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Phylogeography of Austral soil invertebrates

A Thesis submitted in fulfillment of the requirements

for the degree of Doctor of Philosophy

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For Mother

Declaration Statement

The work presented here is, to the best of my knowledge and belief, original except as acknowledged in the text. I hereby declare that I have not submitted this thesis, either in full or in part, for a degree at this or any other institution.

Giles M. Ross

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Abbreviations

ACC	Antarctic Circumpolar Current
ASPA	Antarctic Specially Protected Areas
BEAST	Bayesian Evolutionary Analysis Sampling Trees
BLAST	Basic Local Alignment Search Tool
BOLD	Barcode of Life Database
COI	Cytochrome Oxidase Subunit I
c-p	Colonizer-persister scale
DEC	Disperal-Extinction-Cladogenesis process
DNA	Deoxyribose Nucleic Acid
Ga	Billions of Years
GDM	Generalised Dissimilarity Model
GenAlEx	Genetic Analysis in Excel
Ka	Thousands of Years
LGM	Last Glacial Maximum
Ma	Millions of Years
MAP	Mean Annual Precipitation
MAT	Mean Annual Temperature
MEGA	Molecular Evolutionary Genetics Analysis
MT	Morphotype
MtDNA	Mitochondrial DNA
OTU	Operational Taxonomic Unit
RASP	Reconstruct Ancestral State in Phylogenies

rRNA	Ribosomal ribonucleid acid
SOM	Soil Organic Matter
VL	Victoria Land

Abstract

Soil invertebrates are terrestrial animals belonging to ancient phyla that emerged almost half a billion years ago. They have since spread throughout all known landmasses, with contemporary distributions governed by geological and environmental change across spatial and temporal gradients across the globe. However, limited knowledge of southern hemisphere (Austral) species hampers our ability to discern the general patterns of distribution and speciation. The lack of robust taxonomic information has also constrained our understanding of the evolutionary relationships and functional roles of the diverse soil fauna. This thesis capitalises on the development in molecular tools and improved sequence libraries to explore the factors that define the distribution and diversity of common soil invertebrates, specifically oribatid mites (Acari), springtails (Collembola) and nematodes (Nematoda). I investigated communities at continental-scales from maritime Antarctica and Australia to enable greater resolution of the drivers of distribution that might be applicable to southern hemisphere taxa more broadly.

In a literature review I introduce the bioinformatic approaches using phylogeography to resolve evolutionary theories concerning soil fauna indigenous to Antarctica. Phylogenetic evidence supports most soil faunal groups as having ancient origins, refugial survival and repeated colonisation, whilst also highlighting the benefits of comparative analyses over larger scales. In addition, I show in a perspectives paper that morphological and functional traits are phylogenetically constrained in nematodes and springtails, allowing function to be partially conferred for ‘unknown’ species using sequencing approaches.

Baseline biodiversity across a transect through maritime Antarctica found contrasting distributions of mites and springtails and the influences of climatic factors at broad scales and soil microhabitat conditions at local scales. Detailed population genetic analysis of genotypes of the oribatid mites *Podacarus auberti* and *Membranoppia loxolineata* alongside the springtail

Cryptopygus antarcticus revealed the importance of multiple dispersal events in their ancestral past, supporting theories of refugial survival. Comparative analysis of phylogeographic reconstructions with an analogous Australian transect highlighted that the importance of dispersal differs among mites and springtails and supported the influences of climate and edaphic factors on assemblage structure. These different influences of biogeography and climatic variability related to inherent morphological and physiological traits of the study organisms demonstrate potentially contrasting responses to future episodes of environmental change. With such knowledge, conservation strategies of Austral soil fauna can be re-focussed to ensure their continued persistence in terrestrial ecosystems.

This thesis comprises two literature reviews with one having been published in the peer-reviewed journal *Austral Ecology* (Chapter 3). This chapter largely relied on publicly available data but alse included data collected by my co-authors M. Berg and S. Salmon who provided feedback on the manuscript before publication. Data chapters 4 and 5 analyse soil faunal samples collected by U. Nielsen and colleagues. However, I counted, sorted, sequenced and analysed all invertebrates. Environmental datasets were kindly supplied by B. Ball. I collected and analysed all invertebrate and environmental data in chapter 6.

Chapter 1 - Introduction

1.1 Soil fauna and belowground ecosystems

Since the first scientific descriptions of soil, its myriad of microbes and fauna have been regarded as a constituent factor of the complex organo-mineral substrate that supports all terrestrial ecosystems (Darwin, 1881; Dokuchaev, 1883). The structure and functioning of soil systems rests on the delicate balance between the vegetation aboveground and the biota belowground. Since their emergence over 450 million years ago (Ma), soil fauna have populated every continent, including Antarctica (Nielsen, 2019). They now represent some of the most biodiverse terrestrial communities, although current estimates indicate that most species remain undescribed (Mora et al., 2011). Soil is estimated to support a quarter of all multicellular biota found on Earth (Coleman and Whitman, 2005). However, their minute sizes belie their significant contribution to important processes, such as decomposition and nutrient cycling, making them central to ecosystem functioning (Bardgett and van der Putten, 2014; Nielsen et al., 2015).

Soil fauna range in size from the microscopic nematodes and protozoa to mesofauna including the arthropod mites and springtails, that are often classified as measuring between 0.1 mm and 2 mm in width. Larger macrofauna over 2 mm in width and 1 cm in length include ants, beetles and earthworms act as ecosystem engineers (Briones, 2014; Nielsen, 2019). Whilst the intermediate class of mesofauna are visible to the naked eye, magnification is still required for accurate identification. Their small size, poor morphological differentiation between species and cryptic speciation (i.e. nearly identical species) poses challenges regarding their accurate identification. Soil fauna are known to contribute to multiple processes including decomposition and nutrient cycling that are essential for ecosystem functioning (Bardgett and Van der Putten,

2014). Whilst we have a firm knowledge of their ecological importance, gaps remain in our ability to assess their distribution and diversity at broad and fine scales, as well as linking species assemblage composition with morphological traits and ecosystem functionality. Applying genetic measures of taxonomic distance to species identification have assisted in overcoming these issues, and have allowed the biodiversity, functional taxonomy and evolutionary history of soil fauna to be addressed in far greater resolution (Avise, 2000; Beheregary et al., 2008; Vamosi et al., 2009). With only about 14% of all soil faunal species having been described, and limited sequence coverage of southern hemisphere taxa, there is an opportunity to expand our phylogeographic knowledge of soil fauna in general. Hence, using molecular approaches to establish diversity from a rigorous sampling campaign can provide robust measures of baseline biodiversity, and identify the general drivers of soil faunal biogeography.

