GENETIC DIVERSITY AND PHYLOGEOGRAPHY OF NEW ZEALAND AND ANTARCTIC ARTHROPODS

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ABSTRACT

Historical climatic and geological changes and ecological factors (e.g. population size, dispersal abilities) are conducive to shaping genetic differentiation among natural populations. This thesis examines these issues in a phylogeographic context by looking at patterns of genetic diversity and dispersal of arthropods in two geographic regions.

In southern New Zealand and its outlying islands, regional genetic divergence was revealed using mtDNA (COI) analysis for populations of idoteid isopods. Divergence within *Austridotea benhami* was < 2.0%. However, divergence within *A. lacustris* reached up to 10% with four main groupings: 1) Chatham Islands; 2) Campbell Island; 3) Fiordland; and 4) east coast South Island and Stewart Island. Similarly, *A. annectens* showed two main groups (4.4% divergent): 1) Chatham Islands; and 2) east coast South Island and Stewart Island. These patterns are likely to be the result of geographical isolation, with some populations showing divergence corresponding to the availability of habitat (e.g. the divergence of *A. lacustris* and *A. annectens* on Chatham Islands may relate to the availability of this habitat ~ 4 Mya). Additionally, divergence of *A. lacustris* on Campbell Island and Fiordland may indicate a rare founder event or environmental change that resulted in population isolation. Overall, genetic data and geological history indicated that rare dispersal events (or range expansion and population extinction), particularly during the Pliocene, may have played an important role in shaping the present-day distribution

and genetic structure of freshwater idoteid isopods throughout New Zealand and its outlying islands.

In Antarctica, analysis of patterns of mtDNA (COI) diversity and the geographic distribution of haplotypes among populations of the springtail *Gomphiocephalus hodgsoni* and the mite *Stereotydeus mollis* also revealed (congruent) patterns of genetic sub-structuring. Furthermore, genetic discontinuities suggested limited dispersal opportunities and isolation-by-distance for these taxa. Haplotype diversity within Wright and Victoria Valleys (St. John's, Olympus and Asgard ranges) was found to be higher here than in other areas and the distribution of haplotypes across sites was very heterogeneous. *G. hodgsoni* and *S. mollis* populations harboured eight and 23 new mtDNA haplotypes, respectively and showed links to previously sampled populations across southern Victoria Land. Accordingly, it is possible that this region may represent a primary location of a refugial source population from which many extant populations in southern Victoria Land have expanded since the Pleistocene and Holocene.

Collectively, these studies revealed limited current dispersal and high levels of regional genetic differentiation for three arthropod taxa from fragmented habitats in both New Zealand and Antarctica. It provided insight into the biogeographic processes underlying modern distribution patterns of these taxa and highlighted the utility of the phylogeographic approach in reconstructing biotic history to further knowledge of the relationships that exist between population processes and patterns of species diversity. Additionally, genetic analyses such as those performed here provided a basis to re-evaluate populations from a biodiversity perspective.

Specifically, the New Zealand study clarified specific and generic relationships for potential reassessment of conservation status, while the Antarctic study identified biodiversity hotspots and priorities for conservation strategies (e.g. specially protected areas).

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say that I couldn't have done this without you. Any accomplishments I have achieved are yours to be proud of too.

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THESIS INTRODUCTION

Phylogeography is an integrative discipline involving the study of the geographical distribution of genetic evolutionary lineages (Avise 1998; Ebach 2003). Microevolutionary (e.g. molecular/population genetics) and macroevolutionary processes (e.g. historical biogeography) are looked at together to establish the evolutionary, geographical and geological history of a particular taxon or, in the case of comparative phylogeography, taxa (Bermingham & Moritz 1998). Over the last 20 years, the field of phylogeography has provided considerable insight into historical effects on species distributions and diversification patterns. Indeed, it allows us to 'see genes in space and time' (Hewitt 2001).

Current patterns of species' distributions and genetic diversity provide information on population history, when molecular divergence values are compared to known historical geological events (Brower 1994; Fleischer *et al.* 1998; Roslin 2001). Vicariance and dispersal are two contrasting explanations for patterns of biotic diversity, and phylogeographic data can help us to distinguish between these two processes (Wolf *et al.* 2001). For example, in the simplest case, the taxonomic group of interest may be too young to have been affected by the presumed vicariant (biogeographic) barrier, in which case dispersal is indicated as the primary force shaping population structure. Thus, answering phylogeographical questions enhances our understanding of the underlying processes that form species distributions and maintain biological diversity (e.g. dispersal ability, local deme formation, metapopulation effects (van der Wurff *et al.* 2003)).

In addition, the genetic legacy left behind from historical biogeographic events can lead to insights regarding species origins and aid in determining geographical, ecological and environmental features that serve as dispersal barriers for taxa. This is useful from a conservation perspective since a primary objective of conservation biology is to maintain the evolutionary processes that shape population structure (Garrick *et al.* 2004).

Mitochondrial DNA is widely used for such phylogeographical analyses because it is usually only maternally inherited. Accordingly, individuals can belong to either of two bifurcating genealogical lineages but they cannot be genealogically intermediate. Thus, matrilineal genealogies can be reconstructed to show clear relationships among individuals (Irwin 2002) (e.g. through the haplotype variation we see today, which represents an accumulation of mutations through time (Hewitt 2001)). Additionally, mitochondrial DNA evolves quickly enough to show population differences, but slowly enough to avoid mutational saturation.

In this thesis, I examine phylogeographical distributions of southern New Zealand and southern Victoria Land (Antarctica) arthropod populations. Long term isolation in both regions provides an opportunity to examine the effects of potentially limited dispersal and habitat fragmentation on regional genetic differentiation. The thesis consists of two chapters, dealing with the New Zealand and Antarctic ecosystems, respectively.

Chapter I focuses on three species of freshwater/estuarine idoteid isopod (Crustacea). In general, only limited knowledge of the genetic structures of aquatic invertebrates is available in New Zealand (e.g. Schnabel *et al.* 2000; Smith & Collier

2001; Hogg *et al.* 2002; Stevens & Hogg 2004). Accordingly, this study addresses the paucity of genetic data for New Zealand aquatic invertebrates by studying patterns of diversity throughout the island archipelago geography of this region. Island archipelagos provide a system of spatial simplicity and temporal certainty for the study of biological diversification and evolution (Hewitt 2001; Emerson 2002). In particular, New Zealand, with its extensive coastline, is ideal for investigating patterns of diversification and dispersal of aquatic taxa that use ocean currents to move among habitat patches, and provides an ideal opportunity for examining the effects of vicariance and dispersal on the distribution of fauna because the geological ages of its main islands are well established (Juan *et al.* 1996).

In New Zealand, idoteid isopods are found in fresh and brackish waters on distant off-shore Chatham and Campbell Islands; Stewart Island; and east and west coastal regions of the South Island. Given the widespread but seemingly disjunct distribution patterns of the three New Zealand species (*Austridotea lacustris*, *A. annectens*, *A. benhami*), this group represents a good candidate for testing a model of vicariance versus dispersal and for re-evaluating historic debates about the taxonomic relationships of *Austridotea* (Nicholls 1937; Poore 2001).

The underlying hypotheses of this research were: 1) that genetic differences would exist among geographic regions; and 2) that the magnitude of any genetic divergences would correspond to habitat availability across geological timescales. To address these questions, mitochondrial DNA from the three *Austridotea* species taken from sites across their entire known natural ranges was analysed. Phylogenetic analysis of mtDNA sequences enabled clarifying statements to be made as to the

generic and specific relationships among the taxa. Additionally, the combination of genetic and regional geological information (i.e. phylogeographic analysis) allowed inferences to be made regarding historical (Pliocene) population movement in relation to habitat availability, and the main factor (i.e. dispersal vs. vicariance) shaping the current distribution of these isopods.

The second chapter of the thesis also looks at resolving variation in present-day species distribution patterns. It focuses on a comparative study of the genetic diversity of the springtail *Gomphiocephalus hodgsoni* (Collembola: Hypogastruridae) and the mite *Stereotydeus mollis* (Acari: Penthalodidae) in Wright and Victoria Valleys, southern Victoria Land, Antarctica. Potentially limited dispersal opportunities and correspondingly low levels of gene flow are expected for both taxa which should result in high levels of regional population sub-structuring (Stevens & Hogg 2003). Geographic barriers (e.g. glaciers) such as those seen in the Antarctic, may also be conducive to processes of speciation (Slatkin 1993). Geological events such as continental drift and mountain uplift have predictable consequences for evolutionary lineages because they often result in physical obstacles to gene flow and subsequent formation of monophyletic groups on either side of the barrier (Rosen 1978; Avise 1994). A less understood but equally important historical event is climate change. While species tend to respond in a somewhat uniform manner to geological change, they can respond differently to the effects of climate change (Delcourt & Delcourt 1991). For instance, range expansion or contraction may occur at different rates and in different directions (Ayoub & Riechert 2004). Furthermore, phylogeographic breaks have been shown to arise in the absence of geographic

barriers to gene flow, especially if individual dispersal distances and/or population sizes are small (Irwin 2002). Thus, looking at a species history and ecology together can provide information on the relative influences of geological and climatic events on population diversification (e.g. Masta 2000; Knowles 2001; Ayoub & Riechert 2004). Furthermore, the comparative approach employed here should enhance conclusions as to which features may be important in determining species history if the patterns across taxa are congruent (Hewitt 2001).

One climatic event that has dramatically influenced the distribution of biota is the glacial cycling that has occurred over the last ~ 2 My (Seddon *et al.* 2001). This is of particular interest, given that many extant species' distributions are the result of range expansion from glacial refugia following the end of the Pleistocene glaciation ~ 16,000 years ago (Hewitt 1996; Klicka & Zink 1997; Avise *et al.* 1998; Avise 2000; Seddon *et al.* 2001). The genetic consequences of glacial cycles and the subsequent spatial variability in climate typically result in regional genetic structuring (i.e. population fragmentation) and/or decreased genetic diversity. This is because populations can become isolated in allopatric glacial refugia during glacial maxima and then experience founder effects as individuals representing a subset of the gene pools' total diversity found new populations through expansion out of the glacial refugium when conditions allow (Tremblay & Schoen 1999; Abbott *et al.* 2000; Weider & Hobæk 2003). In this case, molecular markers can be used to identify similarities between the populations stemming from one haploytpe expansion and hence locate its likely refugium (Hewitt 2001).

Recent research on the terrestrial fauna of Antarctica has indicated highly fragmented population structures (e.g. Courtright *et al.* 2000; Fanciulli *et al.* 2001; Frati *et al.* 2001; Stevens & Hogg 2003). To investigate this pattern further, the study presented in Chapter II involved an extension of previous sampling in southern Victoria Land, where both *G. hodgsoni* and *S. mollis* occupy the same widespread but fragmented range. The primary aims of this project were to: 1) accurately assess biodiversity in the region; and 2) to determine if this predominantly ice-free area has served as a refuge in the past (e.g. during Pleistocene/Holocene glacial maxima) and as a subsequent source for the recolonisation of surrounding areas. Accordingly, higher levels of genetic diversity and/or unique genetic resources would be expected.

Mitochondrial DNA (COI) analyses were applied to individuals from throughout southern Victoria Land, Antarctica. In order to more fully assess the processes that shape population structure in these Antarctic terrestrial invertebrates, "network analyses" were also incorporated into the study. The application of this relatively new statistical phylogenetic technique (Templeton 1998, 2004) provides a spatio-temporal statistical interpretation of the geographical associations of haplotypes, and the mutational sequence changes that accumulate with time (Templeton *et al.* 1995; Hewitt 2001; Seddon *et al.* 2001). When employed in conjunction with traditional molecular methodology (Ayoub & Reichert 2004; Templeton 2004), network analyses allow clearer differentiation of the different historical (i.e. biogeographic) and contemporary (i.e. ecological) processes contributing to population structure (e.g. gene flow, range expansion, colonisation, fragmentation) because each of these

processes is expected to produce distinct patterns in the distribution of alleles/haplotypes and the relationships between them (Irwin 2002).

Collectively, the two chapters of this thesis address phylogeographical questions across two ecological groups (aquatic and terrestrial arthropod taxa) and two geological locations, thus providing both taxonomic and geographic comparisons. This work demonstrates how genetic data may contribute to our understanding of the patterns and processes (e.g. dispersal, vicariance/climatic change) that form species distributions, and highlight particular regions and/or taxa for conservation priority. The thesis concludes with an overall summary, and then addresses priorities for future research.

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CHAPTER I

GENETIC DIVERGENCE OF THREE FRESHWATER ISOPOD SPECIES FROM SOUTHERN NEW ZEALAND: EVIDENCE FOR PLIOCENE DISPERSAL[†]

Key Words: Austridotea, mitochondrial DNA, New Zealand, phylogeography

[†]to be published under the same title as: Angela McGaughran, Ian D. Hogg, Mark I. Stevens, W. Lindsay Chadderton, Michael J. Winterbourn

ABSTRACT

We used molecular data to examine the phylogeography of three freshwater isopod species (*Austridotea annectens, A. lacustris, A. benhami*) from southern New Zealand and its outlying islands (Campbell and Chatham Island) in order to test the hypotheses that genetic differences would: 1) exist between geographic locations; and 2) correspond to known geological events (e.g. emergence of islands leading to availability of habitat).

Using mitochondrial DNA (COI) sequence analyses, three main clades corresponding to the three species were resolved, with 16% sequence divergence between *A. annectens* and *A. benhami*, and 31% divergence between these species and *A. lacustris*. Divergence within *A. benhami* was < 2.0%. However, divergence within *A. lacustris* reached up to 10% with four main groupings: 1) Chatham Islands; 2) Campbell Island; 3) Fiordland; and 4) east coast South Island and Stewart Island. Similarly, divergence within *A. annectens* reached up to 4.4% with two main groupings: 1) Chatham Islands; and 2) east coast South Island and Stewart Island.

Patterns of genetic divergence were most likely the result of geographical isolation among *A. lacustris* and *A. annectens* populations. In particular, the divergence of *A. lacustris* and *A. annectens* on Chatham Islands may correspond to the availability of this habitat approximately four million years ago. Additionally, divergence of *A. lacustris* on Campbell Island and Fiordland may indicate either a rare founder event or a change in oceanic circulation that resulted in isolation of regions from a formerly more widespread gene pool.

Thus, range expansion and population extinction, or rare dispersal events, particularly during the Pliocene, appear to have played an important role in

shaping the observed present-day distribution and genetic structure of freshwater idoteid isopods throughout New Zealand and its outlying islands.

INTRODUCTION

Vicariance and dispersal provide contrasting explanations for patterns of biotic diversity (Hausdorf, 2000; Schulte *et al.,* 2000; Zink *et al.* 2000; Ketmaier *et al.,* 2003). A vicariance hypothesis may be tested by comparing times of genetic divergence to known geology-based historical events, and the lack of a correlation may rule out vicariance in favour of dispersal (see de Queiroz 2005). For example, the taxonomic group of interest may be too young to have been affected by a presumed vicariant event (e.g. Estabrook 2001, Sanmartin 2003, Winkworth *et al.* 2002, Perrie *et al.* 2003). Nevertheless, the acceptance of a vicariance hypothesis to explain a biological pattern need not necessarily eliminate the role of dispersal. Phylogenetic data can be very useful for distinguishing between the two processes (Wolf *et al.* 2001, de Queiroz 2005).

For example, Trewick (2000) used mitochondrial DNA sequence data for four genera of large flightless insects (Orthoptera: Rhaphidophoridae) and showed that genetic distances between the New Zealand mainland and the Chatham Islands, 700 km to the east, ranged from 2.8 – 11.2%. This finding was interpreted to indicate phylogenetic separation of New Zealand and Chatham Island lineages in the Pliocene (2 - 6 Mya), much too recently to be explained by vicariant (tectonic) processes (Trewick, 2000). Instead, a single over-sea dispersal event of insects from New Zealand, followed by *in situ* speciation, was proposed. By contrast, Williams *et al.* (2003) presented a molecular phylogeny for the gastropod subfamily Littorininae based on sequence data for two nuclear and two mitochondrial genes. While they concluded that trans-Pacific dispersal seemed a likely explanation for the distribution of five members of *Austrolittorina*, estimated divergence of the South American *A. araucana* from four species in

Australia, New Zealand and eastern Pacific Islands was estimated to have been much earlier (40 – 73 Mya) and was attributed to vicariance associated with a change in climate and/or the final break-up of Gondwana.

The New Zealand archipelago provides an ideal opportunity for examining the effects of vicariance and dispersal on the distribution of fauna because the geological ages of its main islands are well established (Juan *et al.* 1996; de Queiroz 2005; Trewick & Morgan-Richards 2005). Certainly, the means by which a range of invertebrates may have colonised remote oceanic islands remains a subject of debate (e.g. Greenslade *et al.* 1999) and warrants further investigation.

Freshwater isopods *Austridotea lacustris*, *A. annectens* and *A. benhami* of the family Idoteidae are a group of species whose distribution encompasses distant offshore islands and east and west coastal regions of mainland (South Island) New Zealand. Chadderton *et al.* (2003) suggested that this group could be a likely candidate for testing a model of vicariance versus dispersal given their widespread but seemingly disjunct distribution patterns. They also noted the presence of isolated inland populations of *A. lacustris,* which might have accumulated genetic differences. To examine these issues, and to re-evaluate historic debates about generic and specific relationships of *Austridotea* (Nicholls 1937; Poore 2001), we utilised mitochondrial DNA from these three fresh and brackish water isopods, across their known ranges within New Zealand and its outlying islands.

We tested the hypotheses that genetic differences would exist among different geographic regions and ecological habitats (i.e. fresh- versus brackish-water). To further understand the diversification within species, we examined the magnitude of any genetic divergences to determine if these corresponded to habitat

availability (i.e. colonisation and/or isolation of islands) across geological timescales, and thus indicated a role for either dispersal or vicariance.

METHODS

Study taxa and collection of samples

Specimens of *Austridotea annectens*, *A. lacustris* and *A. benhami* (Isopoda: Idoteidae) were collected from the entire range currently known for each species (Chadderton *et al.* 2003) from streams and estuaries in southern South Island, Stewart Island, Campbell Island and Pitt Island (Chatham Islands) (Table 1, Fig. 1). *A. lacustris* has the widest geographic distribution of the three species and was obtained from 12 sites across its geographical range, whereas *A. benhami* is found only on and in the vicinity of the Otago Peninsula, south-east South Island, and was collected from three sites. *A. annectens* (n = 5 sites) and *A. lacustris* are both present in South Island, Stewart Island and Pitt Island (Chatham Islands), but *A. lacustris* is the only species found on the west coast of South Island (Fiordland) and Campbell Island (Fig. 1). All three species live in coastal streams and the lower reaches of larger rivers, although *A. lacustris* and *A. annectens* also inhabit brackish lagoons and estuaries of fluctuating salinity. They inhabit various substrata including sand, mud, gravel, cobbles, submerged branches and logs, and eat plant detritus, algae and small invertebrates. Further details on the ecology and known distribution of these species are provided by Chadderton *et al.* (2003). Isopods were collected by hand and 'kick-nets' and individuals were preserved in the field using 95% ethanol. Species were identified using the criteria given by Chadderton *et al.* (2003).

TABLE 1. Sampling locations for *Austridotea* throughout southern New Zealand (see also Chadderton *et al.*, 2003).

