Heilbron (1925) for Ecuadorian cushion plants, including *Azorella* species. In the Andes of southern Peru 'individual' plants of *Azorella compacta* Phil., 1891 have been reported to spread over areas of 30m^2 (Ralph 1978), although this conclusion was based entirely on anecdotal observation. It therefore seems that the question has not yet been satisfactorily answered. Given the complexity and extensive nature of *A. selago*'s stem and root structure (Huntley 1972), and the destructive sampling required to examine it, the use of molecular techniques provide a potentially effective tool for understanding the growth dynamics of the species. In addition, an understanding of the growth dynamics of *Azorella* in mat form is critical for population genetics and phylogeographic studies since both these investigations assume sample independence, i.e. samples must be confidently attributed to distinct individuals (rather than clones or individuals that have been formed vegetatively). Our first aim was therefore to investigate the growth dynamics of *A. selago* in mat form.

At a larger, geographic scale, environmental factors (especially high speed winds) in combination with historical events (glaciation and volcanism) and local topography have been shown to significantly influence the genetic population structure of the mite *E. minutus*, one of the species inhabiting and sampled from *A. selago* cushions across Marion Island (Mortimer & Jansen van Vuuren 2007). Genetically unique populations were found between the south-western and south-eastern sides of the island for this mite species. However, it remains uncertain whether the phylogeographic pattern of inhabitant species (like *E. minutus*) coincides with the pattern of the host plant (*A. selago*). Our second aim was thus to describe the phylogeographic population structure of *A. selago* across Marion Island and to compare this to the patterns previously described for *E. minutus*.

Chloroplast intergenic spacer regions have been used to investigate intraspecific genetic variation in plants, for example in various flowering plants (see for example Kress *et al.* 2005; Lorenz-Lemke *et al.* 2006), soybeans (Xu *et al.* 2001) and tropical canopy trees (Hamilton 1999; Hamilton *et al.* 2003). These non-coding regions experience limited selective pressure which lead to the accumulation of polymorphisms, which make them ideal markers to use in population level studies (Hamilton 1999). Amplified fragment length polymorphism (AFLP), which generates an anonymous multilocus DNA profile (fingerprint) for each individual (Vos *et al.* 1995), has been shown to be useful for individual identification (Rosendahl & Taylor 1997; Majer *et al.* 1998), studies of relatedness and parentage (Krauss 2000; Madden *et al.* 2004) as well as at the population (Shim & Jorgensen 2000; Tremetsberger *et al.* 2003) and species level (Ishida *et al.* 2003; Pfosser *et al.* 2006). Therefore we used sequence (*trn*H-psbA chloroplast intergenic region) as well as AFLP data to address the aims of this study, i.e. (1) to examine the growth dynamics of *Azorella* in mat form and (2) to assess the phylogeography of *A. selago* across Marion Island.

MATERIALS AND METHODS

Sampling

To investigate the growth dynamics of mats, we selected a large *A. selago* mat on the south-western side of Marion Island at Swartkops Point (see Figure 5.1), and sampled plant material from systematically positioned stations on the mat. A portion of a large mat (>5m x 10m) growing between two black lava ridges was sampled (4.5m x 7.5m sampled). The sampled portion, situated in the middle of the mat, was divided into 1.5m x 1.5m (or 2.3m^2) grids (see Figure 5.2). Leaves were sampled from within each grid (n=15). In the absence of any comparable data for *A. selago*, and to determine the range of genetic similarities for known individuals, we included an additional five discrete cushion individuals from the same locality. We specifically chose individuals from the same locality as the mat to minimize the influence of environmental and other demographic factors (such as past population expansions or bottlenecks) on the genetic variation present in different populations. The objective was to use the genetic similarity of known, unrelated and discrete individual plants as a benchmark against which to identify potential individual plants within the mat. Plants were collected at least 5 meters apart to maximize the chance that they represented discrete individuals (genotypes).

The work reported here forms part of a larger project aiming to document the spatial distribution of both genetic and ecological variation for various species (plants and invertebrates) across Marion Island. Specifically, our aim here is to provide an initial framework for a larger and in depth study that will document genetic and ecological variation for *Azorella* at various spatial and hierarchical scales. For phylogeographic study, we included 42 individuals from eight sampling localities across Marion Island. These were Blue Petrel Bay (n=10), the hydro-electrical dam (n=7), the Meteorological Station at Transvaal Cove (n=2), Stoney Ridge (n=4), Kildalkey Bay (n=2), Watertunnel (n=3), Swartkops Point (n=5) and Mixed Pickle (n=11) (see Figure 5.1). Within a locality, care

was taken to select individual cushions which were at least 5 meters apart. All the plant material was dried and stored with silica gel at room temperature until assayed in the laboratory.