In this thesis, I use molecular approaches to determine the phylogenetic relatedness, population haplotype structure and genetic distances associated with communities of mites, springtails and nematodes to resolve the drivers of their distribution, speciation, contributions to ecosystem functioning and phylogeography of southern hemisphere ecosystems. Soil fauna from maritime Antarctica are compared with those from the Australian Eastern Seaboard given similarities in geographical constraints and environmental influences. Earlier studies suffered from limited sampling opportunity due to the harsh regional conditions and fragmented landscape (Chown et al., 2015; Convey, 2011), and either clustered around research stations or easily accessible coastal areas. I capitalised on access to samples collected as part of large-scale survey of soils in maritime Antarctica and carried out a complementary survey of similar scale on an analogous transect in Australia.

1.2 Soil faunal genetics

Molecular tools have greatly improved our ability to identify species and assess biodiversity, particularly where classification is hampered by poor morphological differentiation, limited taxonomic expertise and a high number of undescribed species (Orgiazzi et al., 2015), which includes most soil fauna. Similarly, identifying early developmental stages of preserved taxa can be problematic; for example, larval stages of Ameronothroidae oribatid mites are often indistinguishable (Wallwork, 1967; Marshall and Convey, 2002). These issues contribute to soil fauna being understudied with low conservation priority. Their role in ecosystem functioning such as organic matter breakdown and nutrient cycling should be researched further to truly understand their contribution to sustaining ecosystem integrity. Considering their importance to ecosystem functioning and use as bioindicators (Dopheide et al., 2020), reliable and repeatable measures of their biodiversity are required, which molecular approaches provide. Detailing biodiversity and population structures across spatial and temporal gradients can substantiate past responses to environmental change that can help predict potential scenarios involving multiple, simultaneous, global change drivers (Rillig et al., 2019).

Molecular approaches to discerning biodiversity

DNA sequencing provides a subjective, data-driven means by which species can be defined, well suited to the challenging soil invertebrate species (Czechowski et al., 2016). However, the best phylogenetic outcomes are subject to selection of suitable genetic markers and bioinformatic pipelines (Ruvolo, 1997; Avise, 2016). I use a combination of mitochondrial COI and nuclear 28S rRNA sequences as they can determine both recent and ancient differences between species and provide broad coverage for increased matching with previously sequenced reference data.

Applying molecular information towards increased resolution of species at the genetic level, contributes towards the phylogeographic and evolutionary understanding of soil fauna. The combination of phylogenetic signals with geological, climatic and environmental datasets can be used to explore the evolutionary histories of species distributions with known timeframes (Avise, 2000). Despite the availability of molecular approaches, knowledge gaps regarding their distribution, diversity and speciation in Antarctic and Australian soil fauna remain.

Existing biogeographic knowledge based on morphological means of taxonomic identification can be verified with sequencing data. These can then be assigned to populations across environmental gradients to determine the factors most influential to species ranges, with high taxonomic resolution. Novel molecular tools integrating trait-based and phylogenetic data have been promoted as a framework for assessing phylogeography, dispersal models and functional roles (Wall et al., 2010; Pey et al., 2014; Gratton et al., 2017). Phylogeography applies phylogenetic data to identify the drivers of speciation using statistical tests of the most likely circumstances that led to current distributions (Graham and Fine, 2008). This information can then be applied to construct distribution models based on species divergence and gene flow within a climatic, geographical, and historical context (Avise, 2000). Expanding sequence datasets from southern hemisphere taxa will also enhance future species verification and biodiversity monitoring, alongside traditional taxonomies associated with morphological, ecological and functional traits.

Taxonomic nomenclature

The taxonomic identification of species has developed over the years, starting with the earliest approaches that relied strictly on morphological differences. These have gradually been used alongside molecular information since the more wide-spread availability of affordable barcoding

technology that occurred around the turn of the new millennium. These contributed to a broader community of researchers to study ecological questions with a reduced reliance on exhaustive taxonomic expertise. However, it is imperative to retain maximum confidence as to the correct identification of taxa. All sequences resolved to species level that have been uploaded to global databases are verified against published, referenced or other source specimens that have been slide-mounted and traditionally identified using keys. To ensure a consistent approach to systematics, pipelines and protocols are applied consistently for clarity and repeatability. Throughout this thesis, all reference to specimens have been applied using the same strict requirements as detailed in the International Code of Zoological Nomenclature (Ride, 1999). However, as sequence analysis and slide mounting were not possible to perform on the same specimens, species identification was performed using genetic markers. Thresholds used for confidence in matching with species were >99.0%. All specimens that cannot be verified to species level with molecular information alone have been replaced with the nearest known taxonomic level e.g. family or genus. If taxonomic thresholds are not met, specimens are referenced as genotypes/morphospecies. All specimens have a type specimen stored in 70% Ethanol for future slide mounting if necessary. Molecular identification based on sequences matched against global databases (NCBI) that have verified species names, often included in published journal articles. Genetic markers used to define specific definitions were used to designate members of species complexes and genotypes. Delineations based on nuclear and mitochondrial markers have specific rates of divergence and background mutation that is described in detail prior to sequence usage in the review chapters (Ch. 2 and 3), and data chapters (Ch. 4, 5 and 6).

The evolutionary history of mites and springtails

In this thesis, I focus on mites (Acari) and springtails (Collembola) as they are among the most widespread faunal groups, represent multiple trophic levels and functional traits, and are involved in multiple ecosystem processes. Specifically, I explored evolutionary histories of mites and springtails along latitudinal transects in maritime Antarctica and Australia to determine the interactions among geographical, climatic and environmental influences on their distribution, diversity and speciation.

Prior to studying more recent evolution, it helps having a firm grasp of species origins and how they fit into the traditional taxonomic structure. Both taxa have ancient origins with early fossil evidence of 470 Ma for mites (McNamara and Selden, 1993) and ~380 Ma for springtails (Hirst and Mulik, 1926), with a widespread Gondwanan distribution with the last common arthropod ancestor, potentially a shrimp-like *Kylinxia*, around 518 Ma (Zeng et al., 2020). From this ancestor evolved the diversity of arachnids with the primary distinction between spiders and the terrestrial mites (acari) with no 'waist' between the thorax and abdomen. Perhaps evolving from an intertidal mite, they diversified to the orders: Parasitiformes Leach, 1815 which includes the predatory Mesostigmatida, the Sarcoptiformes Reuter, 1909 which includes the ecologically important Oribatida Dugès 1833 that include the diverse Astigmatina Canestrini, 1891, and the Trombidiformes Reuter, 1909 which includes the Prostigmata Kramer, 1877. Over 50,000 mite species have been described globally (Halliday et al., 2000), although there are considered to be over a million species of mites globally (Decaëns, 2010; Rupert et al., 2004). With over 10,000 described prostigmatid and 4,500 astigmatid of an estimated global total of over 150,000 species, a large proportion of southern hemisphere species remain undescribed (Coleman et al., 2004).

