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PHYLOGEOGRPAHY AND GENETIC DIVERSITY OF TERRESTRIAL ARTHROPODS FROM THE ROSS DEPENDENCY, ANTARCTICA

A thesis

submitted in partial fulfillment

of the requirements

of

Master of Science in Biology

in Biological Sciences

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by

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ABSTRACT

The pattern of genetic diversity in many species observed today can be traced back to historic ecological events that influenced the distribution of species not only on a global but also a local scale. For example, historical events such as habitat fragmentation, divergence in isolation, and subsequent range expansion, can result in a recognisable pattern of genetic variation which can be used to infer ecological factors (e.g. effective population size, dispersal capacity), as well as those affecting speciation processes. This thesis examines these issues from a phylogeographic and phylogenetic perspective by analysing patterns of variation in the mtDNA *cytochrome c oxidase sub-unit 1* (COI) gene in two co-occurring Antarctic endemic arthropods in Southern Victoria Land, Ross Dependency.

Within the Southern Victoria Land Dry Valleys of Garwood, Marshall and Miers, populations of the springtail *Gomphiocephalus hodgsoni* (Collembola: Hypogastruridae) and mite *Stereotydeus mollis* (Acari: Prostigmata) revealed consistently dissimilar patterns of genetic structure. COI divergence within *G. hodgsoni* was < 0.7%, while divergence within *S. mollis* reached upwards of 17%. Within our study area *G. hodgsoni* and *S. mollis* harboured 10 and 22 haplotypes, respectively and showed links to previously sampled populations across Southern Victoria Land. The distribution of *G. hodgsoni* haplotypes across sites was homogenous while those of *S. mollis* were distinctly heterogenous. The extremely low genetic variation and links to previously sampled populations suggest that *G. hodgsoni* is a relatively recent colonist within our study area and/or the victim of an extreme bottleneck event. On the other hand, the extreme levels of

genetic diversity observed for *S. mollis*, and the occurrence of two highly divergent haplotypes that were unique to our study area, suggest that: (1) *S. mollis* may have had a longer association in isolation with our southern study area; and/or (2) *S. mollis* has colonised our study area on more than one occasion via multiple extant refugial populations.

Throughout its entire Southern Victoria Land range *S. mollis* is characterised by extremely high levels of mtDNA (COI) divergence (>17%), suggesting a possible multi species complex. To examine this issue, I used both Neighbour Joining (NJ) and Maximum Likelihood (ML) methods to construct a phylogeny utilising all 50 known unique *S. mollis* sequences with other Victoria Land congeners including an available *S. belli* sequence, and several new *S. shoupi* sequences. Both NJ and ML analyses revealed significantly congruent trees with strong bootstrap support. The morphologically similar *S. shoupi* was placed as a monophyletic sister group, basal to *S. mollis* in both analyses with strong support. However, there was disagreement between the two methods in the placement of the single *S. belli* sequence within in the resulting phylogenies which was not possible to resolve with the current data. Despite this latter uncertainty, the possibility of cryptic species within *S. mollis* remains.

Collectively, these studies have demonstrated differences in the genetic structure between two co-occurring species and suggested how similar historic processes, combined with differing life history attributes can lead to that differentiation. Furthermore, genetic analyses were used to identify isolated and unique populations, which are likely to be of high conservation value.

PREFACE & ACKNOWLEDGEMENTS

Finding the proper amount of space, time and wording to thank those who have helped along the way is arguably the most difficult portion of this thesis. Fortunately not because of a lack of help and encouragement throughout, but rather an overabundance. I would like to start by thanking my supervisor Dr. Ian Hogg for giving me the opportunity to broaden my scientific horizons and pursue a branch of research that very few people ever get to participate in, let alone truly experience. The last 18 months, with two Antarctic field seasons thrown in, have come and gone in the blink of an eye. I have truly had an incredible time along the way and I look forward to working with you in the future.

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thank you for the friendships that began during my fellowship and came to fruition on the Ice; I can't say I have danced in a gorilla suit under the Antarctic midnight sun with many other people.

For financial and logistical support, which really is the bottom line of Antarctic research, I would like to thank the Foundation for Research and Science Technology (FRST), the Scientific Committee for Antarctic Research (SCAR), the Latitudinal Gradient Project (LGP), the New Zealand Terrestrial Antarctic Biocomplexity Survey (NZTABS), Antarctica New Zealand, Sierra Nevada Brewing Co. and of course all of the wonderful staff at Scott Base and McMurdo Station. K. Grossman you will be happy to know there is a black Sierra Nevada Brewing Co. hat now residing at Scott Base.

In the end, when it seems like there is no one else, there is always family. To my mother Susan and my father Jeff, thank you for providing me with the nothing but love and encouragement. I may have slipped here and there along the way but you never let me fall. I hope that I have made you proud. To my maternal grandparents Howard and Eunice, thank you for all of the support, particularly financial, but most importantly the love and admiration only grandparents can give. Grandpa, I still remember your words of advice to me as a young boy, "You never learn anything by only talking to people who agree with you." Something I hope my reviewers will keep in mind. Grandma, I carry with me your mtDNA; it's served you well, it's served me well and I promise to take good care of it because as an only grandchild its reached the end of the line. Don't worry, I promise to explain that last part next time I come home. To my paternal grandparents Waunita and Leo, though you are gone, you are not forgotten. I know you are right alongside me every step of the way.

Finally, my wife Morgan, without you absolutely nothing that I have accomplished over the past two and a half years would have been possible. The fact that you still love me after I dragged you half way around the world to New Zealand, then left you on your own for months at a time while I went off and played explorer still amazes me. You are not only the love of my life but my best friend. Everything that I have accomplished could not have been possible without you.

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THESIS INTRODUCTION

Mites (Acari: Arachnida) and springtails (Collembola: Hexapoda) are among the oldest and most widespread invertebrates found on the planet (Kethley *et al.* 1979, Greenslade 1992). With a distribution ranging from the tropics to both polar regions, mites and springtails have diversified to fill a wide range of habitats. Mites, with over 48,000 described species, have successfully colonised every known terrestrial habitat from alpine extremes to the depths of caves (Halliday *et al.* 1997, Ducarme 2004). Springtails, with a more modest 6500 described species, are one of the most successful terrestrial hexapods with densities often reaching up to several million individuals per cubic meter of soil (Rusek 1998). Together, both free living soil mites and springtails are an important component of the mesofauna which contribute to the breakdown of detritus, formation of soil and recycling of nutrients on a global scale (Scheu & Shultz 1996, Rusek 1998,).

In the Antarctic, springtails and mites are the dominant macroscopic soil invertebrates and inhabit both the maritime and continental ice free regions (Marshall & Pugh 1996, Hogg *et al*. 2006). In conjunction with the microscopic soil invertebrates (i.e. nematodes, tardigrades and rotifers) springtails and mites play a major role in the recycling of nutrients and formation of soil in these extremely nutrient poor, simple ecosystems (Wall & Virginia 1999). Despite over 50 years of intermittent research, new findings on the biology, distribution, origins and the interactions between Antarctic invertebrates and the continent's complex glacial history are continually being uncovered.

Previous knowledge of Antarctic terrestrial invertebrates, prior to the end of the 20th century, held that the extreme glacial cycling which reached its peak within the last 10 million years resulted in the extirpation of macro-invertebrate organisms from the continent and that the distribution of much of the biota seen today arose from post glaciation colonists (Gressitt 1967, Crowley & North 1988). However, the advent of and widespread application of molecular techniques in Antarctic research towards the end of the 20th century began to challenge some of these long-held beliefs (Frati *et al.* 1999, Vincent 2000, Barnes *et al.* 2006, Stevens & Hogg 2003, Stevens & Hogg 2006, McGaughran *et. al* 2008; 2010).

Using sequence information from the mtDNA *cytochrome c oxidase subunit I* (COI) gene Stevens *et al.* (2006) demonstrated that species of springtail present on the Antarctic peninsula were most likely to have diverged and speciated from each other between ~23-5 MYA while isolation of continental Antarctic springtails since at least ~21-11 MYA appears extremely likely. In addition, molecular studies have suggested that the Antarctic mite *Stereotydeus mollis* (Acari: Prostigmatidae) has been associated with the Southern Victoria Land Ice free areas for a similar period of time based upon observed levels of extreme intra-specific divergence of mtDNA COI sequences (Stevens & Hogg 2006, McGaughran *et al.* 2008).

The use of molecular information, through phylogeographical investigations, has also revealed a pattern of vicariance and postglacial dispersal of Antarctic arthropods that has shaped their population genetic structure to this day (Frati *et. al* 2001, Fanciulli *et al.* 2001, Stevens & Hogg 2003). Stevens & Hogg (2006) and McGaughran *et al.* (2008) used a combined phylogeographic approach for the Southern Victoria Land endemic

springtail *Gomphiocephalus hodgsoni* and the co-occurring mite *Stereotydeus mollis* to determine that both species shared a common phylogeographic pattern that was primarily shaped by mio-pliocene glacial cycling. From the observed geographical patterns of mtDNA variation it was concluded that both species responded in similar fashion to a combination of habitat fragmentation into isolated refugia during periods of glacial expansion and subsequent range expansion during interglacial cycles. However, a limited sample size for *G. hodgsoni* and especially *S. mollis* from the southern Dry Valleys (e.g. Miers and Garwood Valleys) may not have accurately represented the true phylogeographic pattern of both species in this isolated ice-free area.

In chapter 1 of this thesis, I will examine the fine-scale phylogeographical pattern of mtDNA (COI) genetic variability for both *G. hodgsoni* and *S. mollis* in the southern Dry Valleys of Miers, Marshall and Garwood Valleys. I will then contrast these findings with those of Stevens & Hogg (2006) and McGaughran *et al.* (2008) to further test whether *S. mollis* and *G. hodgsoni* share a common phylogeographic history in Southern Victoria Land or if previous findings were influenced by a restricted sample size.

Several factors of a species life history (i.e. mode of dispersal, dispersal capability, breeding system, etc.) can potentially determine how it will respond to vicariant events and will often result in a distinct pattern of the genetic variation in extant populations (Ibrahim 1996, Hewitt 1996). This pattern of genetic variation is often best observed in the spatial distribution and variability of mtDNA haplotypes because of its usual maternal mode of inheritance. Accordingly, individuals can belong to either one of two bifrucating lineages but not both. For this reason, matrilineal mtDNA genealogies have been used extensively to show clear relationships between individuals (Irwin 2002).

The second chapter of this thesis will examine all of the known mtDNA haplotypes for *S. mollis, S. belli* and *S. shoupi*, as well as several new sequences, in an attempt to construct an accurate phylogeny of the three endemic *Stereotydeus* of Southern Victoria Land and the Queen Maud Mountains. Mitochondrial DNA, especially COI, has been found to perform very well in Acarine phylogenies at both the inter-specific and intra-specific level (Cruickshank 2001, Navajas & Fenton 2002, Dabert 2006).

S. mollis, which has been well studied throughout the northern portion of its range (Stevens & Hogg 2006, McGaughran *et al.* 2008) is characterised by extremely high levels of mtDNA variation (up to 17% uncorrected-*p* distance) and may constitute a species complex made up of several divergent lineages which have diverged in isolation. However, previous attempts to accurately place *S. mollis* in a phylogeny of co-occurring *Stereotydeus* from Southern Victoria Land as well as those from the southern Queen Maud Mountains have been hampered by a lack of available sequences from closely related sister taxa (Stevens & Hogg 2006). By adding intra-specific sequence information from closely related, co-occurring sister taxa, I aim to better resolve the placement of several highly divergent haplotypes which have been assigned as belonging to *S. mollis.*

Together, the two chapters in this thesis address both phylogeographical and phylogenetic questions of two co-occurring species of Antarctic arthropods from an area of Southern Victoria Land that has received considerably less attention than more northerly habitats. Thus, it will provide both geographic and taxonomic comparisons that may be used to compare or contrast similar processes in a range of organisms across similar habitats. The thesis concludes with a summary, and provides possible priorities for future research.

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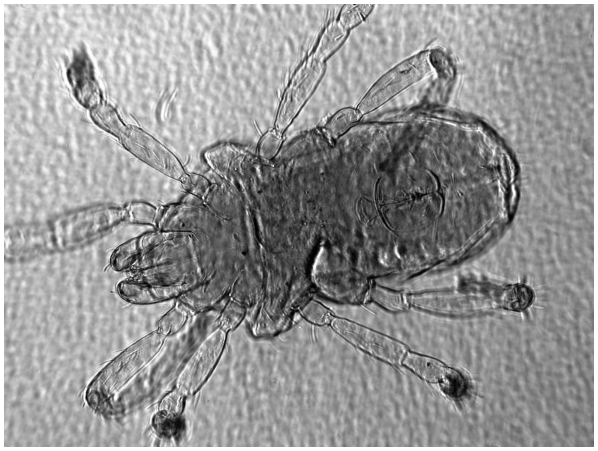
Chapter I

FINE-SCALE DISTRIBUTION AND GENETIC DIVERSITY OF mtDNA HAPLOTYPES FOR ARTHROPOD TAXA WITHIN THE SOUTHERN MOST DRY VALLEYS, SOUTHERN VICTORIA LAND, ANTARCTICA

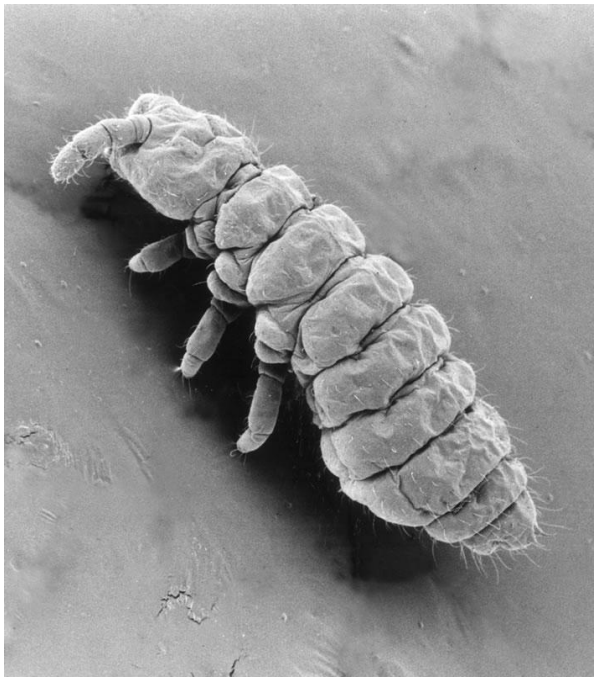
Keywords: mtDNA, *Gomphiocephalus*, springtails, *Stereotydeus*, mites, Antarctica



Stereotydeus mollis (Acari: Prostigmata)

600X Magnification

Photo Credit: Nicholas J. Demetras



Gomphiocephalus hodgsoni (Collembola: Hypogastruridae)

300X Magnification

Photo Credit: Pietro Paolo Fanciulli

INTRODUCTION

The historic effect of glacial cycling as an important determinant of the present day distribution and genetic structure of many temperate and polar species is well recognised (Kinloch *et al.* 1986, Hewitt 1996, Hewitt 2000). The isolation of populations into fragmented habitats or "refugia" with little or no gene flow during periods of glaciation will lead to stochastic and selective effects on population genetic structure. This will result in the loss of alleles due to bottleneck events, lineage and population extinctions and the selection of beneficial mutations (Hewitt 2004). Subsequently, during interglacial periods, habitat expansion can lead to the spread of refugial populations in two ways: 1) rapid long range dispersal from the leading edge (i.e. leptokurtic) or 2) successive dispersal events over several generations (i.e. stepping stone) (Hewitt 1996). Invariably, the way in which populations in refugia respond to interglacial habitat expansion will result in very different patterns of genetic diversity in newly established populations. Rapid, long range leptokurtic dispersal from refugial populations will result in considerable genetic homozygosity throughout newly established populations (Hewitt 1996, Nei *et al.* 1975). Conversely, successive dispersal events over several generations are expected to result in populations with increased levels of genetic diversity, representative of the original population(s) from which they were derived (Ibrahim *et al* 1996).

The well studied glacial history of Southern Victoria Land, Antarctica and the disjunct distribution of the endemic terrestrial soil fauna provides an ideal opportunity to study the way in which organisms respond to habitat fragmentation over evolutionary

time-scales (Armstrong 1978, Denton *et al.* 1984, Denton *et al.* 1991, Hall & Denton 1999, Marchant *et al.*1996, Anderson *et al.* 2002, Stevens *et al.* 2006). The fact that Antarctica has experienced more than 10 major glacial cycles over the last one million years bp and that many terrestrial habitats have only become available within the last 17,000 years bp has resulted in the evolution of terrestrial life in extreme isolation (Hays, 1976, Huiskes *et al.* 2006). This is particularly true for the free living springtails (Collembola) and mites (Acari) from the Trans-Antarctic mountains which represent a Gondwanan relict fauna distantly related to other Southern hemisphere taxa and who have persisted on the Antarctic continent for millions of years bp (Stevens *et al*. 2005). Three species each of springtail and mites are known from the southern Victoria Land region. For the springtails, only one species, *Gomphiocephalus hodgsoni* (Hypogastruridae) is found throughout the Dry Valleys. For the mites four species have been described from the Dry Valleys with one species, *Stereotydeus mollis* (Prostigmatidae) dominant throughout (Sinclair & Stevens 2006).

The phylogeography of the Antarctic springtail *G. hodgsoni* has been extensively studied throughout its southern Victoria Land range (Stevens & Hogg 2003; Nolan et al. 2006; McGaughran et al. 2008; 2010). However, the bulk of the specimens collected thus far have been from the Granite Harbour and the central Dry Valleys regions including Taylor, Wright and Victoria Valleys, encompassing the northern and middle portion of the species' range respectively. In comparison, the phylogeography of the mite *S. mollis* has received even less attention. Only two studies, using combined datasets, are available (Stevens & Hogg 2006; McGaughran et al. 2008). These two studies primarily focused on the more northern Dry Valleys of Southern Victoria Land (i.e. Victoria, Wright & Taylor

Valley) and the off shore islands of the Ross sea region. Of the 61 individuals analysed by McGaughran *et al*. (2008) and the 41 individuals by Stevens and Hogg (2006), only two individuals from one of the southern Dry Valleys (both from Miers Valley) were included. Due to previous logistical constraints, no comprehensive, fine-scale collections of either *G. hodgsoni* or *S. mollis* from the Southern most Dry Valleys had been made.

The purpose of this study was to investigate the phylogenetic structure of both *G. hodgsoni* and *S. mollis* towards the southern range of the species' distribution and contrast this with data from their middle and northern range. We also examined the fine scale phylogeography of both species to identify any local scale processes, which may have influenced the distribution of mtDNA lineages within our study area. Finally, we compared the mtDNA (COI) divergence values between mites and springtails, on a much smaller spatial scale than previously recorded, in an attempt to clarify whether previously observed differences were artefacts of previous sampling distributions and/or the presence of cryptic species

MATERIALS AND METHODS

Molecular Analyses

Study Area, taxa and sample collection

The Dry Valleys of Southern Victoria Land encompass approximately 4800 km^2 and are the largest ice-free areas in continental Antarctica. Mean annual temperatures range from -14.8°C to -30.0°C while available annual precipitation data suggest that <100 mm of water equivalent is received primarily as snow (Doran et al., 2002; Bromley, 1985). Measured ablation rates in the Dry Valleys by Hendersen et al. (1965) and Clow *et al.* (1988) ranged from 150 to >1000 mm a^{-1}, greatly exceeding annual levels of precipitation, resulting in a hyper arid, cold-polar desert environment.

The southern most Dry Valleys, encompassing a series of smaller valleys including the main Miers, Marshall, and Garwood Valleys, are roughly located between 78.00° and 78.15° S and 163.60° and 164.35° E (Fig. 1 & 2). They cover in excess of 250 km^2 or roughly 5% of the total area of the McMurdo Dry Valleys. Positioned in an east-west orientation Miers, Marshall and Garwood Valleys are bounded to the North by the Blue Glacier to the east by the junction of the Koettlitz Glacier and the Ross Ice-shelf, by Hidden Valley to the south, and by the Denton Hills and the Royal Society Range to the west (Fig. 2). Beyond the heads of the respective valleys to the west of Marshall Valley, to the north of Miers Valley and the South of Garwood Valley is a small sheltered area of approximately 18 km^2 called Shangri La. As the name suggests, Shangri La is surrounded

by mountains that effectively isolate it from the other valleys except for a narrow northern passage that connects to upper Garwood Valley. The Joyce Glacier and Lake Buddha, which drains through the northern passage into upper Garwood Valley, are dominant features of the Shangri La landscape (Fig. 3)

Structured sampling at over 600 sites was undertaken to collect terrestrial arthropods from Miers, Marshall and Garwood Valleys as well as Shangri La and including a range of altitudes. *G. hodgsoni* and *S. mollis* were both generally constrained to areas of high soil moisture with access to water (snow patches, lake edges and/ or streams). The relative abundance of terrestrial arthropods was visually determined at each site by turning over stones and counting the number of individuals present for a set ten-minute period (Caruso & Bargagli 2007). Individuals from each species were collected from the underside of stones using a modified aspirator (see Stevens & Hogg 2002) and immediately placed in 95% ethanol. From a total of 633 sites, springtails were observed at 77 sites and mites at 44 sites, and of these, springtails were recovered from 35 sites and mites from 22 sites (Figure 3 & Table 1).