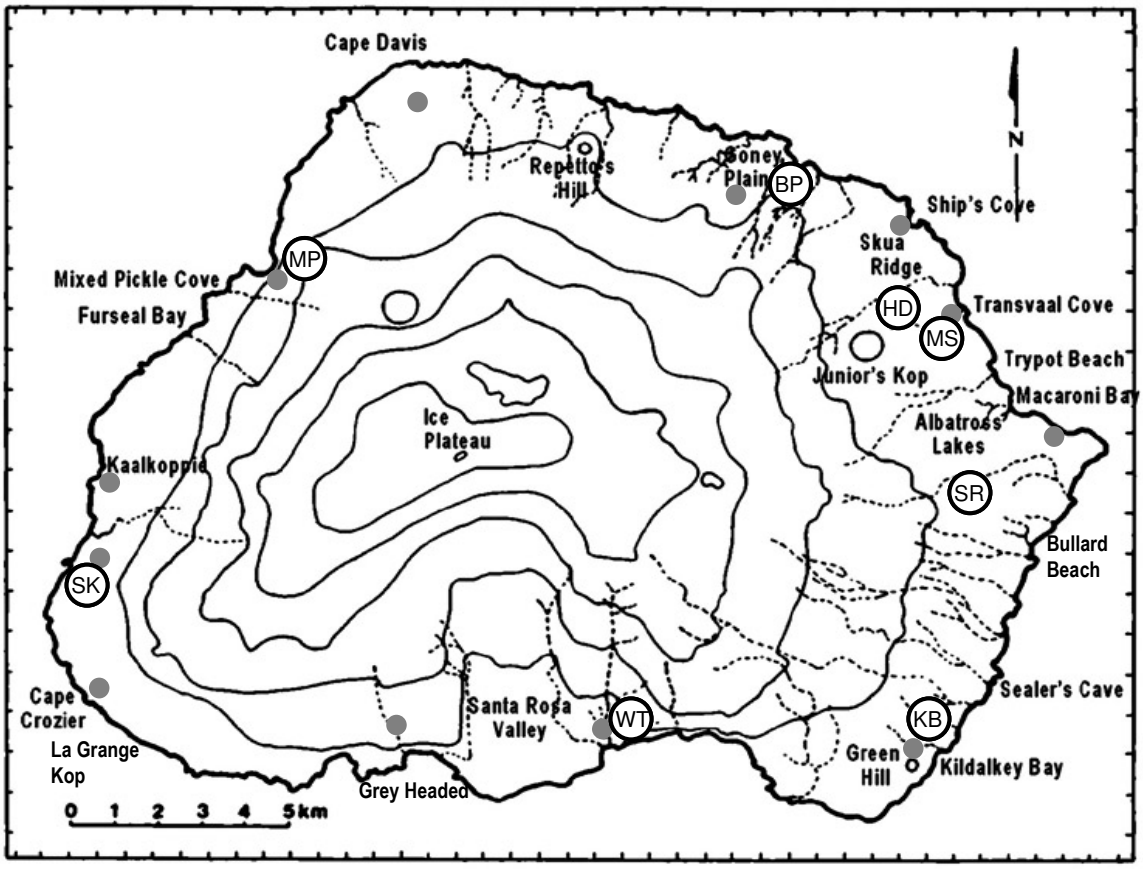

Figure 5.1 Positions of the sampling sites of *Azorella selago* specimens across Marion Island, namely Blue Petrel Bay (BP), the hydro-electrical dam (HD), the Meteorological Station (MS), Stoney Ridge (SR), Kildalkey Bay (KB), Watertunnel (WT), Swartkops Point (SK) and Mixed Pickle (MP). The mat was also sampled at SK. The sampling sites of the previous phylogeographic study on *Eupodes minutus* are indicated in grey (Mortimer & Jansen van Vuuren 2007). The map was adapted from Smith and Gremmen (2004).

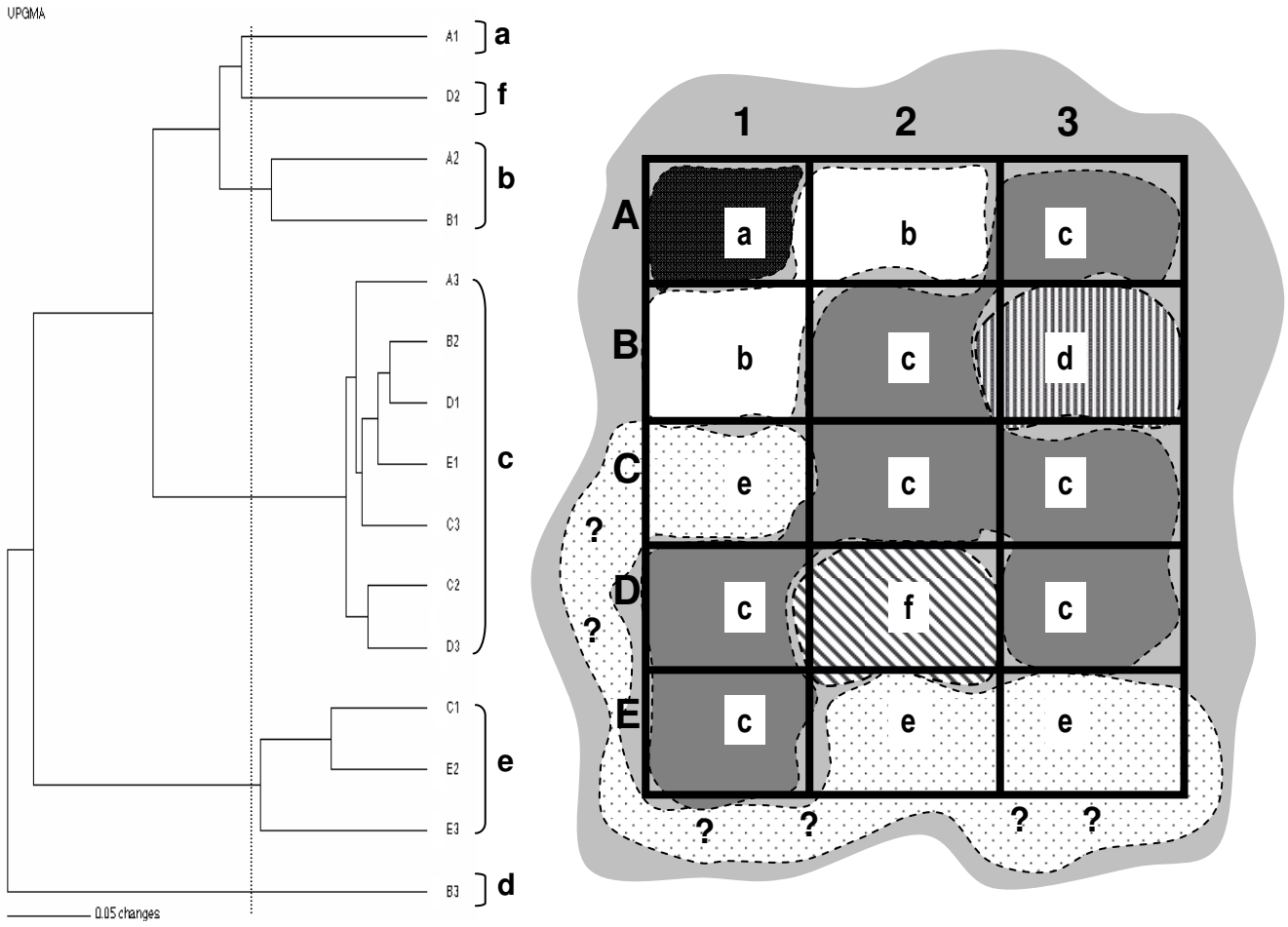

Figure 5.2 UPGMA tree constructed for the *Azorella selago* mat from Swartkops Point. The dotted line indicates the genetic cut-off value for individuals. Also shown, is the transect with the 2.3m^2 grids situated in the middle of the mat. The corresponding individuals (a – f) are indicated on both the tree as well as the grid. Based on the tree results, potential *A. selago* individuals (genotypes) in the mat are indicated with different shading. The "?" symbolize hypothetical growth of individual (e).

DNA extraction

To extract genomic DNA from dried *A. selago* leaves, we followed the CTAB protocol described by Doyle and Doyle (1987). In short, leaves were submerged in liquid nitrogen and ground with a mortar and pestle. Following this, plant material was incubated in 500µl of CTAB buffer (100mM Tris-HCl, ph8.0; 1.4M NaCl, 20mM EDTA; 2% CTAB; 0.2% mercaptoethonal) at 65°C for one hour. An equal volume of chloroform-isoamylalcohol (24:1) was added, followed by DNA precipitation with absolute ethanol. The DNA pellet was rinsed with a wash buffer (1 part ammonium acetate: 3 parts ethanol) and resuspended in 200µl deionised distilled water.