Developments in molecular approaches to taxonomy have been shown as consistent with morphological studies and resulted in the re-classifications of certain taxa. For example, the

Astigmatina (also called Astigmata) was once considered an ancestral lineage to the Oribatida, but recent molecular evidence supports the Astigmatina as belonging to a derived clade (Desmonomatides) within the Sarcoptiformes order (Krantz and Walter, 2009; Arribas et al., 2020b). Whilst these are a diverse and ecologically relevant group, in this study I count the number of astigmatids, but they are not identified to higher resolution owing to the limited number of reference sequences and species identifications. Hence, I am not considering all true members of Oribatida in this thesis. Metagenomic evidence has shown that members of the sister clade Eriophyoidea nalepa, 1898 may be a basal clade to the Sarcoptiformes (that includes the diverse and ecologically important order Oribatida order) and the Trombidiformes that includes the diverse Prostigmata (Arribas et al., 2020b). The predatory Mesostigmata are within the parasite and ticks of the Parisitiformes.

Another branch of the arthropod phylum contained the pancrustacean hexapod springtails and insects. Springtails have diversified into around 9300 described species with an estimated total approaching 50,000 species globally (Potapov et al., 2020). They have been categorised into four main orders: Entomobryomorpha, Poduromorpha, Symphypleona and Neelipleona (Bellinger et al. 1996-2021; Hopkin, 1997; Leo et al., 2019). Springtails have a global distribution and inhabit a range of positions in the ecosystem, from aboveground plant feeders, to litter and edaphic species that consume organic matter and graze on microbes (Leo et al., 2019). Whilst it is well-established that soil invertebrate's contribution to ecosystem functioning, the influence of ecosystem processes and the evolution of morphological and functional traits are not fully understood. Examining the links between genetic diversity with morphological and functional traits in terrestrial soil fauna can shed light on this topic.

1.3 Southern hemisphere terrestrial ecosystems

An array of soil faunal groups inhabits southern hemisphere landmasses, including both the ice-free regions of Antarctica and the terrestrial soil ecosystems of Australia. Relatively thorough morphological surveys of the indigenous soil fauna have been conducted in specific areas along the Antarctic Peninsula (Wallwork, 1967; Marshall and Convey, 2002) and Australia (Greenwood et al., 2004; Colloff and Halliday, 2013); however, these are far from complete, with a lack of broad latitudinal studies. Antarctic and Australian soil fauna includes representatives of all mite orders, whilst Antarctic springtails are limited to members of the Entomobryomorpha and Poduromorpha. Whilst records of biodiversity have been compiled based on morphological records, most biogeographic findings have yet to be verified using molecular taxonomy.

As part of Gondwanaland, the landmasses that eventually became Antarctica and Australia have undergone significant climatic and environmental changes since their inception. Antarctica's climate shifted dramatically following the Gondwanan separation (~150 Ma), resulting in an associated change from a dominance of tropical rainforests during the Cretaceous period to the polar desert seen today (Rozadilla et al., 2015). The harsh environmental conditions formed considerable barriers to dispersal and contributed to the restricted ranges of indigenous species (Convey et al., 2014), promoting a shift in biodiversity. During the latter stages of Gondwanan separation around 35 Ma, Australia split from the southern portion of the supercontinent and moved swiftly northwards into temperate latitudes (Wei, 2004; Hassold et al., 2009). In the intervening period, climatic conditions diverged significantly between the two continents, with Australia maintaining rainforest vegetation for a considerable portion of the Eocene and Oligocene periods. From the Miocene era (~23Ma), ecosystems underwent further change as rainforests shrank amidst increasing aridity in Australia's central and north-western regions (Mooney et al. 2017), whilst maintaining a relatively stable vegetation dominated by bryophytes and ferns in the

193 south and Eastern Seaboard up until the Last Glacial Maximum, LGM (Kemp, 1978). These
194 changes are suspected causing extinction of cold-tolerant species in Australia (Christophel and
195 Greenwood, 1989; Greenwood et al., 2004).

196 Since separation, a series of dramatic glacial cycles in Antarctica influenced the dispersal
197 opportunities of all local biota up to the LGM that occurred between 26,500 and 12,000 years ago
198 (Heroy and Anderson, 2005). These geological changes had profound impacts on soil fauna, with
199 extensive evidence from traditional morphological studies of highly restricted distributions,
200 assuming complete sampling coverage (Convey et al., 2014). Similarly, Australian islands formed
201 because of rising sea-levels at the end of the LGM. This created dispersal barriers among once
202 contiguous populations with assumed uniform genetic diversity. However, the shared Gondwanan
203 history of Antarctica and Australia remains evident from the common ancestry of their
204 contemporary biota (Convey et al., 2008; Baker et al., 2020).

205 Climate predictions have indicated the likelihood of increased frequency of extreme
206 weather events in Australia, with terrestrial ecosystems prone to both extreme drought and
207 inundation (Ummenhofer and Meehl, 2017). Similarly, the Antarctic Peninsula is experiencing the
208 most rapid warming in the polar region, with the potential of rapid “greening” and potential shift
209 to belowground biodiversity requires a robust investigation (Convey et al., 2012; Chown et al.,
210 2022). Therefore, establishing a firm baseline of soil faunal biodiversity can help future monitoring
211 programs in both regions.

212

213 *Southern hemisphere soil fauna richness and distribution patterns*

214 Terrestrial ecosystems in the southern hemisphere regions of maritime Antarctica and Australia
215 have considerable differences in climatic and environmental conditions, that cause a distinction in
216 the dominant vegetation types specific in each region. Both regions support diverse communities

of soil fauna that display restricted geographic ranges and high levels of species endemism. For example, diverse communities along maritime Antarctica's peninsula (Convey et al., 2014) reflect similar patterns along Australia's Eastern Seaboard (Keast, 2013). These shared biotic patterns make them ideal counterparts for comparative study of the multiple drivers of biotic distribution, with an alternate geographical location to highlight patterns that may be applicable to soil fauna generally, regardless of parameters specific to a single geographic location.

Well-documented records based on morphological identification have shown large differences in species richness between the regions. In Australia, an estimated 2,600 mite species are present, although a large proportion of species remain undescribed (Halliday, 1998; Niedbala, 2006; Colloff and Halliday, 2013). Similarly, some 400 springtail species are known from Australia, but total richness is estimated at around 2000 species (Chapman, 2006; Greenslade et al., 2014). This is far greater than the 105 known Antarctic mite species (Schatz, 2004; Mortimer et al., 2011) and 25 recorded springtail species (Collins et al., 2019; Ch. 2). Despite these differences, the underlying factors that govern the distribution and diversity of soil fauna across temporal and spatial scales are thought to be shared.