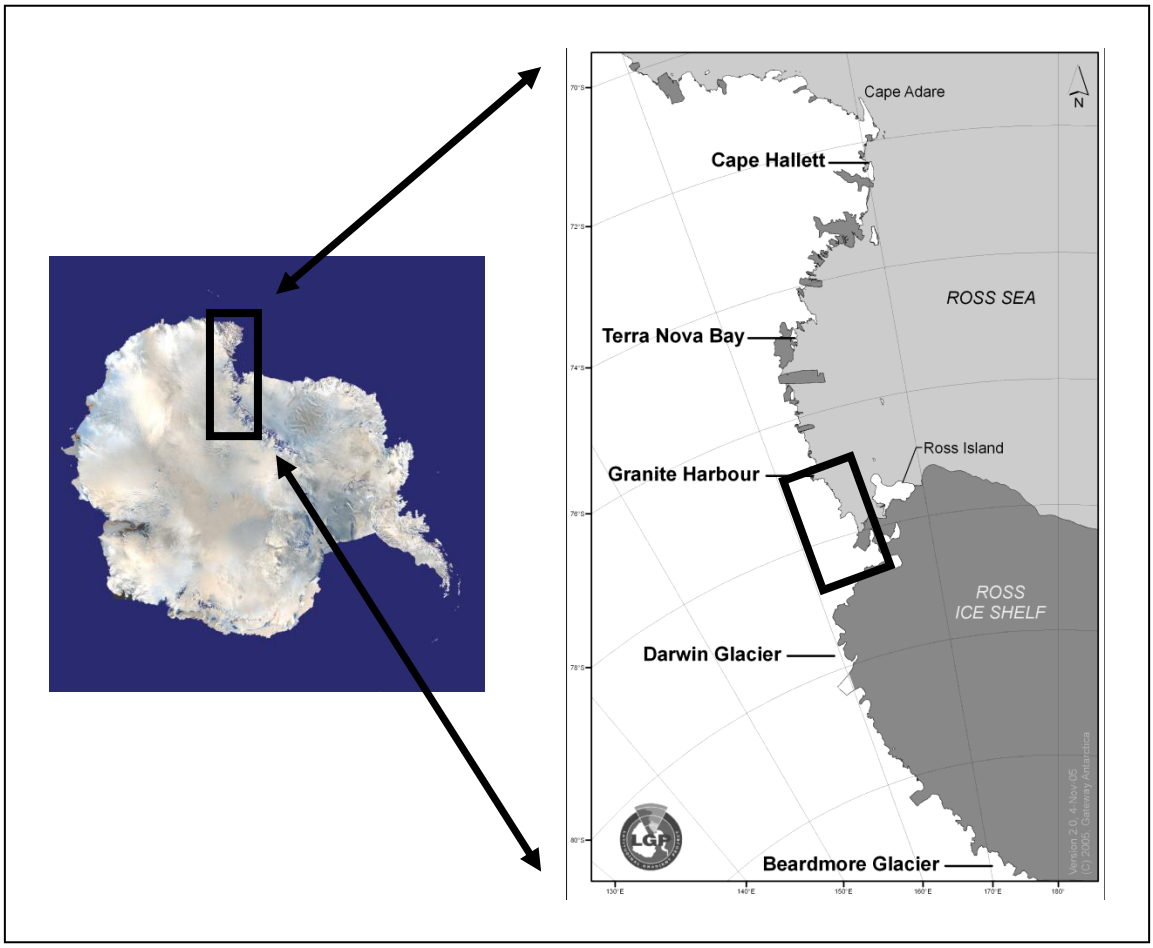


FIGURE 1. Location of the Victoria Land coast in relation to the Antarctic continent. Inset location (right) outlines the area of Southern Victoria Land and the McMurdo Dry Valley region.

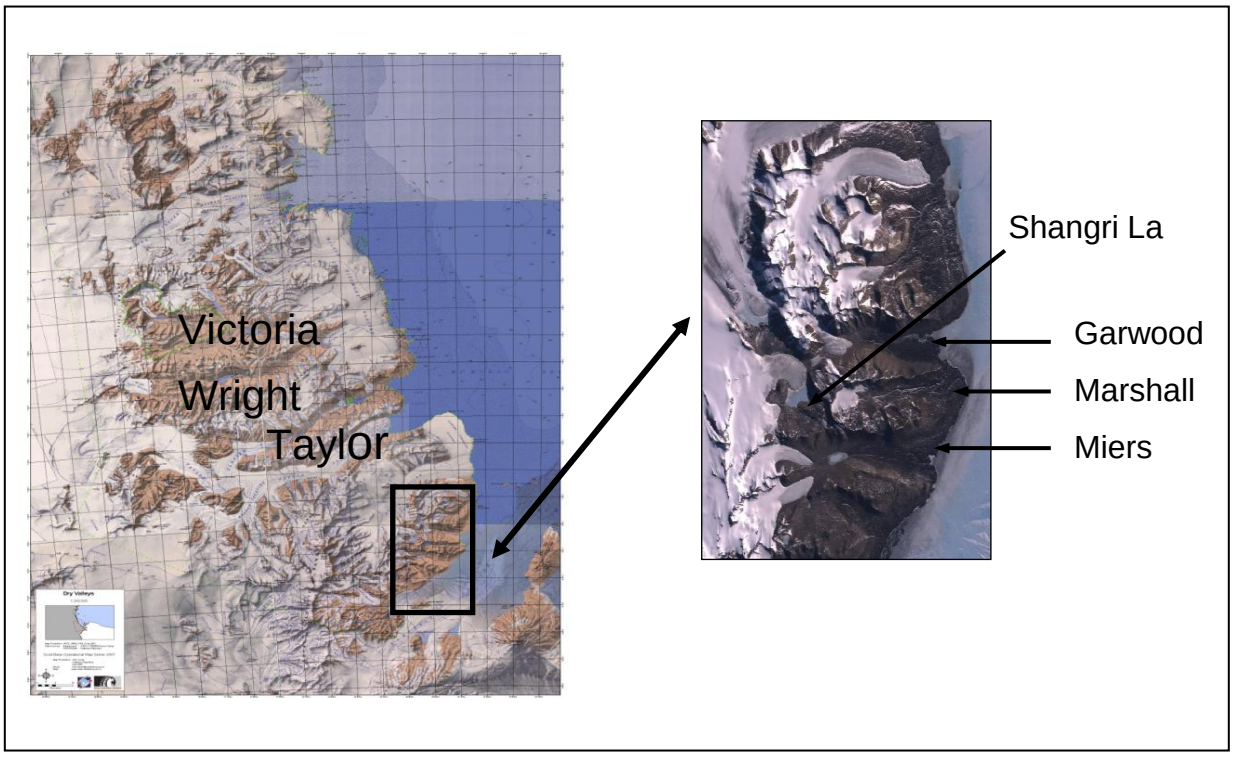


FIGURE 2. Location of our southern Dry Valley study area (inset) in comparison to the larger northern Dry Valleys of Victoria, Wright and Taylor.

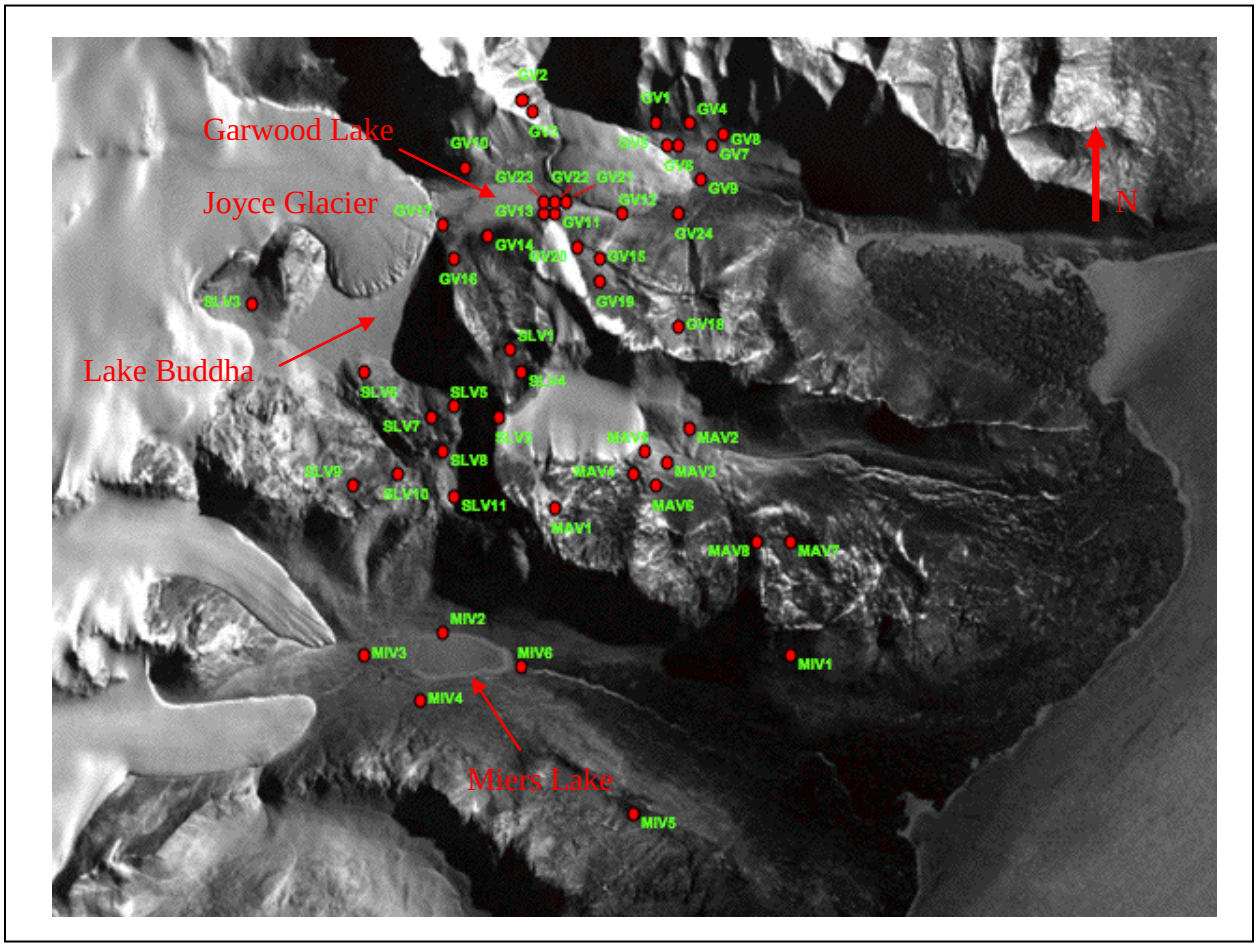


FIGURE 3. Map of study area showing locations of individual sampling sites (red circles) referred to in text and associated tables.

Table 1. Sampling locations and site codes for *G. hodgsoni* and *S. mollis* throughout Garwood, Shangri La, Marshall and Miers Valleys

Location	Lat. (S)	Long. (E)	Species	Site	*G. hodgsoni* (n)	*S. mollis* (n)
Garwood Valley	78° 0' 38.70"	163° 59' 18.30"	*G. hodgsoni*	GV1	6	
	78° 0' 32.28"	163° 54' 21.96"	*G. hodgsoni*	GV2	5	
	78° 0' 30.96"	163° 54' 21.60"	*G. hodgsoni*	GV3	3	
	78° 0' 42.72"	164° 01' 24.06"	*G. hodgsoni*	GV4	3	
	78° 00' 47.10"	164° 00' 30.90"	*G. hodgsoni*	GV5	1	
	78° 0' 46.38"	164° 00' 40.02"	*G. hodgsoni*	GV6	2	
	78° 0' 49.44"	164° 01' 47.22"	*G. hodgsoni*	GV7	3	
	78° 0' 48.42"	164° 01' 50.16"	*S. mollis*	GV8		2
	78° 1' 04.20"	164° 01' 51.60"	*S. mollis*	GV9		2
	78° 0' 59.33"	163° 50' 07.20"	*G. hodgsoni*	GV10	1	
	78° 1' 27.78"	163° 55' 02.40"	Both species	GV11	2	3
	78° 1' 30.06"	163° 58' 11.10"	*G. hodgsoni*	GV12	1	
	78° 1' 26.40"	163° 54' 25.20"	*G. hodgsoni*	GV13	3	
	78° 1' 38.04"	163° 51' 04.32"	*G. hodgsoni*	GV14	3	
	78° 1' 41.46"	163° 55' 52.26"	*G. hodgsoni*	GV15	3	
	78° 1' 49.86"	163° 49' 40.62"	*G. hodgsoni*	GV16	4	
	78° 1' 44.34"	163° 49' 13.68"	*G. hodgsoni*	GV17	2	
	78° 2' 34.14"	164° 01' 17.82"	*G. hodgsoni*	GV18	1	
	78° 1' 45.96"	163° 55' 43.14"	*G. hodgsoni*	GV19	3	
	78° 1' 38.22"	163° 55' 30.06"	*G. hodgsoni*	GV20	4	
	78° 1' 27.54"	163° 55' 24.84"	Both species	GV21	4	3
	78° 1' 26.04"	163° 55' 21.84"	Both species	GV22	4	3
	78° 1' 23.40"	163° 55' 17.70"	*G. hodgsoni*	GV23	3	
	78° 1' 30"	163° 53' 27.60"	*S. mollis*	GV24		2
	78° 00' 42.54"	163° 58' 56.40"	*S. mollis*	GV25		1
Shangri La	78° 2' 26.04"	163° 53' 31.32"	*S. mollis*	SLV1		2
	78° 2' 11.22"	163° 52' 48.84"	*G. hodgsoni*	SLV2	2	
	78° 2' 26.28"	163° 40' 56.64"	*G. hodgsoni*	SLV3	2	
	78° 2' 39.90"	163° 54' 13.74"	*G. hodgsoni*	SLV4	3	
	78° 3' 13.86"	163° 49' 37.98"	Both species	SLV5	1	2
	78° 3' 11.88"	163° 46' 07.61"	*G. hodgsoni*	SLV6	1	
	78° 3' 21.06"	163° 48' 56.16"	*G. hodgsoni*	SLV7	1	
	78° 3' 54.06"	163° 50' 15.72"	*G. hodgsoni*	SLV8	2	
	78° 4' 12.66"	163° 45' 53.70"	Both species	SLV9	1	2
	78° 4' 17.10"	163° 48' 19.26"	*S. mollis*	SLV10		2
	78° 4' 27.24"	163° 50' 35.52"	*G. hodgsoni*	SLV11	3	
Marshall Valley	78° 4' 32.94"	163° 56' 22.80"	*S. mollis*	MAV1		5
	78° 3' 38.04"	164° 02' 37.14"	*G. hodgsoni*	MAV2		
	78° 4' 10.50"	164° 01' 28.26"	*S. mollis*	MAV3		1
	78° 4' 3.36"	163° 59' 01.74"	*S. mollis*	MAV4		2
	78° 4' 3.84"	164° 00' 37.14"	*G. hodgsoni*	MAV5	3	
	78° 4' 19.98"	163° 59' 07.38"	*G. hodgsoni*	MAV6	3	
	78° 4' 42.48"	164° 06' 41.70"	*S. mollis*	MAV7		1
	78° 4' 41.10"	164° 05' 08.94"	*G. hodgsoni*	MAV8	1	

Table 1 Cont. Sampling locations and site codes for *G. hodgsoni* and *S. mollis* throughout Garwood, Shangri La, Marshall and Miers Valleys

Location	Lat. (S)	Long. (E)	Species	Site	*G. hodgsoni* (n)	*S. mollis* (n)
Miers Valley	78° 5' 24.66"	164° 7' 2.76"	*S. mollis*	MIV1		1
	78° 5' 35.88"	163° 50' 09.60"	*S. mollis*	MIV2		10
	78° 5' 51.78"	163° 46' 23.04"	*Both species*	MIV3	4	2
	78° 6' 26.94"	163° 49' 27.84"	*G. hodgsoni*	MIV4	1	
	78° 7' 28.68"	164° 05' 24.24"	*S. mollis*	MIV5		1
	78° 5' 54.96"	163° 53' 45.60"	*S. mollis*	MIV6		3

mtDNA extraction, amplification and sequencing

Total genomic DNA was extracted from 90 *G. hodgsoni* individuals and 52 *S. mollis* individuals using the SIGMA REDExtract-N-Amp™ Tissue PCR Kit. Due to the small size of individual animals (0.5-1.4 mm), the manufacturers recommended volume of extraction buffer was reduced by 90% to concentrate the resulting DNA extract. Following extraction, a 710-bp fragment of the mitochondrial cytochrome *c* oxidase (COI) gene was amplified using the universal primers LCO1490 and HCO2198 (Folmer *et al.* 1994) for *G. hodgsoni* and COI-2R and COI-2F for *S. mollis* (Otto & Wilson 2001). PCR amplification of each individual was carried out in a 20 μL reaction containing 4 μL of extracted DNA (unquantified), 1.0 μM of each primer (HCO/LCO and COI-2R/COI2F for *G. hodgsoni* and *S. mollis* respectively) and 10 μL of *i*-Taq™ 2X PCR master mix (iNtRON Biotechnology, Gyeonggi-do, Korea). Thermocycling conditions for *G. hodgsoni* were: 94°C for 4 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). For *S. mollis*, thermocycling conditions 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 (McGaughran *et al.* 2008).

All PCR products were purified using SAP/EXO (USB Corp., Cleveland, OH, USA). Sequencing used both forward and reverse primers and was performed directly on

a capillary electrophoresis ABI 3130XL genetic analyser (Applied Biosystems Inc., Foster City, CA) at the University of Waikato DNA sequencing facility.

Phylogenetic analyses

Individual sequences were confirmed as being derived from applicable taxa using the GenBank BLAST algorithm and aligned using Geneious Pro v4.7.6 (Drummond *et al.* 2009). PAUP* v4.0b10 (Swofford 2002) was used to perform all phylogenetic analyses. Sequences from the prostigmatid mite *Eriohynchus sp.* (GenBank accession No. AF142135; Otto and Wilson 2001) and the Antarctic hypogastrurid *Biscoia sudpolaris* were used as outgroup taxon (GenBank accension No. DQ309568; Stevens and Hogg 2006). χ^2 tests as employed in PAUP* were used to determine whether the assumption of equal base frequencies among sequences was violated on all sites, parsimony-informative sites and third codon positions only. Modeltest ver 3.7 (Posada & Crandall 1998) was used to ascertain the correct substitution model of evolution for maximum likelihood (ML) heuristic searches (using all unique sequences). 1024 bootstrap replicates were performed to assess confidence in ML analyses. For the mites, the appropriate model selected was HKY+I+Γ (-lnL = 2339.87 (AIC); Ti/tv ratio = 2.6878 I = 0.6006 Γ = 1.9686: with base frequencies set to A = 0.3583 C = 0.1340 G = 0.1150 T = 0.3927). For the springtails the appropriate model was GTR+Γ (-lnL = 1273.47 (AIC); rate matrix: A-C = 2.0127 A-G = 4.0901 A-T = 5.0355 C-G =0.6658 C-T = 8.8873 G-T = 1.0000 Γ = 0.4843: with base frequencies set to A = 0.29719 C = 0.1979 G = 0.1744 T = 0.3558. All

other options in PAUP* remained as default. Distance matrices of pairwise nucleotide sequence divergences were calculated using all unique sequences (Table 2a & b).