Region	Site name	Co-ordinates	Species	Habitat*	Code	GenBank accession
Campbell Island	Hooker Stream	52°28'S 169°12'E	*lacustris*	fw	C1	(to be added)
	Camp Stream	52°33'S 169°07'E	*lacustris*	fw	C2	
Stewart Island	Rakeahua River	46°58.9'S 167°51.5'E	*lacustris*	fw	ST4	
	Little River	46°51.8'S 168°06.6'E	*lacustris*	tidal	ST2	
	Maori River	46°51.2'S 168°04.5'E	*lacustris*	tidal	ST1	
	Horseshoe Bay Stream	46° 52.3'S 168° 07.6'E	*lacustris* *annectens*	tidal	ST3 ST5	
Southland	Tahakopa River	46° 33.7'S 169° 28.5'E	*lacustris*	tidal	S8	
	Waikawa River	46°35.7'S 169°08.0'E	*lacustris*	fw	S9	
	Fortrose Creek	46°33.6'S 168°47.4'E	*annectens*	tidal	S10	
Otago	Sawyers Bay Creek	45°49.2'S 170°36.3'E	*lacustris*	tidal	S6	
	Tomahawk Creek	45°54.0'S 170° 32.2'E	*lacustris*	fw	S5	

	Waipori River	45° 59.6'S 170° 07.8'E	*annectens*	tidal	S7
	Greenacres Stream	45°52.3'S 170°36.4'E	*benhami*	fw	S2
	Lindsay Creek	45°49.9'S 170°33.1'E	*benhami*	fw	S3
	Deborah Bay Stream	45°47.7'S 170°37.5'E	*benhami*	fw	S4
Canterbury	Waikewai Creek	43°51.5'S 172°21.6'E	*annectens*	fw	S1
Pitt Island (Chatham Islands)	Flower Pot Stream	44°14.9'S 176°14.9'W	*lacustris* *annectens*	fw	P1 P2

* fw = freshwater; tidal sites are exposed to at least occasional incursions of saline water.

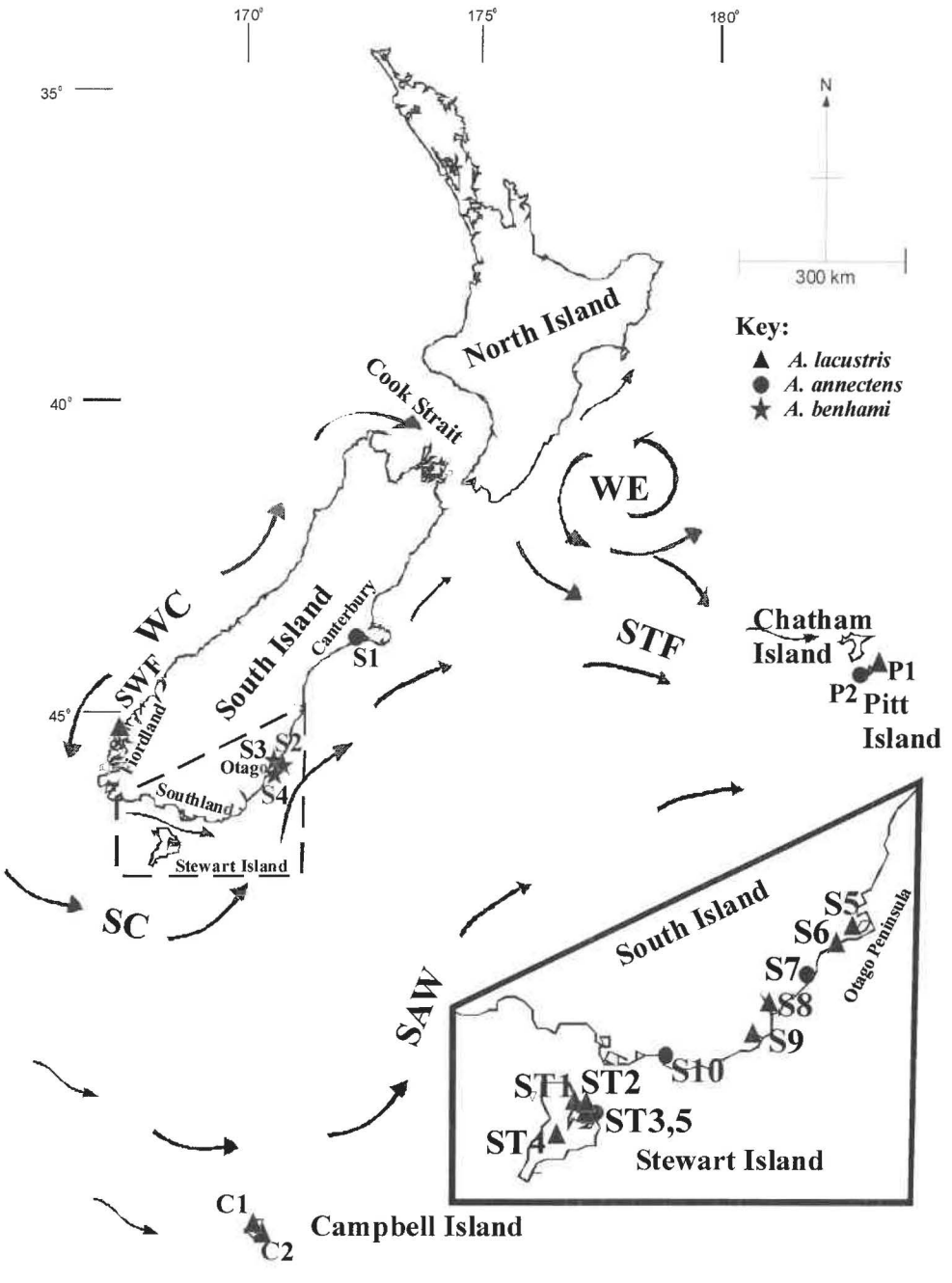

FIGURE 1. Locations of the examined populations of *Austridotea lacustris* (triangles, 12 sites), *A. annectens* (circles, 5 sites), and *A. benhami* (stars, 3 sites) in southern New Zealand (codes correspond to those in Table 1). Arrows indicate predominant ocean circulation features (Carter *et al.* 1998) including the main water currents (SAW = Sub-Antarctic Water; SC = Southland Current; WE = Wairarapa Eddy, and STF = Sub-tropical Front). Inset: shows the cluster of southern east-coast and Stewart Island sites, excluding *A. benhami* locations.

Total DNA was extracted from one to three individuals from each location (Table 1). Extractions consisted of homogenizing approximately five legs from an individual following the standard protocol for animal tissues using the DNeasy Tissue Kit (Qiagen), with the only modification an overnight incubation at 56°C.

PCR amplification (Saiki *et al.* 1988) was carried out using a 50 µl reaction volume consisting of 3 µl of DNA (not quantified), 1 x PCR buffer (Roche), 2.2 mM $MgCl_2$, 0.2 mM of each dNTP (Boehringer Mannheim), 1.0 µM of each primer and 1.0 unit of *Taq* DNA polymerase (Roche) on an Eppendorf Mastercycler gradient thermocycler or a Biometra T1 thermocycler (Whatman Biometra). A 680-bp fragment of the mitochondrial (mt) cytochrome *c* oxidase I (COI) gene was amplified (via PCR) using the primers COI-2F (5'- tty gay cci dyi ggr gga gga gat cc -3'); and COI-2R (5'- ggr tar tcw gar taw cgt ncg wgg tat -3') (Otto & Wilson 2001). The thermal cycling conditions were: initial denaturation at 94°C for 60 s; followed by 40 cycles of denaturation at 94°C for 20 s; annealing at 50°C for 30 s; and extension at 72°C for 90 s; with a final extension at 72°C for 5 min. Reaction products were then cleaned using a Qiaquick PCR Purification Kit (Qiagen) or if necessary band-excised using the Perfectprep gel cleanup kit (Eppendorf). Purified PCR products were sequenced (using the COI-2R primer) directly on a MegaBACE DNA Analysis System (Amersham Biosciences) at the University of Waikato DNA sequencing facility. Alternatively, for some sequencing reactions we used the Big Dye mix (Applied Biosystems) using a Biometra T1 thermocycler (Whatman Biometra) (thermal cycling conditions were: 26 cycles of 96°C for 10 s; 50°C for 5 s; and 60°C for 4 m). Dye terminators were removed by sodium acetate/ethanol purification (2 µl EDTA

(125mM), 2 µl NaOAc (3M), 50 µl 100% ethanol, incubated at room temperature for 15 min, centrifuged at 4°C for 30 min, washed with 200 µl 70% ethanol, centrifuged at 4°C for 5 min, and air-dried), before automated sequencing on a capillary ABI3730 genetic analyser (Applied Biosystems Inc.) at the Allan Wilson Centre Genome Service, Massey University.

Sequences were verified as being derived from isopod DNA using the GenBank BLAST algorithm, and aligned using SEQUENCHER (Gene Codes ver. 4.2) sequence editor. Sequence data were analysed with PAUP* ver. 4.0b10 (Swofford 2002). Initial phylogenetic analysis used the sphaeromid isopod *Sphaeroma terebrans* (GenBank accession AF447859; M. Baratti & G. Messana, unpubl. data) as an outgroup taxon, and showed *A. lacustris* to be the sister group to *A. annectens* and *A. benhami*. Subsequent phylogenetic reconstructions used only *Austridotea* spp. and mid-point rooting. χ^2 tests, as implemented in PAUP*, were used to determine whether the assumption of equal base frequencies among sequences was violated on all sites and using third codon positions only. Modeltest ver. 3.5 (Posada & Crandall 1998) was used to determine the appropriate substitution model for Maximum Likelihood (ML) heuristic searches (using all unique sequences). The model selected was HKY+G (-lnL = 2117.5, ti/tv = 4.2778, G = 0.1829; with base frequencies set to A = 0.4277, C = 0.2205, G = 0.1186, T = 0.2332); all other options in PAUP* remained as default. Distance matrices of pairwise nucleotide sequence divergence were calculated using the HKY85 model. Alternative tree reconstructions utilised Neighbour-Joining (with HKY85) and Maximum Parsimony and Maximum Likelihood analyses used the default options in PAUP*. Confidence in the cladistic analyses was assessed by estimation of the g_1 skewness statistic from 100,000 random tree length distributions (Hillis & Huelsenbeck 1992), and by bootstrap analysis with 500

replicates (Felsenstein 1976). Comparisons of log likelihood scores (using χ^2 tests) for trees, with and without a molecular clock enforced, indicated that these sequences were evolving in a "clock-like" manner. Subsequently, we estimated the age among lineages using the common molecular clock calibration of 2–2.3 % divergence per million years derived from comparisons between geological evidence and invertebrate mitochondrial data (e.g. Brower 1994; Folmer *et al.* 1994; Juan *et al.* 1996; Roslin 2001; Trewick & Morgan-Richards 2005).

RESULTS

A 546-bp (184 codons) segment of unambiguous alignment (no insertions or deletions) from a total of 54 individual *Austridotea* sequences was used for all analyses. Nucleotide composition averaged over all sequences showed an A-T bias of 64% (A = 40%, T = 24%, C = 23%, G = 13%). Base frequencies were homogeneous among sequences for all sites ($\chi^2_{72} = 14.36$, $p = 1.000$), and for the 182 third codon positions ($\chi^2_{72} = 26.68$, $p = 0.999$). There were 182 variable sites and 173 parsimony-informative nucleotide substitutions.

The parsimony analysis found 54 equally parsimonious trees (tree length = 300, C.I. = 0.78, R.I. = 0.91), and the consensus tree was found to have identical topology to the Neighbour-Joining analysis (using HKY85, which suitably accounts for the variable composition rate (A-T bias) across the data set), the MP, and the ML analyses shown in Figure 2. Both a strong phylogenetic signal ($g_1 = -1.125$, $g_{crit} = -0.09$, $p < 0.01$) and bootstrap analysis support the tree topologies in Figure 2. The ML analysis resolved three main clades corresponding to the three currently recognised *Austridotea* species (Fig. 2).

Sequence divergence (using HKY85) between *A. annectens* and *A. benhami* was 16%, and between these species and *A. lacustris*, divergence was 31% (Table 2). Divergence among the three populations of *A. benhami* (S2,3,4) was < 2.0%. However, comparison among the 12 populations of *A. lacustris* revealed four main subdivisions: 1) Campbell Island (C1,2): 9-11% divergence from the other *A. lacustris* groups; 2) Pitt Island (Chatham Islands, P1): 9-11% divergence; 3) Fiordland populations (SFW): 6-10% divergence; and 4) east coast South Island sites (S5,6,8,9) and Stewart Island (ST1,2,3,4): 7-11% divergence (see Table 2, Fig. 2). Within each of these groupings, divergence was low (< 2.5%).

Divergence was also very low within, and among *A. annectens* populations (< 0.5%), except for individuals from Pitt Island (Chatham Islands, P2), which were 4.4% divergent from *A. annectens* collected on South (S1,7,10) and Stewart (ST5) Islands.

We found that patterns of genetic distance (Table 2) and the grouping of populations in the ML analysis (Fig. 2) corresponded to geographic location. For example, specimens of *A. lacustris* and *A. annectens* from the Chatham Islands (800 km east of mainland New Zealand) differed by 6-9% and 4.4%, respectively, from east coast South Island individuals of the two species. Similarly, individuals of *A. lacustris* from Campbell Island were 9-11%, and Fiordland 6-10% divergent from their east coast South Island counterparts. However, we found no differences in sequence divergence corresponding to brackish vs. freshwater locations.

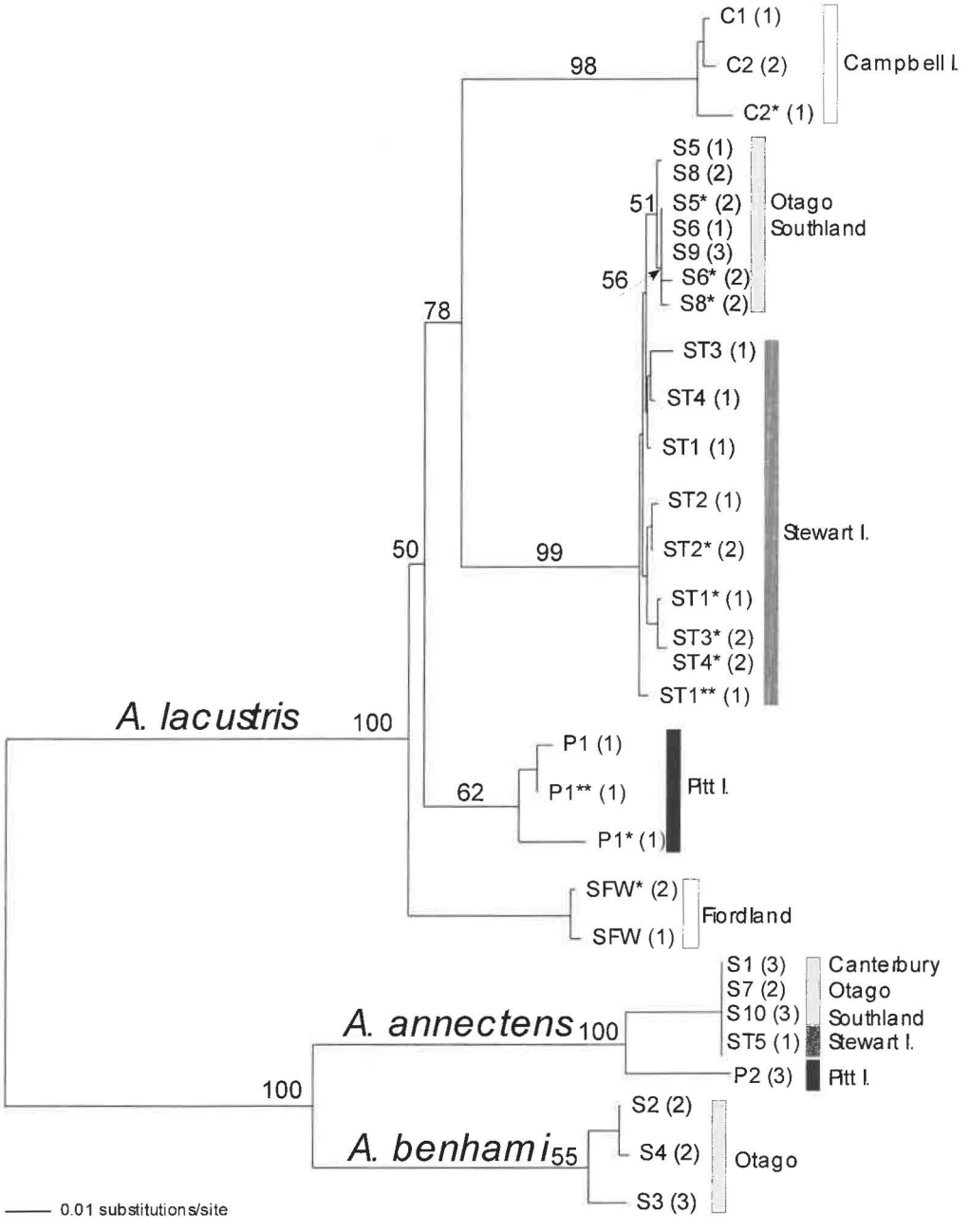

FIGURE 2. Maximum-Likelihood phylogram based on the substitution model HKY+G (ti/tv = 4.2778, G = 0.1829; base frequencies set to A = 0.4277, C = 0.2205, G = 0.1186, T = 0.2332) derived from Modeltest (see methods), using a 546-bp fragment of the mitochondrial DNA (COI) gene using only unique sequences. Bootstrap confidence limits (500 replicates) shown above nodes. Codes are those used in Tables 1 and 2, and Fig. 1; '*' identifies each multiple different haplotype present at a site; the number of identical haplotypes present at any site is given in parentheses.

TABLE 2. Genetic distances (using the HKY85 parameter) based on sequence variation in the mtDNA (COI) sequences (546 aligned sites) among 20 populations of *Austridotea* throughout southern New Zealand. Only unique sequences (25 unique mtDNA (COI) haplotypes) were included in the analysis. Codes refer to those used in Table 1 and Figs. 1 and 2; '*' identifies each multiple different haplotype present at one site, number of identical haplotypes present at any site is identified in the parentheses.

Locations		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
		Campbell I.			Otago/Southland				Stewart I.								Pitt I.			Fordland			Pitt I.	Otago	
1 **C1** (1)																									
2 **C2** (2)		0.004																							
3 **C2*** (1)		0.013	0.009																						
4 **S8** (2), **S5** (1)		0.098	0.100	0.102																					
5 **S6** (1), **S9** (3), **S5*** (2)		0.098	0.098	0.100	0.002																				
6 **S6*** (2)		0.100	0.100	0.102	0.004	0.002																			
7 **S8*** (2)		0.095	0.095	0.098	0.004	0.002	0.004																		
8 **ST2** (1)		0.100	0.102	0.104	0.007	0.009	0.011	0.011																	
9 **ST2*** (2)		0.097	0.100	0.102	0.006	0.007	0.009	0.009	0.002																
10 **ST1**** (1)		0.095	0.098	0.100	0.006	0.007	0.009	0.009	0.006	0.004															
11 **ST1*** (1)		0.102	0.104	0.107	0.007	0.009	0.011	0.011	0.004	0.006	0.006														
12 **ST3*** (2), **ST4*** (2)		0.102	0.104	0.107	0.009	0.011	0.013	0.013	0.006	0.007	0.007	0.002													
13 **ST3** (1)		0.100	0.100	0.102	0.009	0.007	0.009	0.009	0.009	0.011	0.011	0.009	0.007												
14 **ST1** (1)		0.098	0.098	0.100	0.006	0.004	0.006	0.006	0.006	0.004	0.004	0.006	0.007	0.007											
15 **ST4** (1)		0.098	0.098	0.100	0.007	0.006	0.007	0.007	0.007	0.006	0.006	0.007	0.006	0.006	0.002										
16 **P1** (1)		0.097	0.100	0.111	0.077	0.079	0.082	0.082	0.075	0.073	0.075	0.077	0.079	0.084	0.075	0.077									
17 **P1*** (1)		0.095	0.098	0.109	0.073	0.075	0.077	0.077	0.071	0.069	0.071	0.073	0.075	0.080	0.071	0.073	0.004								
18 **P1**** (1)		0.099	0.102	0.113	0.088	0.090	0.092	0.088	0.085	0.083	0.085	0.088	0.090	0.094	0.085	0.088	0.019	0.022							
19 **SFW** (2)		0.091	0.093	0.091	0.092	0.094	0.096	0.092	0.090	0.092	0.090	0.092	0.090	0.094	0.094	0.092	0.075	0.075	0.063						
20 **SFW*** (1)		0.091	0.093	0.095	0.094	0.096	0.099	0.094	0.092	0.094	0.092	0.094	0.092	0.096	0.096	0.094	0.073	0.073	0.063	0.004					
21 **S1** (3), **S7** (2), **S10** (3), **ST5** (1)		0.296	0.300	0.303	0.299	0.302	0.305	0.305	0.296	0.296	0.299	0.299	0.299	0.296	0.299	0.299	0.283	0.277	0.288	0.286	0.286				
22 **P2** (3)	***A. annectens***	0.303	0.306	0.309	0.299	0.302	0.305	0.299	0.296	0.296	0.299	0.299	0.299	0.296	0.299	0.299	0.288	0.283	0.288	0.286	0.286	0.044			
23 **S2** (2)		0.279	0.282	0.288	0.280	0.283	0.286	0.280	0.271	0.274	0.272	0.275	0.277	0.277	0.277	0.280	0.261	0.255	0.250	0.253	0.253	0.153	0.160		
24 **S4** (2)	***A. benhami***	0.282	0.285	0.291	0.283	0.286	0.289	0.283	0.274	0.277	0.274	0.277	0.280	0.280	0.280	0.283	0.263	0.258	0.253	0.255	0.255	0.155	0.162	0.002	
25 **S3** (3)		0.287	0.291	0.293	0.280	0.283	0.280	0.280	0.271	0.274	0.271	0.274	0.277	0.277	0.277	0.280	0.266	0.261	0.255	0.258	0.258	0.156	0.163	0.015	0.017

A. lacustris

Canterbury
Otago
Southland
Stewart I.