Mites and springtails are predominantly found to inhabit coastal terrestrial ecosystems that harbour aboveground vegetation with adequate soil moisture in both regions. The known links between species diversity with elevation and latitude are features common to both Antarctic and Australian continental ranges (McKenzie et al., 2004; Chown and Convey, 2007; Maunsell et al., 2013)). Climatic factors have also been shown as the principal drivers of broad-scale distribution in both Antarctic and Australian soil fauna. Temperature and rainfall shape aboveground vegetative cover in Antarctic ecosystems (Chown and Convey, 2007), and have also been linked to driving soil properties including moisture, pH and nutrient loads that are important to Australian soil faunal distributions (Petersen and Luxton, 1982; Lee and Foster, 1991). Whilst most studies

241 on Australian fauna have been based on morphological studies, advancing our detailed knowledge
242 of the factors that influence species diversity rests upon robust measures of biodiversity (Wu et
243 al., 2011). As such, molecular work has been found to show similar distinctions and high levels of
244 genetic diversity in populations of mites within maritime Antarctica (Van Vuuren et al., 2018), as
245 found in arid ecosystems of Western Australia (Guzik et al., 2021).

246 In addition to comparing geographic regions to gain insight into the drivers of soil faunal
247 distribution, comparative analysis between faunal groups can also indicate the factors that drive
248 distribution and diversity that are independent of geography or climatic variation. Differences in
249 dispersal ability, life cycles and cold and desiccation tolerance between soil faunal groups such as
250 mites and springtails can be used to explore these influences on distribution and evolution. The
251 results discern unique patterns of distribution and evolutionary history that can inform us about
252 their responses to environmental change. Whilst springtails have the ability for local motility and
253 long-term hibernation, mites are capable of long-distance wind or water-borne dispersal (Coulson
254 et al., 2002; Hawes et al., 2007, 2008). This is reflected in contrasting patterns of distribution and
255 diversity dependent on their biotic interaction with climatic and landscape effects involving
256 barriers to dispersal. Furthermore, the fine-scale drivers of assemblage composition are less clear.

257 Studies of species distribution across landscape scales can correlate biotic and abiotic
258 measurements to give insights into whether deterministic factors, such as climate and vegetation
259 cover, stochastic variables including dispersal (Mortimer et al., 2011), natural events or biotic
260 interactions (Caruso et al., 2019; Lee et al., 2019) drive patterns of distribution and diversity. These
261 findings can improve our understanding of the response of soil fauna to historic climatic shifts and
262 ecosystem fragmentation, giving us a better idea of how they may respond to future change.

263 In this thesis, I collect empirical evidence from transects through maritime Antarctica and
264 Australia (Fig. 1.1). Specifically, I investigate distribution and diversity of mites and springtails

from islands along transects in the maritime Antarctica (60º–72ºS) and the Australian Eastern Seaboard (23º–41ºS). Contrasting Antarctic and Australian ecosystems will provide fundamental insight into the distribution of soil fauna in the southern hemisphere.

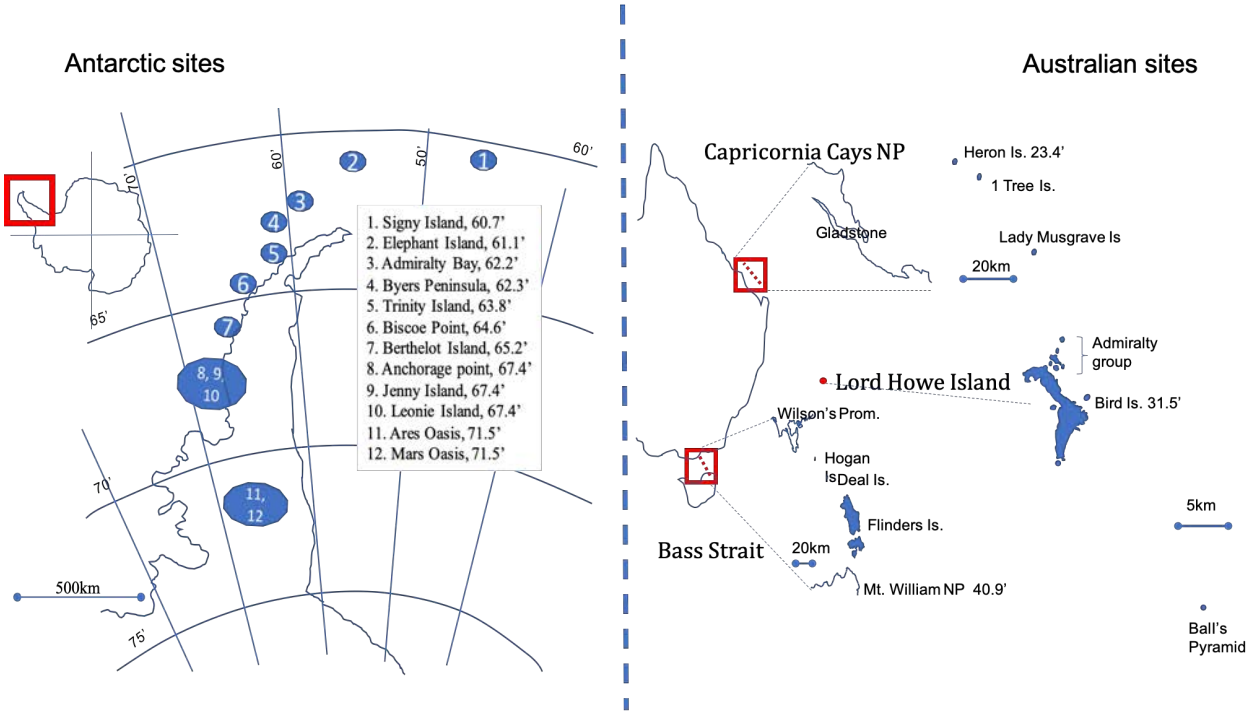

Figure 1.1 Left Panel: Location of sampling island sites along Antarctic Peninsula and their southerly latitude, made during expeditions in 2014-2016. Right Panel: Australian island and mainland sites were used as an independent complementary transect for comparing distribution, diversity and speciation of soil fauna along an equally broad latitudinal transect collected between 2019-2021.

1.4 Thesis aims and structure

This thesis applies molecular tools towards the study of soil invertebrate distribution and diversity at i) local scales in response to environmental variation, ii) regional scales focussing on climatic influences and gene flow; and, iii) temporal scales discerning patterns of evolution and dispersal. By combining molecular and morphological approaches, the spatio-temporal variability that effects soil faunal assemblages can be more clearly observed, with insight into their responses to

historic climatic and environmental changes. The degree by which their phylogenetic relatedness can be linked to evolution and biogeographic context is an important step in understanding the drivers of speciation and responses to fragmented habitats. This knowledge will improve predictions of potential responses to future habitat changes.