Sequencing of *trn*H-psbA

The *trn*H-psbA chloroplast intergenic region was selected given that it has a moderate to high rate of evolution (Hamilton *et al.* 2003). The utility of this marker for fine-scale genetic analyses of *A. selago* was determined by selecting a few random samples for sequencing. For the mat (at Swartkops Point), five samples were selected, namely A1, B2, C3, D2 and E1 (see Figure 5.2). An additional two samples from the region of the Meteorological Station on the island were included to determine if adequate genetic variation exist in this chloroplast intergenic region for subsequent phylogeographic analyses (these two sites are at opposite sides of Marion Island) (Figure 5.1). A 412bp fragment was amplified and sequenced using the primer pair *trn*H (GUG) and pbsA (Hamilton 1999). PCR reactions were carried out using 10ng of genomic DNA under the following cycling conditions: 94°C for 1 minute, (94°C for 30 seconds, 55°C for 30 seconds and 72°C for 45 seconds) for 35 cycles with a final extension step at 72°C for 5 minutes. The PCR products were purified with the Wizard SV Gel and PCR clean-up system (Promega, Madison, USA) following the manufacturer's instructions. The forward primer (*trn*H (GUG)) was used for sequencing with half-reactions of BigDye® Terminator 3.1 mix (Applied Biosystems, Warrington, UK). Purified products were run on an ABI 3100 automated sequencer (Applied Biosystems, Warrington, UK). Sequence electropherograms were checked and edited with BioEdit 7.0.5 (Hall 2005).

AFLP fingerprinting

The advantages of the AFLP technique are that it requires only small quantities of DNA, no prior knowledge of the genome size and in contrast to DNA fingerprinting, is a reliable and repeatable method (see Mueller & Wolfenbarger 1999; Meudt & Clarke 2007). AFLP represents a dominant marker system where alleles are scored as present (1) or absent (0). Perhaps the most serious limitation of this technique is that error rates are noticeably high (1.9% - 2.5%) (Bensch & Akesson 2005). Such high error rates create problems when the objective is individual identification (as is the case here) and in this respect, one has to expect mismatches when assigning genotypes. To compensate for the error rate in our analyses of growth dynamics, we estimated genetic similarity of unrelated discrete individuals (from the same locality) to be a benchmark against which to distinguish individual plants in the mat.

For the AFLP benchmarking, we used a commercial kit supplied by Applied Biosystems (Warrington, UK). The genomic DNA (500ng) was digested and ligated for 3 hours at 37°C in the presence of 1U MseI, 5U EcoRI, 1U T4 ligase, 10X DNA ligase buffer with ATP, 0.5M NaCl, 1mg/ml BSA and the double-stranded adaptors. A small quantity (4µl) of the undiluted ligated DNA fragments was pre-amplified. The preselective amplification reaction was diluted 10-fold and used in the selective amplification step. Initially, 24 primer combinations were screened. For the analyses of the mat, we selected eight primer combinations (Eco-ACC/Mse-CAA, Eco-ACC/Mse-CAC, Eco-ACC/Mse-CAG, Eco-ACC/Mse-CTA, Eco-ACC/Mse-CTC, Eco-ACC/Mse-CTG, Eco-ACC/Mse-CTT and Eco-ACT/Mse-CTC). For the phylogeographic question, we selected four reproducible primer combinations (Eco-ACC/Mse-CAC, Eco-ACC/Mse-CTA, Eco-ACC/Mse-CTG and Eco-ACC/Mse-CTT). The fluorescently labelled selective amplification products together with an internal size standard (500 Rox, Applied Biosystems, Warrington, UK), were run on an ABI 3100 automated sequencer (Applied Biosystems, Warrington, UK). The raw data were manually checked and edited with Genemapper 3.7 (Applied Biosystems, Warrington, UK). We verified the reproducibility of our results (both the population structure as well as the mat) by repeating all benchmarking experiments for 17% of all individuals (i.e. these individuals were independently extracted, digested, ligated and amplified twice for all primer combinations).

Data analysis

The *trn*H-pbsA sequence data were aligned with BioEdit 7.0.5 (Hall 2005) and alignments were confirmed by eye. To verify the authenticity of our sequence data, sequences were compared to those deposited in GenBank through BLAST searches. The presence of indels and / or nucleotide substitutions was assessed using PAUP* 4.0b10 (Swofford 2000).

For AFLP fingerprinting, data matrices were constructed using Genemapper 3.7 (Applied Biosystems, Warrington, UK). To calculate the error rate associated with scoring, all *A. selago* individuals were scored twice (by EM) and the resultant two data matrices compared. Additionally, 16% of all data were scored independently by BJvV and EM. The error rate associated with AFLP data is an important consideration, especially when attempting to identify individuals and describe spatial genetic variation. For both data sets, an error rate of 2.1% occurred when 16% of the raw data were compared. Although noticeably high, this rate falls within those reported in the literature (e.g. ~1.3-2.6% error rate, Bonin *et al*. 2004; 1.9%-2.5% error rate, Bensch & Akesson 2005).

<u>*Azorella selago* mat – AFLP data</u>

The uncorrected pairwise genetic distances separating the 15 samples taken from the mat were calculated using PAUP* 4.0b10 (Swofford 2000). These distances (Nei 1978) were then used to construct an ultrametric tree using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA). Individuality within the mat was based on a comparison to the genetic distances separating known individuals, i.e. discrete cushion plants a minimum of 5m apart (n=5, Swartkops Point). The lowest genetic distance separating all known individuals was used as the cut-

off value to assign genetic identity to samples taken within the mat. In other words, samples within the mat were assumed to be from a single *Azorella* plant if they were genetically more similar than the lowest genetic distance separating any of the known individuals.

Phylogeography – AFLP data

Although our aim was not a comprehensive phylogeographic study across Marion Island, the inclusion of geographically distant samples (close to the maximum distance possible between sites on the island) does allow us some inference about the genetic population structure of the species across the island. To determine whether our data were more structured than random data, we calculated the overall F_{ST} as well as population pairwise F_{ST} values in Arlequin 3.1 (Excoffier *et al.* 2005). A hierarchical Analysis of Molecular Variance (AMOVA) provided information on how the overall variation was partitioned within and among populations. Significance values for the *F*-statistics were obtained from 1000 random permutations of the data. Genetic distances (Nei 1978) as well as gene flow between sampling localities were calculated in POPGENE 1.32 (Yeh *et al.* 1999). Because the small sample sizes did not always allow meaningful calculations of standard diversity indices at the population level, we also combined all samples and regarded Marion Island as a single population. We estimated expected heterozygosity (H_E) and gene diversity for all samples in Arlequin 3.1.