DISCUSSION

The three currently recognised *Austridotea* species were clearly resolved by mtDNA (COI) sequencing, with *A. lacustris* being the most genetically divergent species (~31% from *A. benhami* and *A. annectens*). This finding is consistent with the view expressed by Nicholls (1937) who proposed that *A. lacustris* be placed in a separate genus from the other two, and does not support the earlier opinion of Chilton (1891) that *A. benhami* is a variety of *A. lacustris*. Although divergence among the three populations of *A. benhami* was low (< 2.0%) (*A. benhami* has a distribution restricted to the Otago Peninsula (S2,3,4); Fig. 1), genetic divergence within the other two species was high (up to 11.3%). This level of divergence is similar to the divergences (11.2%) reported by Hebert *et al.* (2003) in their survey of congeneric species pairs covering a wide range of invertebrate and other animal taxa.

The magnitude of intraspecific genetic divergence we found raises the question as to whether *A. lacustris* and *A. annectens* are actually morphologically cryptic species complexes, as found in some other crustacean groups, including amphipods and decapods (e.g. Hogg *et al.* 1998; Witt & Hebert 2000; Hurwood *et al.* 2003; Stevens & Hogg 2004). Our data is strongly suggestive of up to four genetically divergent populations within *A. lacustris* and two in *A. annectens*. However, congruent evidence from nuclear loci or corresponding morphological differences would be required for designation of species status. Nevertheless, protection of these divergent populations would be consistent with sound conservation practice (Moritz 1994). Chadderton *et al.* (2003) commented on the conservation status of all three idoteid species and concluded that, because of its restricted distribution, only *A. benhami* could be considered range-restricted and

at risk. Our genetic analyses provide a basis to re-evaluate populations of *A. lacustris* and *A. annectens* on Campbell and Chatham Islands, and Fiordland (Fig. 2). *A. lacustris* is abundant and widespread on Campbell Island (Joy & Death 2000) and both species appear to be common on Pitt Island in the Chatham Islands. However, on current threat classification criteria (Molloy *et al.* 2002), populations of *A. lacustris* in Fiordland would appear to warrant either data-deficient or "at risk - range restricted" threatened species status. The *A. lacustris* Fiordland population is presently only recorded from two small streams.

Chadderton *et al.* (2003) also speculated on whether inland versus coastal populations of *A. lacustris* have diverged genetically as a result of greater adaptation to freshwater as opposed to estuarine environments. However, we found no differences in sequence divergence corresponding to distance of collecting sites from the sea. For example, individuals of *A. lacustris* from sites 11 km inland (Rakeahua River, ST4), and 5 km inland (Waikawa River, S9), showed little divergence from each other, or from individuals in nearby coastal populations that are estuarine or directly above the normal upper limit of saltwater incursion (and hence exposed periodically to brackish water). Accordingly, genetic differences are more likely to be the result of geographic isolation than environmental adaptation.

The pattern of genetic divergence between Stewart Island and Southland populations of *A. lacustris* was consistent with that exhibited by two non-migratory species in the *Galaxias vulgaris* complex (Allibone & Wallis 1993, Waters & Wallis 2001); a pattern these authors attributed to historic river connections during glacial periods when Stewart Island was connected to the mainland (8 - 14,000 years ago). By contrast, the lack of genetic divergence among Southland, Otago and Canterbury populations of *A. annectens* and *A.*

lacustris was consistent with that found in migratory, diadromous galaxiids with marine dispersal phases. The absence of geographic structure in *Austridotea* spp. on the east coast of South Island was also consistent with patterns documented by Stevens & Hogg (2004) for the corophiid amphipod *Paracorophium excavatum,* and may indicate genetic transfer mediated by periodic dispersal via coastal currents. Conversely, the presence of genetically divergent populations of *A. lacustris* in Fiordland suggests there is a significant oceanic barrier to dispersal between Fiordland and east coast Southland and Stewart Island populations.

However, other studies have shown that the New Zealand land mass during the Pliocene appears to have provided few barriers to aquatic dispersal, and the presence of ephemeral islands throughout the Pliocene may even have allowed for more frequent dispersal (Stevens & Hogg 2004). Oceanic circulation patterns around southern New Zealand, notably the Southland Current and the Subtropical Front (Fig. 1), may provide a mechanism for the dispersal of isopods seen here, assuming that comparable ocean circulation existed at appropriate times in the past. Furthermore, because *Austridotea* species are often found aggregating in cracks and grooves in wood, and *A. lacustris* can tolerate sea water for at least a week without feeding (Chadderton *et al.* 2003), they seem well-suited for successful oceanic dispersal. Indeed, a role for dispersal from southern New Zealand to Chatham Islands in particular via ocean currents has been postulated (e.g. Knox 1960, Trewick 2000, Stevens & Hogg 2004) and other studies have indicated strong marine connectivity along the New Zealand subcontinent and Chatham Rise (e.g. Stilwell 1997; Glasby & Alvarez 1999). In particular, paleographic reconstructions suggest that the most favorable 'invasion period' for organisms to Chatham Islands was probably during the late Pliocene, when periodic cold seawater incursions east of northern South Island would have been

most effectively focused into an east coast current (Nelson *et al.* 1999). Such a dispersal mechanism has been invoked to explain the strong sister relationships (3.6% mitochondrial sequence divergence) found between non-diadromous Canterbury (*Neochanna burrowsius*) and Chatham Islands mudfish (*N. rekohua*) (J.M. Waters & R. McDowall, unpubl. data) and comparable distribution patterns to those of *A. lacustris* and/or *A. annectens* have been described for several groups of marine algae and invertebrates. For example, several marine red algae (Rhodophyceae) and the southern spider crab *Jacquinoyia edwardsii* (Majidae) have very similar distributions to *A. lacustris*, which incorporate southern South Island, Stewart Island, Campbell Island and Chatham Islands (Moore 1961; McLay 1988).

The magnitude of genetic divergence we found in *Austridotea* species on offshore islands appears to correspond to the potential availability of these habitats for colonisation. For example, based on the most commonly used invertebrate molecular clock calibration of 2–2.3% divergence per million years (Brower 1994; Folmer *et al.* 1994; Juan *et al.* 1996; Roslin 2001; Trewick & Morgan-Richards 2005), the divergence of *A. lacustris* and *A. annectens* on Chatham Islands (6-9% and 4.4%, respectively) indicates that potential migration events from mainland New Zealand could have occurred approximately 2.6 – 4.5 Mya and 1.9 – 2.2 Mya, respectively. This is similar to the 4 Ma estimate of Stevens & Hogg (2004) for the brackish water amphipod *Paracorophium excavatum* (using an invertebrate allozyme clock calibration), and compares with the 2 – 6 Ma proposed by Trewick (2000) for four flightless insect genera. Sea level changes and geological history can be used to suggest the earliest and latest possible times that Chatham Islands land surface was above sea level and therefore available for colonisation. The geological record for Chatham Islands

shows that deep marine deposits dominated during the Opoitian Stage (3.6 – 5.2 Mya) indicating that the islands were completely submerged at some time during this period (Campbell *et al.* 1993). The water depth during this period was 50-200 m, and the rate of uplift in areas distant from plate boundaries may be expected to be around one metre per 10,000 years (P. Kamp, pers. comm.). If we assume that 100 m of total uplift was required, then the land surface of Chatham Islands would have been above sea level, and therefore potentially available for colonisation, as early as 4.2 Mya and as late as 2.6 Mya (Campbell *et al.* 1993). Similarly, the divergence of *A. lacustris* on Campbell Island (9-11%) corresponds to a potential dispersal event 3.9 – 5.5 Mya via oceanic currents or a significant isolating mechanism, such as a change in ocean currents that essentially isolated the island from other populations (see Fig. 1). Campbell Island was submerged throughout the early Oligocene, and a volcanic rise above the surface in the late Miocene or early Pliocene would have provided habitat that subsequently has been affected geologically only by periodic volcanism and the Pleistocene glaciations (Fleming 1954).

Finally, although the genus *Austridotea* has a moderately wide distribution in southern South Island and Stewart Island, it is absent north of Banks Peninsula (Canterbury) and from North Island. One possible explanation for this distribution pattern is that isopods were once more widespread in the past and have since become extinct on the North Island (see also de Queiroz 2005). This alternative explanation could also be broadened to suggest that those lineages now present only on Chatham Islands, Campbell Island, and in Fiordland, were also once more widespread throughout greater New Zealand. Extinction may have been caused by higher competitive pressures, unsuitable habitat or other extrinsic factors. Alternatively, water temperature may restrict their distribution to more

southern habitats, or oceanic circulation patterns may have restricted northward dispersal. However, other aquatic invertebrate studies have shown that the Cook Strait region in particular may have posed a significant barrier between North Island and South Island (e.g. Apte & Gardner 2002; Stevens & Hogg 2004).

CONCLUSION

The divergences in mtDNA sequences we found among *Austridotea* populations in southern New Zealand are likely to be the result of dispersal followed by geographical isolation. Such diversification in fast-changing environments and newly opened island habitats is not uncommon (Gorog *et al.* 2004), and highlights the evolutionary importance of recent, long-distance dispersal and/or isolating mechanisms in the origin of extant New Zealand taxa (see also Hurr *et al.* 1999; Winkworth *et al.* 1999, 2002; de Queiroz 2005; Trewick & Morgan-Richards 2005).

The pattern of genetic differentiation found, combined with the geological history of the region implies that Pliocene dispersal and/or isolating events have played the major role in determining the present distribution of these idoteid isopods. Furthermore, the genetic differences among species and among locations suggest that present-day long-distance oceanic dispersal is negligible.

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CHAPTER II

PHYLOGEOGRAPHIC PATTERNS FOR SPRINGTAILS AND MITES THROUGHOUT SOUTHERN VICTORIA LAND, ANTARCTICA: A PLEISTOCENE AND HOLOCENE LEGACY OF REPEATED CYCLES OF GLACIAL REFUGIA AND RANGE EXPANSION[†]

Keywords: glacial refugia, mitochondrial DNA, mites, springtails, phylogeography

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ABSTRACT

We collected individuals of the springtail *Gomphiocephalus hodgsoni* (Collembola: Hypogastruridae) and the mite *Stereotydeus mollis* (Acari: Penthalodidae) from sites in Wright and Victoria Valleys in southern Victoria Land, Antarctica. Our primary aim was to test the hypothesis that this large, ice-free area has served as a refugium throughout historical glaciations and subsequently acted as a source for re-colonisation of surrounding regions. We predicted that this region would harbour higher levels of genetic diversity and/or unique genetic stock relative to previously sampled locations. Traditional, morphology-based taxonomic approaches for assessing biodiversity were applied in conjunction with mtDNA analyses and associated statistical techniques (e.g. phylogenetic and nested clade analyses). Individuals from sites within Victoria and Wright Valleys were compared to previous samples taken from throughout the known range of both species in southern Victoria Land. Analysis of *G. hodgsoni* and *S. mollis* individuals showed a congruent pattern of population sub-structuring and haplotype heterogeneity among sites. We found eight and 22 new mtDNA haplotypes for *G. hodgsoni* and *S. mollis*, respectively. For both species, we found haplotype links to previously sampled populations in the Ross Sea sector. Furthermore, haplotype diversity was higher in Wright and Victoria Valleys relative to any previously sampled locations. Accordingly, it is possible that this region may represent one of the primary refugial source populations from which many of the extant populations in southern Victoria Land have expanded throughout the Pleistocene and more recently in the Holocene.

INTRODUCTION

Phylogeography is the study of the patterns and processes governing the geographical distribution of genealogical lineages (Avise 1998). It looks at processes both within and between species through a combination of historical biogeography (macroevolution) and population genetics (microevolution) (Bermingham & Moritz 1998), and so provides data that is an important component in the interpretation of evolutionary relationships that exist within and among taxa (Avise 1998; Irwin 2002). The spatial assortment of genetic markers has allowed estimates to be made of dispersal rates (i.e. gene flow) among populations, which in turn can be used to reveal the processes and timing of speciation, adaptive radiation and extinction (Bermingham & Moritz 1998). Thus, the biogeographic history of a species can be reconstructed, furthering our knowledge of the relationships that exist between population processes and patterns of species diversity.

Antarctica is an ideal location to study phylogeographic processes and the effects of repeated glacial cycles upon population structure and species distributions. Since the isolation of the Antarctic continent after the Gondwanan break-up, the Antarctic fauna has been exposed to a climate of increasing severity. Prolonged low temperatures and a terrestrial surface that has been dominated by more than ten glacial cycles over the last million years (Hays *et al.* 1976), have meant that many regions of Antarctica have been, or are isolated (< 2% of the continent is ice-free today), with survival of taxa only possible in ice-free refugia (Wise 1967; Hogg & Stevens 2002, 2005). Re-colonisation from these refugia has in many cases only been possible within the current inter-glacial (<17,000 years) as terrestrial habitats have become available. Furthermore, terrestrial colonisation from outside Antarctica

is unlikely due to the isolating oceanic belt (ca. 40°S - 66°S) surrounding the continent (Schultz 1995; Lawver & Gahagan 1998). Thus, the Antarctic landscape, with its unique collection of endemic terrestrial life (Beyer & Bölter 2002), and in particular, southern Victoria Land with its extensive ice-free regions, may be used to test hypotheses related to dispersal and processes of re-colonisation, as well as evolutionary history of taxa relative to environmental change (Stevens & Hogg 2003).

Southern Victoria Land is a region that provides habitat for three species of springtail (*Gomphiocephalus hodgsoni, Neocryptopygus nivicolus*, *Antarcticinella monoculata*), and four species of mite (*Stereotydeus mollis, Coccorhagidia keithi*, *Tydeus setsukoae*, *Nanorchestes antarcticus*). We selected *Gomphiocephalus hodgsoni* Carpenter, 1908 (Collembola: Hypogastruridae) and *Stereotydeus mollis* Womersley and Strandtmann, 1963 (Acari: Penthalodidae) for a comparative study because they are the most numerically dominant and widespread of the seven species, are both endemic, and are found together throughout their distributional ranges. Their habitats in Antarctica are restricted to areas where water is present (e.g. lake edges, snow patch edges, moist river beds, glacier foregrounds) (Kennedy 1993), and when present, both species generally occur in the soil and on the underside of rocks, which provide shelter from wind desiccation (Stevens & Hogg 2002). Because springtail and mite taxa may both have limited dispersal capabilities, they are likely to have a pre-Pliocene origin and be relics of an ancient Gondwanan fauna (Stevens & Hogg 2003, 2005). Studying two unrelated taxa with common distributions within a phylogeographic context, provides an opportunity to examine common historical patterns and processes relevant to the metapopulation structure of Antarctic biota. Furthermore, the influence of the extreme Antarctic environment in isolating

genetically distinct subpopulations, combined with the low dispersal abilities of these organisms, allows study of phylogeography and microevolutionary processes in a natural model system.

Rand (1948, 1954) first noted the relationship between biological diversity and glacial refugia based on the modern distributions of bird and mammal species in North America - specifically, the existence of several refugia located south of continental ice in the Alaska-Bering Sea and high arctic areas. As populations become restricted to ice-free refugia, divergence between populations proceeds in isolation, leading potentially to speciation and often to population sub-structuring/differentiation. The role of glacial refugia in the shaping of species distributions has enjoyed widespread attention, with studies encompassing both the Northern and Southern Hemispheres, and a wide range of taxa (see Hewitt 2000 and references therein). In particular, Pleistocene glaciations over the last ~ 2 My (Edmands 2001) and more recent (~18,000 yrs) Holocene glaciations are likely to have had marked effects on species ranges and population sizes (e.g. Trewick 2001; Trewick & Wallis 2001; Knowles 2001; Weider & Hobæk 2003; Galbreath & Cook 2004; Rowe *et al.* 2004). For example, Garrick *et al.* (2004), found intense levels of population sub-structuring in an Australian saproxylic 'giant' springtail that conformed to known geological/climatic effects of the last glacial, while van der Wurff *et al.* (2003) found patterns of population sub-structuring across distances as short as 50 m in the springtail *Orchesella cincta* in the Netherlands.

In Antarctica, patterns of genetic differentiation within populations over small spatial scales have been found for several terrestrial invertebrates. For instance, Stevens & Hogg (2003) found high levels of genetic diversity throughout the continental and island sites they sampled. Across 45 *G. hodgsoni* sequences, they

found 14 mtDNA (COI) haplotypes, among which divergence was 0.2 – 2.0%, and correlated to spatial patterns. Similar results were found for the mite *S. mollis* although levels of divergence were much higher (17%) (M.I. Stevens & I.D. Hogg, unpubl. data). Fanciulli *et al.* (2001) also showed a pattern of high differentiation and grouping of allele combinations with geographical proximity for the springtail *Gressittacantha terranova* in northern Victoria Land, while Frati *et al.* (2001) found 18 mtDNA (COII) haplotypes in 40 individuals (17 of which were in single populations) of another springtail *Isotoma klovstadi*. Additionally, historical biogeography and ecology of the continental Antarctic mite genus *Maudheimia* (Acari, Oribatida) was examined by Marshall & Coetzee (2000) and evidence of speciation among populations was found. Collectively, these studies have suggested that the Antarctic environment provides suitable conditions to promote micro-evolutionary processes. By contrast, Courtright *et al.* (2000) found no such geographical grouping of haplotypes for the nematode *Scottnema lindsayae*. However, this species undergoes an an-hydrophobic life stage, such that dispersal via wind/water could act as an homogenising force (Stevens & Hogg 2003). Thus, these patterns of genetic differentiation found within and across springtail and mite populations may reflect common historical patterns of isolation (e.g. via long-term barriers among populations) and re-colonisation throughout Victoria Land.

Stevens & Hogg (2003) speculated that the Taylor Valley region contained a pre-Pleistocene refugial population, from which some extant populations of *G. hodgsoni* in southern Victoria Land may have spread. This region contained the highest levels of diversity and harboured two different haplotypes occurring sympatrically, possibly representing reproductively isolated species. A similar pattern was also found for *S. mollis* (M.I. Stevens & I.D. Hogg unpubl data), indicating that these two species may

be ideal candidates for a comparative phylogenetic study. However, despite a large proportion of the total haplotype diversity for both species being found in Taylor Valley, not all was accounted for. Thus, we targeted an extensive ice-free area to the north of Taylor Valley, in the Dry Valley region, encompassing the St. John's, Olympus and Asgard Ranges of Wright and Victoria Valleys (Fig. 1).

We hypothesised that this area would: 1) have higher levels of genetic diversity and/or unique genetic resources compared to previously sampled locations due to its greater ice-free area; and 2) have served as a source from which extant populations have spread (i.e. contain pre-Pleistocene refugial populations), in which case, we would expect to see reduced levels of genetic variation in eastern (coastal) populations due to expansion from western (continental) refugia (sensu Rowe *et al.* 2004). Here, we used molecular analyses to determine phylogeographic patterning. Nested clade analysis was also utilised to allow an objective statistical interpretation of the geographical association of haplotypes in order to elucidate past evolutionary and population events that may have shaped current genetic structure.