Population structure analyses

ARLEQUIN ver 3.11 (Excoffier et al., 2005) was used to identify the genetic characteristics of, and to investigate the presence of genetic structure for, both *G. hodgsoni* and *S. mollis* within our study area. Within the study area haplotype (*h*) and nucleotide (π) diversity indices (Nei, 1987) were calculated for each valley. As well as a hierarchical analysis of molecular variance (AMOVA) comparing each valley as a unique geographic location, with statistical significance of variance components tested with 16,000 permutations was computed. The number of pairwise differences (φ-$_{st}$ values) between haplotypes for both springtails and mites were calculated to investigate whether a deviation from equilibrium was due to past demographic changes (Rogers and Harpending 1992; Morimer and van Vuureen 2007). Tajima's *D* (Tajima, 1989) and Fu's *F*-statistic (Fu, 1997) were calculated using Arlequin ver 3.11 to investigate whether haplotype frequencies deviated from equilibrium.

Due to small sample sizes from many locations throughout the study site (e.g fewer than 3 individuals from 16 of the 35 sample sites for *G. hodgsoni* and 14 of the 22 sites for *S. mollis* (Table 1), it was necessary to pool data into sample locations corresponding to the individual valleys in our study area (Weir & Cockerman, 1984; McGaughran *et al.* 2008).

Haplotype networks

The program TCS ver. 1.21 (Clement et. al 2000) was used to estimate a haplotype network for both *G. hodgsoni* and *S. mollis* with a connection limit of 95% for both species. *G. hodgsoni* had a maximum number of mutational steps less than four and was statistically significant with 95% confidence. However, a connection limit of 95% was unable to connect all groups of *S. mollis* and required a maximum number of mutational steps of 80 to estimate all possible points where distinct groups may be connected.

RESULTS

Molecular Analyses

mtDNA sequence variability

A 599 bp (199 codons) portion, from an unambiguous alignment (no insertions or deletions), of COI from a total of 90 *G. hodgsoni* individuals resulted in 10 haplotypes and a 622 bp (207 codons) alignment for 50 *S. mollis* individuals resulted in 22 haplotypes. The nucleotide composition averaged over all sequences showed an A-T bias of 63.4% (A = 27.0%, T = 36.4%, C = 19.8%, G = 16.8%) for *G. hodgsoni*, and an A-T bias of 69.7% (A = 32.6%, T = 37.1%, C = 14.7%, G = 15.6%) for *S. mollis.* Base frequencies were homogenous among sequences for all sites (*G. hodgsoni* (χ^2 = 0.118, *p* = 1.00, d.f = 27) and *S. mollis* (χ^2 = 5.588, *p* =1.00 d.f. = 63), for parsimony informative sites (*G. hodgsoni* (2 sites χ^2 = 1.00, *p* = 0.66, d.f.= 18) and *S. mollis* (159 sites χ^2 = 21.059, *p* =1.00, d.f. = 63) and for third codon positions (*G. hodgsoni* (200 sites, A-T = 82.85%) χ^2 = 1.085 *p* =1.00 d.f. = 27) and *S. mollis* (208 sites, A-T = 82.9%) χ^2 = 20.662, *p* = 1.00, d.f. = 63).

Amongst all *G. hodgsoni* sequences there were 588/11 constant/variable sites resulting in 10 haplotypes (TABS_Gh1 – TABS_Gh10) with extremely low sequence divergence ranging from 0.17% to 0.67% (uncorrected *p*-distance) (Table 1.2a). All 11 nucleotide substitutions occurred at third codon positions only resulting in no amino acid

changes between haplotypes. Only two of the 11 substitutions between haplotypes were parsimony informative.

Among all *S. mollis* sequences there were 452/170 constant/variable sites resulting in 22 haplotypes (TABS_Sm1 – TABS_Sm22) with high sequence divergence ranging from 0.2% to 17.7% (uncorrected *p*-distance) (Table 1.2b). Of the 170 variable sites, 159 were parsimony informative. Nucleotide substitutions were present at all codon positions and resulted in 29 amino acid substitutions. *S. mollis* haplotypes TABS_Sm1 – 16 ranged from 0.2 – 2.1% sequence divergence (uncorrected *p*-distance) with 592/30 constant/variable sites. Twenty nine nucleotide substitutions occurred at third codon positions resulting in one amino acid change, while one nucleotide substitution occurred at a second codon position resulting in an amino acid change. Haplotypes TABS_Sm17-18 showed very low pair wise sequence divergence (0.2%) but were 14.3 – 15.4% divergent from all other haplotypes with 530/92 constant/variable sites. Fourteen nucleotide substitutions occurred at first codon positions, five at second codon positions and 73 at third codon positions resulting in 16 amino acid substitutions. Haplotypes TABS_Sm19-20 also showed very low pairwise sequence divergence (0.2%) but were 13.7-14.5% divergent from all other haplotypes with 534/88 constant variable sites. Nine nucleotide substitutions occurred at first codon positions, five at second codon positions and 74 at third codon positions resulting in 11 amino acid substitutions. Haplotypes TABS_Sm21-22 showed 1.9% pair wise sequence divergence and were 13.2 - 17.7% divergent from all other haplotypes respectively. Six nucleotide substitutions occurred at first codon positions, two at second codon positions and 82 at third codon positions resulting in 10 amino acid substitutions for the haplotype TABS_Sm21. For the haplotype

TABS_Sm22, seven nucleotide substitutions occurred at first codon positions, one at second codon positions and 75 at third codon positions resulting in 10 amino acid substitutions, one of which was unique.

Table 2a. *Gomphiocephalus hodgsoni* genetic distance (uncorrected) based on mtDNA COI (599 aligned sites) sequence variation among haplotypes

Haplotype	TABS_Gh1	TABS_Gh2	TABS_Gh3	TABS_Gh4	TABS_Gh5	TABS_Gh6	TABS_Gh7	TABS_Gh8	TABS_Gh9	TABS_Gh10
TABS_Gh1										
TABS_Gh2	0.003									
TABS_Gh3	0.002	0.005								
TABS_Gh4	0.002	0.005	0.003							
TABS_Gh5	0.002	0.005	0.003	0.003						
TABS_Gh6	0.002	0.005	0.003	0.003	0.003					
TABS_Gh7	0.002	0.005	0.003	0.003	0.003	0.003				
TABS_Gh8	0.002	0.005	0.003	0.003	0.003	0.003	0.003			
TABS_Gh9	0.002	0.005	0.003	0.003	0.003	0.003	0.003	0.003		
TABS_Gh10	0.007	0.007	0.008	0.008	0.008	0.008	0.005	0.008	0.008	
Biscoia sudpolaris DQ309568	0.198	0.199	0.198	0.196	0.197	0.198	0.198	0.196	0.198	0.199

Table 2b. *Stereotydeus mollis* genetic distance (uncorrected) based on mtDNA COI (622 aligned sites) sequence variation among the mite haplotypes

Haplotype	TABS_sm1	TABS_sm2	TABS_sm3	TABS_sm4	TABS_sm5	TABS_sm6	TABS_sm7	TABS_sm8	TABS_sm9	TABS_sm10	TABS_sm11
TABS_Sm1											
TABS_Sm2	0.003										
TABS_Sm3	0.002	0.002									
TABS_Sm4	0.010	0.010	0.008								
TABS_Sm5	0.011	0.011	0.010	0.002							
TABS_Sm6	0.003	0.003	0.002	0.006	0.008						
TABS_Sm7	0.005	0.005	0.003	0.008	0.010	0.005					
TABS_Sm8	0.011	0.011	0.010	0.011	0.013	0.011	0.010				
TABS_Sm9	0.005	0.005	0.003	0.011	0.013	0.005	0.006	0.010			
TABS_Sm10	0.016	0.016	0.014	0.013	0.014	0.013	0.014	0.018	0.018		
TABS_Sm11	0.016	0.016	0.014	0.013	0.014	0.013	0.014	0.018	0.018	0.003	
TABS_Sm12	0.013	0.013	0.011	0.010	0.011	0.010	0.011	0.014	0.014	0.003	0.003
TABS_Sm13	0.013	0.013	0.011	0.010	0.011	0.010	0.011	0.014	0.014	0.006	0.006
TABS_Sm14	0.021	0.021	0.019	0.024	0.026	0.021	0.019	0.026	0.023	0.023	0.023
TABS_Sm15	0.019	0.019	0.018	0.023	0.024	0.019	0.018	0.024	0.021	0.021	0.021
TABS_Sm16	0.021	0.021	0.019	0.024	0.026	0.021	0.019	0.026	0.023	0.023	0.019
TABS_Sm17	0.150	0.150	0.148	0.151	0.153	0.150	0.146	0.153	0.150	0.150	0.146
TABS_Sm18	0.151	0.151	0.150	0.153	0.154	0.151	0.148	0.154	0.151	0.151	0.148
TABS_Sm19	0.143	0.143	0.141	0.145	0.143	0.143	0.141	0.145	0.143	0.141	0.138
TABS_Sm20	0.141	0.141	0.140	0.143	0.141	0.141	0.140	0.143	0.141	0.140	0.137
TABS_Sm21	0.146	0.146	0.145	0.148	0.150	0.146	0.143	0.148	0.146	0.151	0.148
TABS_Sm22	0.135	0.135	0.133	0.137	0.138	0.135	0.132	0.141	0.135	0.140	0.137
Eriohynchus sp. AF142135	0.170	0.170	0.169	0.167	0.165	0.170	0.167	0.174	0.169	0.170	0.167

Table 2b cont. *Stereotydeus mollis* genetic distance (uncorrected) based on mtDNA COI (622 aligned sites) sequence variation among the mite haplotypes

Haplotype	TABS_sm12	TABS_sm13	TABS_sm14	TABS_sm15	TABS_sm16	TABS_sm17	TABS_sm18	TABS_sm19	TABS_sm20	TABS_sm21	TABS_sm22
TABS_Sm13	0.003										
TABS_Sm14	0.019	0.019									
TABS_Sm15	0.018	0.018	0.002								
TABS_Sm16	0.019	0.019	0.006	0.005							
TABS_Sm17	0.146	0.148	0.145	0.143	0.143						
TABS_Sm18	0.148	0.150	0.146	0.145	0.145	0.002					
TABS_Sm19	0.141	0.145	0.141	0.140	0.141	0.138	0.140				
TABS_Sm20	0.140	0.143	0.140	0.138	0.140	0.140	0.141	0.002			
TABS_Sm21	0.148	0.148	0.146	0.145	0.143	0.175	0.177	0.146	0.145		
TABS_Sm22	0.137	0.137	0.135	0.133	0.132	0.167	0.169	0.141	0.140	0.019	
Eriohynchus sp. AF142135	0.167	0.167	0.167	0.165	0.165	0.169	0.169	0.165	0.164	0.178	0.170

Geographic distribution of mtDNA haplotypes

The most common mtDNA COI haplotype for the springtail *G. hodgsoni* was the widely distributed TABS_Gh1. Of the 90 individuals sequenced, 52 individuals (58%) from 29 of 35 sample sites were found to have this haplotype. The second most common haplotype, TABS_Gh2, was observed in 18 individuals from 12 sites in Garwood Valley with a single specimen found at sample site MAV5 in Marshall Valley. The third most abundant haplotype, TABS_Gh3, was identified from 10 individuals all from Garwood Valley. The haplotype TABS_Gh4 was identified from three individuals, two of which were located in Garwood Valley and again a single individual from sample site MAV5 in Marshall Valley. Haplotypes, TABS_Gh5-10 were represented by single individuals only. Three of these (TABS_Gh5, 6 & 10), were found in Garwood Valley, one in Miers Valley (TABS_Gh7), one in Marshall Valley (TABS_Gh8), and one in Shangri La (TABS_Gh9).

The distribution of mtDNA COI haplotypes for the mite, *S. mollis* was much different than that of *G. hodgsoni*. Of the 22 haplotypes observed, a majority (12) came from single individuals from 8 sample sites spread throughout the study area. The most common haplotype, TABS_Sm17 was observed in nine individuals from eight sample sites (four in Garwood Valley and four in Miers Valley). The second most common haplotypes were TABS_Sm10 and TABS_Sm17 with six individuals found for each. Haplotype TABS_Sm10 was found at six sample sites, two in Garwood Valley, two in Marshall Valley and two in Shangri La. In contrast, TABS_Sm17 was only found at two sample sites in Marshall Valley and one site in Miers Valley where it was numerically

dominant. The third most common haplotype, TABS_Sm9 came from four individuals from two sample locations in Garwood Valley. Six haplotypes (TABS_Sm2, 3, 4, 6, 14 & 20) occurred in only two or three individuals. TABS_Sm3 was found at two sample sites in Garwood Valley while the closely related haplotypes TABS_Sm4 and TABS_Sm6 were found at two sample sites in Marshall Valley. TABS_Sm14 was found at one sample site in Garwood Valley and one in Shanrgi La while the highly divergent haplotype TABS_Sm20 was found at two sites restricted to Miers Valley. Overall, there was no obvious pattern to the distribution of *S. mollis* haplotypes throughout our study area other than the restriction of the highly divergent TABS_Sm17-22 to the southern portion of our study area in Miers Valley and the southern Marshall/Miers Valley ridge.

Phylogenetic analyses

The *G. hodgsoni* Neighbour joining (NJ) tree based on uncorrected *p*-distances showed the most resolution with all haplotypes grouped into one closely related clade (Fig. 4). Haplotypes TABS_Gh1, 3, 6, 9 and 5 grouped together into one subclade with extremely low divergence (0.2%) while haplotypes TABS_Gh2, 10 and 7 grouped into another subclade, again with very low divergence (0.2 – 0.8%). A single nucleotide substitution at bp 172, which matched that of the outgroup taxon *Biscoia sudpolaris* resulted in this haplotype TABS_Gh8 being identified as the most basal. Maximum Parsimony (MP) and Maximum Likelihood analysis (ML), of the 10 *G. hodgsoni* haplotypes found throughout the entire study area identified a highly monomorphic tree topology with no resolution. Very low bootstrap support and resolution of the MP and

ML trees was due to the very low number of variable sites (11) separating *G. hodgsoni* haplotypes. It seems likely that MP/ML jacknife/bootstrapping methods are too demanding for the present data set to return any meaningful phylogenetic signal due to the extremely low mtDNA sequence variation found in the study area.

S. mollis NJ, MP & ML tree topologies were significantly congruent (Fig. 5a, b & c). All trees showed support for the placement of all *S. mollis* 22 haplotypes into 4 divergent clades with several bifurcation groups. Haplotypes TABS_Sm1-16 grouped into one clade (2.6% intra-clade divergence) with significant sub structuring while TABS_Sm17 & 18, 19 & 20 and 21 & 22 grouped into distinct individual clades respectively. Overall, there was strong support for the NJ, MP and ML tree topology with bootstrap confidence limits (1024 replicates) above nodes $\leq 50\%$

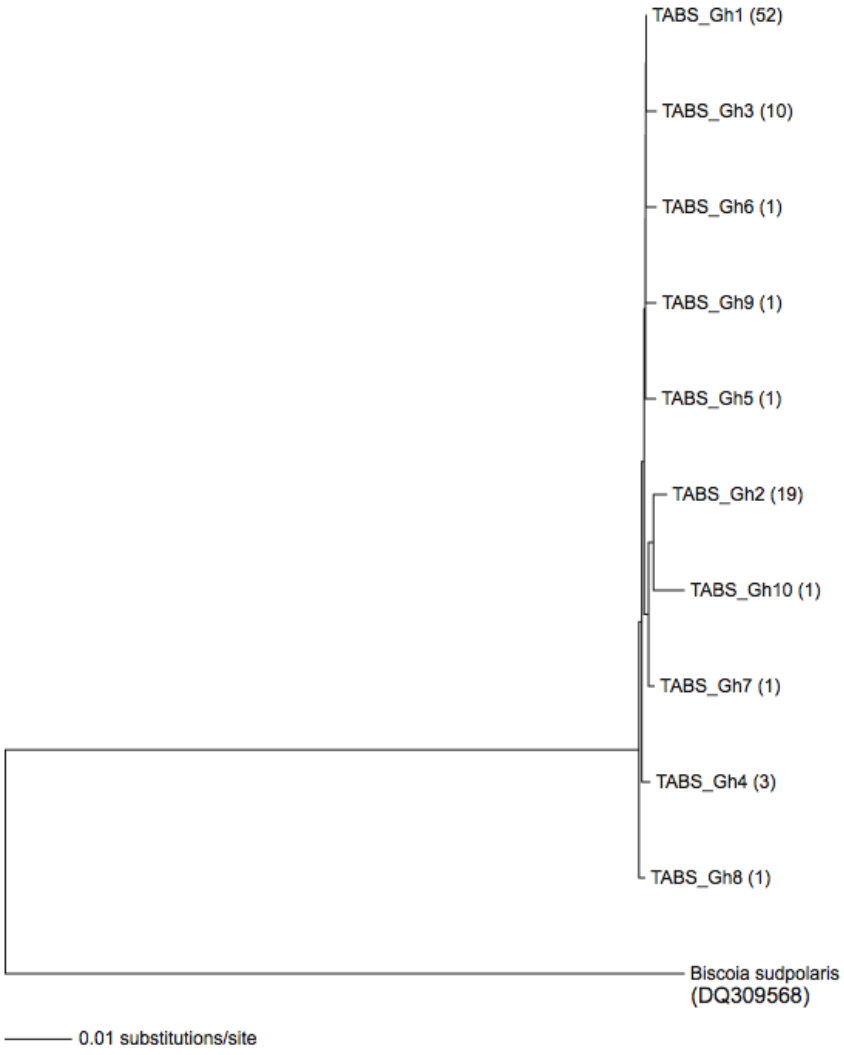


FIGURE 4. Neighbour joining phylogram for *Gomphiocephalus hodgsoni* based upon a 599 bp fragment of the mtDNA (COI) gene using only unique sequences. The number of identical haplotypes present at any given site is indicated in parentheses. Bootstrap support values were extremely low and are not reported.

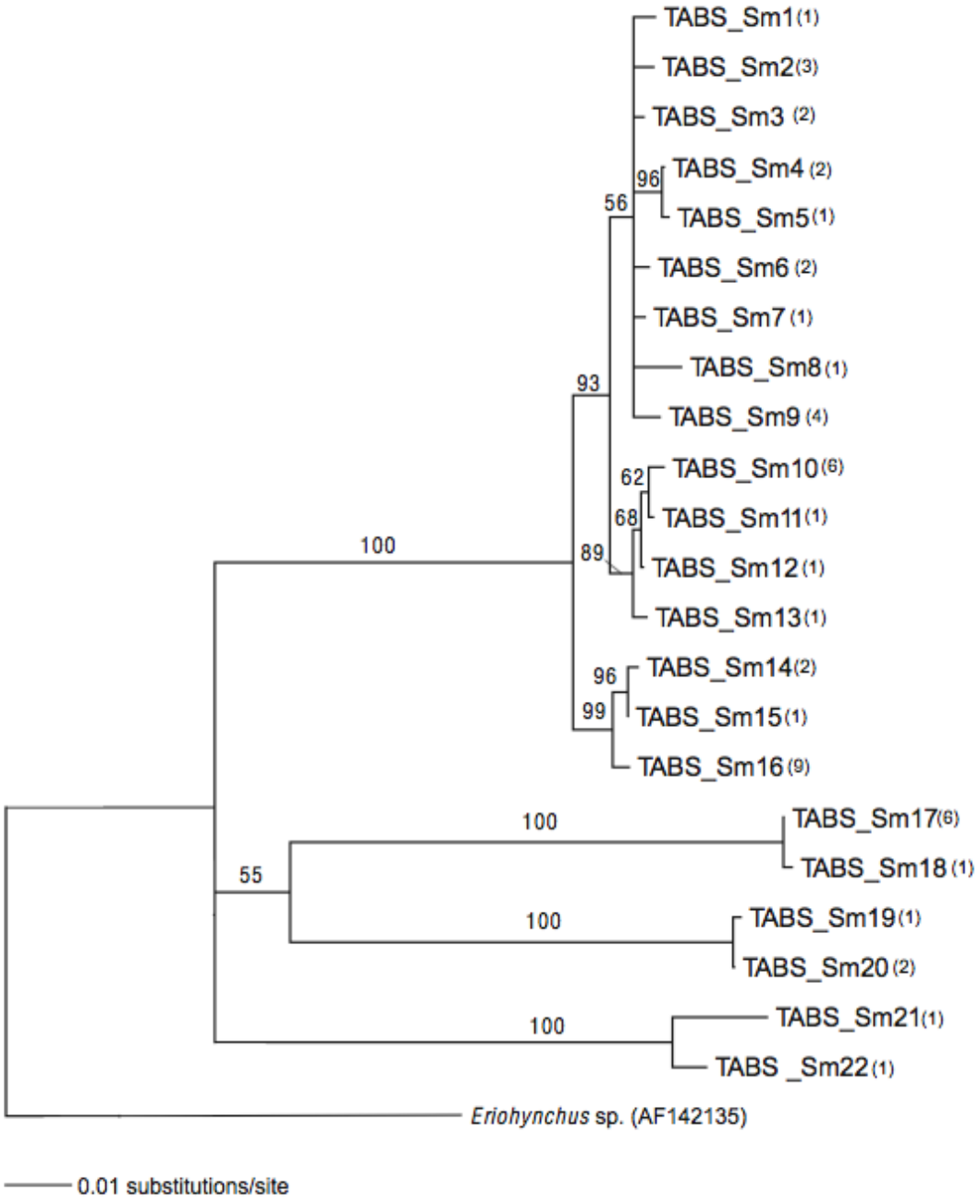


FIGURE 5a. Neighbour joining phylogram for *Stereotydeus mollis* using only unique sequences based upon a 622 bp fragment of the mtDNA (COI) gene. Bootstrap support values are given above nodes. The number of identical haplotypes present at any given site is indicated in parentheses.