Using these approaches, I aim to address the following research questions:

1. How have molecular phylogenies advanced knowledge of the origins and distribution of Antarctic soil fauna?
2. Can phylogenetic signals be used to predict morphological traits as a proxy for ecosystem functionality in soil invertebrate assemblages?
3. What are the patterns of broad and fine scale distribution and diversity of mites and springtails across maritime Antarctica?
4. Do mites and springtails show contrasting phylogeographic histories in maritime Antarctica?
5. Are there common principles that drive distribution and dispersal in southern hemisphere soil invertebrates?

The thesis is organised into five chapters written in manuscript style, aligned with the five research questions above. The objectives for each chapter are described in more detail below. Author contributions are clearly defined following each chapter introduction.

Chapter 2

In this chapter, I present the findings of a literature review that synthesized contemporary phylogeographic knowledge of Antarctic invertebrates. I specifically sought to reveal patterns of

distribution and diversity of soil fauna based on available studies in the region. Antarctic soil faunal biogeography was shown to be structured by a complex combination of ancient and recent events. Molecular data has helped resolve evolutionary histories beyond traditional morphological studies, indicating that repeated dispersal events from multiple refugial locations dispersed throughout the regions combined with colonisation events of species from other continents have contributed to current distribution. However, several gaps in the understanding of the origins and evolution of the indigenous taxa are also presented. There is a clear need for sampling at both broad and fine scales across environmental gradients targeting lesser-known areas to counter the data deficiencies in order to establish the relative importance of climatic and environmental drivers of distribution. This chapter is written in manuscript form with contributing edits from my PhD supervisory panel: A/Prof. Uffe Nielsen, Prof. James Cook, and A/Prof. Paul Rymer.

Chapter 3

In Chapter three, I demonstrate how molecular information can provide insight into the potential contributions of soil fauna to ecosystem functioning. This published work linked springtail and nematode phylogenies with functional and ecological traits (Ross et al., 2022). This chapter provides another aspect of molecular information that contributes to the research questions of the thesis, namely the drivers of soil invertebrate evolution and distribution. Comparing the phylogenetic clustering and relatedness of functionally similar taxa provides a conceptual framework for identifying the functional roles of unknown species based on genetic sequences alone. Functional attributes were collated from multiple sources and highly cited databases, of which the owners were co-authors in the study as noted in the author contributions. The study

329 demonstrates the strengths and limitations of the bioinformatic approach, with the necessity to
330 integrate existing taxonomies into phylogenetic studies and highlights the uneven distribution of
331 global soil invertebrate sequences, with emphasis on increasing numbers of southern hemisphere
332 sequences to assist future monitoring efforts. This chapter was published as a review in *Austral*
333 *Ecology*. I conceived and led the data collection, analysis and writing of the first draft of the
334 manuscript. Datasets for springtail morphology were provided by S. Salmon and M. Berg.
335 Manuscript writing was supervised by U. Nielsen whilst all authors contributed to editing.
336
337 *Chapter 4*
338 In Chapter four, I explored the distribution and diversity of mites and springtails collected across
339 a significant climatic and environmental gradient in maritime Antarctica utilising preserved
340 specimens (60º-74ºS; Fig. 1.1). Antarctica is an ideal environment to study drivers of distribution
341 and diversity due to: i) relatively uniform ecosystem types common across the transect; and, ii)
342 limited disturbance and minimal influence of invasive species and human-assisted migration.
343 Distribution of mites was fairly consistent with five of seven oribatid mite and three of five
344 identified springtail species being present at most sites. However, diversity was much lower at the
345 inland southerly oases with limited species sharing between the northern and southern regions
346 conforming with previous work. Springtail and oribatid mite abundance and diversities were
347 related to temperature and rainfall climatic variables across the latitudinal gradient, with some
348 evidence of spatial isolation playing a minor role. At finer scales (i.e. within sites), mite and
349 springtail assemblage structure was related to vegetation cover and soil pH and nutrient content,
350 conforming with the hypothesis that different variables govern distribution at large and fine
351 scales. I carried out all counting, sequencing and analysis of soil fauna in this study. Samples were
352 collected by my principal supervisor Assoc. Prof. U. Nielsen during a US NSF-funded expedition

(2014-2016) led by Prof. B. Ball. Environmental datasets were provided by Prof. Becky Ball who will be a co-author on resulting manuscripts.

Chapter 5

In Chapter five, my aim was to explore the phylogeography of mites and springtails in maritime Antarctica using the same framework as in Chapter four. The two groups have differing habitat preferences and dispersal abilities indicating that divergent phylogeographic patterns are likely. Earlier studies show evidence of contrasting patterns of dispersal between oribatid mite species associated with refugia along the Antarctic peninsula, alongside strong associations between genetic diversity and dispersal between sub-Antarctic islands. In this study, I present the findings of a comparative phylogenetic analysis of mites and springtails along a broad transect of maritime Antarctica.

Comparison of molecular variance and phylogeographic reconstructions for the oribatid mite genotypes of *Podacarus auberti* Grandjean, 1955 and *Membranoppia loxolineata* Wallwork, 1965 and the springtail *Cryptopygus antarcticus* Willem, 1901 revealed contrasting episodes of dispersal, vicariance and extinction. Differences between the two taxa indicated that dispersal was more common in springtails, suggesting that greater sensitivity to environmental conditions may drive localised extinctions. However, springtails' greater dispersal abilities may also enable more recolonisation events that lead to increased levels of genetic recombination in more isolated sites far from known refugia. By contrast, both mite species displayed higher levels of molecular variance among populations that suggest restricted mixing of populations once long-range dispersal occurs. This demonstrated the importance of dispersal ability in invertebrate distributions throughout maritime Antarctica with implications for potential responses to future

shifts in environmental conditions. I carried out all sample sorting, sequencing and phylogeographic analysis in this study using the same samples described in Chapter 4.

Chapter 6

In Chapter six, I investigate the distribution, diversity and phylogeography of Australian soil faunal communities as an analogue to the latitudinal transect in maritime Antarctica. Australian mites and springtails were collected along a continental-scale latitudinal gradient (23º–41ºS; Fig 1.1), within a uniform ecosystem type. Mites and springtails were found to have restricted distributions along the transect, with only two mite and one springtail species found at all sites. Abundance and species richness were related to climatic drivers such as rainfall and soil parameters including soil pH. Additionally, biogeographic context was also shown to have some influence on genetic diversity suggesting effects of distance to source populations from the mainland and period of isolation. This indicates how passive dispersal acts as a driver of widespread distributions. These have implications for monitoring soil faunal diversity across a global perspective. I designed the study, collected the samples, sorted, counted, and undertook all specimen sequencing and laboratory analyses in this study.