RESULTS AND DISCUSSION

Sequence data revealed genetic invariance for *A. selago* (GenBank accession number: EF614999) collected from two geographically distant localities, despite the *trn*H-psbA chloroplast intergenic region having a moderately high rate of evolution (Hamilton *et al*. 2003). In the absence of any insertions / deletions or nucleotide substitutions, we conclude that the resolution obtained from this marker was insufficient to allow fine-scale genetic analyses. As such, the *trn*H-psbA region was excluded from further analyses. In contrast, the AFLP data provided sufficient resolution and, based on this marker system, we clearly show that the *A. selago* mat consists of multiple individuals rather than a single individual. Also, significant population substructure exists within in *A. selago* across Marion Island. Both these findings have significant implications, and are discussed in more detail below.

Stevens *et al*. (2007) have recently highlighted the potential for natural cross contamination, an important consideration when studying genetic patterns. Specifically, in addition to unexpectedly high levels of genetic variation, these contaminants would result in erroneous spatial patterns of genetic variation. Whereas such natural contaminants are readily detected with sequence data, it remains impossible to reliably exclude these from fragment data (see Stevens et al. 2007). With respect to the present study, we argue that our results are free of the confounding effects for natural contaminants for several reasons. First, individual *Azorella* plants (both from the mat (individual (a), (c) and (f); Figure 5.2) as well as from a different locality (Meteorological Station) on Marion Island) were sequenced for a chloroplast marker. When our sequences were compared to sequences in

GenBank (BLAST searches), our sequences were most similar to members of the plant order Apiales as would be expected. Secondly, the levels of genetic variation observed in our study was not unexpectedly high and fell within the range reported for other vascular plants (see for example Pfosser *et al*. 2006).

Azorella selago mat

The eight AFLP primer pairs produced 112 reliable polymorphic bands for the 15 samples taken from the mat (fragment sizes ranged from 75bp to 500bp). Uncorrected pairwise distances separating these samples ranged from 0.05 (D1 and E1) to 0.61 (A3 and C1). When considering the five known discrete individuals from Swartkops Point, the eight primer pairs produced 137 polymorphic bands. The uncorrected pairwise distance between these five *Azorella* plants ranged from 0.21 to 0.75. Given that the lowest uncorrected sequence divergence separating known individuals was 0.21, and that the error rate estimated for our data were 2.1%, we used this value (0.21) as the cut-off point below which samples were assumed to belong to the same plant (thus being genetically more similar than the lowest divergence separating known individuals as well as that a difference of 2.1% may be accounted for by the error rate in the scoring of polymorphic bands). Applying this value to the mat, six distinct genotypes were identified (see Table 5.1 and Figure 5.2). The highest genetic distance separating samples from within the mat (0.61 between C1 and A3; see Table 5.1) is comparable to the highest distance separating known individuals from Swartkops Point (0.75). In addition, when reanalyzing the mat data for the identical four markers included in the phylogeographic study, divergences within the mat ranged from 0.05 (between C3 and B2; C3 and D1; C3 and E1 as well as C2 and D3) to 0.63 (C1 and C2), which is comparable to values estimated for individuals across Marion Island (range from 0.05 to 0.70).

Table 5.1 Uncorrected pairwise genetic distances between Swartkops Point *Azorella selago* mat samples. Single individuals have a *p*-distance larger than 0.21

	A1	A2	A3	B1	B2	B3	C1	C2	C3	D1	D2	D3	E1	E2	E3
A1															
A2	0.25														
A3	0.32	0.30													
B1	0.22	0.18	0.33												
B2	0.31	0.35	0.08	0.34											
B3	0.38	0.39	0.55	0.37	0.56										
C1	0.39	0.43	0.61	0.37	0.58	0.57									
C2	0.35	0.38	0.12	0.34	0.09	0.58	0.60								
C3	0.35	0.40	0.09	0.32	0.07	0.56	0.60	0.10							
D1	0.36	0.36	0.09	0.33	0.04	0.54	0.59	0.10	0.08						
D2	0.22	0.28	0.31	0.25	0.25	0.44	0.38	0.29	0.30	0.28					
D3	0.37	0.37	0.12	0.34	0.07	0.54	0.60	0.07	0.09	0.06	0.27				
E1	0.36	0.36	0.07	0.33	0.06	0.55	0.61	0.12	0.08	0.05	0.3	0.10			
E2	0.35	0.40	0.58	0.39	0.54	0.54	0.12	0.57	0.57	0.56	0.32	0.54	0.58		
E3	0.27	0.34	0.48	0.28	0.47	0.52	0.23	0.47	0.46	0.46	0.33	0.46	0.50	0.17	

We argue that two, not necessarily mutually exclusive, processes can be proposed to explain the growth dynamics of *Azorella* mats as uncovered by our genetic analyses. These are the process of fragmentation and interdigitated growth of individuals. Most cushion plants are known to experience fragmentation over time (Armesto *et al.* 1980). Within the dead part of the cushion, the older stems ultimately disintegrate and are blown away, leaving two separate parts of the original cushion that are of course genetically identical. The availability of open areas within and between cushion individuals allows other individuals (with different genotypes) to establish between these fragments and subsequently merge into a mat form (Armesto *et al.* 1980). Alternatively, it is possible that over time a high density of individual cushions merely merge to form a continuous structure, or mat, without fragmentation being part of the process. To illustrate these two processes, we refer to individual (e) in Figure 5.2. Individual (e) is found in grids C1, E2 and E3. This individual could have been separated due to fragmentation, thus, individuals (c) and (f) established within the dead parts of individual (e). However, since the samples were collected from the middle of a large mat (as opposed to the edge of the mat), we cannot rule out that individual (e) may form an enlarged cushion that grows around the borders of the sample grid (see Figure 5.2). Our sampling of this mat therefore does not allow us to distinguish these two processes, and further investigations at a much larger scale are needed. Nonetheless, these results clearly demonstrate that mats are formed by more than a single individual and, at least in this case, by multiple genotypes.