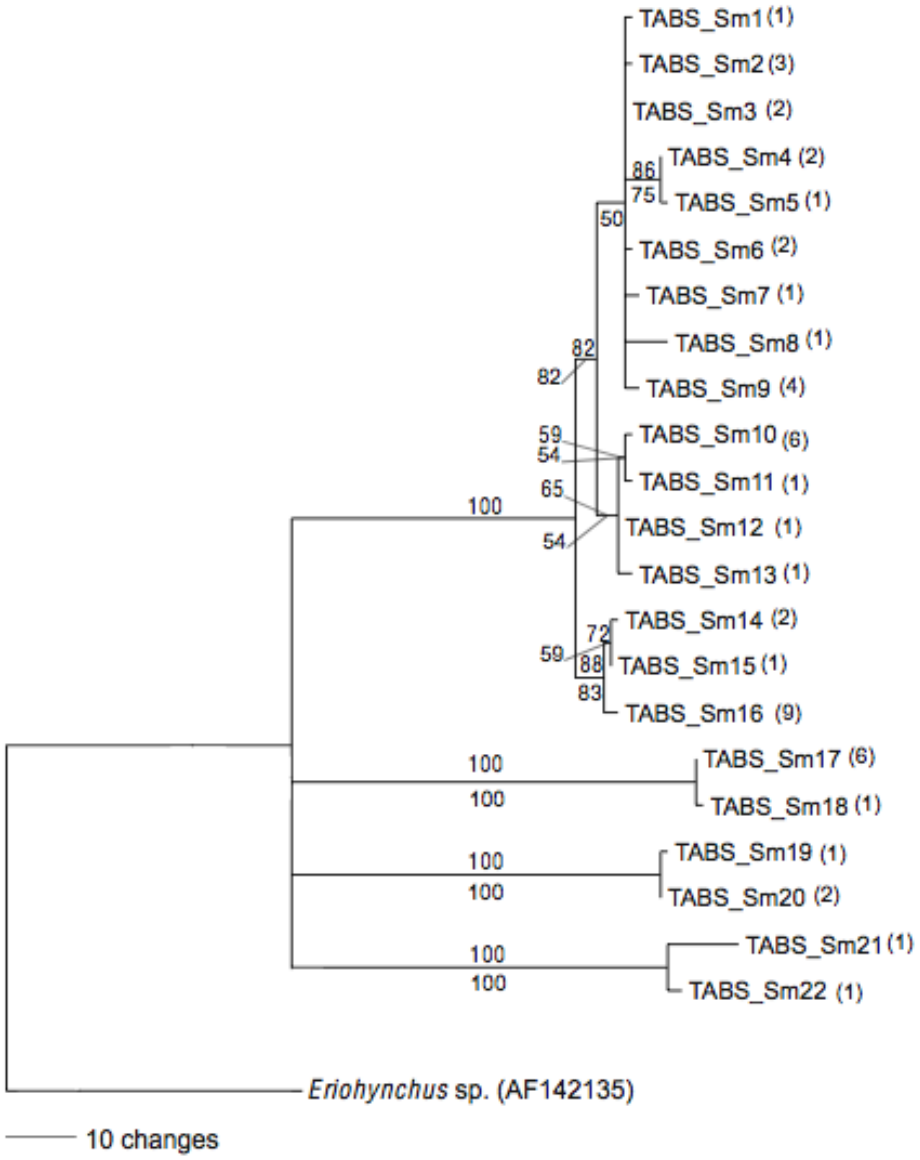


FIGURE 5b. 50% majority rule consensus phylogram of 3 Maximum-pasimony trees for *Stereotydeus mollis* estimated using the default options in PAUP* and only unique sequences. 50% majority rule values are shown below nodes and bootstrap confidence limits (1024 replicates) are shown above nodes. The number of identical haplotypes present at any given site is indicated in parentheses.

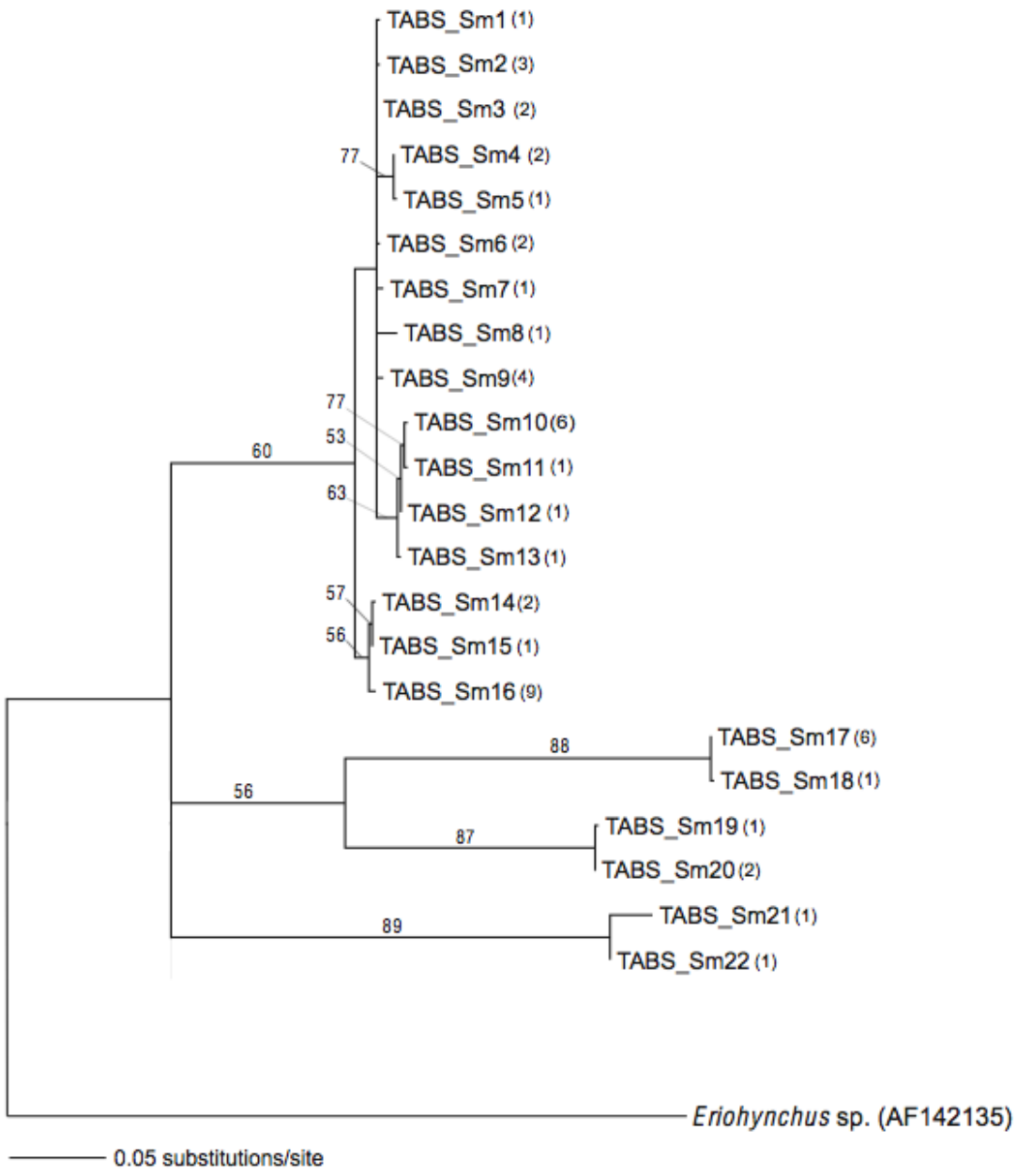


Figure 5c. Maximum likelihood phylogram for *S. mollis* based upon the model HKY+*I*+*Γ* with the settings Ti/tv ratio = 2.6878 *I* = 0.6006 *Γ* = 1.9686: with base frequencies set to *A* = 0.3583 *C* = 0.1340 *G* = 0.1150 *T* = 0.3927. ML-bootstrap values (1024 replicates) are shown above nodes.

Population structure analyses

Haplotype diversity (*h)* for *G. hodgsoni* ranged from 0.13 in Shangri La to 0.71 in Marshall Valley and 0.68 and 0.33 in Garwood and Miers Valleys, respectively (Table 3). However, Miers Valley having the second lowest haplotype diversity also had the second smallest sample size (n = 5). Due to the uneven sample sizes and its potential effect on haplotype diversity, it was necessary to apply the re-sampling method as described by Leberg (2002) to obtain a statistically powerful comparison of haplotype frequencies between sample locations. This re-sampling method involves making a random, multiple reduction of *n* for each location, which corresponds to the minimum sample size collected (in this case Miers Valley). By repeating the random reduction of *n* for each location 100 times, the information contained within the original sample is still available (Leberg 2002; Haavie *et al.* 2000).

After re-sampling, haplotype diversity for *G. hodgsoni* (*h*) ranged from 0.0 in Shangri La to 0.70 in both Marshall and Garwood Valleys. Due to the extremely low mtDNA (COI) sequence divergence observed, *h* after resampling was not significantly different (p = 0.33, paired t - test) than *h* calculated before re-sampling. This suggests that within our study area a random sample size of just five individuals is adequate to describe the mtDNA (COI) diversity per location.

Nucleotide diversity (π) for *G. hodgsoni*, averaged over all haplotypes, ranged from 0.00021in Shangri La to 0.0023 in Garwood valley and was calculated at 0.0019 in Marshall Valley and 0.00056 in Miers Valley (Table 3). AMOVA revealed a very low level of population structure, with only 9% of the molecular variation assigned among

geographic locations (Table 4). φ-$_{st}$ values were small and significant which indicates limited isolation and/or a high degree of gene flow between geographic populations within the study area. Tajima's *D* and Fu's F_s tests did not detect any departures from neutrality and all results were non-significant.

For *S. mollis,* haplotype diversity (*h*) ranged from 0.875 in Garwood Valley to 0.964 in Shangri La and was 0.917 and 0.904 in Marshall and Miers Valleys, respectively (Table 3). *S. mollis*, unlike, *G. hodgsoni*, did not exhibit increased levels of *h* with increasing sample sizes and it was not necessary use re-sampling technique applied to *G. hodgsoni*. Nucleotide diversity (π) ranged from 0.105 in Miers Valley to 0.014 in both Garwood and Shangri La. π in Marshall Valley was lower than that in Miers Valley but still much greater than π in both Garwood Valley and Shangri La at 0.086 (Table 3). AMOVA revealed a higher degree of population structuring than *G. hodgsoni*, with 17.72% of the molecular variation apportioned among geographic locations. φ-$_{st}$ values were larger and significant, indicating a greater degree of isolation and/or more limited gene flow between geographic populations of *S. mollis* compared to those of *G. hodgsoni* (Table 4). Tajima's *D* and Fu's F_s tests did not detect any departures from neutrality and all results were non-significant.

Table 3. Population statistics and genetic variation of sampled locations throughout southern Dry Valleys for *Gomphiocephalus hodgsoni* and *Stereotydeus mollis* mtDNA COI sequences

Species	Genetic population	n	x	No. of Polymorphic sites	*h* (SD)	π (SD)	Θ (SD)	Distribution of Haplotypes
G. hodgsoni	Garwood Valley	61	6	9	0.685 (.0378)	0.002 (0.002)	1.366 (0.855)	TABS_Gh1-6, 10
	Shangri La	16	2	2	0.125 (0.106)	0.000 (0.000)	0.125 (0.202)	TABS_Gh1, 9
	Marshall Valley	8	4	4	0.714 (0.181)	0.002 (0.002)	1.143 (0.835)	TABS_Gh1, 2, 4, 8
	Miers Valley	5	2	2	0.333 (0.215)	0.001 (0.001)	0.333 (0.380)	TABS_Gh1, 7
S. mollis	Garwood Valley	16	7	22	0.875 (0.050)	0.014 (0.008)	8.533 (4.165)	TABS_Sm2, 3, 9, 10 , 12 ,14 ,16
	Shangrila Valley	8	7	22	0.964 (0.077)	0.014 (0.008)	8.964 (4.625)	TABS_Sm2, 6 ,7 ,10, 13, 14,16
	Marshall Valley	9	6	141	0.917 (0.073)	0.086 (0.047)	53.361 (25.540)	TABS_Sm4, 6, 10, 11, 17, 22
	Miers Valley	17	10	166	0.904 (0.050)	0.105 (0.219)	65.125 (29.570)	TABS_Sm1, 5, 8, 15, 16, 17, 18, 19, 20, 21

n, number of individuals; x, number of haplotypes; *h*, haplotype diversity; π, nucleotide diversity; Θ, mean number of pairwise differences

Table 4. Analysis of molecular variance (AMOVA) results for *Gomphicephalus hodgsoni* and *Stereotydeus mollis* as calculated in Arlequin ver. 3.11 (Excoffier *et.al* 1992)

Source of Variation	d.f.	Sum of Squares	Variance components	Pecentage of variation	Signifcance
G. hodgsoni					
Among Locations	3	4.039	0.05412 Va	9.16	$p < 0.02$
Within Locations	86	46.183	0.53701 Vb	90.84	
Total	89	50.222	0.59113		
F_{st}				0.09155	
S. mollis					
Among Locations	3	194.801	3.88264 Va	17.72	$p < 0.002$
Within Locations	46	829.819	18.03955 Vb	82.28	
Total	49	1024.62	21.92579	100	
F_{st}				0.17725	

Haplotype networks

For the springtail *G. hodgsoni*, a connection limit of 95% was able to connect all haplotypes into a tightly grouped star pattern in which haplotypes TABS_Gh3 – 7 and TABSGh9 - 10 were connected to the dominate haplotype TABS_Gh1 through one mutational step. Haplotype TABS_Gh2 was connected to haplotype TABS_Gh1 by two mutational steps and haplotype TABS_Gh8 was connected to haplotype TABS_Gh7 by three mutational steps (Fig. 6a). The resulting star shaped pattern of the haplotype network for *G. hodgsoni*, where all other haplotypes observed radiated from the numerically dominant haplotype TABS_Gh1, is highly indicative of recent range expansion (Mortimer & van Vuuren 2007, Avise 1994, Conroy & Cook 2000, Jolly *et al.* 2005).

For the mite *S. mollis*, a connection limit of 95% confidence was able to successfully connect haplotypes TABS_Sm1 – 13, TABS_Sm14 – 16, TABS_Sm17 – 18, TABS_Sm19 – 20 and TABS_Sm21 - 22 into 5 separate groups (Fig. 6b). However, a connection limit of 95% confidence was not able to connect the resulting groups. A connection limit of 11 mutational steps was required to estimate the connection of groups TABS_Sm1 – 13 to TABS_Sm14 – 16, 83 steps to connect clades TABS_Sm19 -20 to TABS_Sm1 – 13, 85 steps to connect clades TABS_Sm17 -18 to TABS_Sm19 – 20 and 79 steps to connect TABS_Sm21 - 22 to TABS_Sm14 - 16 .

It was possible to resolve several loops within the *S. mollis* haplotype network using most parsimonious methods. However, a single mutational loop for haplotype

TABS_Sm8, which branched off equidistant between both haplotypes TABS_Sm7 and 9, was left unresolved due to unknown parsimony (Fig. 6b).

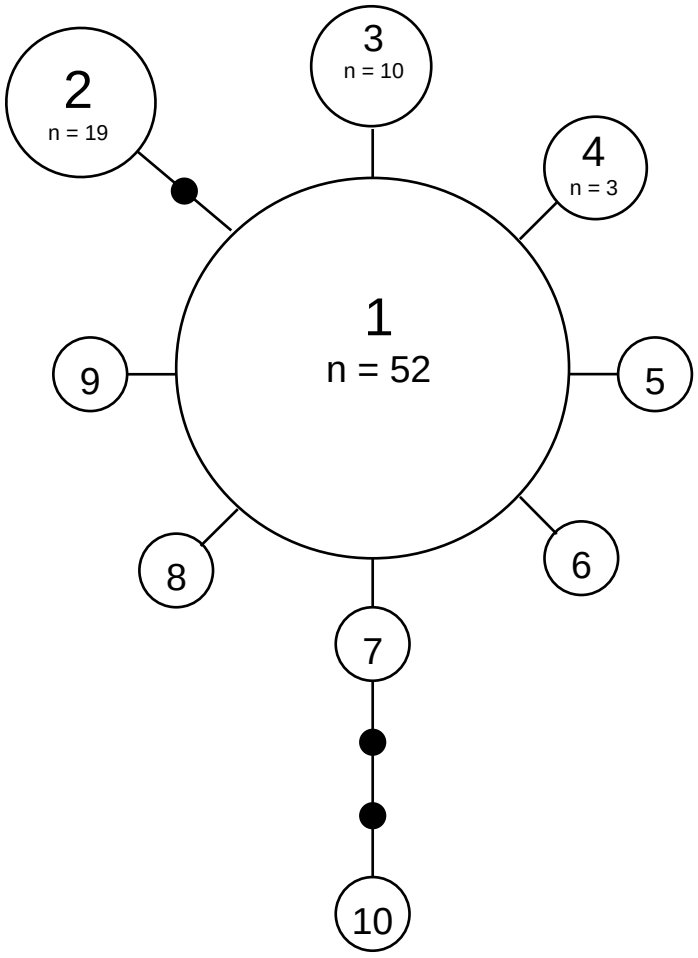


FIGURE 6a. Haplotype network for the 10 *Gomphicephalus hodgsoni* mtDNA COI haplotpyes from 90 individuals (TABS_Gh prefix omitted). Missing mutational steps between haplotypes are indicated by a '•'. Haplotype frequencies > 1 are indicated below the haplotype number and by relative size of circle.

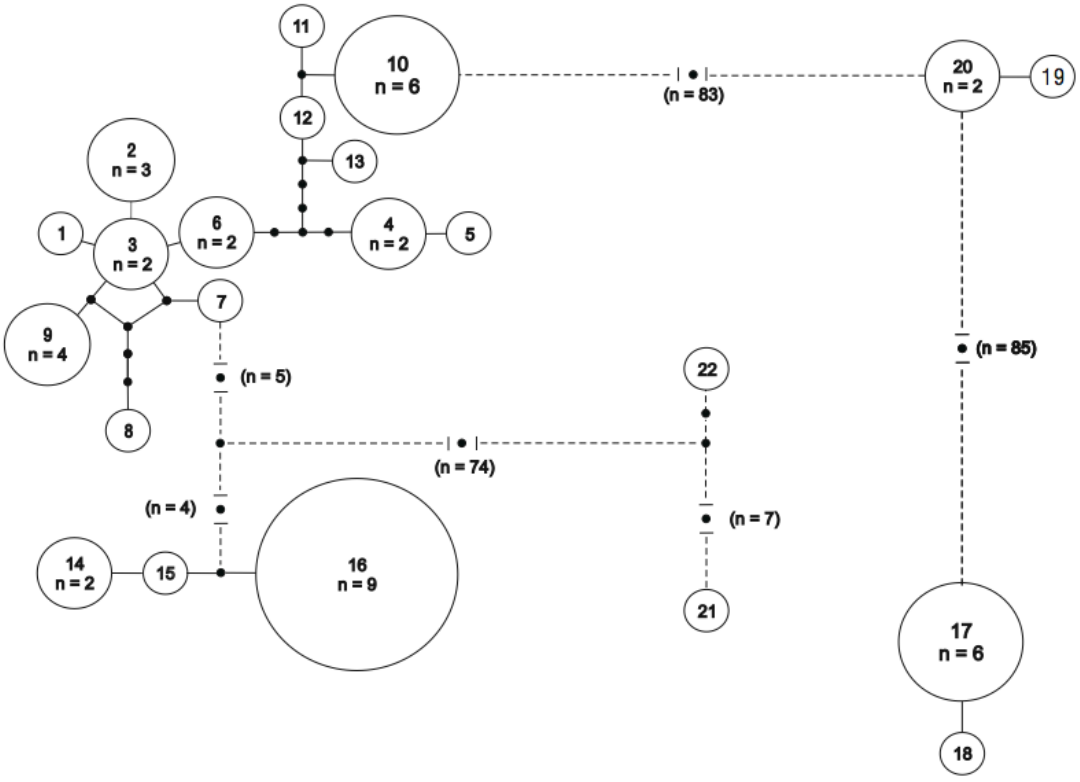


FIGURE 6b. Haplotype network for the 22 *Stereotydeus mollis* mtDNA COI haplotypes from 50 individuals (TABS_Sm prefix omitted). Missing mutational steps between haplotypes indicated by a '•'. Where distinct groups are found not to connect with 95% confidence (see methods) proposed links are indicated by "---| • |--- ". Haplotype frequencies >1 are indicated below the haplotype number and by relative size of circle.