MATERIALS and METHODS

Study area, taxa and collection of samples

The Dry Valleys consist of some 4,000 km^2 of high relief ice-free mountain polar desert topography and, with a mean annual temperature of -20°C and a precipitation level of ~ 80 mm water equivalent, the area is described as a hyper-arid cold polar desert (Marchant & Denton 1996). Taylor, Wright and Victoria are the main valleys in the region and these are separated by the Kukri Hills, and the Asgard and Olympus Ranges (Fig. 1). The mountains are highest in altitude further inland (> 2000 m), and become progressively lower towards coastal regions (Marchant & Denton 1996).

Sampling covered several sites in Wright and Victoria Valley floors and the surrounding Asgard, Olympus and St. John's ranges (Fig. 1). We sampled in excess of 40 sites (Table 1) throughout Wright and Victoria Valleys, and collected specimens from seven locations for *G. hodgsoni*, and 14 locations for *S. mollis*. Individuals of each species were collected from the underside of stones, which were always located in close proximity to moisture (e.g. snow packs, streams, glacier foregrounds), using a modified aspirator to collect samples (see Stevens & Hogg 2002 for complete details). Upon collection, samples were stored in 95% ethanol and returned to the laboratory for further analyses.

G. hodgsoni and *S. mollis* specimens were identified to the lowest taxonomic level possible using original and/or relevant identification keys (e.g. Strandtmann 1967; Wise 1971).

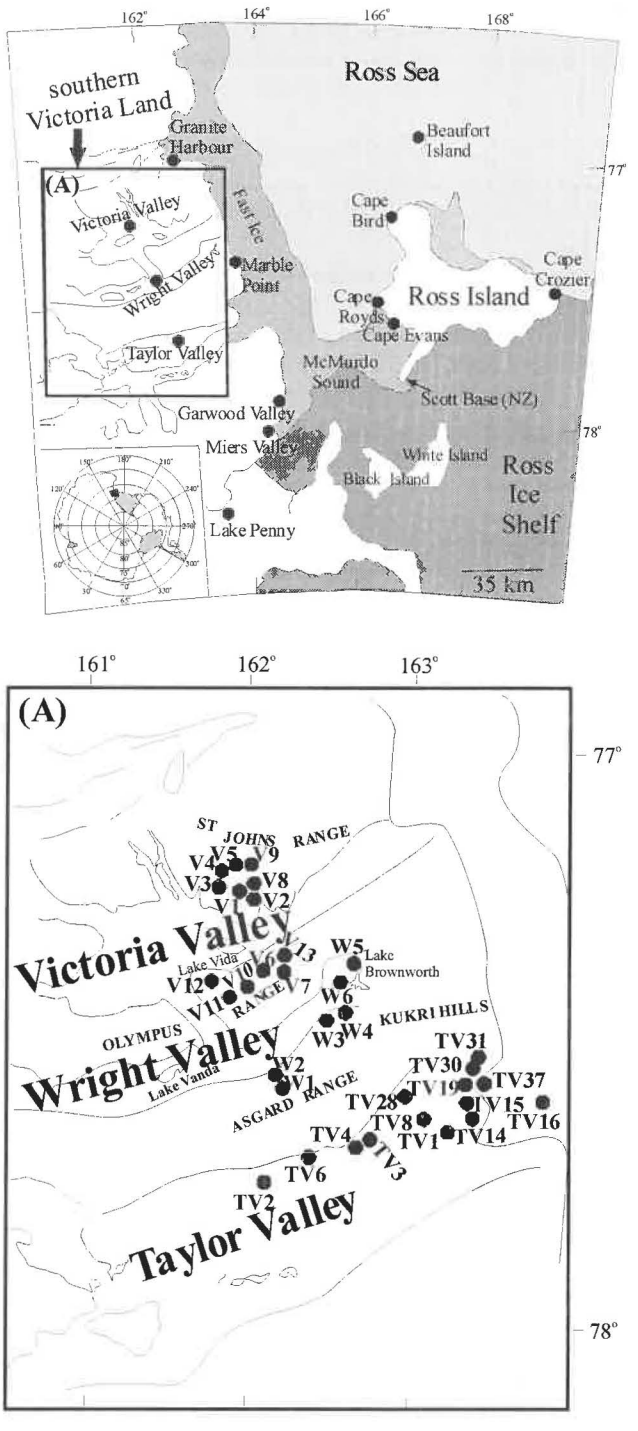

FIGURE 1. Sampling locations (solid circles) for *Gomphiocephalus hodgsoni* and *Stereotydeus mollis* in the Ross Sea region. Inset: distribution of sampling sites for *G. hodgsoni* and *S. mollis* within Victoria, Wright and Taylor Valleys in southern Victoria Land, Antarctica. Codes correspond to those given in Table 1.

TABLE 1. Sampling locations for *G. hodgsoni* and *S. mollis* throughout southern Victoria Land, Antarctica.

Region	Coordinates	Species	Location	NCA Haplotype	GenBank Accession
Wright Valley	77°30.52(S)				(to be added)
	162°10.13(E)	*S. mollis*	W1-1	W1	
	77°31.09(S)				
	162°11.26(E)	*S. mollis*	W2-2	W1	
	77°27.50'(S)				
	162°33.38'(E)	*S. mollis*	W3-1	W3a	
			W3-2	W3b	
	77°26.44'(S)				
	162°42.52'(E)	*G. hodgsoni*	W4-1	W4	
			W4-2	W4	
			W4-3	W4	
			W4-4	W4	
			W4-5	W4	
	77°26.50'(S)				
	162°35.44'(E)	*S. mollis*	W5-1	W5a	
			W5-2	W5b	
			W5-3	W5c	
			W5-4	W5d	
	77°27.44'(S)				
	162°36.40'(E)	*G. hodgsoni*	W6	V13a	
Victoria Valley	77°19.31'(S)				
	161°53.54'(E)	*G. Hodgsoni*	V1-1	V10a	
			V1-2	V10a	
			V1-3	V10b	
			V1-4	V10a	
		S. mollis	V1-5	V1a	
			V1-6	V1b	
			V1-7	V1c	
	77°21.43'(S)				
	162°06.06'(E)	*S. mollis*	V2-1	V2a	
			V2-2	V2b	
			V2-3	V2c	
			V2-4	V2d	
	77°19.31'(S)				
	161°52.17'(E)	*G. hodgsoni*	V3-1	W4	
			V3-2	V10a	
			V3-3	W4a	
	77°25.15'(S)				
	161°48.33'(E)	*G. hodgsoni*	V4-1	V13a	
		S. mollis	V4-2	V4	
	77°33.15'(S)				
	161°48.20'(E)	*S. mollis*	V6-1	V6	
	77°42.05'(S)				
	161°57.23'(E)	*S. mollis*	V7-1	V7	
			V7-2		

Region	Coordinates	Species	Location	NCA Haplotype GenBank Accession
	77°31.03'(S)			
	161°50.28'(E)	*G. hodgsoni*	V8-1	V8a
			V8-2	V8b
			V8-3	V8c
			V8-4	V10a
	77°31.05'(S)			
	161°50.46'(S)	*S. mollis*	V9-1	V9a
			V9-2	V9b
	77°41.55'(S)			
	161°57.20'(E)	*G. hodgsoni*	V10-1	V10a
			V10-2	V10b
			V10-3	V10a
	77°41.55'(S)			
	161°57.20'(E)	*S. mollis*	V11-1	V11
	77°34.05(S)			
	162°04.50'(E)	*G. hodgsoni*	V13-1	V13a
			V13-2	V13b
		S. mollis	V13-3	V13c
			V13-4	V13d
			V13-5	V13e
			V13-6	V13f
Taylor Valley	77°39.44'(S)			
	163°05.50'(E)	*G. hodgsoni*	TV1-1	TV1
			TV1-2	TV19
			TV1-3	TV1
			TV1-4	TV1
			TV1-5	TV1
			TV1-6	TV1
			TV1-7	TV1
			TV1-8	V10a
	77°45.50'(S)			
	162°02.14'(E)	*G. hodgsoni*	TV2-1	TV1
			TV2-2	TV1
			TV2-3	TV1
			TV2-4	TV1
			TV2-5	TV2a
			TV2-6	TV1
			TV2-7	TV1
			TV2-8	TV1
			TV2-9	TV1
		S. mollis	TV2-10	TV2b
			TV2-11	V1a
			TV2-12	TV4
			TV2-13	MPa
	77°38.34'(S)			
	162°46.30'(E)	*G. hodgsoni*	TV3-1	TV3a
			TV3-2	TV1a
		S. mollis	TV3-3	TV3b
			TV3-4	V1a

Region	Coordinates	Species	Location	NCA Haplotype GenBank Accession
	77°38.32'(S)			
	162°46.28'(E)	*S. mollis*	TV4-1	TV4
	77°36.45'(S)			
	162°24.08'(E)	*G. hodgsoni*	TV6-1	TV19
	77°34.08'(S)			
	163°09.21'(E)	*G. hodgsoni*	TV8-1	TV19
	77°38.29'(S)			
	163°17.41'(E)	*G. hodgsoni*	TV14-1	TV19
	77°38.30'(S)			
	163°17.50'(E)	*S. mollis*	TV15-1	TV15a
			TV15-2	TV15b
			TV15-3	V1a
			TV15-4	V1a
			TV15-5	TV4
	77°41.12'(S)			
	163°52.20'(E)	*G. hodgsoni*	TV16	TV19
	77°33.05'(S)			
	163°20.28'(E)	*G. hodgsoni*	TV19	TV19
	77°41.13'(S)			
	162°57.00'(E)	*G. hodgsoni*	TV28	TV1
	77°36.26'(S)			
	163°30.03'(E)	*G. hodgsoni*	TV30-1	TV19
			TV30-2	TV19
	77°36.46'(S)			
	163°31.37'(E)	*G. hodgsoni*	TV31	TV19
	77°37.16'(S)			
	163°29.21'(E)	*G. hodgsoni*	TV37	TV19
Garwood Valley	78°01.11'(S)			
	164°03.24'(E)	*G. hodgsoni*	GV-1	GV
Miers Valley	78°05.46'(S)			
	163°45.32'(E)	*G. hodgsoni*	MV-1	V10a
			MV-2	V10a
			MV-3	V10a
			MV-4	V10a
		S. mollis	MV-5	MVa
			MV-6	MVb
Granite Harbour	77°00.52'(S)			
	162°36.05'(E)	*G. hodgsoni*	GH1-1	W4
			GH1-2	V8c
			GH1-3	V8c
			GH1-4	V1a
	77°00.52'(S)			
	162°36.05(E)	*G. hodgsoni*	GH6-4	V8c
			GH6-5	R1a
			GH6-6	V8c
	77°02.18'(S)			
	162°28.11'(E)	*S. mollis*	GH7-1	GH7

Region	Coordinates	Species	Location	NCA Haplotype GenBank Accession
Marble Point	77°26.07'(S)			
	163°49.34'(E)	*G. hodgsoni*	MP-1	TV19
			MP-2	TV19
			MP-3	TV19
		S. mollis	MP-4	MPa
			MP-5	MPb
Lake Penny	78°18.36'(S)			
	163°24.28'(E)	*G. hodgsoni*	LP-1	LP1
			LP-2	LP1
			LP-3	V10a
			LP-4	V10a
Ross Island	77°13.16'(S)			
	166°26.49'(E)	*G. hodgsoni*	R1-1	R1a
			R1-2	R1b
			R1-3	R1a
			R1-4	V8c
			R1-5	V8c
			R1-6	V8c
		S. mollis	R1-7	V1a
			R1-8	TV15b
	77°32.46'(S)			
	166°09.47'(E)	*G. hodgsoni*	R2-1	R1a
			R2-2	R1a
			R2-3	R3a
			R2-4	R3a
			R2-5	R1a
		S. mollis	R2-6	TV15b
			R2-7	TV15b
	77°38.02'(S)			
	166°26.33'(E)	*G. hodgsoni*	R3-1	R3a
			R3-2	R3a
			R3-3	R3b
		S. mollis	R3-4	TV15b
	77°27.48;(S)			
	169°11.49'(E)	*G. hodgsoni*	R4-1	R1a
			R4-2	R1a
			R4-3	R1a
Beaufort Island	76°55.54'(S)			
	166°54.49'(E)	*G. hodgsoni*	BI-1	BIa
			BI-2	BIa
			BI-3	BIb
		S. mollis	BI-4	BIc
			BI-5	V2a
			BI-6	V2a
			BI-7	V2a
Delta Stream	77°37.26'(S)			
	163°06.34'(E)	*G. hodgsoni*	DS-1	TV1
			DS-2	TV1
			DS-3	TV1
			DS-4	TV1
			DS-5	TV1
Hut Point P.	77°51.10'(S)			
	166°40.48'(E)	*S. mollis*	HPP-1	HPPa
			HPP-2	HPPb
Cape Royds	77°32.46'(S)			
	166°09.47'(E)	*S. mollis*	CR-1	CR

Molecular Analyses

mtDNA extraction, amplification and sequencing

Total DNA was extracted from one to three preserved specimens from each location (Table 1), following the DNeasy extraction protocol for animal tissues set out in the DNeasy Tissue Kit Handbook (Qiagen). Upon extraction, a 710-bp fragment of the mitochondrial cytochrome *c* oxidase (COI) gene was amplified using the universal primers LCO1490 (5' – ggt caa caa atc ata aag ata ttg ga – 3') and HCO2198 (5' – taa act tca ggg tga cca aaa aat ca – 3') (Folmer *et al.* 1994) for *G. hodgsoni*, and COI-2R (5' – ggr tar tcw gar taw cgt ncg wgg tat – 3') and COI-2F (5' – tty gay cci dyi ggr gga gga gat cc – 3') for *S. mollis* (Otto & Wilson 2001). Amplifications for each *G. hodgsoni* and *S. mollis* specimen used a 25 µl reaction volume containing 3 µl of the extracted DNA (unquantified), 1 x PCR buffer (Roche) 2.2 mM $MgCl_2$, 0.2 mM of each dNTP (Boehringer-Mannheim), 1.0 µM of each primer (LCO1490/HCO2198 and COI-2R/COI-2F for *G. hodgsoni* and *S. mollis*, respectively), and 1.0 unit of *Taq* DNA polymerase (Roche). The thermal cycling conditions for *G. hodgsoni* were: 94°C for 1 min followed by five cycles of denaturation and polymerase amplification (94°C for 1 min, 45°C for 1.5 min, 1 min at 72°C) followed by 35 cycles of 94°C for 1 min, 51°C for 1.5 min and 1 min at 72 °C, followed by 5 min at 72°C (Stevens & Hogg 2003); and for *S. mollis* were: initial denaturation at 94°C for 1.5 min followed by 40 cycles of denaturation and polymerase amplification (94°C for 20 s, 55°C for 30 s and then 1.5 min at 72°C), followed by 5 min at 68°C.

All reaction products were purified using the QIAquick PCR Purification Kit (Qiagen) or using SAPEXO (USB Corp.) and a Biometra T1 thermocycler (Whatman

Biometra) (thermal cycling conditions were: a single cycle of 37°C for 30 min, followed by 80°C for 15 min), or if necessary band-excised using the Perfectprep gel cleanup kit (Eppendorf). Sequencing utilised LCO1490 (for *G. hodgsoni*) or COI-2R (for *S. mollis*) primers, and performed directly either on a MegaBACE DNA Analysis System (Amersham Biosciences) at the University of Waikato DNA sequencing facility, or we used BigDye™ Terminator chemistry (Perkin-Elmer Applied Biosystems) and a Biometra T1 thermocycler (Whatman Biometra)(thermal cycling conditions were: 26 cycles of 96°C for 10 s; annealing at 50°C for 5 s; and 60°C for 4 min), followed by sodium acetate/ethanol purification (in brief, by adding 2 µl EDTA (125mM), 2 µl NaOAc (3M), 50 µl 100% ethanol, incubating at room temperature for 15 min, centrifuging at 4°C for 30 min, washing with 200 µl 70% ethanol, centrifuging at 4°C for 5 min and air-drying), before automated sequencing on a capillary ABI3730 genetic analyser (Applied Biosystems Inc.) at the Allan Wilson Centre Genome Service, Massey University.

Phylogenetic analyses

Individual DNA sequences were verified as being derived from the relevant taxa using the GenBank BLAST algorithm, and were aligned using SEQUENCHER (Gene Codes vers. 4.2) sequence editor. Data were then analysed using the computer programme PAUP* (vers. 4.0b10) (Swofford 2002). Preliminary phylogenetic analyses utilized several prostigmatic mites (accession no.s AF142132-AF142143; Otto & Wilson 2001) and hypogasturid springtails as outgroup taxa (see also Stevens & Hogg 2003). We used these analyses to determine which of the *S. mollis* and *G.*

hodgsoni individuals (separately for each species) would form a sister group to the other intra-specific individuals. As previously found for *G. hodgsoni* (Stevens & Hogg 2003), preliminary analyses found several Taylor Valley individuals forming a sister group to all other individuals. Similarly, using the con-familial outgroups, an *S. mollis* individual from Taylor Valley was basal to all other intra-specific *S. mollis* individuals.

As implemented in PAUP*, chi square (χ^2) tests were used to determine whether the assumption of equal base frequencies among sequences was violated on all sites, on parsimony-informative sites, and on third codon positions only. Modeltest vers. 3.6 (Posada & Crandall 1998) was used to determine the appropriate substitution model for Maximum Likelihood (ML) heuristic searches (using all unique sequences). The model selected for *G. hodgsoni* was TrN+I (-lnL = 812.1001; rate matrix: A-C = 1.0000, A-G = 35.2664, A-T = 1.0000, C-G = 1.0000, C-T = 6.0747, G-T = 1.0000; proportion of invariable sites (I) = 0.9218; with base frequencies set to A = 0.2715, C = 0.1934, G = 0.1565, T = 0.3785); and for *S. mollis* was HKY+I+G (-lnL = 1960.1649; ti/tv = 1.5774, I = 0.4853; with base frequencies set to A = 0.3583, C = 0.1385, G = 0.1420, T = 0.3612); all other options in PAUP* remained as default. Distance matrices of pairwise nucleotide sequence divergence were calculated (uncorrected distances) using all unique sequences. Several alternative tree reconstructions were utilised: Neighbour-Joining (with uncorrected distances), Maximum Parsimony, and Maximum Likelihood analyses (using the default options in PAUP*). Confidence in the cladistic analyses was assessed by estimation of the g_1 skewness statistic from 100,000 random tree length distributions (Hillis & Huelsenbeck 1992), and by bootstrap analysis with 500 replicates (Felsenstein 1976).

Comparisons of log likelihood scores (using χ^2 tests) for trees, with and without a molecular clock enforced, indicated that these sequences were evolving in a "clock-like" manner. Subsequently, we estimated the age among lineages using the common molecular clock calibration of 2–2.3 % divergence per million years derived from comparisons between geological evidence and invertebrate mitochondrial data (e.g. Brower 1994; Folmer *et al.* 1994; Juan *et al.* 1996; Roslin 2001; Trewick & Morgan-Richards 2005).

Network analyses

For *G. hodgsoni* and *S. mollis*, the programme TCS vers. 1.18 (Clement *et al.* 2000) was used to estimate a haplotype tree or cladogram using the algorithm of Templeton *et al.* (1992). The cladogram for *G. hodgsoni* had a maximum number of mutation steps less than eight, so was statistically supported with 95% confidence, and for *S. mollis*, the connection limit was fixed at 60 mutational steps. Populations were specified by their GPS coordinates and sample sizes, and then defined into a nested structure including outgroup probabilities (Castelloe & Templeton 1994), following the nesting rules described in Crandall (1996). Briefly, construction of the nested series of clades started from the tips of the network by nesting haplotypes ('zero-step clades') that are by definition separated by one mutational change, within 'one-step clades', and proceeded step by step, to identify higher level clades until the final level of nesting clades included the entire network (Templeton *et al.* 1987; Templeton & Sing 1993; Alexandrino *et al* 2002).