Regardless of which processes are responsible for mat formation, favourable environmental conditions play an important role in the establishment of *A. selago* (Frenot *et al.* 1993; le Roux 2004). We argue that optimum weather and substrate conditions are necessary for individual plants to flourish and essentially merge into a mat form. *Azorella selago*'s range extends from sea level to ~800 meters above sea level, with mat formation being prevalent in drainage lines of mid-altitude fellfield habitats (see also Heilbron 1925), particularly on the western side of the island (M. A. McGeoch, personal observation). In addition, most of the discrete cushions on Marion Island range, on average, from 0.40 to 1.15m in diameter and their size in open fellfield habitat appear to be related to the distance and size of neighbouring cushions (le Roux & McGeoch 2004). The extent of an individual genotype in the mat sampled here stretched across a distance of 7.5m and 4.5m (Figure 5.2, individual or genotype (c)), which is far larger than any discrete cushion plant recorded on the island (le Roux & McGeoch 2004; M. A. McGeoch, personal observation). The largest discrete cushions recoded to date, almost all of which have lost their circular shape and become crescent shaped or irregular, are in the order of 2-3m in maximum diameter. However, fairly narrow (<1.0m) strips of continuous *A. selago* vegetation are also found in association with vegetation banked terraces on some fellfield sites and vegetation strips on scoria cones on the island (Holness 2001; Boelhouwers *et al.* 2003). The AFLP results thus suggest that at least one of the processes proposed above is involved in *A. selago* mat formation, i.e., several different genotypes merge to form the mat. This may result either from interdigitation and / or fragmentation. Furthermore, individual plants in mats grow larger than discrete cushion individuals.

In addition to *A. selago* playing a keystone role on Marion Island, evidence suggests that this species is increasingly susceptible to ongoing climate change in the region (le Roux & McGeoch 2007; McGeoch *et al.* in press). The species is predicted to experience increased competition from faster growing species responding to rising temperatures with more vigorous growth and an upward shift in elevation (le Roux & McGeoch 2007). In addition, *A. selago* has been shown to be drought sensitive with increased stem death predicted under the current drying trend being experienced on the island (le Roux *et al.* 2005; le Roux & McGeoch 2007). Studies such as the one presented here are thus essential to better understand this functionally important and apparently threatened species.

Population structure

Four primer pairs were selected to provide insight into the phylogeographic structure of *A. selago* across Marion Island. These primer pairs produced 120 reliable polymorphic bands for 42 specimens included from eight sampling localities. In general, we found that the genetic diversity of *A. selago* across Marion Island was high. The gene diversity was 1.0 which indicates that all of the individuals included had a unique genotype (see Pfosser *et al*. 2006). This is not surprising given that AFLP data generate a unique fingerprint for each individual, and one would not expect these to be identical unless plants reproduce clonally (see Pfosser *et al.* 2006 for a similar finding). Since special attention was given to sampling individual plants (discrete cushions were sampled at least 5m apart), this analysis essentially confirms the individuality of all our specimens. Genetic distances among individuals ranged between 0.05 (two individuals sampled at the hydro electrical dam and Blue Petrel Bay) and 0.70 (two individuals sampled at the hydro electrical dam and Mixed Pickle). This result also demonstrates that *A. selago* is not commonly clonal, at least not over distances greater than 5m.

Considering all *A. selago* sampled across Marion Island, AMOVA indicated that most of the variation was within populations (sites) (89.9%) with the remaining 10.1% between populations. The distribution of variation for this species is very similar to that reported for *Dystaenia ibukiensis* (Yabe) Kitag., 1937 also a member of the Apiaceae, distributed throughout Japan (Pfosser *et al.* 2006). These authors reported 18.7% of the variation among populations with 81.3% of the variation within populations. Similarly, we argue that the high genetic diversity (genetic distance range of 0.05 to 0.7 as outlined above) and large number of polymorphic fragments found for *Azorella* cushions (gene diversity of 1.0) on Marion Island suggest a high degree of outcrossing and that vegetative reproduction, or fragmentation, may be largely confined to fine scales (< 5m) and possibly within mat growth forms.

F_{ST} was significant (F_{ST}=0.101, p=0.01), indicating population substructure across Marion Island. Although our sample sizes were generally less than 10 plants per population rendering population level analyses problematic, we nonetheless compared all populations in a pairwise manner (see Table 5.2 for pairwise F_{ST} values and genetic distances between populations). Two localities were significantly differentiated; these are Mixed Pickle which

differed from >70% of the populations and Watertunnel which differed from >40% of the populations (Table 5.2). In the region of Mixed Pickle, situated on the western side of the island, multiple volcanic and glacial events have been documented, especially at Triegaardt Bay (geographically <1km from Mixed Pickle). Similar to the western side of the island, multiple catastrophic events also occurred in the vicinity of Crawford Bay (Watertunnel is situated on the bay) (McDougall *et al.* 2001). We argue that these catastrophic events, coupled with harsh environmental conditions and complex topography, have significantly impacted on the population structure of these populations, essentially isolating them from other populations in the region.

The average gene flow across the island was low overall (the number of migrants per generation between the populations sampled estimated at 0.74 (Yeh *et al.* 1999)). However, this ranged from 0.3 (between Kildalkey Bay and the Meteorological Station at Transvaal Cove) to 5.3 (between Blue Petrel Bay and the hydro-electrical dam). Given that the central part of Marion Island (above ~760 m.a.s.l.) is frequently snow-covered (this area is described as a polar desert; Smith & Mucina 2006) and is devoid of any vascular plant growth, it is very unlikely that migration occurs across these high altitude areas. We therefore speculate that most of the migration occurs along the coast. Interestingly, higher levels of gene flow (range from 1.3 to 5.3) were found around the northern side of the island (from Swartkops Point to the Meteorological Station) compared to lower levels (generally less than 1 individual per generation) along the southern side of the island (again measured from Swartkops Point to the Meteorological Station). This could be explained by the topography and history of the island. Although the northern side of the island experienced multiple volcanic eruptions, these are all relatively old (Pleistocene) compared to the southern side of the island where both older as well as very recent eruptions have been documented (Pleistocene and Holocene) (McDougall *et al.* 2001). In terms of topography, the southern side of the island is much more inhospitable compared to the northern side with large areas, including Santa Rosa Valley, which is virtually devoid of vascular vegetation.

Table 5.2 A matrix indicating genetic distances (below the diagonal) and pairwise F_{ST} values (above the diagonal) for all *the A. selago* populations across Marion Island (*p<0.05; **p<0.01).