DISCUSSION

Within all *G. hodgsoni* individuals the mtDNA (COI) sequences were extremely similar with only 11 variable nucleotide sites resulting in 10 haplotypes 599-bp in length. The most distantly related haplotype (TABS_Gh8) was only 0.8% divergent and was represented by a single individual in Garwood valley. All *G. hodgsoni* haplotypes within the study area were likely derived from a single maternal lineage, the numerically dominant and widespread haplotype TABS_Gh1. In comparison, a smaller representative sample size for *S. mollis* revealed 170 variable nucleotide sites that resulted in 22 mtDNA (COI) haplotypes 622-bp in length with levels of haplotype divergence up to 17.7%.

Sequence divergence values for *G. hodgsoni* were similar to those reported by McGaughran *et al.* (2008; 2010) from a smaller sample size taken in the same area. In addition, haplotype TABS_Gh1, which was most numerous in our study area, was identical to the numerically dominant haplotype identified in previous studies (Stevens and Hogg 2003, 2006; McGaughran *et al.* 2008, 2010). Of the 45 haplotypes identified by McGaughran *et al.* (2010), using a 471-bp portion of the mtDNA (COI) gene, three (TABS_Gh1, 4 & 8) were positively identified within our study area while all others were unique to our southern study area. Though the majority of *G. hodgsoni* haplotypes identified in this study (70%) were unique to our southern study area, they were only one mutational step different than other previous haplotypes identified by Stevens and Hogg (2003) and McGaughran *et. al* (2010) from the more northerly Taylor, Victoria and Wright Valleys (Fig. 2). Interestingly, haplotype TABS_Gh8 which was observed in a single individual from Marshall Valley (sample site MAV6), was identical to a 471-bp

mtDNA (COI) haplotype from a single individual found by McGaughran *et. al* (2010) at Mt. Seuss in the Granite Harbour region. Geographically, Mt. Seuss is more than 120 km north of our sample area. This observed haplotype sharing between the two areas may be further evidence of limited long range dispersal for this species as suggested by McGaughran *et al*. (2008; 2010).

Maximum uncorrected sequence divergences for *S. mollis* in this study were higher (17.7% versus 14.5%) than those previously reported by Stevens and Hogg (2006) and McGaughran *et al.* (2008). However, our findings of increased levels of sequence divergence may in part be attributed to the use of a larger portion of the *S. mollis* COI gene than in previous studies (622-bp versus 504-bp respectively). Of the 36, 504 –bp mtDNA (COI) haplotypes identified by Stevens and Hogg (2006) and McGaughran *et al.* (2008), 10 were found in our study area. However, two of the most divergent haplotypes TABSSm_19 and 20 (up to 14.1% uncorrected *p*–distance) were only identified from our southern study area. The remaining eight haplotypes, which were unique to our study area, were all less than 5 mutational steps different than 10 haplotypes identified from previous studies in the more northern Taylor Valley region (Stevens and Hogg, 2006, McGaughran *et al*. 2008).

If the common arthropod molecular clock, 1.4 – 2.3% sequence divergence per million years is used (Brower 1994, Juan *et al.* 1996, Knowlton & Weight 1998, Quek *et al.* 2004), a conservative estimate would suggest that *G. hodgsoni* populations within our study area diverged from a single maternal lineage between approximately 500,000 and 300,000 years bp. In comparison, this would suggest that *S. mollis* populations within our study area diverged within the last 11.8 – 7.08 Ma3. However, it has been suggested that

the extreme differences in mtDNA variation between mites and springtails on the continental Antarctic may be due to a disparity in nucleotide substitution rates (Stevens and Hogg, 2006). An increased mutation rate, which has been suggested for some mite taxa (Murrell *et al.*, 2005), combined with decreased generation times may shed some uncertainty on the common arthropod molecular clock when applied to *S. mollis*. However, the extreme mtDNA (COI) variability differences between mites and springtails within our study area cannot be explained solely by a differing substitution rate. If mites and springtails were to share a common phylogenetic history within our study area, *S. mollis* would be required to have a mtDNA (COI) substitution rate variation approximately 25-50 times faster than *G. hodgsoni*.

It has previously been suggested (Stevens and Hogg 2006) that mites and springtails shared a common phylogeographic history that was primarily shaped by Mio-Pliocene glacial cycling. Similarly, McGaughran *et al.* (2008), using an expanded sample size, found that the distributional pattern of mtDNA (COI) haplotypes for both species throughout Southern Victoria Land were also broadly similar; while at the same time different enough to suggest that other explanations may be valid. We found that the distribution of of *S. mollis* and *G. hodgsoni* haplotypes were distinctly dissimilar, suggesting that the two species do not share a common phylogeographic history within our more southern study area. For example, *G. hodgsoni* showed very reduced genetic variability resulting in a higher degree of haplotype sharing among locations than *S. mollis* (83 % vs. 36% respectively).

AMOVA indicated a higher degree of genetic structure for *S. mollis* than for *G. hodgsoni* due to the latter's highly reduced genetic variability and the restriction of the

most highly divergent *S. mollis* haplotypes to two locations within our study area (Miers and the bordering southern ridge separating it from Marshall Valley) (Fig. 4). Four of the most divergent haplotypes (TABS_Sm17 & 18 and TABS_Sm21 & 22) have been previously identified from other locations within Southern Victoria Land. Using a 504 bp portion of the same COI region Stevens and Hogg (2006) and McGaughran *et al.* 2008 identified four similar haplotypes from the more northern Dry Valleys and Beaufort Island, located off the northern coast of Ross Island to the north east of our study area. However, the two highly divergent haplotypes TABSSm_19 and 20 have only been observed in our southern sampling area. Taken together, this may suggest that *S. mollis* survived a longer period of isolation in multiple refugia throughout their Southern Victoria Land distribution. The divergent haplotypes TABSSm_19 & 20, which are unique to our southern sampling area, may have arisen in isolation from a southern refugial population whose mtDNA lineages are still restricted to this area.

In comparison, *G. hodgsoni* is most likely a recent colonist in our study area and/or the remnant of an extreme bottleneck event. The highly reduced nucleotide diversity and a simple star shaped haplotype network of *G. hodgsoni*, within our study area compared to that from their more northern range, is indicative of recent colonisation and range expansion away from a source population (Avise 1994, Hewitt 1996). The presence and distribution of the *G. hodgsoni* haplotype TABS_Gh3, which is unique to Garwood Valley and restricted to the vicinity of Garwood Glacier, may indicate ongoing and relatively recent *in situ* population genetic differentiation. However, the large Garwood Valley sample size in relation to the other sample locations may warrant caution.

The highly reduced genetic variability observed for all populations of *G. hodgsoni* is consistent with the more extreme nature of past Antarctic glacial cycling in our southern study area as compared to that of the northern Taylor Valley area, which harbours greater levels of genetic diversity and has been previously suggested as an area of past glacial refugia for *G. hodgsoni* (Stevens and Hogg, 2003 and McGaughran, 2008; 2010. The smaller geographic size, lower elevation and formation of large proglacial lakes (Hendy, 2000) during repeated glacial cycles over the last 200,000 years bp were much more severe (Denton *et al.*, 1984, Denton and Marchant, 2000), and likely a more formidable constraint on extant *G. hodgsoni* populations within our study area compared to those in the more northerly Dry Valleys. In comparison, *S. mollis* which has a much higher desiccation tolerance and greater dispersal capability than *G. hodgsoni* (Sinclair and Sjursen, 2001; Sjursen and Sinclair 2001), may have been able to better respond and recover to these successive glacial disturbances within, or in proximity to, our study area thus retaining higher levels of mtDNA (COI) variability. The occurrence of several *S. mollis* haplotypes in both our southern study area and the more northerly Dry Valleys may indicate past links between these two areas or be the result of greater dispersal rates. However, the restriction of the highly divergent haplotypes TABS_Sm19 and 20, as well as other unique derived haplotypes, to our southern study area may indicate a southern area of past glacial refugia and population expansion.

The small size, limited number of morphological characters available and high levels of intra-specific diversity can profoundly complicate the determination of Acari taxa (Navajas and Fenton, 2002). Though no morphological differences were observed in this study, the high levels of mtDNA (COI) sequence divergence found for *S. mollis* may

indicate the presence of a multi-species complex and/or cryptic speciation. Previous work on the genetic diversity of mites has been restricted primarily to species of agricultural, medical and veterinary importance with a worldwide distribution (Navajas and Fenton, 2002). For example, Ros and Breeuwer (2007) found maximum COI sequence divergence rates between several different species of *Tetranychus* spider mites ranged from 1-13% while intra-species divergence ranged from 0 - 7.3%. In comparison, Salomone *et. al* (2002) using a 471 – bp portion of the mtDNA COI gene reported maximum haplotype divergence values for the Orabatid mite *Steganacarus carlosi* from the same Canary Island up to 7.8% and divergence values between *S. carlosi* and *S. terenifensis* ranging from 9.8-15.8%. Surprisingly however, Salomone *et. al* (2002) reported mtDNA COI divergence values ranging from 16.8 -23.4% between *S. carlosi* haplotypes from different islands within the Canary chain.

Within our southern McMurdo Dry Valleys study area, we found very low levels of genetic diversity for the endemic springtail *G. hodgsoni* and very low levels of population substructuring. The low mtDNA sequence variation and haplotype distribution suggest a pattern of recent colonisation and/or a severe bottleneck event followed by subsequent range expansion within our study area. Close genetic links to the predominant mtDNA COI lineage found throughout the northern range of *G. hodgsoni* may suggest a pattern of "leptokurtic" (re)invasion via a northerly route following a period of unsuitable habitat availability and quality due to past glacial cycling. In addition, increased levels of haplotype diversity in Garwood Valley in comparison to the study area as a whole, and the presence of several closely related but unique haplotypes not found elsewhere may be evidence of ongoing divergence in isolation. We found very high levels of mtDNA (COI)

genetic diversity for the endemic mite *S. mollis* within our southern study area. Three of the four divergent mtDNA lineages and several of the individual haplotypes found in our study area have also been collected from the more northern Taylor Valley region. However, the presences of the highly divergent haplotypes TABS_Sm19 and 20, which have not been reported previously, suggest that *S. mollis* may have also survived periods of glaciation in a southern refuge in addition to the northern Dry Valley region.

In summary, the interaction between the geological characteristics, Mio-Pliocene glacial cycling and dispersal potential have resulted in a distinctly dissimilar distributional pattern of mtDNA (COI) haplotypes for both the mite *S. mollis* and the springtail *G. hodgsoni* within our southern study area. This extreme disparity between levels of mtDNA (COI) genetic variability of the two Antarctic arthropod taxa cannot be solely explained by a difference in mutational rates. The presence of two highly divergent *S. mollis* haplotypes (TABS_Sm19, 20) within our study area, which have not been identified elsewhere, suggest that *S. mollis* has had a longer association with our southern study area than *G. hodgsoni* or that this particular lineage has re-colonised from a southern glacial refugia that persisted in extreme isolation.

The presence of several mite lineages with connections back to the more northerly Dry Valleys and a high level of sequence divergence suggest that multiple colonisations of *S. mollis* from the north have also occurred within our study area (Ibrahim *et al.* 1996, Hewitt 1996). In addition, the increased levels of mtDNA (COI) variability of *S. mollis* populations further south, as compared to those of *G. hodgsoni*, predict that the distribution of *S. mollis* in Southern Victoria Land, and most certainly its geographic

center, range extend further south than that of *G. hodgsoni* (Arnaud-Haond *et al.* 2006, Eckert *et al.* 2007).

In order to resolve the relationships between the four highly divergent *S. mollis* mtDNA (COI) lineages, future work may benefit from: 1) The inclusion of nuclear markers like ITS2 in future molecular studies as advocated by Cruickshank (2002); and 2) the inclusion of physical, chemical and environmental data in an attempt to establish whether historic niche conservatism (Peterson et al. 1999) has driven and/or is maintaining the observed high levels mtDNA (COI) diversity in *S. mollis*

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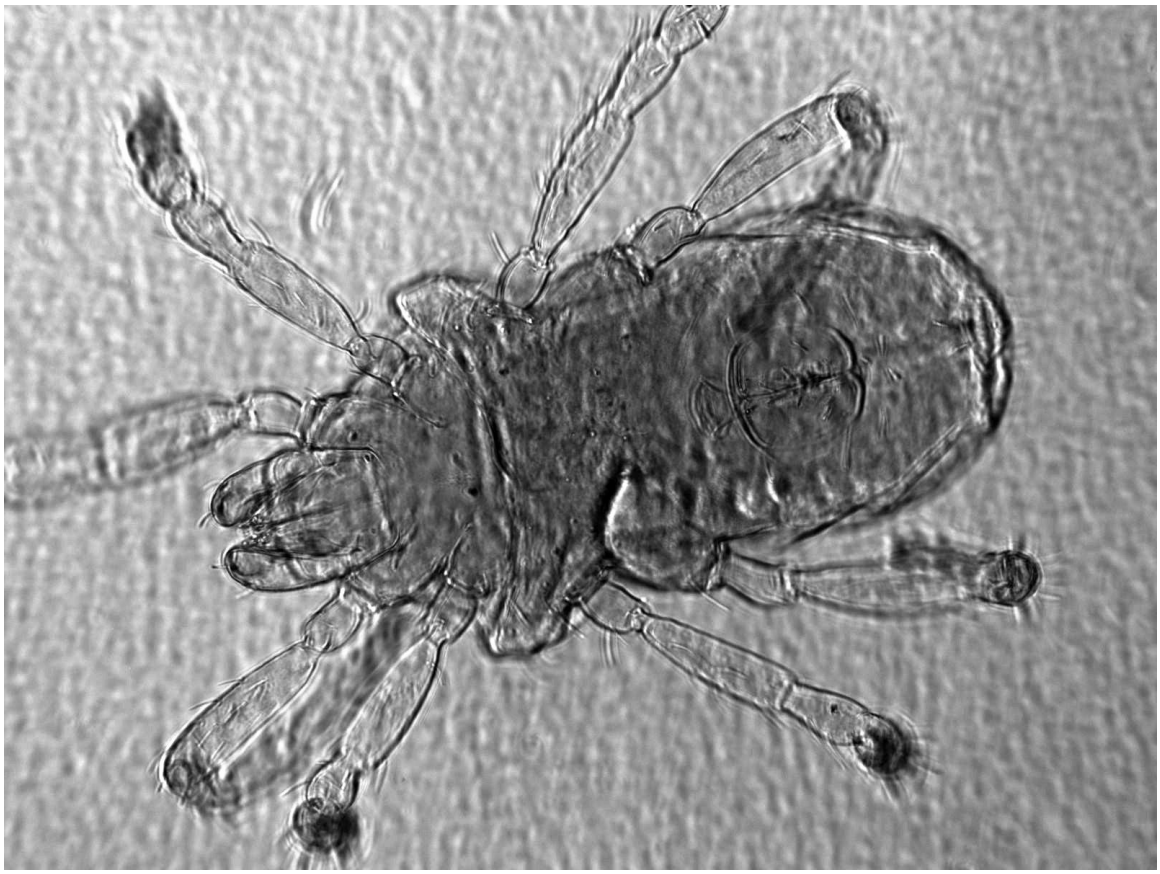
Chapter II

LATITUDINAL DISTRIBUTION AND MOLECULAR PHYLOGENY OF THE PROSTIGMATID MITE GENUS STEREOTYDEUS IN SOUTHERN VICTORIA LAND AND THE QUEEN MAUD MOUNTAINS, ANTARCTICA

Keywords: mtDNA, *Stereotydeus*, mites, Southern Victoria Land, Antarctica



Stereotydeus mollis (Acari: Prostigmata)

600X Magnification

Photo Credit: Nicholas J. Demetras

INTRODUCTION

The free living soil mites *Stereotydeus* Berlese, 1901 (Acari: Prostigmatidae) comprise a circum polar group of with a broad southern hemisphere distribution of ancient Gondwanan origin (Womersly & Strandtmann 1963, Fittkau *et al.*1969, Spain and Luxton 1971, Olivier 2006). Within the continental and maritime Antarctic, *Stereotydeus* is represented by eight species, seven of which are endemic (Marshall and Pugh 1996). Of the eight species, three (*S. belli*, *S. mollis*, *S. shoupi*) are known from Victoria Land and the Queen Maud Mountains (Womersley and Strandtmann 1963, Strandtmann 1967, Sinclair and Stevens 2006).

Stereotydeus belli Trouessart 1902 is common near the transition zone of Northern Victoria Land and Southern Victoria Land. Here, the southern-most distribution of *S. belli* has been reported to overlap with that of the northern-most distribution of *Stereotydeus mollis* Womersley & Strandtmann 1962 (ca. 74° 42’ S) (Caruso & Bargagli 2007). South of the Drygalski Ice tongue, located at 75° 24’ S, *S. mollis* becomes the dominant acarine inhabiting the permanently ice free continental areas and those of the offshore islands in the McMurdo Sound region (i.e. Ross and Beaufort Island) (Womersley and Strandtmann 1963, Sinclair and Stevens 2006). The southern distributional limit of *S. mollis* is somewhat uncertain, possibly due to a lack of sampling effort, but the species has been reported to occur as far south as Mt. Discovery and Minna Bluff (ca. 78° 40’ S) (Gressitt *et al.* 1963, Strandtmann 1967). South of Minna Bluff the available literature on the occurrence and distribution of mites is quite sparse. Spain (1971) carried out the first comprehensive arthropod surveys of the ice free regions in

proximity to Darwin Glacier (ca. 80° S) and reported a complete absence of both Collembola and Acari. However, during the austral summers of 2004 and 2007, individuals of *Stereotydeus* were collected from ice free areas adjacent to the Darwin Glacier (79° 49' S, 159° 26' W) (Hogg, unpublished data). South of the Darwin glacier, *Stereotydeus shoupi* Strandtmann 1967 is known from the ice free areas of the Queen Maud Mountains near the Beardmore and Shackleton Glaciers (ca. 83° - 85° S) (Strandtmann 1967, Stevens & Hogg 2006).

Throughout their respective geographic ranges, *S. belli*, *S.mollis*, and *S. shoupi* all share similar morphological characteristics that often overlap when the range of variation is compared between species (Strandtmann 1967). To compound matters, several developmental stages with differing morphological characteristics, may be present at a particular site or at differing sites due to environmental conditions (Gressitt *et al.* 1964). This can present a challenge when using morphological characteristics to distinguish between species. For example, one of the few defining characteristics between *S. mollis* and *S. shoupi* is the number of microscopic setae present on the genital cover, 6 for *S. mollis* and 7 for *S. shoupi*. However, the number of setae on each genital cover is different during each of the five developmental stages of *S. mollis* (Strandtmann 1967, Pittard 1971). Although no comprehensive studies of *S. shoupi* or *S. belli* has been conducted to date, it is reasonable to assume that similar morphological characteristics are likely to vary during the developmental stages for these species as well.

To compound the subtle inter and intra-specific morphological variation in Antarctic *Stereotydeus* spp., several recent studies have found a strikingly high degree of intra-specific mtDNA (COI) variation in otherwise morphologically similar populations

of *S. mollis* (Stevens & Hogg 2006, McGaughran *et al.* 2008, Chapter 1). Levels of mtDNA COI pairwise sequence divergence up to 17.7% have been reported from a population of *S. mollis* from the southern Dry Valleys of Southern Victoria Land suggesting the possibility of cryptic species (Chapter 1). Preliminary phylogenetic analysis of the Antarctic mite genus *Stereotydeus* by Stevens & Hogg (2006) using a 504 bp portion of the mtDNA COI gene provided some support that *S. mollis* from the Southern Victoria Land Dry Valleys formed a polyphyletic group with *S. shoupi* from the Beardmore Glacier region in the Queen Maud Mountains. However, since this preliminary study, several more mtDNA (COI) lineages of *S. mollis* have been identified which may improve the resolution and phylogenetic relationships among *Stereotydeus* spp. from Victoria Land and the Queen Maud Mountains.