Finally, nested geographical distance analyses were performed using the programme GEODIS ver. 2.2 (with 1000 permutations for a 5% level of statistical significance) (Posada & Crandall 2000; Posada *et al.* 2000). The null hypothesis of no geographical association(s) between clades was tested by assessing the clade distance (Dc) - the geographical range of the clades; the nested distance (Dn) - how far individuals bearing clade *x* haplotype are from individuals bearing clade *y* haplotype for tip and interior clades; and the interior vs. tip distance (I-T) – a contrast of the Dc and Dn values between interior and tip clades which calculates within each nested clade, the difference between the average interior and average tip distances (Templeton *et al.* 1995; Templeton 1998; Creer *et al.* 2001; Alexandrino *et al.* 2002).

Clades with statistically significant values of Dc, Dn or I-T were assessed with the updated inference key of Templeton (http://darwin.uvigo.es/software/geodis.html) to obtain a measure of how any one particular clade was geographically distributed relative to its closest evolutionary sister clades. This allowed us to deduce which factor(s) (e.g. restricted gene flow, past fragmentation, range expansion), caused significant spatial association among haplotypes.

RESULTS

Molecular Analyses

mtDNA sequence variation

Molecular analyses for *G. hodgsoni* and *S. mollis* combined new samples from Wright and Victoria Valleys with previous data obtained by Stevens & Hogg (2003) and M.I. Stevens & I.D. Hogg (unpubl. data) from throughout southern Victoria Land. Here, we used 471-bp (157 codons) of unambiguous alignment (no insertions or deletions) from a total of 95 individuals for *G. hodgsoni* and 504-bp (168 codons) of unambiguous alignment from a total of 61 individuals for *S. mollis* for all analyses. Nucleotide composition averaged over all sequences showed an A-T bias of 64.8% (A = 0.269, T = 0.379, C = 0.192, G = 0.160) and 69.1% (A = 0.358, T = 0.333, C = 0.160, G = 0.148) for *G. hodgsoni* and *S. mollis*, respectively. Base frequencies were homogeneous among sequences for all sites (*G. hodgsoni*: $\chi^2_{282} = 5.91$, $p = 1.00$ for, and *S. mollis*: $\chi^2_{111} = 9.92$, $p = 1.00$), for the parsimony informative sites (*G. hodgsoni* (15 sites): $\chi^2_{282} = 44.15$, $p = 1.00$; and *S. mollis* (101 sites): $\chi^2_{111} = 61.11$, $p = 1.00$), and for third codon positions (*G. hodgsoni* (157 sites, A-T = 83.9%): $\chi^2_{282} = 44.15$, $p = 1.00$; and *S. mollis* (168 sites, A-T = 85.1%): $\chi^2_{111} = 5.269$, $p = 1.00$). There were 20 variable sites and 15 parsimony-informative nucleotide substitutions for *G. hodgsoni*, and 126 variable sites and 101 parsimony-informative nucleotide substitutions for *S. mollis*.

The nucleotide substitutions among haplotypes revealed low divergence among individuals of *G. hodgsoni* (< 2%), but a large degree of population sub-structuring, with 20 well-defined clades or haplotypes, ranging in divergence from 0.2% to 1.9%. The most divergent haplotypes were TV2a (1.8%), TV1 (1.7%), V13c (1.3%) and V8a (1.2%). Twelve of these haplotypes were from previous samples located in continental southern Victoria Land, Ross Island and Beaufort Island; and eight were new haplotypes - seven from Victoria Valley and one from Lake Brownworth (Wright Valley) (Fig. 1). The level of haplotype diversity within Victoria Valley in particular, was almost double that seen in the other previously sampled locations. In addition, the seven haplotypes from Victoria Valley came from just 3 locations: St. John's Range (V8 (3)), Mt. Cerberus (V10 (2)), and Mautrino Peak (V13 (2)), showing that diversity within populations is high. Furthermore, these three locations were all at high altitudes (780 – 1540 m).

Phylogenetic analyses

Maximum Parsimony (MP) analyses found four most parsimonious trees for *G. hodgsoni* (tree length = 29, C.I. = 0.759, R.I. = 0.972, H.I. = 0.241), and 100 most parsimonious trees for *S. mollis* (tree length = 474, C.I. = 0.675, R.I. = 0.898, H.I. = 0.325). For *G. hodgsoni*, the strict consensus tree was found to have identical topology to the Neighbour-Joining (NJ) analysis (using uncorrected distances), but not to the Maximum Likelihood (ML) analyses. Of the four most parsimonious trees found for *G. hodgsoni*, two had the same topology as the NJ tree (see Fig. 2a), while the other two had the same topology as the ML analysis (Fig. 2b). The two

topologies differed only in their placement of haplotype V13c (Fig. 2a,b). Meanwhile, for *S. mollis,* the consensus tree resolved four main clades, with unresolved polytomy in the clade containing individuals from southern Victoria Land continental sites and Cape Royds (Ross Island). MP and ML trees for *S. mollis* are shown in Figure 3 a,b. Both a strong phylogenetic signal (g_1 = -0.552, g_{crit} = -0.09, p < 0.01 for *G. hodgsoni* and g_1 = -0.790, g_{crit} = -0.09, p = < 0.01 for *S. mollis*) and bootstrap analysis support the tree topologies in Figures 2 and 3.

For *G. hodgsoni*, the distribution of haplotypes within clades across the southern Victoria Land populations in general was very heterogeneous (Fig 2a,b). For Victoria Valley, 17 individuals identified from six populations contained eight haplotypes. Haplotypes identical to those in Victoria Valley were also found in individuals from: 1) Taylor Valley; 2) Granite Harbour; 3) Lake Penny; and 4) Ross Island. Haplotypes V8a and V8b cluster together as a sub-clade to another clade which contains eight individuals from Victoria and Wright Valleys, and one individual from Granite Harbour. Closely related to this group was a clade containing another haplotype from Victoria Valley (V8c). In addition, one haplotype in Victoria Valley (V13a) was found in a Ross Island population, and another (V10a) was found in Miers Valley and Lake Penny populations. Haplotype V13c had an unresolved placement in the four MP trees that may or may not link to Beaufort Island directly (Fig 2a,b). Populations in Wright Valley were very homogeneous, possessing only one haplotype (W4) throughout six individuals and two locations (Fig 2a,b). Conversely, the Victoria Valley populations were more heterogeneous, such that each population contained more than one different haplotype. For example, in the most extreme case (site V8), four individuals from the same location had four different haplotypes (Table 1). Thus, *G. hodgsoni* samples from populations in Wright and

Victoria Valleys showed links to nearly all haplotypes from previously sampled locations. The only haplotype not accounted for at this stage was one that is the most divergent, and it is unique to Taylor Valley.

For *S. mollis*, we found a similar pattern of heterogeneity and population sub-structuring, with new samples showing links to most previously collected samples (M.I. Stevens & I.D. Hogg, unpubl. data). Divergence among *S. mollis* individuals was found to be higher (up to 17.1%) than *G. hodgsoni*. We found 5 clades, ranging in divergence from 2.7% to 14.5%, of which one was unique to Wright Valley (W5a). V13f (Mautrino Peak, Victoria Valley) and W5a (Wright Valley) were the most divergent haplotypes (16.9% and 14.5%, respectively). Haplotype diversity was again higher in Wright and Victoria Valleys, with 22 haplotypes found (seven and 15 in Wright and Victoria Valleys, respectively) out of 38 total across all populations. Three haplotypes from Wright Valley were from one location (W5b,c,d), while the other four were from three locations (W2,W3 and W5). Eleven haplotypes from Victoria Valley were from three locations (V1, V2 and V13) and the other four were from three locations (V4, V9, and V11) (Fig 3a,b). Of the 38 haplotypes, only seven were found in more than one individual. For example, haplotype V1a (Victoria Valley), was also present in individuals from Taylor Valley, Ross Island, and Granite Harbour; and haplotype V2a was found in an individual from Beaufort Island. Samples from Wright and Victoria Valleys showed links to those in Marble Point, Taylor Valley, Granite Harbour, Hut Point Peninsula, Miers Valley and Beaufort Island thus, the haplotypes found in Wright and Victoria Valleys account for haplotypes present in all other sampled locations.

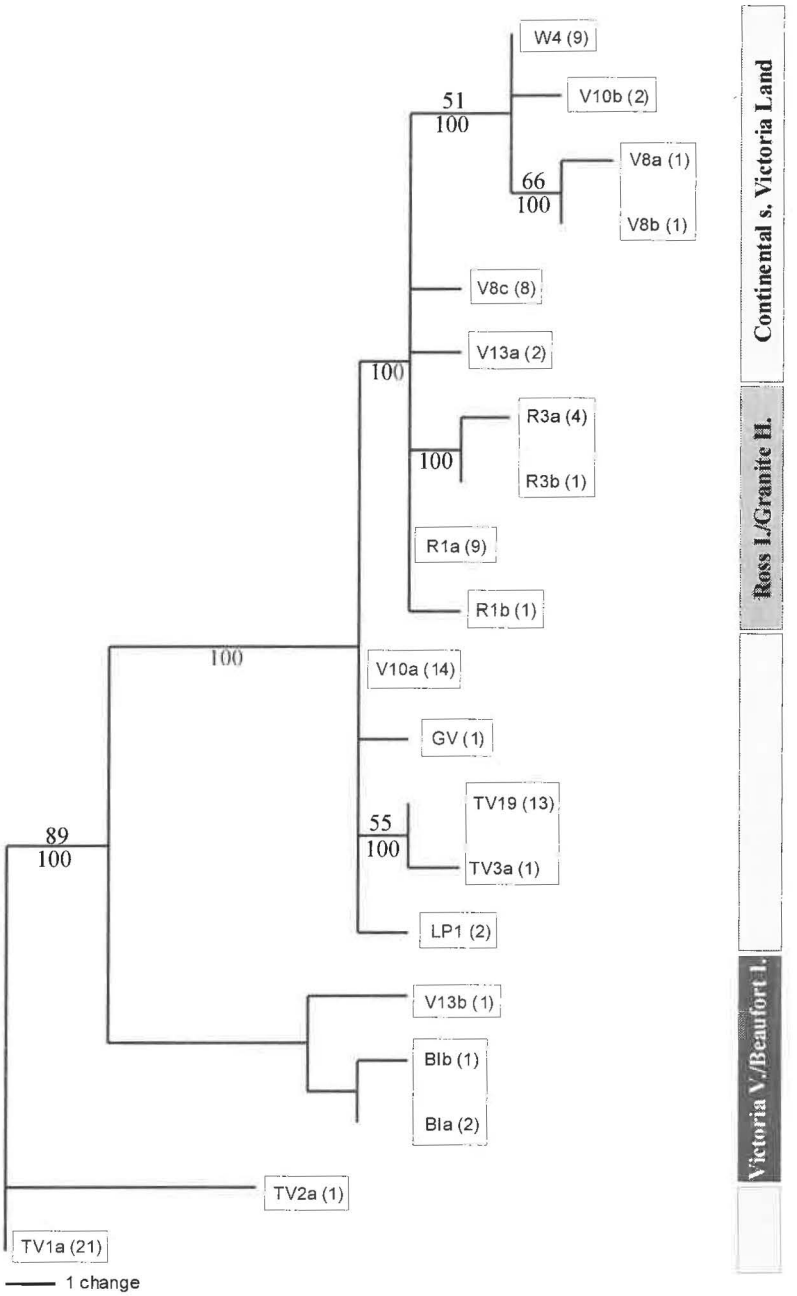

FIGURE 2a. One of the four Maximum-Parsimony phylograms created using the default options in PAUP*, for *G. hodgsoni*, which corresponded to the 50% majority rule consensus and Neighbour-Joining trees. 50% majority rule values are shown below nodes; bootstrap confidence limits (500 replicates) are shown in bold type above nodes; codes are those used in Table 1; the number of identical haplotypes present at any site is given in parentheses.

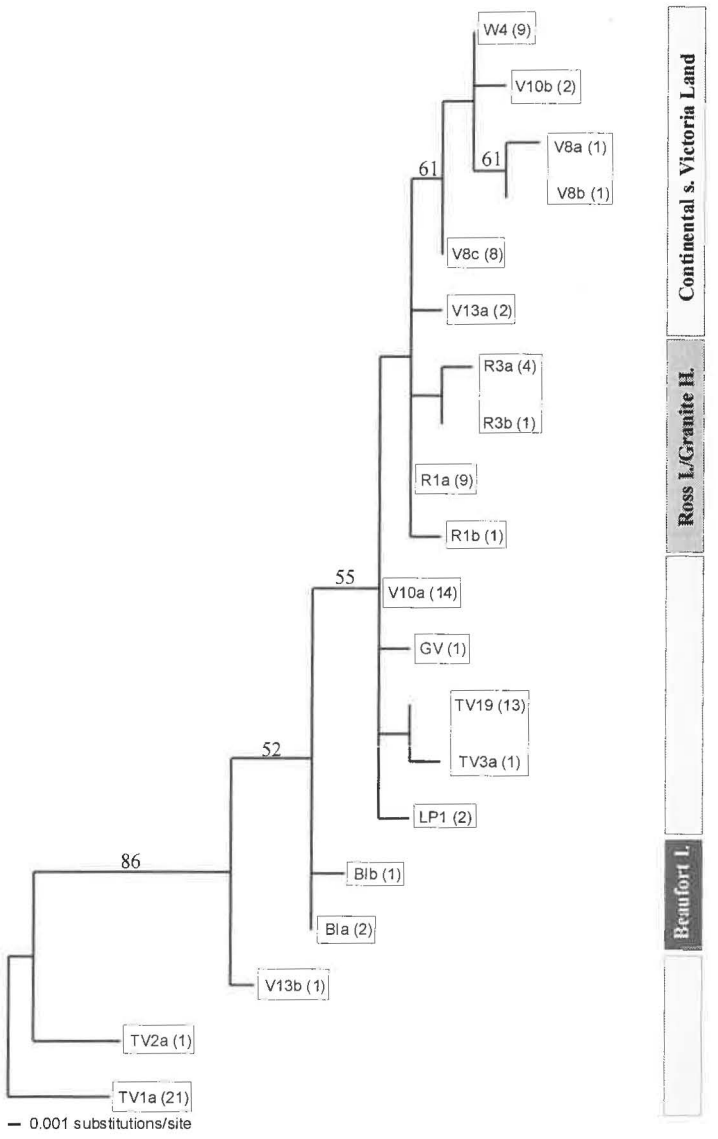

FIGURE 2b. Maximum-Likelihood phylogram for *G. hodgsoni* based on the substitution model TrN+I (-lnL = 812.1001; rate matrix: A-C = 1.0000, A-G = 35.2664, A-T = 1.0000, C-G = 1.0000, C-T = 6.0747, G-T = 1.0000; proportion of invariable sites (I) = 0.9218; with base frequencies set to A = 0.2715, C = 0.1934, G = 0.1565, T = 0.3785) derived from Modeltest (see methods) using a 471-bp fragment of the mitochondrial DNA (COI) gene using only unique sequences. Bootstrap confidence limits (500 replicates) shown above nodes. Codes are those used in Table 1; the number of identical haplotypes present at any site is given in parentheses.

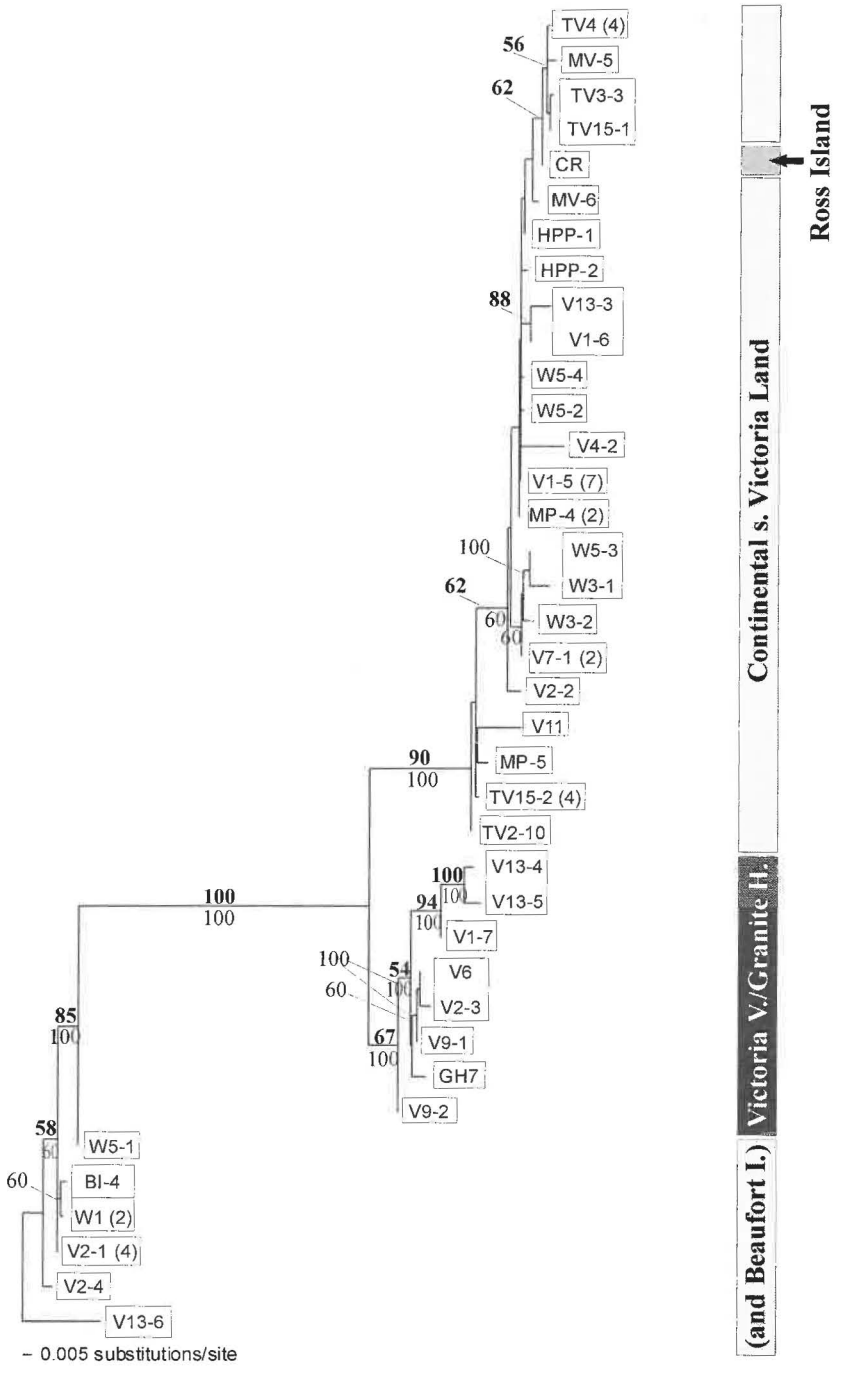

FIGURE 3a. Maximum-Parsimony phylogram for *S. mollis* created using the default options in PAUP*. 50% majority rule values are shown below nodes; bootstrap confidence limits (500 replicates) are shown in bold type above nodes; codes are those used in Table 1; the number of identical haplotypes present at any site is given in parentheses.