	M. Station	Blue Petrel	Hydro. dam	Kildalkey Bay	Mixed Pickle	Swartkops Point	Stoney Ridge	Watertunnel
M. Station		0.013	0	0.362	0.347*	0	0.095	0.519
Blue Petrel	0.138		0	0.105	0.151**	0.010	0.012	0.198*
Hydro. dam	0.184	0.059		0.114	0.245**	0	0.044	0.222*
Kildalkey Bay	0.227	0.239	0.411		0.093	0.022	0	0.183
Mixed Pickle	0.131	0.147	0.266	0.076		0.203**	0.077	0.280**
Swartkops Point	0.160	0.082	0.067	0.272	0.175		0	0.149*
Stoney Ridge	0.175	0.085	0.166	0.121	0.084	0.112		0.074
Watertunnel	0.270	0.236	0.396	0.043	0.097	0.256	0.139	

Substructure was similarly reported for *E. minutus* populations across Marion Island (Mortimer & Jansen van Vuuren 2007). For this microarthropod, the localities of Kildalkey Bay (south-eastern side of Marion Island) and La Grange Kop (south-western side of Marion Island) were significantly different from each other and it is argued that past climatic events, in combination with harsh weather conditions, have played a major role in shaping the genetic diversity across the island (Mortimer & Jansen van Vuuren 2007). Our findings for *Azorella*, although not identical to those reported for *E. minutus*, are nonetheless largely congruent in that climatic and environmental conditions appear to cause population substructure across the island. However, for both these studies robust statistical conclusions are somewhat hampered by small sample sizes. In spite of this limitation, the population substructure found is significant. What is needed is a larger study including more populations and larger sample sizes for *Azorella* to further investigate the phylogeographic patterns of this species across Marion Island.

To conclude, we found that the *A. selago* mat consisted of multiple individuals, some extensive, which we ascribe to either the process of fragmentation and / or to a high density of individuals merging to form the mat. The phylogeographic structure of *A. selago* indicates significant population substructure in the species across Marion Island. Substructure has similarly been described for *E. minutus* (sampled exclusively from *Azorella* cushions) as well as other small invertebrates on the island (*Cryptopygus antarcticus travei* Déharveng, 1981 and *Tullbergia bisetosa* (Börner, 1903) (Myburgh *et al.* 2007)). We suggest that additional studies of a similar nature (including more markers and greater sample sizes) should be implemented to confirm the findings presented here.

REFERENCES

ARMESTO, JJ, ARROYO, MTK, & VILLAGRÁN, C. 1980. Altitudinal distribution, cover and size structure of umbelliferous cushion plants in the high Andes of Central Chile. *Acta Oecologica,* 1, 327-332.

BARENDSE, J, & CHOWN, SL. 2001. Abundance and seasonality of mid-altitude fellfield arthropods from Marion Island. *Polar Biology,* 211, 73-82.

BENSCH, S, & AKESSON, M. 2005. Ten years of AFLP in ecology and evolution: why so few animals? *Molecular Ecology,* 14, 2899-2914.

BOELHOUWERS, J, HOLNESS, S, & SUMNER, P. 2000. Geomorphological characteristics of small debris flows on Junior's Kop, Marion Island, maritime sub-Antarctic. *Earth Surface Processes and Landforms*, 25, 341-352.

BOELHOUWERS, J, HOLNESS, S, & SUMNER, P, 2003: The maritime sub-Antarctic: a distinct periglacial environment. *Geomorphology*, 52, 39–55.

BONIN, A, BELLEMAIN, E, BRONKEN EIDESEN, P, POMPANON, F, BROCHMANN, C, & TABERLET, P. 2004. How to track and assess genotyping errors in population genetics studies. Molecular Ecology, 13, 3261-3273.

DOYLE, JJ, & DOYLE, JL. 1987. A rapid DNA isolation procedure for small amounts of fresh leaf tissue. *Phytochemical Bulletin,* 19, 11-15

CHOWN, SL. 1994. Historical ecology of sub-Antarctic weevils (Coleoptera: Curculionidae): patterns and processes on isolated islands. *Journal of Natural History,* 28, 411-433.

CHOWN, SL, GREMMEN, NJM, & GASTON, KJ. 1998. Ecological Biogeography of Southern Ocean Islands: Species-area relationships, human impacts and conservation. *The American Naturalist,* 152, 562-575.

EXCOFFIER, L , LAVAL, G, & SCHNEIDER, S . 2005. Arlequin ver. 3.1: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*,1, 47-50.

EMERSON, BC. 2002. Evolution on Oceanic Islands: molecular phylogenetic approaches to understanding pattern and process. *Molecular Ecology,* 11, 951-966.

FRENOT, Y, GLOAGEUN, JC, PICOT, G, BOUGERE, J, & BENJAMIN, D. 1993. *Azorella selago* Hook. used to estimate glacier fluctuations and climatic history in the Kerguelen Islands over the last two centuries. *Oecologia,* 95, 140-144.

FRENOT , Y, & GLOAGUEN, JC. 1994. Reproductive performance of native and alien colonizing phanerogams on a glacier foreland, Iles Kerguelen. *Polar Biology*, 14, 473-481.

FRENOT, Y, GLOAGUEN, JC, CANNAVACCIUOLO, M, & BELLIDO, A. 1998. Primary succession on glacier forelands in the subantarctic Kerguelen Islands. *Journal of Vegetation Science,* 9, 75-84.

HALL, T. 2005. BioEdit. *In* Carlsbad: Ibis Therapeutics, a division of Isis Pharmaceuticals.

HAMILTON, MB. 1999. Four pairs for the amplification of chloroplast intergeneic regions with intraspecific variation. *Molecular Ecology,* 8, 521-523.

HAMILTON, MB, BRAVERMAN, JM, & SORIA-HERNANZ, DF. 2003. Patterns and relative rates of nucleotide and insertion/Deletion evolution at six chloroplast intergenic regions in New World species of the Lecythidaceae. *Molecular Biology and Evolution,* 20, 1710-1721.

HÄNEL, C, & CHOWN, S. 1998. An introductory guide to the Marion and Prince Edward Island Special Nature Reserves, 50 years after annexation. Pretoria, Department of Environmental Affairs and Tourism.

HEILBRON, O. 1925. Contributions to the ecology of the Ecuadorian páramos with special reference to cushion plants and osmotic pressure. *Svensk Botanisk Tidskrift,* 19, 153-170.

HOLNESS, SD. 2001. Periglacial slope processes, landforms and environment at Marion Island, martitime Subantarctic. University of Western Cape, Bellville.

HUGO, EA, MCGEOCH, MA, MARSHALL, DJ, & CHOWN, SL. 2004. Fine scale variation in microarthropod communities inhabiting the keystone species *Azorella selago* on Marion Island. *Polar Biology,* 27, 466-473.

HUNTLEY, BJ. 1972. Notes on the ecology of *Azorella selago* Hook.f. *South African Journal of Botany,* 38, 103-113.

ISHIDA, TA, HATORRI, K, SATO, H, & KIMURA, MT. 2003. Differentiation and hybridization between *Quercus crispula* and *Q. dentata* (Fagaceae): insights from morphological traits, amplified fragment length polymorphism markers, and leaf miner composition. *American Journal of Botany,* 90, 769-776.

KRAUSS, SL. 2000. Accurate gene diversity estimates from amplified fragment length polymorphism (AFLP) markers. *Molecular Ecology,* 9, 1241-1245.