Chapter 7

In the final chapter, I synthesize how my research have advanced our phylogeographic understanding of southern hemisphere soil fauna. Overall, southern hemisphere oribatid mites and springtail abundances and species diversity had similar responses to climatic and environmental variability, which demonstrates that fundamental drivers govern their distribution irrespective of geographic location or latitude. Moreover, the two taxa show contrasting dispersal ability, which governs their distribution at landscape scales. In maritime Antarctica, this highlighted refugial

survival followed by multiple dispersal events linked to warming climatic conditions that precipitated glacier melt and revealing more areas viable for colonisation. In the Australian context, isolation of new islands formed by corals contrasted remnant peninsulas that were cut-off from mainland populations following sea-level rises following the LGM. The shared responses of analogous faunal communities to historic climatic variation are discussed with future directions presented that will further our understanding of the drivers of essential soil faunal biodiversity that are under increasing pressure from shifting environmental conditions in both northern and southern hemisphere ecosystems.

Chapter 2 - Antarctic soil faunal phylogeography shows ancient origins, repeated colonisation and recent evolution

Abstract

Antarctica is populated by a diverse array of terrestrial fauna considering the extreme climatic conditions. Their origins and diversity has long puzzled ecologists. Early theory considered contemporary populations as descendants of recent arrivals given the harsh conditions and significant disturbances imposed by repeated glacial cycles. However, mounting evidence points to established populations of most indigenous taxa well before the Last Glacial Maxima (LGM) indicating more ancient origins. Here we present insights into the origins and distribution of endemic terrestrial invertebrates by synthesizing results of phylogeographic studies. Molecular dating shows an ancient origin for most indigenous taxa, including Acari (up to 100 million years ago, Ma), Collembola (21–11 Ma), Nematoda (~30 Ma), Tardigrada (>1 Ma) and Chironomids (>49 Ma), while Rotifera appear to be more recent colonizers (~130 Ka). Subsequent population bottlenecks and rapid speciation is evident from the phylogenies of multiple taxa, with high divergence found, for example, among tardigrades of continental Antarctica and mites in maritime Antarctica, driven by genetic drift during repeated periods of isolation. Limited evidence for gene transfer after the LGM between the continental and maritime regions in springtails and mites indicate local refugia within both Antarctic regions, with repeated wind or water-borne dispersal and colonization of contiguous regions during interglacial periods. By contrast, rotifers show limited evidence of *in situ* speciation following more recent colonization, yet their precise histories remain unresolved. Greater knowledge of Antarctica's fauna will enable us to account for their biodiversity and to focus conservation efforts and ensure their persistence in the case of changing environmental conditions.

2.1 Introduction

Antarctica's terrestrial ecosystems are home to thriving populations of soil invertebrates (Chown and Convey, 2016), that inhabit the seasonally snow and ice-free soils (Chown and Convey, 2007). To date, some 550 species of Antarctic invertebrates have been described, with 170 of these being endemic to the region (Adams et al., 2014; Velasco-Castrillón et al., 2014c). Morphology based taxonomy has advanced the study of their biodiversity and distribution. However, confirming these patterns for all Antarctic taxa has been constrained by the region's limited accessibility (Convey, 2011; Chown et al., 2015) and demanding taxonomic expertise required for microinvertebrate identification. Molecular approaches are now revealing profound insights into the origin, diversity and distribution of terrestrial Antarctic invertebrates confirming certain aspects, such as climatic bioregions (e.g. maritime and continental Antarctica; Pugh and Convey, 2008), and have formed a consensus over the ancient origins and complex evolutionary history for Antarctica's invertebrate biota (Marshall and Pugh, 1996; McInnes and Pugh, 1998), despite early speculation of recent colonisation (Starý and Block, 1998).

Phylogeographic analysis is a powerful tool that can evaluate evolutionary timelines to provide robust understanding of the events that have shaped current distributions. The combination of sequence-based phylogenetic datasets with biogeographic distributions and geological histories can detail both recent and ancient ancestries. Most phylogeographic studies focus on northern hemisphere taxa (Beheregaray, 2008), but a growing list of studies have targeted invertebrates from the Antarctic region. Here we review published phylogeographic studies of Antarctic soil fauna within its three constituent regions, each with distinct climatic and geological histories—i) continental Antarctica, ii) maritime Antarctica, and iii) sub-Antarctic islands (Fig. 2.1).

454

455 **Figure 2.1 Map of the three Antarctic regions, continental, maritime and sub-Antarctic islands**
456 **(Source: Google Earth image based on Landsat and Copernicus satellite images, Maxar**
457 **Technologies). Overlay of circumpolar distribution of molecular studies of springtail specimens**
458 **(yellow triangles) and other invertebrates (blue circles), adapted from McGaughran et al., 2011.**
459 **"Gressitt Line" demarcating the proposed biotic frontier between the peninsula and continent is**
460 **shown by light green line. Red boxes outline areas covered by detailed maps of Victoria Land (Fig.**
461 **2.4) and the Antarctic Peninsula (Graham Land) Fig. 2.5.**

462

463 First, we introduce the geological and climatic histories that have shaped both the region
464 and its terrestrial fauna, before providing a brief overview of the main phylogeographic
465 approaches. We then summarise the general patterns found within and between regions and the
466 dominant invertebrate groups, specifically: mites (Acari), springtails (Collembola), nematodes
467 (Nematoda), rotifers (Rotifera), tardigrades (Tardigrada) and the chironomid midges (Insecta).

Some examples of each group include oribatid mites: *Halozetes belgicae* Michael, 1903 and *Membranoppia loxolineata* Wallwork, 1965; springtail *Cryptopygus cisantarcticus* Willem, 1901; nematode: *Scottnema lindsayae* Timm, 1971; rotifer: *Macrotrachela jankoi* Iakovenko, 2015, and, tardigrade: *Hypsibius exemplaris* Doyère, 1840 (Fig. 2.2). Finally, we focus on the future directions for terrestrial Antarctic invertebrate research and evaluate the capacity to monitor and protect the unique Antarctic ecosystems.

2.1.2 Geological history

Antarctica's current landmass was once part of the early supercontinent Rodinia that existed between 1,300 and 700 million years ago (Ma) (Fuck et al., 2008). This protocontinent underwent separation and reformed as Pangea, which itself began breaking up ~335 Ma. The rifted supercontinent of Laurasia encompassed the North American, European and Asian continents. Meanwhile, the southerly Gondwanan portion contained the Antarctic, South American, African, and Australian continents (McMenamin and McMenamin, 1990). As Pangea separated, Antarctica was already positioned over the south pole (Rogers and Santosh, 2004) but supported lush forests and a thriving dinosaur population (Rozadilla et al., 2015).