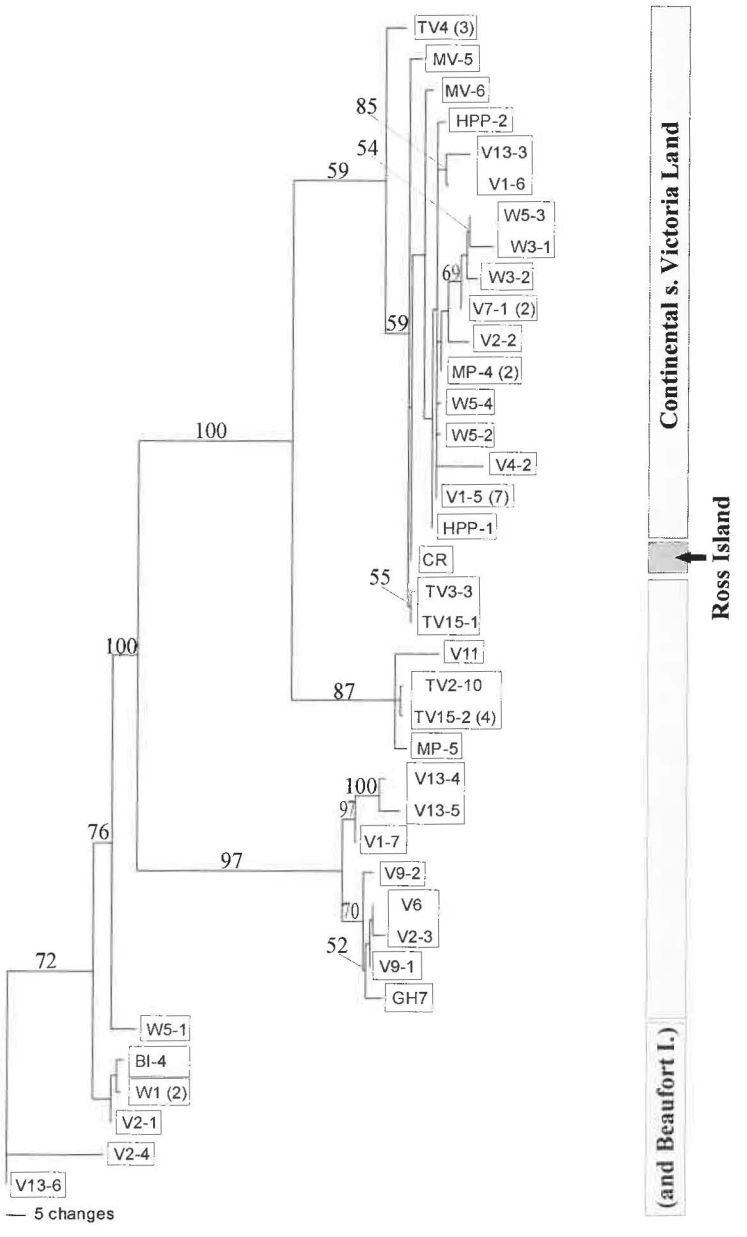

FIGURE 3b. Maximum-Likelihood phylogram for *S. mollis* based on the substitution model HKY+I+G (-lnL = 1960.1649; ti/tv = 1.5774, I = 0.4853; with base frequencies set to A = 0.3583, C = 0.1385, G = 0.1420, T = 0.3612) derived from Modeltest (see methods) using a 504-bp fragment of the mitochondrial DNA (COI) gene using only unique sequences. Bootstrap confidence limits (500 replicates) shown above nodes. Codes are those used in Table 1; the number of identical haplotypes present at any site is given in parentheses.

Network analyses

Analysis of the geographical distribution of haplotypes incorporates the nested relationships of the cladogram to detect signals of population history events at different nesting levels and hence on a relative time scale. The nested cladogram for *G. hodgsoni* contained 20 haplotypes and provided ten 1-step clades, six 2-step clades and two 3-step clades (Fig. 4a). The maximum number of mutational steps between haplotypes was seven. The most divergent haplotypes were shown in the nested clade to be TV1 and TV2a, as these two had the greatest numbers of mutational steps between themselves and the next haplotype, and this agrees with the sequence divergence values and the phylogenetic analyses (Fig. 2a,b; Appendix I). Therefore, these are the most derived haplotypes, with the ancestral haplotype in the nested cladogram being V10-1.

Unfortunately, for *G. hodgsoni*, nested clade analysis provided no insight into processes shaping local population differentiation for haplotypes contained in nesting clades 2-1, 2-3 and 2-4 (i.e. clades 1-1, 1-2, 1-5, 1-6, 1-7 and 1-8) because there was no genetic variation in these samples (i.e. no haplotype frequency information) (Table 2a, 3a). Consequently, we could not estimate distance values (i.e. Dc, Dn, I-T) for these clades and they could not be analysed using Templeton's updated dichotomous inference key. However, nested contingency analysis revealed significant association of clades and sampling locations for four clades across two clade levels. Two 2-step clades (2-2 and 2-6) and two 3-step clade (3-1 and 3-2) showed significant values for Dc, Dn, or I-T within the nesting clade (i.e. allowing rejection of the null hypothesis of no geographical association between haplotypes), and could be analysed using the inference key. Restricted gene flow with isolation-by-distance was inferred for clade

2-2 (which contains samples from Taylor Valley, Victoria Valley, Garwood Valley and Lake Penny), 2-5 (Victoria Valley and Ross Island samples), and 3-2 (Taylor and Victoria Valley and Beaufort Island samples), whereas restricted gene flow/dispersal with some long distance dispersal was the most likely explanation for the patterns observed at nested level 3-1 (Victoria Valley, Wright Valley, Ross Island, Taylor Valley, Garwood Valley and Lake Penny) (Table 4a).

The nested cladogram for *S. mollis* contained 38 haplotypes and provided 20 1-step clades, eight 2-step clades and three 3-step clades (Fig. 4b). The maximum number of mutational steps between haplotypes was 60. The most divergent haplotype was shown in the nested clade to be V13f (Victoria Valley), with associated members of clade 2-7 being next divergent (i.e. W2, W5a - Wright Valley; V2a, V2d - Victoria Valley; and Blc - Beaufort Island), and this corresponds to the sequence divergence and phylogenetic reconstructions (Fig. 3 a,b; Appendix II). Therefore, these are the most derived haplotypes, with the ancestral haplotype in the nested cladogram being V1a. Insight into processes shaping local population differentiation was only gained for haplotypes contained in clades 1-5, 1-18 and 3-1 because there was no genetic variation in the other clades (Table 2b, 3b). However, the analysis revealed significant association of clades and sampling locations for these three clades across two clade levels. For all three clades (1-5, 1-18 and 3-1), restricted gene flow with isolation-by-distance was inferred as the most likely explanation for the patterns observed (Table 4b). These significant clades contained samples from Wright and Victoria Valleys and Hut Point Peninsula (clade 1-5), Wright and Victoria Valleys (clade 1-18), and Wright, Victoria, and Miers Valleys, Hut Point Peninsula and Marble Point (clade 3-2).

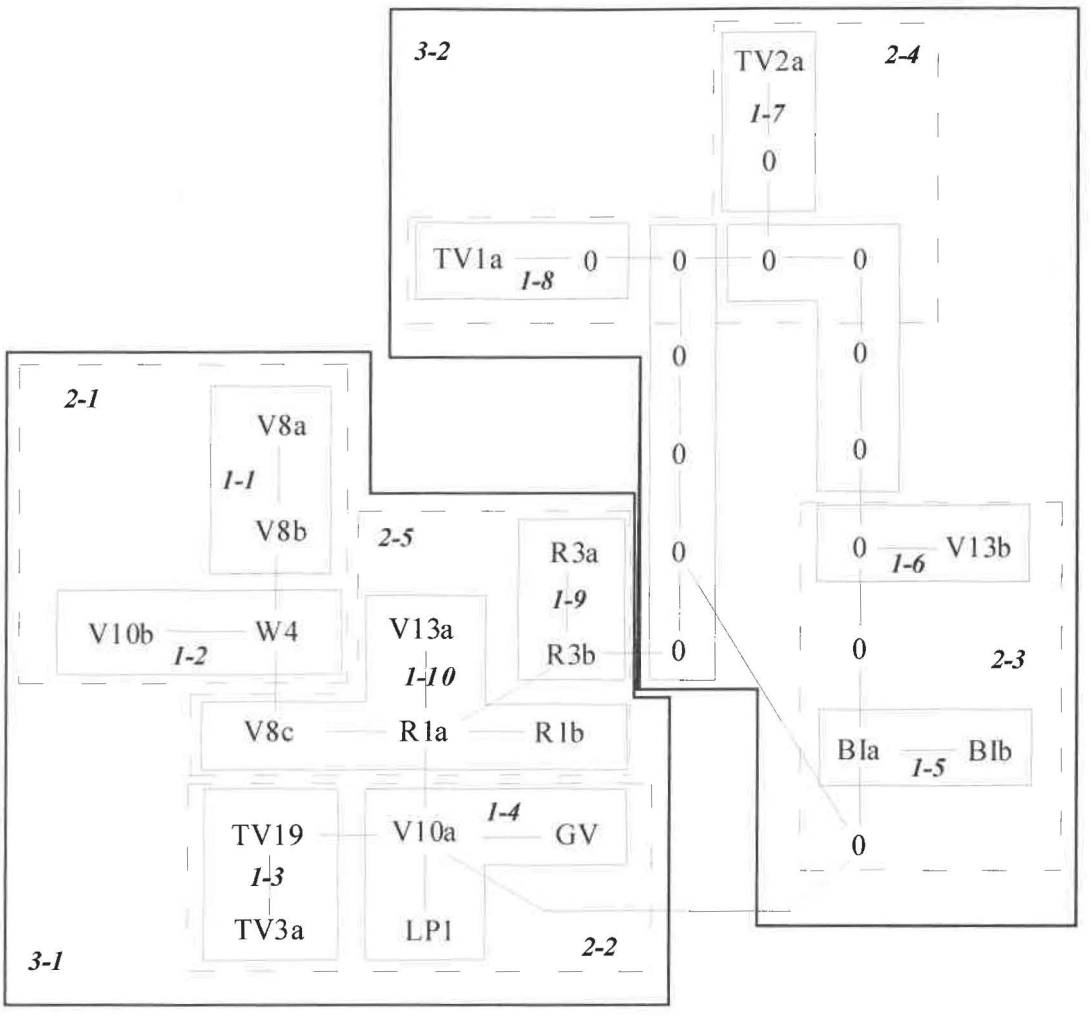

FIGURE 4a. Nested clade design for the mitochondrial DNA (COI) 20 unique haplotypes from 95 individuals of *G. hodgsoni*. Haplotypes are indicated by their codes (as referred to in Table 1). Missing haplotypes/mutational steps are indicated by an '0'. The clades are identified using a two number system, where the first number refers to the nesting hierarchy and the second is an arbitrary, individual clade identifier. Thin-lined polygons enclose 1-step clades, broken lined polygons enclose 2-step clades, thick-lined polygons enclose 3-step clades.

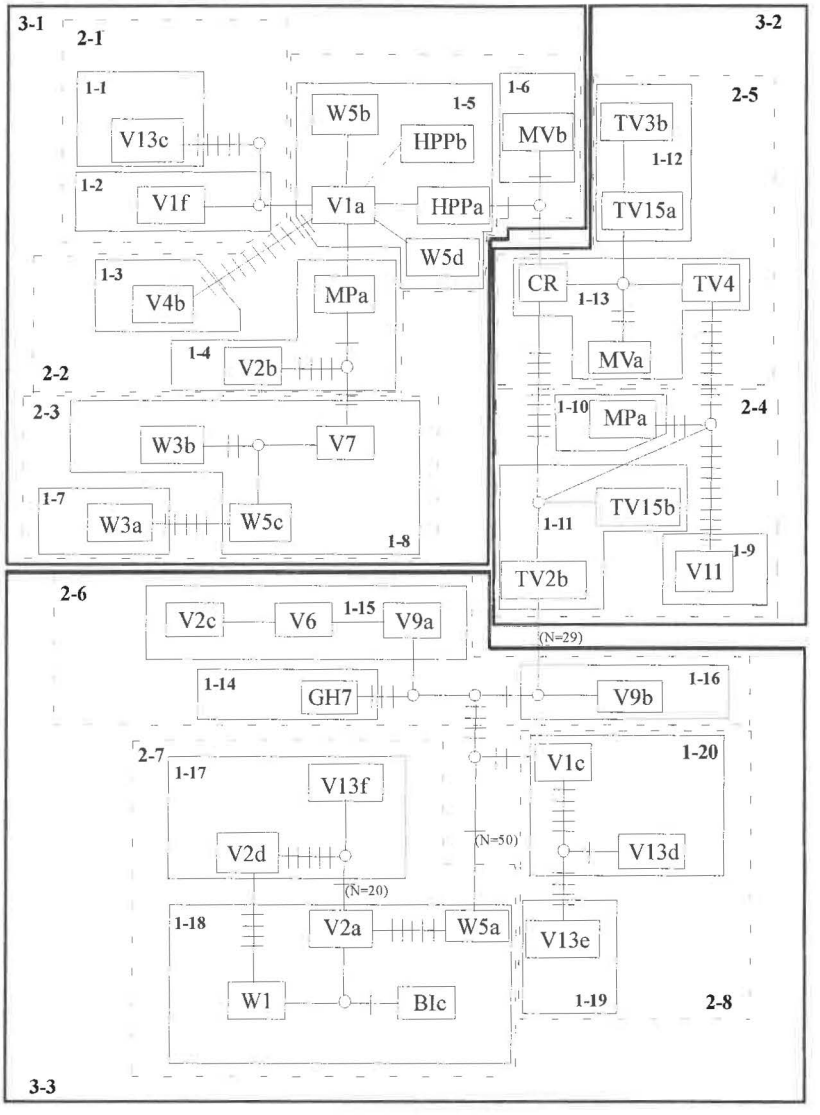

FIGURE 4b. Nested clade design for the mitochondrial DNA (COI) 38 unique haplotypes from 61 individuals of *S. mollis*. Haplotypes are indicated by their codes (as referred to in Table 1). Missing haplotypes/mutational steps, are indicated by an '○' or '—' and in cases where a large number of these exist, the number is given beneath the '—' symbol. The clades are identified using a two number system, where the first number refers to the nesting hierarchy and the second is an arbitrary, individual clade identifier. Thin-lined polygons enclose 1-step clades, broken lined polygons enclose 2-step clades, thick-lined polygons enclose 3-step clades.

TABLE 2. Nested clade distance analysis of cytochrome *c* oxidase I haplotypes observed in: (A) *Gomphiocephalus hodgsoni*, and (B) *Stereotydeus mollis*. Brackets reflect the nesting structure (see Figure 4a,b). Dc and Dn are clade and nested clade distances, respectively (for details see Templeton *et al.* 1995). Interior vs. tip contrasts for Dc and Dn are indicated with 'I-T' in the corresponding clade, with interior clades given in italic type. Superscript S and superscript L indicate that distance measures are significantly smaller and larger, respectively, than expected under random distribution of haplotypes.

(A)

Haplotypes			**1-step clades**			**2-step clades**			**3-step clades**		
Clade	**Dc**	**Dn**	**Clade**	**Dc**	**Dn**	**Clade**	**Dc**	**Dn**	**Clade**	**Dc**	**Dn**
V8-a,b			1-1	0.00	11.90						
V10b	*21.87*	*25.82*				2-1	14.72^{S}	35.68			
W4	*14.20*	*13.30*	*1-2*	*15.34*	*15.04*						
I-T	7.67	12.53	I-T	15.34	3.14						
			1-3	7.57^{S}	12.98^{S}						
			1-4	40.39^{L}	45.57^{L}	*2-2*	24.23^{S}	25.93^{S}	*3-1*	*40.04*	*39.78*
			I-T	32.82^{L}	32.60^{L}						
R3a	5.54	5.27									
R3b	*0.00*	*3.37*	1-9	66.80^{L}	67.91^{L}						
I-T	5.54	1.90				*2-5*	63.53^{L}	70.54^{L}			
			1-10	4.82^{S}	44.84^{S}	I-T	27.05^{L}	10.17			
						2-3	*61.50*	83.78^{L}	3-2	37.82	39.60
						2-4	10.38^{S}	22.06^{S}	I-T	2.22	0.18

(B)

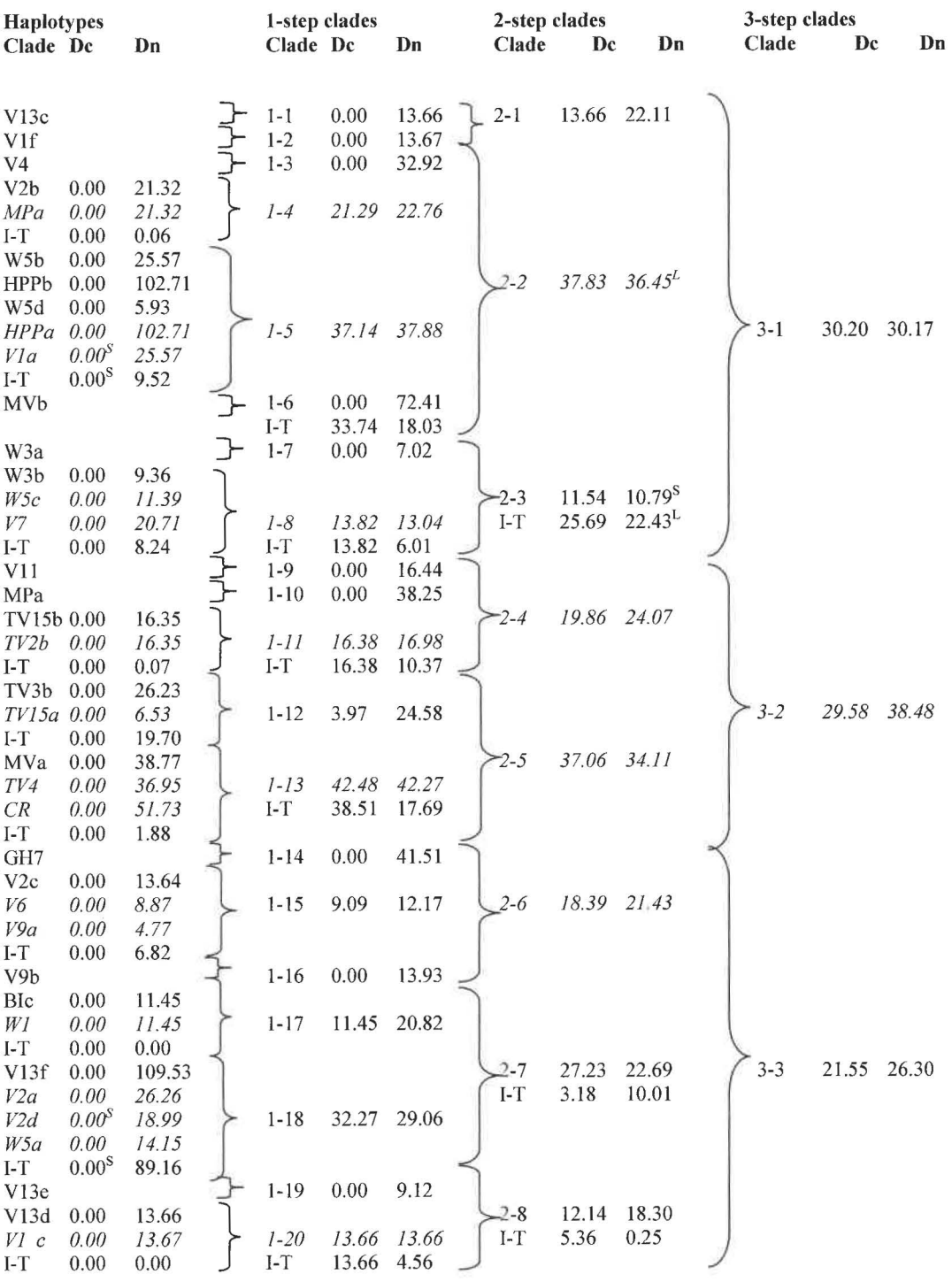
Haplotypes
Clade Dc Dn
V13c
V1f
V4
V2b 0.00 21.32
MPa 0.00 21.32
I-T 0.00 0.06
W5b 0.00 25.57
HPPb 0.00 102.71
W5d 0.00 5.93
HPPa 0.00 102.71
V1a 0.00S 25.57
I-T 0.00S 9.52
MVb
W3a
W3b 0.00 9.36
W5c 0.00 11.39
V7 0.00 20.71
I-T 0.00 8.24
V11
MPa
TV15b 0.00 16.35
TV2b 0.00 16.35
I-T 0.00 0.07
TV3b 0.00 26.23
TV15a 0.00 6.53
I-T 0.00 19.70
MVa 0.00 38.77
TV4 0.00 36.95
CR 0.00 51.73
I-T 0.00 1.88
GH7
V2c 0.00 13.64
V6 0.00 8.87
V9a 0.00 4.77
I-T 0.00 6.82
V9b
BIc 0.00 11.45
W1 0.00 11.45
I-T 0.00 0.00
V13f 0.00 109.53
V2a 0.00 26.26
V2d 0.00S 18.99
W5a 0.00 14.15
I-T 0.00S 89.16
V13e
V13d 0.00 13.66
V1 c 0.00 13.67
I-T 0.00 0.00
1-step clades
Clade Dc Dn
1-1 0.00 13.66
1-2 0.00 13.67
1-3 0.00 32.92
1-4 21.29 22.76
1-5 37.14 37.88
1-6 0.00 72.41
I-T 33.74 18.03
1-7 0.00 7.02
1-8 13.82 13.04
I-T 13.82 6.01
1-9 0.00 16.44
1-10 0.00 38.25
1-11 16.38 16.98
I-T 16.38 10.37
1-12 3.97 24.58
1-13 42.48 42.27
I-T 38.51 17.69
1-14 0.00 41.51
1-15 9.09 12.17
1-16 0.00 13.93
1-17 11.45 20.82
1-18 32.27 29.06
1-19 0.00 9.12
1-20 13.66 13.66
I-T 13.66 4.56
2-step clades
Clade Dc Dn
2-1 13.66 22.11
2-2 37.83 36.45L
2-3 11.54 10.79S
I-T 25.69 22.43L
2-4 19.86 24.07
2-5 37.06 34.11
2-6 18.39 21.43
2-7 27.23 22.69
I-T 3.18 10.01
2-8 12.14 18.30
I-T 5.36 0.25
3-step clades
Clade Dc Dn
3-1 30.20 30.17
3-2 29.58 38.48
3-3 21.55 26.30