Here, I include all known mtDNA (COI) sequences from *S. mollis, S. belli* and *S. shoupi*, and include several new sequences from the Darwin and Beardmore Glacier regions to examine the relationships between the highly divergent lineages of *S. mollis* and other Stereotydeus spp. from Victoria Land and the Queen Maud Mountains

MATERIALS and METHODS

Sample collection

This study includes 38 previously published unique mtDNA (COI) haplotypes for *Stereotydeus mollis* collected in Southern Victoria Land and deposited in GenBank (Stevens & Hogg 2006, GenBank accession numbers: DQ305386-88, DQ305390-97, DQ309572-74; McGaughran *et al.* 2008, GenBank accession numbers: DQ305361-84)(Table II.1) In addition, 12 previously unpublished *S. mollis* mtDNA (COI) haplotypes were identified from specimens collected from the southern Antarctic Dry Valleys (Miers, Marshall, and Garwood Valleys; Shangri La) in January 2009 (Fig. 2). Due to the differing haplotype nomenclature used in previous studies, and to aid in interpretation, these data sets were consolidated and simplified nomenclature assigned. Haplotypes were aligned and renamed using the generic prefix Sm followed by a unique numerical character (i.e. Sm1-50). Table 1 lists all of the unique *S. mollis* haplotypes used in this study and cross references them with those identified by both Stevens & Hogg (2006) and McGaughran *et al.* (2008).

Sequence data for *Stereotydeus shoupi* from the Queen Maud Mountains and for *S. belli* from Northern Victoria Land were obtained from GenBank. (Stevens & Hogg 2006, GenBank accession numbers DQ309576 and DQ309577, respectively). In addition, mites were collected from the Darwin Glacier region (Diamond Hill) in January 2007 and from the Beardmore Glacier region (Ebony Ridge) in January 2010. From these, four

individuals of *Stereotydeus* sp. were sequenced from Darwin Glacier and one from Beardmore Glacier.

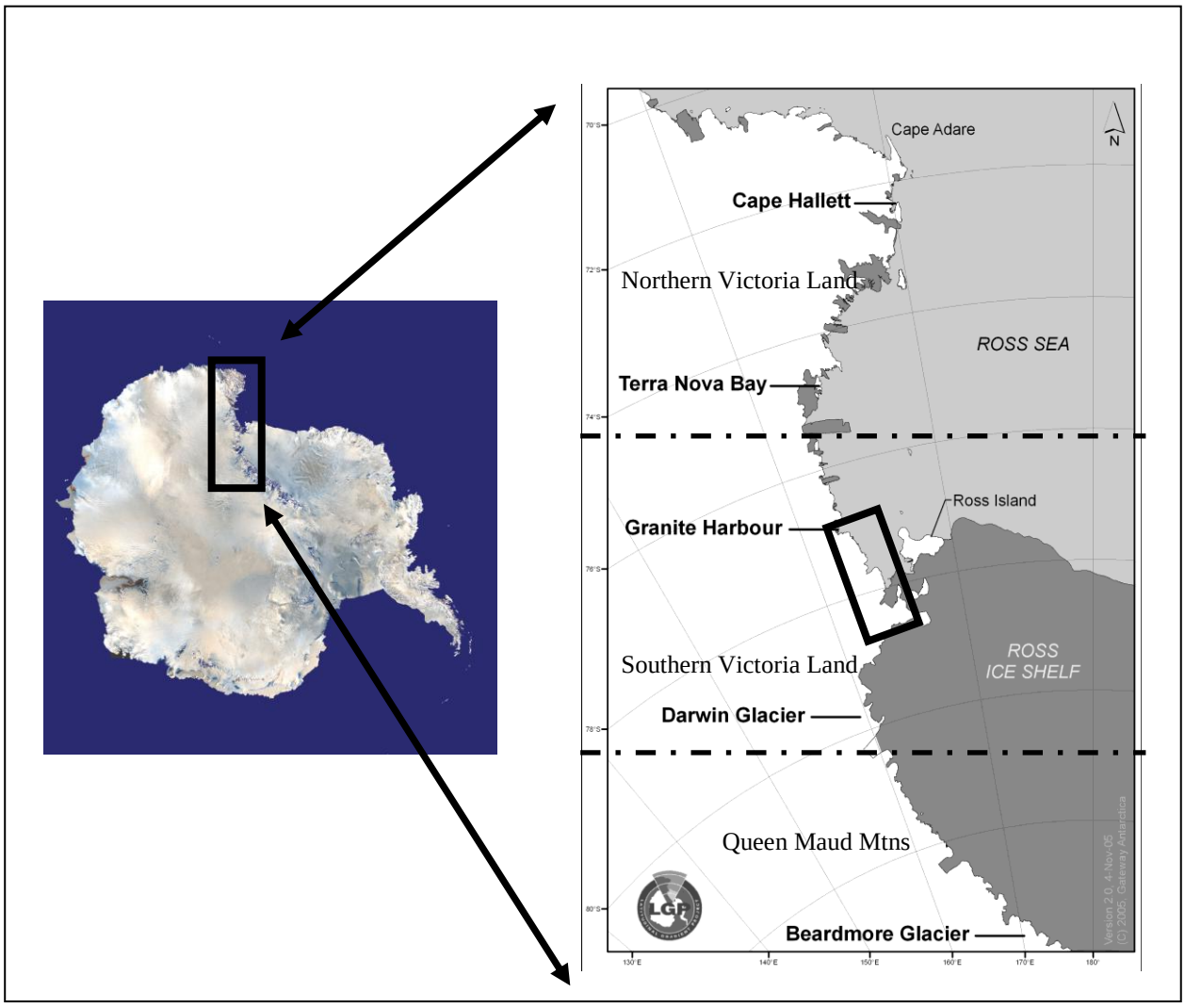


FIGURE 1. Locaton of the Victoria Land coast in relation to the Antarctic continent. Inset location (right) outlines the area of the McMurdo Dry Valley region. Dashed lines delineate the approximate arbitrary borders of Northern Victoria Land, Southern Victoria Land and the Queen Maud Mountains.

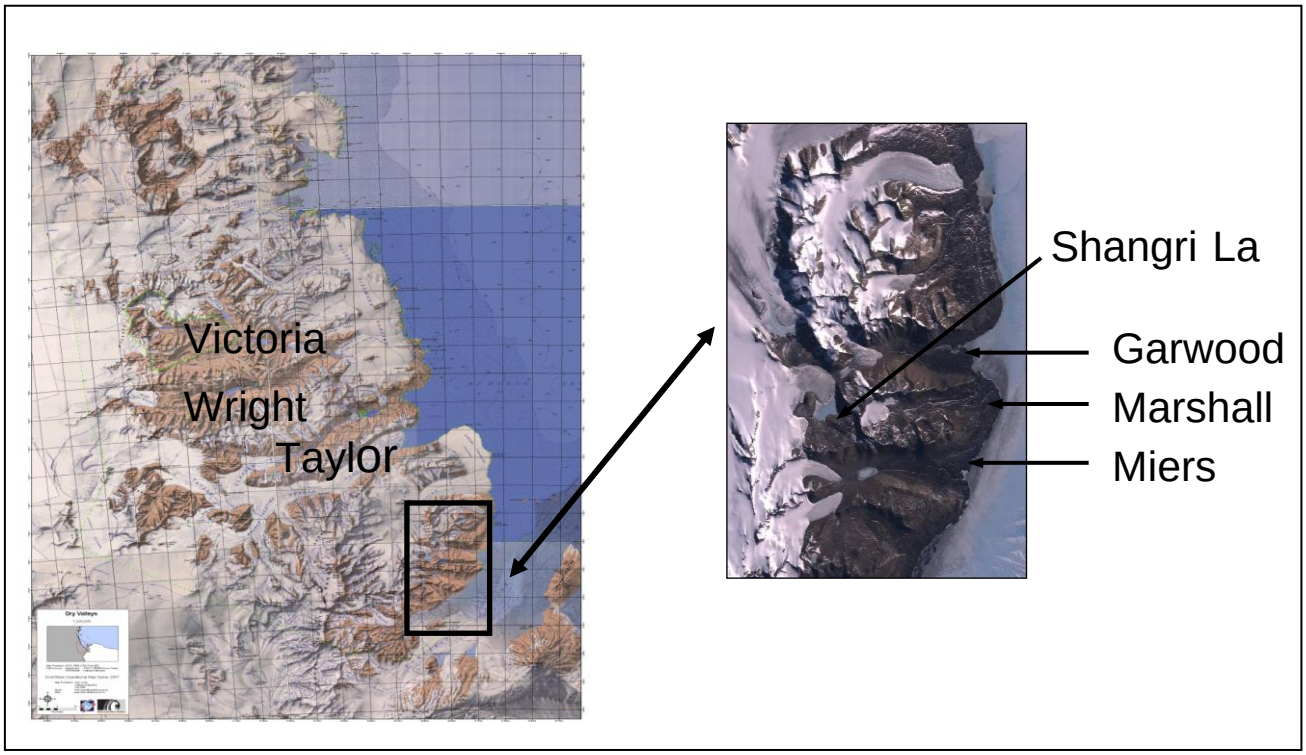


FIGURE 2. Location of the southern Dry Valley study area (inset) in comparison to the larger northern Dry Valleys of Victoria, Wright and Taylor.

Table 1. *Stereotydeus mollis* haplotype codes used with cross reference to previous research.

Haplotype code	Chapter I	Stevens & Hogg (2006)	McGaughran *et al.* (2008)	GenBank Asc. #
Sm1		A		DQ305386
Sm2		B		DQ305389
Sm3		D		DQ305391
Sm4	TABS_Sm13	E		DQ305398
Sm5	TABS_Sm4	F		DQ305392
Sm6	TABS_Sm7	G		DQ305396
Sm7	TABS_Sm3	H		DQ305368
Sm8	TABS_Sm9	I		DQ305387
Sm9	TABS_Sm6	J		DQ305397
Sm10	TABS_Sm16	K		DQ305385
Sm11		L		DQ305390
Sm12		M		DQ305394
Sm13		N		DQ305393
Sm14	TABS_Sm17/18	O		DQ309572
Sm15		P		DQ305395
Sm16		Q		DQ309573
Sm17		R		DQ309574
Sm18			S1	DQ305361
Sm19			S2	DQ305362
Sm20			S3	DQ305363
Sm21			S4	DQ305364
Sm22			S5	DQ305365
Sm23			S6	DQ305367
Sm24	TABS_Sm1		S7	DQ305369
Sm25			S8	DQ309370
Sm26			S9	DQ313371
Sm27			S10	DQ317372
Sm28			S11	DQ321373
Sm29			S12	DQ325374
Sm30			S13	DQ329375
Sm31			S14	DQ333376
Sm32			S15	DQ337377
Sm33			S16	DQ341378
Sm34			S17	DQ345379
Sm35			S18	DQ349380
Sm36			S19	DQ353381
Sm37			S20	DQ357382
Sm38			S21	DQ361383
Sm39			S22	DQ365384
Sm40	TABS_Sm2			To be added later
Sm41	TABS_Sm5			To be added later
Sm42	TABS_Sm8			To be added later
Sm43	TABS_Sm10			To be added later
Sm44	TABS_Sm11			To be added later

Table 1 cont. *Stereotydeus mollis* haplotype codes used with cross reference to previous research.

Chapter II	Chapter I	Stevens & Hogg (2006)	McGaughran *et al.* (2008)	GenBank Asc. #
Sm45	TABS_Sm12			To be added later
Sm46	TABS_Sm14			To be added later
Sm47	TABS_Sm15			To be added later
Sm48	TABS_Sm19/20			To be added later
Sm49	TABS_21			To be added later
Sm50	TABS_22			To be added later

mtDNA extraction, amplification and sequencing

Total genomic DNA was extracted from individual animals using the SIGMA REDExtract-N-Amp™ Tissue PCR Kit. Due to the small size of individual animals (0.5-0.75 mm), the manufacturers recommended volume of extraction buffer was reduced by 90% to concentrate the resulting DNA extract. Following extraction, a 675-bp fragment of the mitochondrial cytochrome *c* oxidase (COI) gene was amplified using the mite specific primers COI-2R and COI-2F (Otto & Wilson 2001). PCR amplification was carried out in a 20 μL reaction containing 4 μL of extracted DNA (unquantified), 1.0 μM of each primer and 10 μL of *i*-Taq™ 2X PCR master mix (iNtRON Biotechnology, Gyeonggi-do, Korea). Thermocycling conditions 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 (McGaughran *et al.* 2008).

All PCR products were purified using SAP/EXO (USB Corp., Cleveland, OH, USA). Sequencing used both forward and reverse primers and was performed directly on a capillary electrophoresis ABI 3130XL genetic analyser (Applied Biosystems Inc., Foster City, CA) at the University of Waikato DNA sequencing facility.

Phylogenetic analyses

Individual sequences were confirmed as being derived from applicable taxa using the GenBank BLAST algorithm. We also compared pairwise sequence alignments with those from the distantly related, co-distributed, prostigmatid mite *Nanorchestes antarcticus* (Demetras and Hogg unpubl. data) in order to verify that no *N. antarcticus* were erroneously assigned as *Stereotydeus* sp. All sequences were aligned using Geneious Pro v4.7.6 (Drummond *et al.* 2009). PAUP* ver.4.0b10 (Swofford 2002) was used to perform neighbour joining (NJ) analysis. Due to the number of sequences (n=58) and available computing power, the Genetic Algorithm for Rapid Likelihood Inference as implemented in the computer program GARLI ver0.951 (Zwickl 2006) was used for Maximum Likelihood analysis (ML). Several runs were performed in GARLI as suggested by the author in order to obtain the corresponding ML tree of best fit (Zwickl 2006). The prostigmatid mite *Eriohynchus sp.* (GenBank accession No. AF142135; Otto and Wilson 2001) was used as an outgroup taxon as it was the most closely related taxon amongst prostigmatic species available on GenBank (Stevens and Hogg 2006). χ^2 tests as employed in PAUP* were used to determine whether the assumption of equal base frequencies among sequences was violated on all sites and third codon positions only. jModeltest ver 3.7 (Posada and Crandall 1998) was used to ascertain the correct substitution model of evolution for maximum likelihood (ML) heuristic searches (using all unique sequences). Bootstrap replicates (n=1024) were performed to assess confidence in both NJ and ML analyses. The appropriate ML model selected was HKY+I+Γ (-lnL = 3158.0623 (AIC); Ti/tv ratio = 2.8729 I = 0.5320 Γ = 0.7250: with base frequencies set

to A = 0.4094 C = 0.0919 G = 0.1180 T = 0.3807). All other options in GARLI remained as default. Distance matrices of pairwise nucleotide sequence divergences were calculated in PAUP* using all unique sequences (Appendix I).

RESULTS

mtDNA sequence variability

A 504 bp (168 codons) portion of unambiguous alignment (no insertions or deletions) of the COI gene, was used to match the existing data set as reported by Stevens & Hogg (2006) and McGaughran *et al.* (2008), resulted in a combined data set of 50 haplotypes for the mite *S. mollis*. Four 504 bp haplotypes were obtained from the four *Stereotydeus* sp. collected from the Darwin Glacier ice free region and a single haplotype from one *Stereotydeus shoupi* collected from the Beardmore Glacier ice region. The nucleotide composition averaged over all sequences showed an A-T bias of 69.0-71 % for all species (A = 35.8%, T = 33.2%, C = 16.1%, G = 14.9% for *S. mollis*; A = 35.7%, T = 35.5%, C = 16.1%, G = 12.9% for *S. shoupi*; A = 35.9%, T = 34.8%, C = 15.8%, G = 13.5% for *Stereotydeus* sp. from the Darwin Glacier; and A = 35.3%, T = 34.1%, C = 15.7%, G = 14.9% for *S. belli*). Base frequencies were homogenous among all sites (χ^2 = 20.6141, p = 1.00 , d.f = 168), and for third codon positions (168 sites, A – T = 82.02%; χ^2 = 21.451, p = 1.00 , d.f = 168), across all taxa.

Among all the *Stereotydeus* sequences there were 326/168 constant/variable sites. Sequence divergence for the fifty COI haplotypes of *S. mollis* ranged from 0.2% to 17.5% (uncorrected *p*-distances). The two *S. shoupi* sequences from the Beardmore Glacier were identical and were 13.7%-17.9% divergent relative to the 50 *S. mollis* haplotypes. The four *Stereotydeus* sp. sequences from the Darwin Glacier (79.5°S) were 0.2%- 0.6% divergent from each other and were 8.5%-8.7% divergent from the two *S. shoupi*

collected from the Beardmore Glacier (83.5°S) and 14.3-18.1% divergent in comparison to the 50 *S. mollis* haplotypes. The single *S. belli* sequence from Cape Hallett (71°S) was 14.3%-14.5% divergent from the four *Stereotydeus* sp. from the Darwin Glacier, 13.3% divergent from the two *S. shoupi* from the Beardmore Glacier and 9.5%-14.1% divergent in comparison to the 50 *S. mollis* haplotypes (Appendix 2.1).

Nucleotide substitutions were present at all codon positions in all taxa resulting in 35 unique amino acid substitutions among *S. mollis* and nine unique amino acid substitutions amongst *S. shoupi* and *Stereotydeus* sp. A single amino acid polymorphism separated the two *S. shoupi* sequences from the Beardmore Glacier from the four individuals of *Stereotydeus* sp. from the Darwin Glacier. For the single *S. belli* sequence, the majority of nucleotide substitutions (98% or 1/47) occurred at "silent" third codon positions and resulted in only one amino acid polymorphism when compared to *S. mollis* haplotypes.

Phylogenetic analyses

Both the NJ and ML trees were significantly congruent and placed 48 of the 50 *S. mollis* haplotypes into five divergent clades with bootstrap support $\geq$50%. There was extremely strong bootstrap support in both the NJ and ML analyses for the placement of *S. shoupi* as basal to all other taxa analysed. In addition, both NJ and ML consistently grouped all four *Stereotydeus* sp. collected from the Darwin Glacier with *S. shoupi* collected from the Beardmore Glacier; $\geq$97% bootstrap support for both NJ and ML analyses (Fig. 3a & b)

In both the NJ and ML analyses there was considerable disagreement in the placement of the highly divergent (up to 17.5 %) *S. mollis* haplotype Sm48 and the single available *S. belli* sequence. NJ analysis placed both *S. belli* and the *S. mollis* haplotype Sm16 within a highly divergent clade with low bootstrap support (<50%) and Sm48 as basal to all other *S. mollis* haplotypes with very strong bootstrap support (97%) (Fig. 3a) Conversely, ML analysis grouped Sm48 and Sm16 together into a similar highly divergent clade with low bootstrap support (<50%) while placing *S. belli* as basal to all *S. mollis* haplotypes with strong bootstrap support (72%) (Fig. 3b).