Gondwana began to break up ~175 Ma, with Australia and South America beginning to separate 85-60 Ma before moving swiftly northwards by 35 Ma. These final movements created the channels on either side of the Antarctic continent (Wei, 2004; Hassold et al., 2009) allowing the establishment of the Antarctic Circumpolar Current (ACC; Siegert et al., 2008). This formed a profound physical barrier and affected the region's climate, limiting biotic crossings and suitable habitat (Pugh and Convey, 2008).

2.1.3 Past and current Antarctic climate

Since Antarctica's separation from Gondwanaland, the climate has gradually cooled until low temperatures precipitated the southern ice cap by 44 Ma (Ehrmann and Mackensen, 1992), followed by repeated cycles of glaciation and ablation occurring over the last two million years (2 Ma) until the Last Glacial Maxima (LGM, 22–12 thousand years ago, Ka) (Davies et al., 2012). During the repeated glaciation, ice-sheets covered most of maritime and continental Antarctica, reaching a depth of 7 km on the continent. This would have led to extirpation (local extinction) of many indigenous floral and faunal species (Pugh and Convey, 2008). Throughout the Pleistocene (~2.5 Ma–12 Ka), at least eight glacial cycles occurred, with colder temperatures and greater snowfall producing ice sheets. The episodic glaciations had a pronounced 100 Ka cycle between the earlier periods (0.74–0.43 Ma; EPICA, 2004), accelerating into a 40 Ka cycle in the last four glaciations starting from 430 Ka (Augustin et al., 2004). Interglacial periods brought about more favourable temperatures and greater water availability allowing vegetation and soil faunal communities to become established.

Continental Antarctic temperatures are cold and dry all year round in the high plateaus (Mean Annual Temperature: MAT: -55°C) with milder conditions in coastal regions (MAT: -10°C) (Turner et al., 2005; SOE, 2011). Yet summer temperatures and increased solar radiation thaw soils in some regions facilitating biological activity. In maritime areas, summer temperatures can reach 15°C, but generally remain below 0°C, whilst more rainfall is common at lower altitudes along the Antarctic peninsula. The maritime region is bounded by the extent of the ice-shelves that extends from the western side of the peninsula and encompasses the adjacent islands below 60°S and extending up to the Antarctic Polar Front. Despite the lower latitudes, the South Shetland and Orkney Islands are exposed to strong winds that keep MAT to below -4°C. Further away, the sub-

Antarctic islands lie above the limit of sea-ice with MAT ~2°C, and occasional rain during the summer (Turner et al., 2005).

Rapid deglaciation in the last century on the peninsula's west coast (Cook et al., 2005, 2016; Çiner et al., 2019) is undoubtedly linked to contemporary increases in temperatures, estimated at 0.56ºC every decade in the latter half of the 20th century (Turner et al., 2005; 2009), with additional pronounced peninsular warming since 2000 (Siegert et al., 2019). This has led to accelerated greening of sub-Antarctic islands with expected susceptibility to invasive species (Cannone et al., 2022). Further local warming associated with the diminishing Antarctic ozone hole (Solomon et al., 2016) has been predicted to enhance glacier melt, with a 25% increase in ice-free land along the eastern continental Antarctic coastline and peninsula by 2100 (Lee et al., 2017). These climate scenarios are expected to expand habitable areas in the maritime and peninsular regions (Turner et al., 2009), with larger ranges of continental species (Barrett et al., 2006). These changes to vegetation and soil microhabitat may influence the distribution and diversity of native fauna (Wall and Moore, 1999), or have to contend with greater colonisation rates and competition, reinforcing the need for ecosystem monitoring to inform conservation efforts (Parmesan and Yohe, 2003; Nielsen et al., 2011b).

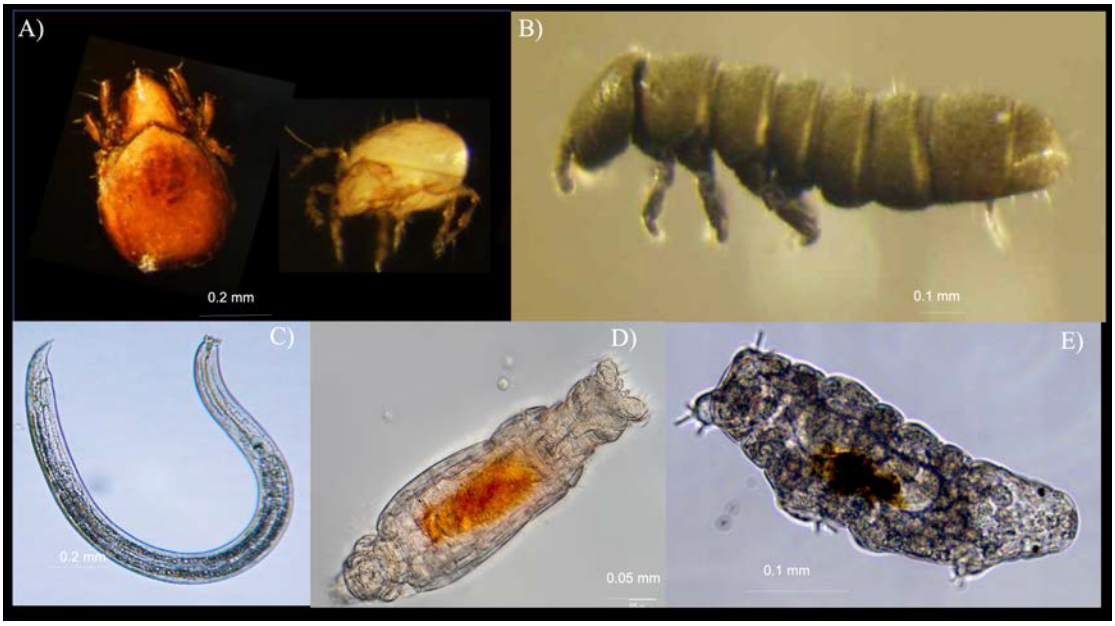

Figure 2.2 Photographs of the main groups of Antarctic soil fauna: A) Oribatid mites: Ameronothridae and Oppiidae families (source: GMR); B) Springtail *Cryptopygus cisantarcticus* (source: GMR); C) Nematode: *Scottnema lindsayae* BOLD systems (uncredited); D) Rotifer: *Macrotrachela jankoi* (source: Iakovenko, 2015; NERC Open Access Research Archive (NORA), http://nora.nerc.ac.uk/), and E) Tardigrade: *Hypsibius exemplaris* (source: Jönsson, 2019).