KRESS, WJ, WURDACK, KJ, ZIMMER, EA, WEIGT, LA, & JANZEN, DH. 2005. Use of DNA barcodes to identify flowering plants. *Proceedings of the National Academy of Sciences USA*, 102, 8369-8374.

LE ROUX, PC. 2004. *Azorella selago* (Apiaceae) as a model for examining climate change effects in the sub-Antarctic. University of Stellenbosch, Stellenbosch.

LE ROUX, PC, & MCGEOCH, MA. 2004. The use of size as an estimator of age in the sub-Antarctic cushion plant, *Azorella selago* (Apiaceae). *Arctic and Antarctic Alpine Research,* 36, 509-517.

LE ROUX, PC, MCGEOCH, MA, NYAKATYA, MJ, & CHOWN, SL. 2005. Effects of a short-term climate change experiment on a sub-Antarctic keystone plant species. *Global Change Biology,* 11, 1628-1639.

LE ROUX, PC, & MCGEOCH, MA. 2007. Changes in climate extremes, variability and signature on sub-Antarctic Marion Island. *Climatic Change,* DOI 10.1007/s10584-007-9259-y.

LORENZ-LEMKE, AP, MADDER, G, MUSCHNER, VC, VALERIA, C, STEHMANN, JR, BONATTO, SL, SALZANO, FM, & FREITAS, LB. 2006. Diversity and natural hybridization in a highly endemic species of *Petunia* (Solanaceae): a molecular and ecological analysis. *Molecular Ecology*, 15, 4487-4497.

MADDEN, JR, LOWE, TJ, FULLER, HV, COE, RL, DASMAHAPATRA, KK, AMOS, W, & JURY, F. 2004. Neighbouring male spotted bowerbirds are not related, but do maraud each other. *Animal Behaviour,* 68, 751-758.

MAJER, D, LEWIS, BG, & MITHEN, R. 1998. Genetic variation among field isolates of *Pyrenopeziza brassicae. Plant Pathology,* 47, 22-28.

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

MCGEOCH, MA, LE ROUX, PC, HUGO, EA, & CHOWN, SL. 2006. Species and community responses to short-term climate manipulation: Microarthropods in the sub-Antarctic. *Austral Ecology,* 31, 719-731.

MCGEOCH, MA, LE ROUX, PC, HUGO, EA, & NYAKATYA, MJ. (in press). Spatial variation in the terrestrial biotic system. In: *The Prince Edward Islands: Land-Sea Interactions in a Changing Ecosystem* (eds SL. Chown and PW. Froneman). African SunMedia, Stellenbosch.

MEUDT, HM, & CLARKE, AC. 2007. Almost forgotten or latest practice? AFLP applications, analyses and advances. *Trends in Plant Science*, 12, 106-117.

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.

MUELLER, UG, & WOLFENBARGER, LL. 1999. AFLP genotyping and fingerprinting. *Trends in Ecology and Evolution,* 14, 389-394.

MYBURGH, M , CHOWN, SL, DANIELS, SR, & JANSEN VAN VUUREN, B. 2007. Population structure, propagule pressure and conservation biogeography in the sub-Antarctic: lessons from indigenous and invasive springtails. *Diversity and Distributions*,13, 143-154.

NEI, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics,* 89, 583-590.

PFOSSER, M, JAKUBOWSKY, G, SCHLÜTER, PM, FER, T, KATO, H, STUESSY, TF, & SUN, BY. 2006. Evolution of *Dystaenia takesimana* (Apiaceae), endemic to Ullung Island, Korea. *Plant Systematics and Evolution,* 256, 159-170.

RALPH, CP. 1978. Observations on *Azorella compacta* (Umbelliferae), a tropical Andean cushion plant. *Biotropica*, 10, 62-67.

ROSENDAHL, S, & TAYLOR, JW. 1997. Development of multiple genetic markers for studies of genetic variation in arbuscular mycorrhizal fungi using AFLP. *Molecular Ecology,* 6, 821-829.

SCOTT, L. 1985. Palynological indications of the Quaternary vegetation history of Marion Island (sub-Antarctic). *Journal of Biogeography,* 12, 413-432.

SHIM, SI, & JORGENSEN, RB. 2000. Genetic structure in cultivated and wild carrots (*Daucus carota* L.) revealed by AFLP analysis. *Theoretical and Applied Genetics,* 101, 227-233.

SMITH, VR, & GREMMEN, NJM. 2004. *The Flora of Marion and Prince Edward Island.* The Netherlands.

SMITH, VR, & MUCINA, L. 2006. Vegetation of Subantarctic Marion and Prince Edward Islands. In: *The Vegetation of South Africa, Lesotho and Swaziland* (eds L. Mucina and C. Rutherford), pp 701 – 723. Pretoria, South African National Biodiversity Institute.

STEVENS, MI, HUNGER, SA, HILLS, SFK, & GEMMILL, CEC. 2007. Phantom hitch-hikers mislead estimates of genetic variation in Antarctic mosses. *Plant Systematics and Evolution*, 263, 191-201.

SWOFFORD, D. 2000. *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other methods).* Sinauer.

TREMETSBERGER, K, STUESSY, TF, GUO, YP, BAEZA, CM, WEISS, H, & SAMUEL, RM. 2003. Amplified Fragment Length Polymorphism (AFLP) variation within and among populations of *Hypochaeris acaulis* (Ateraceae) of Andean southern South America. *Taxon,* 52, 237-245.

VERWOERD, WJ. 1971. *Marion and Prince Edward islands. Report on the South African biological and geological expedition 1965-19*66 (ed. EM Zinderen-Bakker). Cape Town, A.A. Balkema Press.

VOS, P, HOGERS, R, BLEEKER, M, REIJANS, M, VAN DE LEE, T, HORNES, M, FRIJTES, A, POT, J, PELEMAN, J, KUIPER, M, & ZABEAU, M. 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic Acid Research,* 23, 4407-4414.

XU, DH, ABE, J, KANAZAWA, A, GAI, JY, & SHIMAMOTO, Y. 2001. Identification of sequence variations by PCR-RFLP and its application to the evaluation of cpDNA diversity in wild and cultivated soybeans. *Theoretical and Applied Genetics*, 102, 683-688.

YEH, F, YANG, R, & BOYLE, T. 1999. POPGENE. *In* Edmonton, Canada: Department of Renewable Resources, University of Alberta.