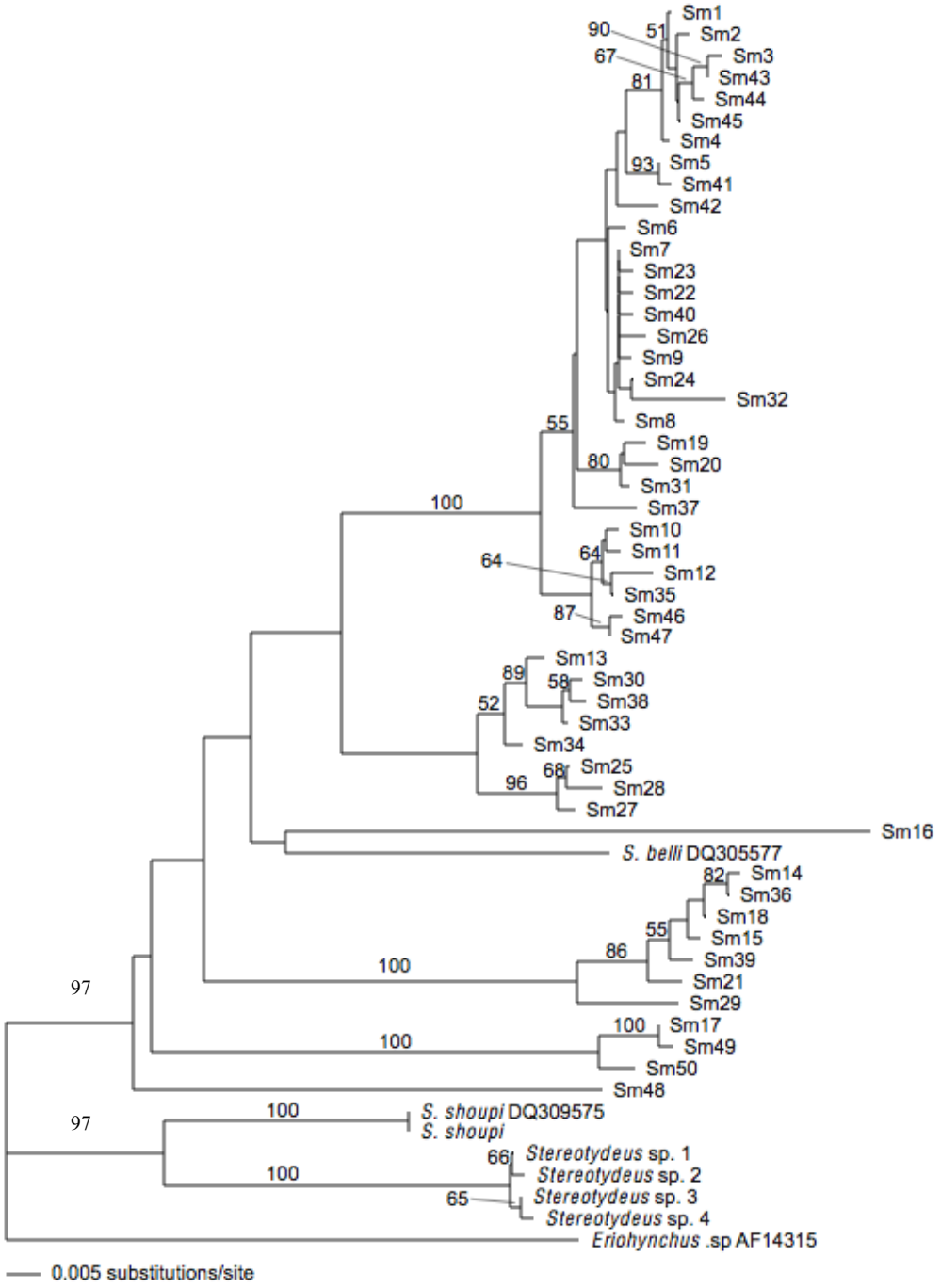


FIGURE 3a. Neighbour joining phylogram based upon all available *Stereotydeus* sequences from Southern Victoria Land and the Queen Maud Mountains using a 504 bp fragment of the mtDNA (COI) gene. Bootstrap confidence limits (1024 replicates) shown above nodes. Codes are those used in Table 1.

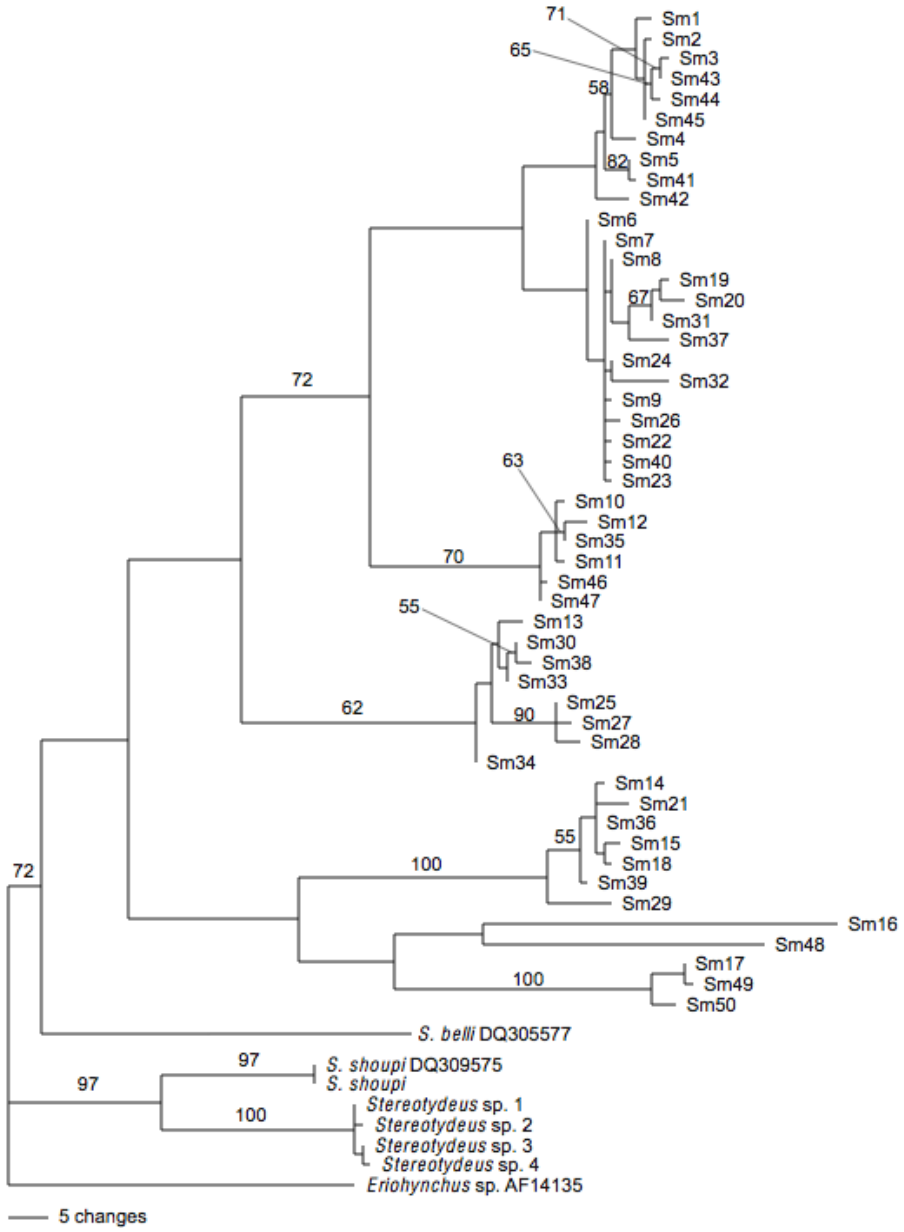


FIGURE 3b. Maximum likelihood phylogram based upon the substitution model HKY+I+Γ (-lnL = 3158.0623 (AIC); Ti/tv ratio = 2.8729 I = 0.5320 Γ = 0.7250: with base frequencies set to A = 0.4094 C = 0.0919 G = 0.1180 T = 0.3807) derived from jModeltest (see methods), using a 504-bp fragment of the mtDNA (COI) gene from all available *Stereotydeus* sequences from Southern Victoria Land. Codes are those used in Table 1.

DISCUSSION

The 504-bp portion of the mtDNA (COI) gene from the 50 known haplotypes of *S. mollis* used in this study showed very high levels of intra specific divergence (0.2 - 17.5% uncorrected-*p* distance). These levels of divergence exceeded the levels of inter-specific divergence found between the three recognised species of Southern Victoria Land *Stereotydeus*; *belli, mollis* and *shoupi*. Both NJ and ML analyses revealed a complex tree topology with five well supported *S.mollis* and two discrete, well supported clades which grouped the *S. shoupi* sequences from the Beardmore Glacier with the four *Stereotydeus* sp. from the Darwin Glacier in both the NJ and ML trees. However, both NJ and ML analyses disagreed on the placement of both *S. belli* and the highly divergent *S. mollis* haplotype Sm48. This may be an artefact of the current data set due to the use of only a single *S. belli* sequence in the analyses and not necessarily the utility of the mtDNA (COI) gene at the species level (Linares *et al.* 2009). For example, the inclusion of additional *S. mollis* and *S. shoupi* sequences, along with the four individuals of *Stereotydeus* sp. from the Darwin glacier, increased the resolution of the phylogeny by placing *S. shoupi* as a monophyletic sister taxa with strong bootstrap support basal to all *S. mollis* haplotypes. This is in contrast to the position of *S. shoupi* as a paraphyletic group within *S. mollis* as reported by Stevens & Hogg using a more limited data set (2006).

The mtDNA (COI) gene has been widely accepted as a suitable molecular marker for the phylogenetic study of mite taxa and for investigating both the intra-specific relationships of populations at the species level as well as the inter-specific relationships

of closely related species (Navajas & Fenton (2002), Cruickshank (2002), Dabert (2006). Boyer *et al.* (2007) found a similar pattern of extremely high COI variability (up to 19.2% uncorrected-*p* distance) in the Arachnid *Aoraki denticulata denticulata* endemic to the South Island of New Zealand. The inclusion of several sequences from the subspecies *A. denticulata major*, as well as other closely related sister taxa within the genus *Aoraki*, resulted in a deep branching phylogeny which was well supported by bootstrap analysis and suggested the presence of several cryptic species. These results were in agreement with previous research which has found increased resolution of high diversity phylogenies with the inclusion of a representative range of molecular data from closely related taxa (Talavera & Castresana 2007).

Despite being unable to completely resolve the phylogenetic relationships of the three Southern Victoria Land *Stereotydeus*, due to the conflicting placement of *S. belli* and the highly divergent *S. mollis* haplotype Sm48, the probability of cryptic species occurring within *S. mollis* remains. The extreme glacial cycling of Southern Victoria Land and isolating conditions of Antarctica may have led to the high levels of genetic divergence observed and without resulting in reproductive isolation.

Based on these mtDNA COI sequence data, I conclude that the northern range of *S. shoupi* extends into Southern Victoria Land (Darwin Glacier) and that *S. shoupi* is a monophyletic sister taxa of *S. mollis*. The availability of only a single *S. belli* COI sequence severely limited the ability to accurately place it within the phylogeny as evidenced by the low bootstrap values and differing placements within the NJ and ML trees. The addition of further *S. belli* sequences, from across its distribution, may help resolve its evolutionary relationship with that of the highly divergent lineages of *S.*

mollis. Sequence data from other Antarctic *Stereotydeus* sp. from across the continent holds the promise of a more comprehensive understanding of the phylogeny and evolutionary history of Antarctic *Stereotydeus*.

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APPENDIX I. Genetic distance (uncorrected) base upon the variation in the unique mtDNA COI (504-bp aligned sites) of the *S. mollis*, *S. belli*, *S. shoupi* & *Stereotydeus* sp. as reported in Chapter II. Codes are those referred to in Table 1

Haplotype	Sm1	Sm2	Sm3	Sm4	Sm5	Sm6	Sm7	Sm8	Sm9	Sm10	Sm11	Sm12	Sm13	Sm14	Sm15	Sm16	Sm17
Sm1																	
Sm2	0.004																
Sm3	0.008	0.008															
Sm4	0.004	0.004	0.008														
Sm5	0.014	0.014	0.018	0.010													
Sm6	0.012	0.012	0.020	0.012	0.010												
Sm7	0.012	0.016	0.020	0.012	0.010	0.004											
Sm8	0.014	0.018	0.022	0.014	0.012	0.006	0.002										
Sm9	0.014	0.014	0.018	0.010	0.008	0.006	0.002	0.004									
Sm10	0.022	0.026	0.030	0.022	0.030	0.024	0.024	0.026	0.026								
Sm11	0.022	0.026	0.030	0.022	0.030	0.024	0.024	0.026	0.026	0.004							
Sm12	0.024	0.028	0.032	0.028	0.036	0.030	0.030	0.032	0.032	0.010	0.010						
Sm13	0.075	0.075	0.083	0.077	0.075	0.069	0.071	0.069	0.073	0.062	0.065	0.067					
Sm14	0.139	0.139	0.147	0.141	0.147	0.141	0.143	0.145	0.145	0.137	0.141	0.137	0.121				
Sm15	0.131	0.131	0.139	0.133	0.139	0.133	0.135	0.137	0.137	0.129	0.133	0.129	0.117	0.008			
Sm16	0.145	0.141	0.147	0.141	0.143	0.141	0.145	0.147	0.143	0.143	0.147	0.151	0.147	0.171	0.167		
Sm17	0.145	0.145	0.153	0.147	0.147	0.141	0.143	0.145	0.145	0.141	0.145	0.141	0.121	0.161	0.159	0.173	
Sm18	0.133	0.133	0.141	0.135	0.141	0.135	0.137	0.139	0.139	0.131	0.135	0.131	0.115	0.006	0.006	0.165	0.157
Sm19	0.020	0.024	0.028	0.020	0.026	0.020	0.016	0.014	0.018	0.028	0.028	0.034	0.069	0.141	0.137	0.145	0.143
Sm20	0.020	0.024	0.028	0.020	0.026	0.020	0.020	0.018	0.022	0.024	0.024	0.030	0.069	0.139	0.135	0.147	0.143
Sm21	0.129	0.129	0.137	0.131	0.137	0.131	0.133	0.135	0.135	0.127	0.131	0.131	0.111	0.010	0.014	0.161	0.157
Sm22	0.014	0.018	0.022	0.014	0.012	0.006	0.002	0.004	*0.004*	0.026	0.026	0.032	0.073	0.145	0.137	0.143	0.143
Sm23	0.014	0.018	0.022	0.014	0.012	0.006	0.002	0.004	0.004	0.026	0.026	0.032	0.073	0.145	0.137	0.147	0.145
Sm24	0.014	0.018	0.022	0.014	0.012	0.006	0.002	0.004	0.004	0.026	0.026	0.032	0.073	0.145	0.137	0.143	0.145
Sm25	0.075	0.075	0.083	0.077	0.079	0.073	0.075	0.077	0.077	0.069	0.073	0.075	0.024	0.119	0.111	0.153	0.121
Sm26	0.016	0.020	0.024	0.016	0.014	0.008	0.004	0.006	0.006	0.028	0.028	0.034	0.071	0.143	0.135	0.145	0.143
Sm27	0.075	0.075	0.083	0.077	0.079	0.073	0.075	0.077	0.077	0.073	0.077	0.079	0.028	0.123	0.115	0.149	0.121
Sm28	0.081	0.081	0.089	0.083	0.085	0.079	0.081	0.083	0.083	0.075	0.079	0.077	0.026	0.117	0.113	0.159	0.123
Sm29	0.127	0.127	0.135	0.129	0.135	0.129	0.131	0.133	0.133	0.125	0.129	0.125	0.109	0.028	0.032	0.181	0.155

APPENDIX I. Cont. Genetic distance (uncorrected) base upon the variation in the unique mtDNA COI (504-bp aligned sites) of the *S. mollis*, *S. belli*, *S. shoupi* & *Stereotydeus* sp. as reported in Chapter II. Codes are those referred to in Table 1.

Haplotype	Sm18	Sm19	Sm20	Sm21	Sm22	Sm23	Sm24	Sm25	Sm26	Sm27	Sm28
Sm19	0.135										
Sm20	0.133	0.008									
Sm21	0.012	0.131	0.129								
Sm22	0.139	0.018	0.022	0.135							
Sm23	0.139	0.018	0.022	0.135	0.004						
Sm24	0.139	0.018	0.022	0.135	0.004	0.004					
Sm25	0.113	0.077	0.077	0.109	0.077	0.077	0.077				
Sm26	0.137	0.020	0.024	0.133	0.006	0.006	0.006	0.075			
Sm27	0.117	0.077	0.077	0.113	0.077	0.077	0.077	0.004	0.075		
Sm28	0.111	0.079	0.079	0.111	0.083	0.083	0.083	0.006	0.081	0.010	
Sm29	0.030	0.131	0.127	0.034	0.133	0.133	0.133	0.109	0.131	0.113	0.105

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Haplotype	Sm1	Sm2	Sm3	Sm4	Sm5	Sm6	Sm7	Sm8	Sm9	Sm10	Sm11	Sm12	Sm13	Sm14	Sm15	Sm16	Sm17
Sm30	0.081	0.081	0.089	0.083	0.081	0.075	0.077	0.075	0.079	0.067	0.071	0.073	0.010	0.127	0.119	0.149	0.123
Sm31	0.020	0.024	0.028	0.020	0.022	0.016	0.012	0.010	0.014	0.028	0.028	0.034	0.071	0.143	0.139	0.149	0.145
Sm32	0.024	0.028	0.032	0.024	0.024	0.020	0.016	0.018	0.018	0.034	0.034	0.040	0.073	0.135	0.131	0.143	0.141
Sm33	0.079	0.079	0.087	0.081	0.079	0.073	0.075	0.073	0.077	0.065	0.069	0.071	0.008	0.125	0.117	0.151	0.121
Sm34	0.071	0.071	0.079	0.073	0.071	0.065	0.067	0.065	0.069	0.062	0.065	0.067	0.012	0.121	0.113	0.143	0.119
Sm35	0.018	0.022	0.026	0.022	0.030	0.024	0.024	0.026	0.026	0.004	0.004	0.006	0.063	0.139	0.131	0.147	0.143
Sm36	0.137	0.137	0.145	0.139	0.145	0.139	0.141	0.143	0.143	0.135	0.139	0.135	0.119	0.002	0.006	0.169	0.161
Sm37	0.020	0.020	0.028	0.024	0.026	0.016	0.016	0.014	0.018	0.024	0.028	0.030	0.063	0.137	0.129	0.145	0.141
Sm38	0.081	0.081	0.089	0.083	0.081	0.075	0.077	0.075	0.079	0.071	0.075	0.077	0.014	0.129	0.121	0.149	0.121
Sm39	0.131	0.131	0.139	0.133	0.139	0.133	0.135	0.137	0.137	0.129	0.133	0.129	0.113	0.008	0.012	0.163	0.155
Sm40	0.014	0.018	0.022	0.014	0.012	0.006	0.002	0.004	0.004	0.026	0.026	0.032	0.071	0.145	0.137	0.147	0.145
Sm41	0.016	0.016	0.020	0.012	0.002	0.012	0.012	0.014	0.010	0.032	0.032	0.038	0.077	0.149	0.141	0.145	0.149
Sm42	0.014	0.018	0.022	0.014	0.012	0.010	0.010	0.008	0.012	0.030	0.030	0.032	0.075	0.147	0.139	0.149	0.145
Sm43	0.006	0.006	0.002	0.006	0.016	0.018	0.018	0.020	0.016	0.028	0.028	0.030	0.081	0.145	0.137	0.147	0.151
Sm44	0.006	0.006	0.006	0.006	0.016	0.018	0.018	0.020	0.016	0.024	0.028	0.030	0.077	0.141	0.133	0.143	0.147
Sm45	0.002	0.002	0.006	0.002	0.012	0.014	0.014	0.016	0.012	0.024	0.024	0.026	0.077	0.141	0.133	0.143	0.147
Sm46	0.022	0.026	0.030	0.022	0.030	0.024	0.024	0.026	0.026	0.008	0.008	0.014	0.065	0.139	0.131	0.147	0.145
Sm47	0.020	0.024	0.028	0.020	0.028	0.022	0.022	0.024	0.024	0.006	0.006	0.012	0.063	0.137	0.129	0.145	0.143
Sm48	0.139	0.139	0.141	0.143	0.145	0.141	0.141	0.143	0.143	0.141	0.143	0.143	0.131	0.133	0.133	0.157	0.139
Sm49	0.147	0.147	0.155	0.149	0.149	0.143	0.145	0.147	0.147	0.143	0.147	0.143	0.123	0.163	0.161	0.175	0.002
Sm50	0.141	0.141	0.149	0.143	0.143	0.137	0.139	0.141	0.141	0.137	0.141	0.141	0.117	0.157	0.155	0.171	0.014
S. shoupi 1	0.147	0.145	0.153	0.149	0.147	0.147	0.147	0.149	0.147	0.147	0.151	0.145	0.137	0.167	0.167	0.179	0.153
S. shoupi 2	0.147	0.145	0.153	0.149	0.147	0.147	0.147	0.149	0.147	0.147	0.151	0.145	0.137	0.167	0.167	0.179	0.153
Stereotydeus sp.1	0.163	0.163	0.171	0.165	0.171	0.167	0.167	0.169	0.169	0.161	0.165	0.165	0.145	0.173	0.169	0.179	0.167
Stereotydeus sp.2	0.165	0.165	0.173	0.167	0.173	0.169	0.169	0.171	0.171	0.163	0.167	0.167	0.147	0.175	0.171	0.181	0.169
Stereotydeus sp.3	0.165	0.165	0.173	0.167	0.173	0.169	0.169	0.171	0.171	0.163	0.167	0.167	0.147	0.171	0.167	0.179	0.169
Stereotydeus sp.4	0.167	0.167	0.175	0.169	0.175	0.171	0.171	0.173	0.173	0.165	0.169	0.169	0.149	0.173	0.169	0.181	0.171
S. belli AF14135	0.111	0.115	0.119	0.111	0.109	0.107	0.103	0.105	0.105	0.105	0.109	0.113	0.099	0.141	0.137	0.131	0.139
Eriohynchus sp. AF142135	0.171	0.171	0.179	0.173	0.173	0.173	0.175	0.177	0.177	0.171	0.173	0.175	0.163	0.167	0.171	0.194	0.181