TABLE 3. Nested contingency analysis of geographical associations for mitochondrial DNA (cytochrome *c* oxidase I) data from: (A) *Gomphiocephalus hodgsoni*, and (B) *Stereotydeus mollis*. Clades not showing genetic or geographic variation are excluded (no test is possible within such nested categories)

(A)

Clade	Permutational X^2 statistic	Probability
1-2	10.000	0.119
1-9	0.833	1.000
2-1	13.000	0.104
2-2	28.981	0.000*
2-5	17.500	0.007*
3-1	107.880	0.000*
3-2	25.000	0.000*
Total Cladogram	82.423	0.000*

*Significant at the 0.05 level

(B)

Clade	Permutational X^2 statistic	Probability
1-4	3.000	0.328
1-5	21.000	0.028*
1-8	8.000	0.144
1-11	5.000	0.184
1-12	2.000	1.000
1-13	10.000	0.108
1-15	6.000	1.000
1-17	2.000	1.000
1-18	24.000	0.002*
1-20	2.000	1.000
2-1	2.000	1.000
2-2	48.000	0.000*
2-3	1.875	1.000
2-4	14.000	0.134
2-5	6.000	0.267
2-6	6.667	0.906
2-7	5.000	0.650
2-8	0.750	1.000
3-1	30.655	0.030*
3-2	10.800	0.092
3-3	26.300	0.008*
Total Cladogram	**84.946**	**0.000***

*Significant at the 0.05 level

TABLE 4. Demographic inferences from the nested clade distance analysis (Templeton *et al.* 1995, Templeton 1998) in: (A) *Gomphiocephalus hodgsoni* and (B) *Stereotydeus mollis*

(A)

Clade	Inference chain	Inferred pattern
Haplotypes nested in clade 2-2	1-2-3-4-NO	Restricted gene flow with isolation-by-distance
Haplotypes nested in clade 2-5	1-2-11-17-4-NO	Restricted gene flow with isolation-by-distance
Haplotypes nested in clade 3-1	1-2-3-5-6-7-YES	Restricted gene flow / dispersal with some long-distance dispersal
Haplotypes nested in clade 3-2	1-2-11-17-4-NO	Restricted gene flow with isolation-by-distance

(B)

Clade	Inference chain	Inferred pattern
Haplotypes nested in clade 1-5	1-2-3-4-NO	Restricted gene flow with isolation-by-distance
Haplotypes nested in clade 1-18	1-2-3-4-NO	Restricted gene flow with isolation-by-distance
Haplotypes nested in clade 3-1	1-2-11-17-4-NO	Restricted gene flow with isolation-by-distance

DISCUSSION

The mtDNA (COI) gene was relatively homogeneous among the *G. hodgsoni* sequences, with only 20 positions exhibiting different nucleotides in one or more sequences across the 471-bp. By contrast, 126 sites exhibited nucleotide variation in *S. mollis* sequences. *G. hodgsoni* and *S. mollis* populations within Wright and Victoria Valleys were both characterized by high levels of sub-structuring and local mitochondrial diversity, with St. John's Range, Mt. Cerberus and Lake Brownworth in particular, appearing to be regions of high genetic diversity (i.e. biodiversity hotspots). This finding is in accordance with previous studies which found high levels of regional genetic divergence in *G. hodgsoni* (Stevens & Hogg 2003) and *S. mollis* (M.I. Stevens & I.D. Hogg, unpubl data) populations throughout the locations across southern Victoria Land they sampled, particularly in *G. hodgsoni* Taylor Valley and Beaufort Island clades (1.7% and 1.3% respectively, compared to < 0.6% among all other populations).

Here, we found low levels of divergence among *G. hodgsoni* haplotypes (< 2%). However, the number of haplotypes found for *G. hodgsoni* was relatively high (20 haplotypes from 95 individuals), which indicates high levels of heterogeneity among individuals. Stevens & Hogg (2003) found that haplotype sharing among locations occurred in only three instances. *G. hodgsoni* populations within Victoria and Wright Valleys also showed that, while haplotypes were dispersed across locations, few populations (seven) actually shared common haplotypes. For example, samples taken from the same location in St. John's Range (V8) have haplotypes also found in individuals within the clades from Victoria Valley, Granite Harbour, Ross Island, Lake Penny, Miers Valley and Taylor Valley (Table 1, Fig. 2a,b). These findings are

in agreement with other work on Antarctic terrestrial invertebrates (Fanciulli *et al.* 2001; Frati *et al.* 2001; Stevens & Hogg 2003), showing that patterns of high haplotype diversity are common for terrestrial arthropods in Antarctica, and potentially reflecting common phylogeographic histories.

While the Dry Valley region is extensively ice-free throughout the year, the area has not always been ice-free on a geological time scale (Thompson *et al.* 1971). Accordingly, suitable sites for survival of terrestrial arthropods in Antarctica are not constant even on small temporal and spatial scales. When present, such sites are often separated by large unsuitable areas, such that geographic distance alone can be an efficient isolating factor. Geographic barriers such as glacial tongues, which slope down towards the floors of the valleys, and areas of barren rock, also influence the limits of distribution of the different species (Frati *et al.* 2001; Fanciulli *et al.* 2001; Stevens & Hogg 2002). High altitude sites (i.e. > 1500 m) may have provided refugial habitat above the reach of glaciers for these taxa. For example, St John's Range sites approached 1550 m in altitude and also harboured high levels of diversity. Thus, it is likely that populations in Antarctica have been structured through both modern and historical geological effects.

Molecular clock calibration of 2 - 2.3% divergence per million years, derived from geological evidence and invertebrate mitochondrial data (e.g. Brower 1994; Folmer *et al.* 1994; Juan *et al.* 1996; Roslin 2001; Trewick & Morgan-Richards 2005), suggests that groups in this study have most likely diverged within the last one million years. Because even the most genetically distinct lineages of this study (and that of Stevens & Hogg 2003) are closely related (< 2.0% divergence), Pleistocene and/or Holocene glaciation is implicated as the primary determinant of present-day phylogeographical patterns. Indeed, levels of divergence among *G. hodgsoni* (Stevens & Hogg 2003)

and *Isotoma klovstadi* (Frati *et al.* 2001) conformed to that reported for other arthropod species with a Pleistocene coalescence.

In particular, genealogical data has been widely used to deduce the impact of Pleistocene biogeography on speciation processes and many studies have found that the Pleistocene glaciations had considerable impact on phylogeographic patterns, within vertebrate (Hewitt 1996; Klicka & Zink 1997; Avise *et al.* 1998) and invertebrate taxa (Marshall & Coetzee 2000; Buckley *et al.* 2001; Trewick & Wallis 2001; Garrick *et al.* 2004). One main finding of these studies has been that there appears to be greater genetic diversity at unglaciated sites such as nunataks (i.e. localised ice-free regions) relative to glaciated sites, and that often postglacial colonization has been from multiple (vs. single) refugia (Church *et al.* 2003; Weider & Hobæk 2003; Ayoub & Riechert 2004; Rowe *et al.* 2004) with zones of secondary contact between haplotypes from different refugia (e.g. Tremblay & Schoen 1999; Abbott *et al.* 2000; Stevens & Hogg 2003). Thus, the existence of multiple glacial refugia can play a substantial role in determining present-day species distributions, as well as initiating or modifying genetic variation in taxa through glacial vicariance (Holder *et al.* 1999; Weider & Hobæk 2003; Galbreath & Cook 2004).

The pattern we found was that of a large western (continental) refugium, and suggests that Victoria and Wright Valleys may be the site of an *in situ* surviving remnant source population left over from Pleistocene glacial refugia, from which extant populations in the region were able to expand and re-colonise virgin habitat during one, or several of the inter-glacials throughout the Pleistocene or Holocene. For *G. hodgsoni*, the nested clade analysis showed V10a (Mt. Cerberus, Victoria Valley) to be the most ancestral haplotype, while the most ancestral haplotype for *S. mollis* was V1a (St. John's Range, Victoria Valley) (Fig. 4a,b). This reinforces the

hypothesis of the existence of an ancestral source population in Victoria Valley in particular, since ancestral haplotypes should be closer to the origin of range expansion (i.e. haplotypes should become more derived west to east). Indeed, we found high levels of genetic variation at the more western (inland) sites, which supports the idea of expansion of populations from western refugia following an eastern colonization route (Hewitt 2001; Knowles 2001; Ayoub & Riechert 2004; Rowe *et al.* 2004). In addition, the nested clade analysis found that restricted gene flow with isolation-by-distance and restricted gene flow/dispersal but with some long distance dispersal best explained the observed patterns of haplotype distribution in both *G. hodgsoni* and *S. mollis* populations (Table 4a,b). This agrees with the geographic history of the region (i.e. vicariant Pleistocene climate change and the availability of refugia coupled with habitat diversity on a micro-scale across the landscape) and the biological history of the taxa (e.g. their low dispersal ability).

While additional (i.e. multiple) refugia may have existed (e.g. in Taylor Valley, Cape Bird, Granite Harbour, Beaufort Island (Stevens & Hogg 2003)), there does appear to have been fragmentation of a single large ancestral source population located in Wright and Victoria Valleys. For *G. hodgsoni*, all but the most divergent haplotype (unique to Taylor Valley), and for *S. mollis*, all haplotypes, were represented in the Wright and Victoria Valley samples (Fig. 2a,b). However, because we are unable to account for the unique Taylor Valley haplotype for *G. hodgsoni*, we cannot be sure whether this haplotype originated in Taylor Valley (thus widening the location of the ancestral source population to include Taylor Valley and therefore the entire ice-free Dry Valley region), or is sourced elsewhere. Further sampling in the region (specifically in areas of the Asgard Ranges, which dissect Wright and Taylor Valleys (Fig. 1)) may be helpful in this regard.

Interestingly, the nested cladograms for both taxa show more than one route for some haplotypes to neighbouring haplotypes (Fig. 4 a,b) (e.g. for *G. hodgsoni*, clade 3-2 to clade 3-1; for *S. mollis*, clade 2-4 to clades 2-5 and 2-6; clade 2-7 to clades 2-6 and 2-8) which indicates unresolved areas within the networks, or potentially, reticulate patterns of evolution (i.e. homoplasy). Reticulate evolution occurs when hybridization, horizontal gene transfer and/or recombination have taken place in an evolutionary lineage (Posada *et al.* 2002; Beroni *et al.* 2003) with recombination perhaps being the most likely for these sexually reproducing species, as has been shown for other animal mitochondrial DNA (Ladoukakis & Zouros 2001). This can, in some cases, confound attempts to reconstruct accurate evolutionary trees and infer phylogenetic history (Templeton & Sing 1993; Xu 2000; Posada *et al.* 2002). In our study, the unresolved branches were mostly located in clades without significant Dc, Dn and/or I-T values where they did not affect final inferences. However, clade 3-2 for *G. hodgsoni* and clade 1-18 for *S. mollis* (both of which contain Beaufort Island populations) did contain significant Dc, Dn and/or I-T values (Table 4a,b), so inferences regarding these clades must be interpreted with caution.

A possible explanation for the pattern of reticulation we see within *G. hodgsoni* may be found from the Beaufort Island population (BIa and BIb). Recombination between Taylor Valley (TV1 and TV2a) and Victoria Valley (V13b) samples potentially could account for the unresolved position of Beaufort Island. Alternatively, recombination could have occurred between Taylor Valley and Beaufort Island samples to give the Victoria Valley haplotype. For *S. mollis*, the patterns of reticulation are more complex and could represent recombination between several populations including Taylor Valley, Cape Royds, Miers Valley, Marble Point, Victoria Valley, Granite Harbour and Beaufort Island (Fig. 4b). Since this

recombination was most likely among populations that are relatively distant geographically, it may further support the idea that these populations were once united in an ancestral refugium.

However, other problems with nested clade analysis have been encountered. For example, loss of accurate haplotype distributional data can occur if some haplotypes have moved spatially during glacial and interglacial periods, or been lost from the refugial population entirely (see Seddon *et al.* 2001; Contreras-Diaz *et al.* 2003). Therefore nested clade analysis results must be interpreted carefully. Nevertheless, statistical phylogeography has made much progress providing objective explanations for patterns of genetic diversity, and in the absence of multiple genetic markers, studying more than one species comparatively offers strength and validity to results (e.g. Irwin 2002). Accordingly, the congruence of results across two unrelated taxa here, provides further support for the hypothesis that the patterns observed in the mtDNA phylogeny have been driven by recent (< 2 My) climatic change.

Interestingly, Janetschek (1967) reported life cycle differences between populations of *G. hodgsoni* living at high and low altitudes. *G. hodgsoni* samples were essentially absent from the valley floors, but *S. mollis* individuals were occasionally found. This may suggest that *G. hodgsoni* populations have yet to re-colonise the valley floors, while *S. mollis* populations may be expanding their ranges at a quicker rate. Additionally, for *S. mollis*, all haplotypes throughout southern Victoria Land were represented in Wright and Victoria Valleys, implying that greater dispersal in this species may be producing an homogenising pattern. Collectively, these findings may offer further support to the theory that barriers created isolation which led to diversification in subsequently recolonised areas (as populations historically moved upwards in the face of glacial advance, and then back down as

glaciers receded), and may explain the finding of higher diversity in more western regions since altitude decreases towards the coast (Marchant & Denton 1996).

However, there are alternative explanations to that of glacial refugia for the pattern of disjunct mixing of genetic diversity across populations found in this study. One possibility that is often excluded from consideration is that the phylogeographic breaks were created as a result of stochastic events rather than having a specific biogeographic cause. Indeed, Irwin (2002) has shown that phylogeographic breaks can occur in the absence of geographic barriers to gene flow. Another possibility is that of recent passive or assisted dispersal. Stevens & Hogg (2002) provided new distributional records for springtails and mites in southern Victoria Land, and found both *G. hodgsoni* and *S. mollis* at sites where they had been absent some 40 years ago, suggesting recent range expansion (i.e. local dispersal). However they found no evidence for passive dispersal over longer distances, which is perhaps expected since dry Antarctic winds are thought to cause desiccation in Antarctic terrestrial invertebrates (Gressitt *et al.* 1960; Frati *et al.* 2001). Dispersal assisted by birds and/or humans is a stronger possibility (Strong 1967; Tilbrook 1967; Fanciulli *et al.* 2001; Stevens & Hogg 2005) and Pugh & Convey (2000) suggest that dispersal through water may also be possible for mite species. However, assisted dispersal is perhaps unlikely, or at least limited, in this case because it would presumably remove geographic barriers (Stevens & Hogg 2003, 2005). Instead, present-day gene flow among geographic regions is likely to be restricted because, for both taxa, few haplotypes were shared among populations, indicating a lack of current gene flow. Nevertheless, the frequency and success of assisted dispersal events would be more accurately assessed through population genetic studies, where evaluation of allele frequencies at gene and/or allozyme loci would better quantify dispersal rates among

habitats (Frati *et al.* 1997; Fanciulli *et al.* 2001; Hogg & Stevens 2002; Stevens & Hogg 2003).

Morphological analyses suggested that the exact chaetotaxy of *G. hodgsoni* adults is quite variable, but there were no qualitative differences between individuals from different sites. In other morphological characters, there was no detectable variation between samples of *G. hodgsoni* taken from different locations. However, morphological variation has been identified in *S. mollis* from Cape Royds, Cape Roberts and Wright Valley (Womersley & Strandtmann 1963) and this study found high genetic divergence amongst *S. mollis* clades (up to 14.5%). Interestingly, Antarctic mites and springtails have different life cycles. For example, variable activity levels induced by the Antarctic climate (springtails maintain diurnal activity, while mites are active over a 24-hour period (M.I. Stevens & I.D. Hogg, unpubl. data) may drive metabolic differentiation between these two taxa and may lead to differences in mutation rates.

CONCLUSION

The degree of mtDNA sequence divergence, combined with the geographical positioning of haplotypes (i.e. the distribution of diversity revealed by nested clade analysis), and the presence of rare genetic resources were used to identify specific areas as potential past refugia for *G. hodgsoni* and *S. mollis* populations in southern Victoria Land, Antarctica.

The phylogeographic congruence we found for *G. hodgsoni* and *S. mollis* and the alignment of our results with those of previous studies, indicates that these taxa have attained a common pattern of geographical and genetic subdivision due to their common biogeographic history of range expansion, and spatial and temporal variation in habitat structure and quality. Furthermore, they appear to have diversified genetically without morphological variation. This divergence appears to be associated with founder events during recolonisation of previously glaciated areas throughout the Pleistocene and Holocene, resulting in marked genetic structuring across their geographic ranges across southern Victoria Land. Therefore, genetic divergences among *G. hodgsoni* and *S. mollis* populations in southern Victoria Land appear to have coincided with the Pleistocene/Holocene glaciations. Moreover, this historical period of alternating glacial cycles, combined with the presence of current geographic barriers (i.e. the influence of the Antarctic environment), appears to have played an active role in shaping modern species distributions and diversification. Thus, it appears that the Antarctic environment provides the conditions necessary for both macro- and micro-evolutionary (speciation) processes to occur.

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APPENDIX I. Genetic distance (using uncorrected distances) based on sequence variation in the mtDNA COI sequences (471 aligned sites) among the 95 individuals of *Gomphiocephalus hodgsoni* analysed. Only unique sequences (20 unique haplotypes) were included in the analysis. Codes are those referred to in Table 1; the number of identical haplotypes present at any site is identified in parentheses.

Locations	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1 W4-1 (9)	Continental s. Victoria Land														Ross I./Granite H.				Beaufort I.
2 V8-1 (1)	0.006																		
3 V8-2 (1)	0.013	0.002																	
4 V8-3 (8)	0.006	0.006	0.004																
5 V10-1 (14)	0.002	0.011	0.008	0.004															
6 V10-2 (2)	0.002	0.006	0.004	0.004	0.004														
7 V13-1 (2)	0.004	0.011	0.008	0.004	0.004	0.008													
8 V13-3 (1)	0.002	0.017	0.015	0.011	0.011	0.011	0.015												
9 TV1-1 (21)	0.008	0.019	0.019	0.019	0.015	0.019	0.019	0.017											
10 TV2-1 (1)	0.008	0.021	0.021	0.017	0.017	0.017	0.021	0.015	0.011										
11 TV3-1 (1)	0.008	0.015	0.013	0.008	0.004	0.008	0.008	0.011	0.019	0.021									
12 TV19 (13)	0.011	0.013	0.011	0.006	0.002	0.006	0.006	0.008	0.017	0.019	0.002								
13 GV (1)	0.004	0.013	0.011	0.006	0.002	0.006	0.006	0.013	0.017	0.019	0.006	0.004							
14 LP-1 (2)	0.021	0.013	0.011	0.006	0.002	0.006	0.006	0.013	0.017	0.019	0.006	0.004	0.004						
15 R1-1 (9)	0.011	0.008	0.006	0.002	0.002	0.006	0.002	0.013	0.017	0.019	0.006	0.004	0.004	0.004					
16 R1-2 (1)	0.006	0.011	0.008	0.004	0.004	0.008	0.004	0.015	0.019	0.021	0.008	0.006	0.006	0.006	0.002				
17 R3-2 (4)	0.011	0.011	0.011	0.006	0.006	0.011	0.006	0.017	0.017	0.019	0.011	0.008	0.008	0.008	0.004	0.006			
18 R3-3 (1)	0.008	0.009	0.009	0.004	0.004	0.009	0.004	0.015	0.013	0.019	0.008	0.006	0.006	0.006	0.002	0.004	0.002		
19 BI-1 (2)	0.006	0.015	0.013	0.008	0.004	0.008	0.008	0.006	0.015	0.017	0.008	0.006	0.006	0.006	0.006	0.008	0.011	0.008	
20 BI-3 (1)	0.019	0.015	0.013	0.008	0.006	0.011	0.008	0.008	0.017	0.019	0.011	0.008	0.008	0.008	0.006	0.008	0.011	0.006	0.002

APPENDIX II. Genetic distance (using uncorrected distances) based on sequence variation in the mtDNA COI sequences (504 aligned sites) among the 61 individuals of *Stereotydeus mollis* analysed. Only unique sequences (38 unique haplotypes) were included in the analysis. Codes refer to those used in Table 1; the number of identical haplotypes present at any site is identified in parentheses.