CHAPTER 6

SUMMARY

SUMMARY

My research contributes substantially to the limited phylogenetic and biogeographic information that currently exists for species from the South Polar Region. The phylogeny of ameronothroid mites (genus *Halozetes*), represents the first molecular study undertaken on this group of unique mites from the South Polar Region (Chapter 2). This research will be submitted to '*Molecular Phylogenetics and Evolution'*. My results are the first molecular evidence to show that the evolution of the ameronothroid mites was habitat specific (i.e. intertidal, supralittoral and terrestrial). I also found that the ameronothroid mites (as well as moss (Skotnicki *et al*. 2004)) share congruent biogeographical patterns with some marine species (notothenioid fish (reviewed by Verde *et al.* 2007) and bivalves (Page & Linse 2002)) even though the marine and terrestrial environments differ considerably (Peck *et al.* 2006). Also, these mites were previously considered to represent an ancient group predating the fragmentation of Gondwana (Wallwork 1973), however, my molecular clock estimations showed that these mites are young (<10myo), which means that their diversification is probably due to glaciation and isolation of the Antarctic region (~10mya) rather than the fragmentation of Gondwana. The taxonomic diversity of *Halozetes* Berlese, 1917 (based on genetic data) was greater than the morphological characters currently used for alpha taxonomy. This implies that the morphological characters currently used in *Halozetes* taxonomy should be reassessed and my molecular findings could provide a guideline for future morphological studies.

The phylogeny project had a few shortcomings and most of them were unavoidable. I included a limited number of samples and localities, which may affect my biogeographic conclusions, but this is due to the difficulty in obtaining samples from these isolated sub-Antarctic (and Maritime) localities. The low sample sizes, in part, are also due to the inaccurate storage of the mite specimens. The specimens were mainly stored in 70% absolute alcohol instead of 100% that made DNA recovery difficult. When considering the taxonomy of the mites, I did not investigate these mites on a morphological level (e.g. electron microscopy), since this research is reserved for a future project (L. Coetzee, personal communication).

Reported herein are also the first phylogeographic study on two mite species (cosmopolitan prostigmatid mite *Eupodes minutus* Strandtmann, 1967 and endemic oribatid mite *Halozetes fulvus* Engelbrecht, 1975) and one plant species (dominant cushion plant *Azorella selago* Hook. f., 1847) from sub-Antarctic Marion Island (Chapter 3 to 5). The research conducted on *E. minutus* was published in '*Polar Biology'* during 2007 (Mortimer & Jansen van Vuuren 2007); while the *A. selago* study was recently accepted in '*Antarctic Science'* (Mortimer *et al*. in press). The *H. fulvus* research will be submitted to '*Molecular Ecology'*. These phylogeographic projects contribute to a larger project that aims to document the spatial distribution of both genetic and ecological differentiation for various species (microarthropods and plants) on Marion Island. When conducting manual comparisons, I found that complex phylogeographic patterns exist for species on the geologically dynamic Marion Island similar to those described from other sub-tropical volcanic islands such as the Hawaiian and the Canary Islands. Also, I found that the phylogeographic patterns of mite species were very similar to indigenous springtail

species, *Cryptopygus antarcticus travei* Déharveng, 1981 and *Tullbergia bisetosa* (Börner, 1903) (Myburgh *et al.* 2007), while the pattern differs slightly for the host plant, *A. selago*. The different pattern described for *A. selago* may be due to the small sample sizes which may have hampered robust statistical inferences. For the mite and plant species, I found unique populations on the western (Kaalkoppie for *H. fulvus*, La Grange Kop for *E. minutus* and Mixed Pickle for *A. selago*), eastern (Bullard Beach for *H. fulvus* and Kildalkey Bay for *E. minutus*), northern (Middelman and Long Ridge for *H. fulvus*) and southern side (Grey Headed for *H. fulvus* and Watertunnel for *A. selago*) of the island. The most noticeable finding was that all species have unique populations on the western side of the island (localities ~5km apart). Nonetheless, findings for both microarthropod and plant species were similar in that historical events (glaciation and volcanism) and environmental conditions shaped their population structure across the island. Also, the two mite species (*E. minutus* and *H. fulvus*) have different microhabitat preferences. Irrespective of these preferences, the molecular evidence indicated that the populations of both mite species are still growing on the island. In addition to the phylogeographic studies, it is the first genetic study in the sub-Antarctic region to examine the growth dynamics of *A. selago* (Chapter 5) since the reproductive biology of this plant is unknown (typified by discrete and mat growth forms) and it was necessary to include distinct individuals in my phylogeographic study. The investigated *A. selago* mat comprised of multiple individuals, which can be attributed to the process of fragmentation and / or to a high density of individuals merging to form the mat.

The shortcomings for the phylogeographic projects on Marion Island were also limited. The sample sizes for both *E. minutus* and *A. selago* (excluding *H. fulvus*) were low, which potentially limited statistical inferences. However, both these studies were conducted to provide an initial framework for more detailed studies to follow later (*E. minutus* for the *H. fulvus* project and *A. selago* for future genetic and ecological spatial variation projects). Due to time constraints, additional markers (mitochondrial and nuclear) were not included in this study but should be considered for future phylogeographic projects on Marion Island.

To conclude, the biodiversity in the South Polar Region appears to be higher than initially anticipated and more detailed studies are necessary to unravel biogeography of this region. Very complex genetic patterns are not only present between species from different sub-Antarctic islands, but also on sub-Antarctic Marion Island. The Prince Edward Island archipelago is South Africa's only Special Nature Reserve, as such, the conservation of biodiversity is of great importance. In this study, conservation "hotspots" were identified that will need strict regulations, especially localities on the eastern and western side of the island. Since trips around the island are currently not monitored which implies that the migration of numerous microarthropod species are mediated, my findings together with previous results (Myburg *et al*. 2007) will directly contribute to an active management plan of the island.

REFERENCES

Mortimer, E. and B. 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., M. McGeoch, S. Daniels and B. Jansen van Vuuren (in press). Growth form and population genetic structure of *Azorella selago* on sub-Antarctic Marion Island. Antarctic Science.

Myburgh, M., S. Chown, S. Daniels and B. Jansen van Vuuren (2007). Population structure, propagule pressure and conservation biogeography in the sub-Antarctic: lessons from indigenous and invasive springtails. Diversity and Distributions 13: 143-154.

Peck, L., P. Convey and D. Barnes (2006). Environmental constraints on life histories in Antarctic ecosystems: tempos, timings and predictability. Biological Reviews 81: 75-109.

Page, T. and K. Linse (2002). More evidence of speciation and dispersal across the Antarctic Polar Front through molecular systematics of Southern Ocean *Limatula* (Bivalvia: Limidae). Polar Biology 25: 818-826.

Skotnicki, M., A. Mackenzie, J. Ninham and P. Selkirk (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.

Verde, C., G. Lecointre and G. di Prisco (2007). The phylogeny of polar fishes and the structure, function and molecular evolution of hemoglobin. Polar Biology 30: 523-539.

Wallwork, J. (1973). Zoogeography of some terrestrial micro-arthropoda in Antarctica. Biological Reviews 48: 233-259.