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Haplotype	Sm18	Sm19	Sm20	Sm21	Sm22	Sm23	Sm24	Sm25	Sm26	Sm27	Sm28	Sm29	Sm30	Sm31	Sm32	Sm33	Sm34	Sm35
Sm30	0.121	0.079	0.079	0.117	0.079	0.079	0.079	0.022	0.077	0.026	0.024	0.111						
Sm31	0.137	0.004	0.008	0.133	0.014	0.014	0.014	0.079	0.016	0.079	0.081	0.131	0.081					
Sm32	0.129	0.032	0.036	0.125	0.018	0.018	0.014	0.077	0.020	0.077	0.083	0.123	0.075	0.028				
Sm33	0.119	0.077	0.077	0.115	0.077	0.077	0.077	0.020	0.075	0.024	0.022	0.109	0.002	0.079	0.073			
Sm34	0.115	0.069	0.069	0.111	0.069	0.069	0.069	0.020	0.067	0.024	0.026	0.107	0.010	0.071	0.069	0.008		
Sm35	0.133	0.028	0.024	0.129	0.026	0.026	0.026	0.071	0.028	0.075	0.077	0.127	0.069	0.028	0.034	0.067	0.063	
Sm36	0.004	0.139	0.137	0.008	0.143	0.143	0.143	0.117	0.141	0.121	0.115	0.026	0.125	0.141	0.133	0.123	0.119	0.137
Sm37	0.131	0.016	0.024	0.127	0.018	0.018	0.018	0.071	0.020	0.071	0.077	0.127	0.069	0.016	0.028	0.067	0.063	0.024
Sm38	0.123	0.079	0.079	0.119	0.079	0.079	0.079	0.020	0.077	0.024	0.022	0.113	0.004	0.081	0.079	0.006	0.010	0.073
Sm39	0.006	0.133	0.131	0.014	0.137	0.137	0.137	0.111	0.135	0.115	0.109	0.026	0.119	0.135	0.127	0.117	0.113	0.131
Sm40	0.139	0.018	0.022	0.135	0.004	0.004	0.004	0.075	0.006	0.075	0.081	0.133	0.077	0.014	0.018	0.075	0.067	0.026
Sm41	0.143	0.028	0.028	0.139	0.014	0.014	0.014	0.081	0.012	0.081	0.087	0.137	0.083	0.024	0.026	0.081	0.073	0.032
Sm42	0.141	0.022	0.022	0.141	0.012	0.012	0.012	0.083	0.014	0.081	0.085	0.135	0.081	0.018	0.026	0.079	0.071	0.030
Sm43	0.139	0.026	0.026	0.135	0.020	0.020	0.020	0.081	0.022	0.081	0.087	0.133	0.087	0.026	0.030	0.085	0.077	0.024
Sm44	0.135	0.026	0.026	0.131	0.020	0.020	0.020	0.077	0.022	0.077	0.083	0.129	0.083	0.026	0.030	0.081	0.073	0.024
Sm45	0.135	0.022	0.022	0.131	0.016	0.016	0.016	0.077	0.018	0.077	0.083	0.129	0.083	0.022	0.026	0.081	0.073	0.020
Sm46	0.133	0.028	0.024	0.129	0.026	0.026	0.026	0.069	0.028	0.073	0.075	0.127	0.071	0.028	0.038	0.069	0.062	0.008
Sm47	0.131	0.026	0.022	0.127	0.024	0.024	0.024	0.067	0.026	0.071	0.073	0.125	0.069	0.026	0.036	0.067	0.060	0.006
Sm48	0.133	0.137	0.137	0.127	0.143	0.143	0.143	0.125	0.137	0.125	0.129	0.145	0.135	0.139	0.141	0.133	0.131	0.141
Sm49	0.159	0.145	0.145	0.159	0.145	0.147	0.147	0.123	0.145	0.123	0.125	0.157	0.125	0.147	0.143	0.123	0.121	0.145
Sm50	0.153	0.139	0.139	0.149	0.139	0.141	0.141	0.117	0.139	0.121	0.123	0.151	0.119	0.141	0.137	0.117	0.115	0.139
S. shoupi 1	0.165	0.149	0.151	0.165	0.149	0.149	0.149	0.145	0.143	0.141	0.147	0.169	0.143	0.151	0.149	0.141	0.133	0.147
S. shoupi 2	0.165	0.149	0.151	0.165	0.149	0.149	0.149	0.145	0.143	0.141	0.147	0.169	0.143	0.151	0.149	0.141	0.133	0.147
Stereotydeus sp.1	0.173	0.167	0.171	0.171	0.169	0.169	0.169	0.149	0.163	0.145	0.155	0.177	0.147	0.171	0.169	0.149	0.141	0.163
Stereotydeus sp.2	0.175	0.169	0.173	0.173	0.171	0.171	0.171	0.147	0.165	0.143	0.153	0.179	0.149	0.173	0.171	0.151	0.143	0.165
Stereotydeus sp.3	0.171	0.169	0.173	0.169	0.171	0.171	0.171	0.147	0.165	0.143	0.153	0.175	0.149	0.173	0.171	0.151	0.143	0.165
Stereotydeus sp.4	0.173	0.171	0.175	0.171	0.173	0.173	0.173	0.149	0.167	0.145	0.155	0.177	0.151	0.175	0.173	0.153	0.145	0.167
S. belli AF14135	0.135	0.103	0.107	0.131	0.105	0.105	0.105	0.095	0.099	0.095	0.099	0.137	0.099	0.107	0.101	0.097	0.091	0.109
Eriohynchus sp. AF142135	0.165	0.175	0.175	0.165	0.173	0.177	0.177	0.165	0.171	0.161	0.165	0.175	0.171	0.179	0.175	0.169	0.163	0.173

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Haplotype	Sm36	Sm37	Sm38	Sm39	Sm40	Sm41	Sm42	Sm43	Sm44	Sm45	Sm46	Sm47	Sm48
Sm37	0.135												
Sm38	0.127	0.073											
Sm39	0.006	0.129	0.121										
Sm40	0.143	0.018	0.077	0.137									
Sm41	0.147	0.028	0.083	0.141	0.014								
Sm42	0.145	0.022	0.081	0.139	0.012	0.014							
Sm43	0.143	0.026	0.087	0.137	0.020	0.018	0.020						
Sm44	0.139	0.022	0.083	0.133	0.020	0.018	0.020	0.004					
Sm45	0.139	0.022	0.083	0.133	0.016	0.014	0.016	0.004	0.004				
Sm46	0.137	0.032	0.071	0.131	0.026	0.032	0.030	0.028	0.028	0.024			
Sm47	0.135	0.030	0.069	0.129	0.024	0.030	0.028	0.026	0.026	0.022	0.002		
Sm48	0.135	0.135	0.133	0.131	0.143	0.143	0.147	0.141	0.137	0.141	0.141	0.139	
Sm49	0.163	0.143	0.123	0.157	0.147	0.151	0.147	0.153	0.149	0.149	0.147	0.145	0.141
Sm50	0.157	0.137	0.117	0.151	0.141	0.145	0.147	0.147	0.143	0.143	0.141	0.139	0.139
S. shoupi 1	0.169	0.145	0.141	0.165	0.149	0.145	0.147	0.151	0.147	0.147	0.149	0.147	0.147
S. shoupi 2	0.169	0.145	0.141	0.165	0.149	0.145	0.147	0.151	0.147	0.147	0.149	0.147	0.147
Stereotydeus sp.1	0.175	0.161	0.147	0.173	0.167	0.169	0.173	0.169	0.165	0.165	0.163	0.161	0.161
Stereotydeus sp.2	0.177	0.163	0.149	0.175	0.169	0.171	0.175	0.171	0.167	0.167	0.165	0.163	0.163
Stereotydeus sp.3	0.173	0.163	0.149	0.171	0.169	0.171	0.175	0.171	0.167	0.167	0.165	0.163	0.163
Stereotydeus sp.4	0.175	0.165	0.151	0.173	0.171	0.173	0.177	0.173	0.169	0.169	0.167	0.165	0.165
S. belli AF14135	0.139	0.107	0.097	0.133	0.101	0.107	0.111	0.117	0.113	0.113	0.109	0.107	0.127
Eriohynchus sp. AF142135	0.169	0.173	0.169	0.165	0.177	0.171	0.179	0.177	0.173	0.173	0.173	0.171	0.165

APPENDIX I. Cont. Genetic distance (uncorrected) base upon the variation in the unique mtDNA COI (504-bp aligned sites) of the *S. mollis*, *S. belli*, *S. shoupi* & *Stereotydeus* sp. as reported in Chapter II. Codes are those referred to in Table 1.

Haplotype	Sm49	Sm50	S. shoupi 1	*S. shoupi* 2	*Stereotydeus* sp. 1	*Stereotydeus* sp. 2	*Stereotydeus* sp. 5	*Stereotydeus sp 6*	S. belli
Sm50	0.016								
S. shoupi 1	0.155	0.153							
S. shoupi 2	0.155	0.153	0.000						
Stereotydeus sp.1	0.169	0.161	0.085	0.085					
Stereotydeus sp.2	0.171	0.163	0.087	0.087	0.002				
Stereotydeus sp.3	0.171	0.163	0.087	0.087	0.002	0.004			
Stereotydeus sp.4	0.173	0.165	0.087	0.087	0.004	0.006	0.002		
S. belli AF14135	0.141	0.137	0.133	0.133	0.143	0.145	0.143	0.145	
Eriohynchus sp. AF142135	0.181	0.175	0.149	0.149	0.147	0.149	0.147	0.149	0.149

THESIS CONCLUSION

Geographic patterns of genetic variation and the structure of intra-specific phylogenetic partitioning are determined by a complex interaction of historical and contemporary processes of which both vicariance and dispersal have long been recognised as important factors (Avise 2000). Through the use of mtDNA molecular data it is possible to describe the geographical distribution, phylogenetic relationships and genetic distances among evolutionary lineages of organisms, which can lead to a better understanding of regional biogeography and areas of endemism (Bermingham & Moritz 1998). The prevailing isolating conditions of the Antarctic and its well studied glacial history over the last ten million years have provided an ideal habitat in which to apply these molecular techniques.

In this thesis I have used genetic data, specifically the mtDNA (COI) gene, to investigate the phylogeographic pattern and origin of two free living Antarctic endemic soil arthropods in the southern Dry Valleys of Victoria Land, Antarctica. In addition, I have also investigated the phylogenetic relationships of three free living mites of the genus *Stereotydeus* (Prostimatidae: Acari), which occur along a latitudinal gradient from Northern Victoria Land south into the Queen Maud Mountains.

In chapter 1, I found extremely low levels of mtDNA genetic variability (0.2%-0.8% uncorrected-*p* distance) for the Southern Victoria Land endemic springtail, *G. hodgsoni* in our study area located in the southern most Dry Valleys (Marshall, Miers, Garwood Valley; Shangri La). Construction of a parsimony-based haplotype network revealed a simple star shaped pattern of nine haplotypes which were connected by less

than two mutational steps to a wide spread, numerically dominant ancestral haplotype. Analysis of molecular variance (AMOVA) found very low support for population genetic structure within our study area as evidenced by the wide spread distribution of the most numerically dominant haplotype TABS_Gh1, which was present at 45 out of 52 sites sampled. A comparison of the mtDNA (COI) haplotypic data from our southern study area with that of Stevens & Hogg (2003; 2006) and McGaughran *et al.* (2008; 2010), from the northern portion of *G. hodgsoni's* range, revealed that the numerically dominant haplotype in our study area was identical to that across *G. hodgsoni's* entire range. It was also noted that the more northern Dry Valley region of Victoria, Wright and Taylor Valleys harboured much higher levels of COI divergence than our more southern study area (up to 2.5% vs. 0.8% respectively) (McGaughran *et al.* 2010).

Taken together, the extremely low genetic variation, absence of population genetic structure and the wide spread distribution of a single common haplotype indicate that *G. hodgsoni* is most likely a recent colonist in our study area with strong links back to populations in the more northern Dry Valley region of Victoria, Wright and Taylor Valleys. This is in agreement with the more severe glacial cycling over the past 200,000 years and relatively low topographical relief of our study area (<1100 m elevation), which most likely restricted *G. hodgsoni* to inland ice free areas of higher elevation further north. Specifically, the inter-valley habitats found in the Asgard Range and the Kukri Hills in the vicinity of Victoria, Wright and Taylor Valleys (Stevens & Hogg 2003, McGaughran *et al.* 2008). Through the process of "leptokurtic" dispersal into suitable habitats during interglacial periods, relatively rare long distance migrants, who successfully dispersed into and colonised our study area resulted in the establishment of

a population with very little genetic differentiation (Ibrahim *et al.* 1996, Hewitt 1996, Huensdorfer *et al.* 2005). Due to the maternally inherited, haplotypic and non-recombining nature of mtDNA, populations that arise via relatively rare leptokurtic dispersal and expand to fill their isolated habitat may become fixed for the founding mtDNA lineage (Carranza & Arribas 2008) as was found in our southern study area.

In comparison, I found extremely high levels of mtDNA genetic variablity (0.8%-17.5% uncorrected-*p* distance) for the Southern Victoria Land endemic mite, *S. mollis* from the same study area. Construction of a parsimony based haplotype network revealed a very disjunct, complex pattern, with five distinct clades separated by up to 85 mutational steps. AMOVA revealed a greater degree of population structure due in part to the restriction of the most divergent haplotypes to the southern portion of our study area. Ten of the 622-bp COI haplotypes found in our study area were identical at 504-bp to the COI haplotypes identified by Stevens & Hogg (2006) and McGaughran *et al.* (2008) from the northern Dry Valleys. In addition, another ten haplotypes identified in our southern Dry Valley study area were less than 5 mutational steps different than those mentioned above. However, the two divergent haplotypes TABS_Sm19 & 20 (up to 14.1% pairwise divergent compared to all other *S. mollis* haplotypes), were unique to our southern study area. Levels of sequence divergence in our southern study area were higher than those found by Stevens & Hogg (2006) and McGaughran *et al.* (2008) from the northern Dry Valleys. However, this can be explained in part by the use of a longer sequence from the mtDNA COI gene (622-bp vs. 504-bp) than in previous studies.

Taken together the high levels of genetic variation, greater degree of population genetic structure and the occurrence of the unique, divergent haplotypes TABS_Sm19 &

20 in our study area suggest that *S. mollis* may have had a much longer association with our southern Dry Valley study area than *G. hodgsoni* and or been recolonised by multiple refugial *S. mollis* populations located throughout Southern Victoria Land. A normal or stepping stone mode of dispersal for *S. mollis* migrants into our study area would result in and maintain the current pattern of high mtDNA genetic variability observed for *S. mollis* in this study (Ibrahim *et al.* 1996, Hewitt 1996, Bohonak 1999). This in agreement with the findings that diel levels of activity are much higher for *S. mollis* than *G. hodgsoni* (Stevens & Hogg (2003) and that *S. mollis* is much more desiccation tolerant than *G. hodgsoni* (Sinclair & Sjursen 2001, Sjursen & Sinclair 2002).

It is clear that the phylogeographic patterns found for both *S. mollis* and *G. hodgsoni* are dissimilar in our southern study area and likely the result of the differing dispersal capabilities between the two species. From a conservation perspective, isolated populations of species who are monophyletic at mtDNA alleles with low dispersal capabilities (as we found for *G. hodgsoni*) may constitute environmentally significant units that may be undergoing speciation in isolation due to genetic drift as well as local selective pressures (Crandall *et al.* 2000, Moritz 1994).

Chapter II aimed to resolve the phylogenetic relationship amongst the three mite species of the genus *Stereotydeus* present in Southern Victoria Land and the Queen Maud Mountains in light of the extreme mtDNA COI genetic diversity observed in *S. mollis* across its entire range (up to 17.5 % uncorrected-*p* distance). Though no morphological differences were detected in Chapter I and the previous studies of Stevens & Hogg (2006) and McGaughran *et al.* (2008) the possibility remained that the extreme sequence divergence observed may be due to a complex of morphologically cryptic

species. To address this, NJ and ML analysis were performed on 39 previously published and publicly available *S. mollis* mtDNA COI haplotypes obtained from GenBank (Stevens & Hogg 2006, McGaughran *et al.* 2008), 11 previously unpublished *S. mollis* haplotypes collected from the southern Dry Valleys of Marshall, Miers, Garwood; Shangri La, 2 *S. shoupi* sequences from the Beardmore Glacier, 4 *Stereotydeus* sp. from the Darwin Glacier and a sequence of *S. belli* from the Northern Victoria Land/Southern Victoria Land transition zone (Stevens & Hogg 2006).

The resulting ML and NJ trees were significantly congruent and were able to successfully place *S. shoupi* as basal to all *S. mollis* haplotypes with very strong bootstrap support. The four individuals of *Stereotydeus* sp. collected from the Darwin Glacier were also placed within a monophyletic clade as a sister taxa to *S. shoupi* with very strong bootstrap support. However, NJ and ML analyses disagreed on the placement of *S. belli* within the resulting phylogenies. NJ analysis placed *S. belli* in the middle of the phylogeny, effectively separating 39 of the least divergent *S. mollis* mtDNA lineages from the 11 most divergent, with bootstrap support well below 50%. Conversely, ML analysis placed *S. belli* as basal to all *S. mollis* haplotypes with bootstrap support well below 50%. This disagreement was likely the result of the availability of a single *S. belli* sequence in both the ML and NJ analyses. As was evident with *S. shoupi,* the inclusion of multiple mtDNA haplotype sequences resulted in intra-specific information, which dramatically increased the power of phylogenetic analyses and resulted in the correct placement of taxa (Moore 1995, Funk *et al*. 1995). Though I was unable to work out the exact placement of *S. belli* amongst the highly divergent *S. mollis* haplotypes, I was able

to report and expand the distribution *S. shoupi* into Southern Victoria Land in the vicinity of the Darwin Glacier

In this thesis, I have used genetic data in a phylogeographic and phylogenetic context to study the dispersal, colonisation and speciation of Antarctic arthropods in light of historical landscape processes. The application of genetic data to highly fragmented Antarctic populations can also be beneficial from a management perspective to identify unique populations of high conservation value and to better understand how they may respond to global climate changes. The use of accurate phylogenetic data will also aid in the definition and delineation of species and a better understanding of current Antarctic biodiversity.

Future Research

The phylogeography of both *G. hodgsoni* and *S. mollis* has been extensively studied throughout their Southern Victoria Land range using the mtDNA (COI) gene (Stevens & Hogg 2003; 2006, McGaughran *et al.* 2008; 2010, Chapter 1). The maternal inheritance, rate of mutation (ca. 1.4 – 2.5% per MYA) and availability of generic primers make mtDNA the marker of choice for investigating historical processes across a landscape wide setting (Avise 2000, Knowlton & Weigt 1998). However, for finer scale analyses of population genetic structure, alternative nuclear markers such as microsatellites or AFLP's, which are bi-parentally inherited with high mutation rates, may be more informative (e.g. Van dur Wurff *et al.* 2003, Hinomoto *et al.* 2009). Nevertheless, such markers do have drawbacks. The isolation and testing of useable microsatellites can be a time consuming and expensive endeavour, while the shear amount of data generated by AFLPs (not all of which is useable), can be daunting. However, the potential gain in knowledge from the use of such molecular markers should compensate for the cost and time required for development. Another potentially fruitful avenue for future research, would be to add sequence data from the rDNA Internally Transcribed Spacer region 1 (ITS1) to the phylogenetic work for *S. mollis*. ITS1 has proven to be very complimentary to COI in the delineation of species within Acari phylogenies and would be a very useful along with the addition of further *Stereotydeus* spp. from across the Antarctic continent (Cruickshank 2002, Navajas & Fenton 2002, Dabert 2006). Together, both of these approaches would provide further insights on historic processes of speciation as well as present day processes of dispersal, gene flow and levels of biodiversity of Antarctic arthropods.

Nicholas J. Demetras; Sierra Nevada Pale Ale in hand hand, Mt. Erebus in background

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