Locations	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1 W1 (2)	Wright Valley							Continental s. Victoria Land															
2 W3-1	0.143																						
3 W3-2	0.135	0.02																					
4 W5-1	0.016	0.135	0.127																				
5 W5-2	0.141	0.03	0.022	0.133																			
6 W5-3	0.137	0.012	0.008	0.129	0.018																		
7 W5-4	0.141	0.03	0.022	0.133	0.004	0.018																	
8 V1-5 (7)	0.139	0.028	0.02	0.131	0.002	0.016	0.002																
9 V1-6	0.145	0.034	0.026	0.137	0.008	0.022	0.008	0.006															
10 V1-7	0.115	0.089	0.077	0.107	0.077	0.077	0.077	0.075	0.081														
11 V2-1 (4)	0.004	0.147	0.139	0.012	0.145	0.141	0.145	0.143	0.149	0.119													
12 V2-2	0.133	0.028	0.024	0.125	0.018	0.016	0.018	0.016	0.022	0.071	0.137												
13 V2-3	0.127	0.093	0.081	0.119	0.081	0.081	0.081	0.079	0.085	0.022	0.131	0.075											
14 V2-4	0.012	0.139	0.141	0.024	0.147	0.143	0.147	0.145	0.151	0.121	0.012	0.139	0.133										
15 V4-2	0.139	0.052	0.044	0.131	0.026	0.04	0.026	0.024	0.028	0.085	0.143	0.036	0.089	0.145									
16 V6	0.123	0.091	0.079	0.115	0.079	0.079	0.079	0.077	0.083	0.022	0.127	0.069	0.006	0.129	0.083								
17 V7	0.139	0.016	0.008	0.131	0.014	0.004	0.014	0.012	0.018	0.079	0.143	0.016	0.083	0.145	0.036	0.081							
18 V9-1	0.121	0.089	0.077	0.113	0.077	0.077	0.077	0.075	0.081	0.02	0.125	0.067	0.008	0.127	0.081	0.002	0.079						
19 V9-2	0.117	0.081	0.069	0.109	0.069	0.069	0.069	0.067	0.073	0.022	0.121	0.063	0.014	0.123	0.077	0.012	0.071	0.01					
20 V11	0.151	0.038	0.046	0.143	0.048	0.05	0.048	0.046	0.048	0.091	0.155	0.046	0.095	0.147	0.063	0.091	0.05	0.089	0.085				
21 V13-3	0.151	0.036	0.036	0.143	0.018	0.032	0.018	0.016	0.014	0.087	0.155	0.032	0.091	0.149	0.04	0.089	0.028	0.087	0.079	0.048			
22 V13-4	0.131	0.093	0.089	0.123	0.089	0.089	0.089	0.087	0.089	0.016	0.135	0.083	0.034	0.129	0.097	0.038	0.091	0.036	0.038	0.089	0.081		
23 V13-5	0.127	0.097	0.093	0.123	0.097	0.093	0.097	0.095	0.095	0.02	0.131	0.091	0.034	0.125	0.105	0.038	0.095	0.036	0.042	0.095	0.085	0.014	
24 V13-6	0.056	0.169	0.163	0.06	0.169	0.167	0.169	0.167	0.169	0.145	0.052	0.163	0.151	0.048	0.167	0.147	0.167	0.145	0.143	0.169	0.161	0.143	0.137
25 TV2-10	0.133	0.04	0.024	0.125	0.026	0.028	0.026	0.024	0.03	0.069	0.137	0.024	0.073	0.139	0.042	0.067	0.028	0.065	0.062	0.026	0.04	0.085	0.089
26 TV3-3	0.137	0.038	0.026	0.129	0.02	0.026	0.02	0.018	0.024	0.077	0.141	0.022	0.085	0.143	0.038	0.083	0.026	0.081	0.073	0.046	0.034	0.089	0.097
27 TV4 (3)	0.135	0.032	0.02	0.127	0.014	0.02	0.014	0.012	0.018	0.075	0.139	0.02	0.083	0.141	0.032	0.081	0.02	0.079	0.071	0.04	0.028	0.087	0.095
28 TV15-1	0.135	0.036	0.024	0.127	0.018	0.024	0.018	0.016	0.022	0.075	0.139	0.02	0.083	0.141	0.036	0.081	0.024	0.079	0.071	0.044	0.032	0.087	0.095
29 TV15-2	0.137	0.04	0.024	0.129	0.026	0.028	0.026	0.024	0.03	0.073	0.141	0.028	0.077	0.143	0.042	0.071	0.028	0.069	0.065	0.026	0.04	0.089	0.093
30 CR	0.137	0.032	0.02	0.129	0.014	0.02	0.014	0.012	0.018	0.077	0.141	0.024	0.085	0.143	0.032	0.083	0.02	0.081	0.073	0.044	0.028	0.089	0.097
31 MV-5	0.143	0.04	0.028	0.135	0.022	0.028	0.022	0.02	0.026	0.083	0.147	0.028	0.091	0.149	0.04	0.089	0.028	0.087	0.079	0.048	0.036	0.095	0.103
32 MV-6	0.143	0.038	0.026	0.135	0.012	0.026	0.012	0.01	0.016	0.079	0.147	0.026	0.083	0.149	0.032	0.081	0.022	0.079	0.071	0.052	0.026	0.091	0.099
33 MP-4	0.141	0.026	0.018	0.133	0.004	0.014	0.004	0.002	0.008	0.077	0.145	0.014	0.077	0.147	0.026	0.075	0.01	0.073	0.065	0.048	0.018	0.089	0.097
34 MP-5	0.133	0.046	0.03	0.129	0.032	0.034	0.032	0.03	0.036	0.075	0.137	0.03	0.079	0.139	0.048	0.073	0.034	0.071	0.067	0.028	0.046	0.091	0.091

APPENDIX II (contd)…

Locations	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
	Wright Valley							Continental s. Victoria Land															
Beaufort Island																							
35 HPP-1	0.141	0.03	0.022	0.133	0.004	0.018	0.004	0.002	0.008	0.077	0.145	0.018	0.081	0.147	0.026	0.079	0.014	0.077	0.069	0.048	0.018	0.089	0.097
36 HPP-2	0.137	0.032	0.02	0.129	0.006	0.02	0.006	0.004	0.01	0.073	0.141	0.016	0.077	0.143	0.028	0.075	0.016	0.073	0.065	0.046	0.02	0.085	0.093
37 GH7	0.119	0.083	0.071	0.111	0.075	0.071	0.075	0.073	0.079	0.026	0.123	0.065	0.014	0.125	0.083	0.012	0.073	0.01	0.016	0.085	0.085	0.038	0.038
38 BI-4	0.006	0.149	0.141	0.018	0.141	0.143	0.141	0.139	0.145	0.117	0.006	0.133	0.129	0.018	0.139	0.125	0.143	0.123	0.119	0.153	0.151	0.133	0.133

APPENDIX II (contd)…

Locations	24	25	26	27	28	29	30	31	32	33	34	35	36	37
	Continental s. Victoria Land													
Beaufort Island														
24 V13-6														
25 TV2-10	0.161													
26 TV3-3	0.165	0.028												
27 TV4 (3)	0.163	0.022	0.006											
28 TV15-1	0.163	0.026	0.002	0.004										
29 TV15-2	0.165	0.004	0.028	0.022	0.026									
30 CR	0.165	0.022	0.006	0.004	0.004	0.022								
31 MV-5	0.171	0.03	0.01	0.008	0.008	0.03	0.008							
32 MV-6	0.171	0.03	0.016	0.014	0.014	0.03	0.01	0.018						
33 MP-4	0.169	0.026	0.02	0.014	0.018	0.026	0.014	0.022	0.012					
34 MP-5	0.161	0.01	0.03	0.024	0.028	0.01	0.028	0.032	0.036	0.032				
35 HPP-1	0.169	0.026	0.016	0.014	0.014	0.026	0.01	0.018	0.008	0.004	0.032			
36 HPP-2	0.165	0.024	0.014	0.012	0.012	0.024	0.012	0.02	0.01	0.006	0.03	0.006		
37 GH7	0.147	0.063	0.079	0.077	0.077	0.067	0.079	0.085	0.077	0.071	0.069	0.075	0.071	
38 BI-4	0.058	0.135	0.139	0.137	0.137	0.139	0.139	0.145	0.143	0.141	0.135	0.141	0.137	0.125

THESIS CONCLUSION

Intraspecific patterns of genetic variation are determined by historical and contemporary processes (Avise 1998). Study of these patterns through the application of molecular techniques defines the field of phylogeography and phylogenetic analysis of the genetic structure of taxa can provide important insights into the roles of past (biogeographic) and modern (ecological) processes in species formation (Bermingham & Moritz 1998). Phylogenetic data can also allow estimates to be made of dispersal rates (i.e. gene flow), range alterations, and refugial origins (e.g. Avise 1998; Bermingham & Moritz 1998). Furthermore, it can be used to distinguish between the processes of dispersal and vicariance (Wolf *et al.* 2001; Ketmaier *et al.* 2003; de Queiroz 2005).

In this thesis, I employed genetic data to examine phylogeographic-based issues for endemic Antarctic and New Zealand arthropods. The phylogeographic patterns found were consistent with (limited) dispersal pathways, (re)colonisation and Pleistocene/Holocene vicariance.

Mitochondrial (COI) DNA sequence divergence analysis for New Zealand idoteid isopods indicated that geographical isolation, and subsequent diversification, was likely to have followed rare Pliocene dispersal events in the New Zealand archipelago for these populations (Chapter I). Diversification in fast-changing environments and newly opened island habitats, such as Chatham Islands is not uncommon (Gorog *et al.* 2004), and highlights the evolutionary importance of recent, long-distance dispersal and/or isolating mechanisms in the origin of extant New Zealand taxa (Hurr *et al.* 1999; Winkworth *et al.* 1999, 2002; de Queiroz 2005; Trewick & Morgan-Richards 2005).

Genetic divergences between Stewart Island and Southland populations of *Austridotea lacustris* and among Southland, Otago and Canterbury populations of *A. annectens* and *A. lacustris* were consistent with those exhibited by other species (e.g. Allibone & Wallis 1993; Waters & Wallis 2001). Meanwhile, the absence of geographic structure in *Austridotea* spp. on the east coast of South Island was also consistent with patterns documented by Stevens & Hogg (2004). Collectively, this lends support to the hypothesis of genetic transfer mediated by periodic oceanic dispersal. In particular, a role for dispersal from southern New Zealand to Chatham Islands by means of coastal ocean currents has been postulated (e.g. Knox 1960; Moore 1961; McLay 1988; Trewick 2000; Stevens & Hogg 2004).

The absence of the *Austridotea spp.* group north of Banks Peninsula (Canterbury) and from North Island may indicate that isopods were once more widespread in the past and have since become extinct in these areas (see also de Queiroz 2005). Indeed, contemporary long-distance oceanic dispersal among these populations was found to be negligible, suggesting that present-day oceanic currents may be sufficient to isolate isopod populations, thus limiting gene flow among biogeographic regions; an occurrence not uncommon in New Zealand taxa (Stevens 2002). The lack of gene flow may result in a long-term reduction in genetic variability in these populations as genetic drift and mutation become the major forces modifying their future structure (Skotnicki *et al.* 2000; Edmands 2001; Frati *et al.* 2001; Fanciulli *et al.* 2001).

In addition to identifying the major force(s) shaping population structure and species distributions in a phylogeographic context, molecular data may also be used to make informed conservation decisions. For example, examination of patterns of genetic diversity among natural populations can identify biodiversity 'hotspots' as areas for protection, while genetic data can also help to elucidate relationships within

and among taxa to prioritise conservation measures based on more accurately described generic and species status. From a biodiversity perspective, the three currently recognised *Austridotea* species (*A. lacustris, A. annectens, A. benhami*) were clearly resolved by mtDNA (COI) sequencing (Chapter I), with *A. lacustris* being the most genetically divergent species (~31% from *A. benhami* and *A. annectens*). The magnitude of intraspecific genetic divergence raised the question as to whether *A. lacustris* and *A. annectens* both consist of morphologically cryptic species complexes similar to other crustacean groups (e.g. Hogg *et al.* 1998; Witt & Hebert 2000; Hurwood *et al.* 2003; Stevens & Hogg 2004). For example, my data suggested that up to four and two genetically divergent populations exist within *A. lacustris* and *A. annectens*, repectively. In this case, congruent evidence from nuclear loci or corresponding morphological differences would be required for designation of species status. Nevertheless, protection of these divergent populations would be consistent with sound conservation practice (Moritz 1994).

Chapter II aimed to establish the degree of genetic contact (i.e. gene flow/dispersal) between fragmented habitats in southern Victoria Land, Antarctica. A comprehensive molecular analysis incorporating mitochondrial (COI) DNA and statistical phylogenetic analyses was carried out on individuals of *Gomphiocephalus hodgsoni* and *Stereotydeus mollis* to evaluate patterns of genetic diversity, refugial origins and extent of gene flow among populations.

Genealogical data has been widely used to deduce and describe the considerable impact of Pleistocene biogeography on speciation processes and phylogeographic distributional patterns within many taxa (e.g. Hewitt 1996; Klicka & Zink 1997; Avise *et al.* 1998; Marshall & Coetzee 2000; Buckley *et al.* 2001; Trewick & Wallis 2001; Garrick *et al.* 2004). Here, examination of spatial genetic structure across the

geographic ranges for *G. hodgsoni* and *S. mollis* revealed divergence associated with extreme isolation through habitat fragmentation and founding/bottleneck events followed by range expansion during recolonisation of previously glaciated areas throughout the Pleistocene and, more recently, during the Holocene.

Levels of mtDNA (COI) divergence among *G. hodgsoni* haplotypes were low (< 2%) and among *S. mollis* populations were relatively higher (up to 14.5%). However, the number of haplotypes found for both species was relatively high (20 and 38 for *G. hodgsoni* and *S. mollis*, respectively). Phylogenetic analyses found that haplotypes showed a heterogeneous distribution throughout populations in southern Victoria Land for both taxa. Additionally, while haplotypes were dispersed across locations, few populations actually shared common haplotypes, while individuals within populations often had different haplotypes.

The geographical positioning of haplotype distribution among *G. hodgsoni* and *S. mollis* populations was revealed by nested clade analysis and used to invoke theories regarding a more accurate definition of the refugial origins of these taxa. The pattern found was that of a large western (continental) refugium, suggesting that Victoria and Wright Valleys (and possibly Taylor Valley) may be the site of a pre-Pleistocene *in situ* remnant source population, from which extant populations in the region recolonised surrounding areas when conditions allowed. Additionally, the nested clade analysis identified restricted gene flow with isolation-by-distance and restricted gene flow/dispersal but with some long distance dispersal as the most parsimonious explanations for the observed patterns of haplotype distribution in both *G. hodgsoni* and *S. mollis* populations. This finding agreed with Antarctica's geographic history of vicariant Pleistocene/Holocene climate change and availability of refugia, coupled with habitat diversity on a micro-scale across the landscape and the biological history

of the taxa (particularly their low dispersal ability). Interestingly, the nested clade analysis also picked up patterns of reticulate species evolution for both *G. hodgsoni* and *S. mollis*. This recombination was most likely among populations that were relatively distant geographically (e.g. Beaufort Island, Taylor Valley and Victoria Valley for *G. hodgsoni*), which may further support the idea that these populations were once united in an ancestral refugium.

Patterns of high haplotype diversity and distributional heterogeneity are not uncommon for populations of terrestrial arthropods in Antarctica, and this may reflect common phylogeographic histories (Courtright *et al.* 2000; Fanciulli *et al.* 2001; Frati *et al.* 2001; Stevens & Hogg 2003). Indeed, as shown here, the Antarctic environment is well structured to promote phylogeographic structure in terrestrial invertebrate populations. Comparative phylogeography using other endemic Antarctic taxa (e.g. nematodes, tardigrades) from southern Victoria Land will enable further examination of colonisation routes, range expansion and long-term persistence of taxa in this extremely fragmented habitat.

Overall, and as for the New Zealand study (Chapter I), dispersal in this study appears to be limited. This pattern has been found for other terrestrial taxa in Antarctica, and may be related to problems with wind dessication (Gressitt *et al.* 1960; Frati *et al.* 2001). Disperal assisted by birds and/or humans may be a possibility (Strong 1967; Tilbrook 1967; Fanciulli *et al.* 2001; Stevens & Hogg 2002, 2005). Meanwhile, based on species distributions in this study, *S. mollis* may be re-colonising the Antarctic landscape at a faster rate than *G. hodgsoni*. The possibility of human-assisted dispersal is particularly important with an increasing human presence in Antarctica (e.g. Strandtmann & George 1973; Broadbent 1994), as is that

of present-day dispersal in general, since modern climatic shifts may influence the potential availability of new habitats.

From a conservation perspective, I suggest that St. John's Range, Mt. Cerberus and Lake Brownworth may be potential biodiversity 'hotspots'. Accordingly, such information may be used to identify priorities for conservation strategies, (e.g. establishment of specially protected areas to limit entrance to and movement within regions of high genetic diversity and/or unique genetic resources).

The issues examined in this thesis illustrate the usefulness of applying a phylogeographic approach to infer various speciation and colonisation mechanisms, particularly when coupled with population genetic, geological and biogeographical data. Meanwhile, the advent of new analytical techniques, such as the nested clade analytical approach employed in Chapter II, allow statistically rigorous separation of current and historical processes that shape genetic variation in populations (Templeton *et al.* 1995; Templeton 2004). Phylogeography as a field of study, has already enjoyed a positive and promising adolescence and should continue to offer new and exciting insights into the dynamics of population structure in the future. Meanwhile, application of genetic data to conservation efforts can assist with informed management decisions based on more accurate definition of both species and population 'conservation units'.

Future Research

Many studies have investigated population structures in a phylogeographic context (e.g. Stevens & Hogg 2003; Ayoub & Riechert 2004; Galbreath & Cook 2004; Neiman & Lively 2004). However, much research in this area has focused on finer-scale spatial areas, especially in invertebrate populations where large genetic divergences can be found over small spatial scales. Future research should therefore target larger scale areas (e.g. greater southern hemisphere and/or northern/southern hemisphere comparisons) to determine the sorts of geographic, ecological and environmental features that serve to shape population structure for terrestrial invertebrate taxa on a global scale.

In particular, a northern/southern hemisphere contrast would be informative given the contrasting geographic history of the opposing polar regions. For example, the southern hemisphere may exhibit a vicariant model of diversity because these invertebrates are likely to have survived long-term habitat fragmentation via refugia. While the northern hemisphere may exhibit similar patterns, dispersal with population expansion/contraction via glacial cycling (which would obscure genetic structure) is perhaps likely to have played a more significant role, given the closer proximity of land masses. Tracking population and species movements since the Mio-Pliocene, through the application of molecular DNA markers and associated statistical techniques (such as network analysis), within respective hemispheres would enhance our understanding of the effects of different processes (e.g. gene flow, fragmentation, range expansion) on population structure (Templeton 2004). Insights gained from such studies will greatly enhance our understanding of processes related

to dispersal pathways (colonisation routes) and associated dispersal barriers for invertebrate taxa, as well as those related to maintenance of biological diversity.

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