2.1.4 The origins and evolution of Antarctic terrestrial fauna

Early theory of invertebrate origins proposed their diversity was a result of rapid evolution following migration from surrounding oceanic islands or continental landmasses after the LGM via wave or wind dispersal (Starý and Block, 1998). Pronounced glaciation during the LGM and evidence of accelerated dispersal, colonisation and speciation following the LGM supported this theory (Peck et al., 2006). Alternatively, evidence from morphologically-derived identifications suggested a far more ancient and vicariant origin for all soil faunal groups, except for rotifers (Fig. 2.3). Here, the ACC can be seen to precede the majority of species radiation. This may be due to the strong physical barriers caused by the current that limits biotic invasion, thereby fostering endemism. It must be noted that whilst dispersal and vicariance can both occur, and potentially produce similar distribution patterns, detailed analyses of molecular markers are able to define the phylogenetic relatedness between species and determine the most statistically likely

phylogeographic history based on calibrated evolutionary models.

For example, high divergence among populations of endemic springtail species indicates rapid speciation *in situ* throughout the pre-Pleistocene (> 3 Ma; Knowles, 2001), while certain indigenous mite taxa show molecular evidence of pre-Gondwanan origins (> 32 Ma; Pugh and Convey, 2008). These ancient origins adhere to the idea of Gondwanan vicariance, whereby populations were split apart following the rifting supercontinent (Krosch et al., 2011). Arguments against the theory of ancient origins point to a lack of strong evidence for refugial locations (Fontaneto et al., 2009). However, molecular analyses support the consensus of ancient origins given the greater availability of relevant data (Pugh and Convey, 2008; Warren et al., 2014).

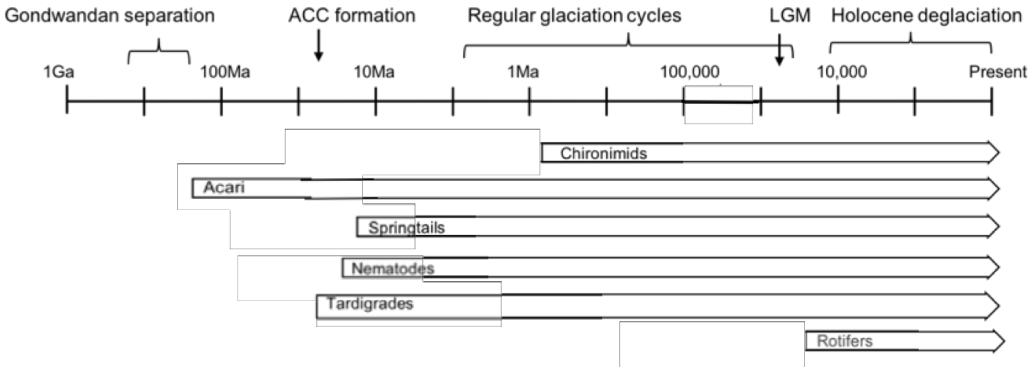

Figure 2.3 Graphical timeline invertebrate groups and earliest indication of endemic Antarctic lineage. Ages based on mean estimated origins from molecular dating studies in continental Antarctica alongside main regional geological events over a logarithmic timeline. 1Ga = 1,000 Ma (Adapted from Convey, 2010).

Early climate change refugees

The current consensus is that many of Antarctica's indigenous invertebrates survived through periods of glaciation *in situ*, relying on suitable refugia to endure the ice-ages (Convey et al., 2008; Hawes, 2015). Despite wide-spread ice-sheet coverage, strong biological and phylogeographic evidence points towards the presence of habitable ice-free areas throughout the past 5 Ma (Prentice et al., 1993; Pugh and Convey, 2008). Such refugia are thought to be associated with geothermal activity, coastal areas, continental Dry Valleys and high-altitude ridges, horns and arêtes,

collectively named ‘nunataks’, that lie above the maximum glacial height (Pugh and Convey, 2008; Fraser et al., 2014, 2018). Freshwater lakes and ponds have also been proposed as key invertebrate refugia (Wagner et al., 2006) before sea-level rises associated with the LGM inundated these sites with seawater (Squier et al., 2002; Cromer et al., 2006). However, it appears that not all proposed refugial sites may be habitable or ice-free after the discovery of barren high elevation hypersaline sites near the continental Beardmore Glacier (Lyons et al., 2016) and glaciated sites along the peninsula (Lau et al., 2020), fuelling ongoing debate about the location of suitable refugia.

Antarctic invertebrates have adapted to local conditions allowing them to undergo desiccation (anhydrobiosis) or entering a state of dormancy to withstand freezing (cryptobiosis). Anhydrobiosis is particularly useful for longer term survival and common to Antarctic springtails (Holmstrup, 2018), tardigrades (Somme, 1996), nematodes (Wharton and Ferns, 1995; McGill et al., 2015), rotifers (Rebecchi et al., 2019) and the sub-Antarctic midge, *Belgica antarctica* Jacobs, 1900 (Benoit et al., 2009). Certain groups can reduce their metabolism to almost zero, including tardigrades (Altiero et al., 2015), and some species of nematodes secrete anti-freezing proteins (Adhikari et al., 2009). Such adaptations are thought to have aided their persistence in refugia during glacial maxima.

2.2 Phylogeographic analyses

Phylogeography combines biogeographical information with phylogenetic analyses to assess patterns of speciation and colonisation (Avise, 2000). The approach has shown how biotic responses to climatic and ecological conditions are constrained by geological context (Graham and Fine, 2008; Smith et al., 2014), such as the strong relationship between refugia and diversity. High

resolution taxonomic data for soil invertebrates have been recorded with multiple molecular studies of springtails and other invertebrates across the region (Fig. 2.1). These molecular studies also supported the demarcations of Antarctic bioregions (Terauds et al., 2012). Among key divisions is the 'Gressitt Line', a proposed biotic frontier separating the Antarctic Peninsula (a.k.a. Graham Land) and continental Antarctica (Pugh and Convey, 2008; Fig. 2.1). This division is accompanied by substantial genetic divergence and limited overlap in species, suggesting the presence of multiple refugia on either side of the division (Chown and Convey, 2007). Rigorous statistical pipelines using intra- and inter-specific differences can assess theories of persistence, divergence and migration (Arbogast and Kenagy, 2001). With genealogies subject to repeated colonisation events at the local scale (< 1 Ma), molecular phylogenetic assessment is the best approach to resolve evolutionary histories. We now introduce methods used for the phylogeographic analysis of Antarctic soil fauna.

2.2.1 Molecular taxonomy

DNA sequencing allows the measurement of genetic divergence within and among species that improves phylogenetic reconstructions (Thomson et al., 2018). DNA barcoding is now commonly applied to identify putative species of soil fauna (Hebert et al., 2003). Commonly used markers are based on mitochondrial DNA (mtDNA) and nuclear DNA that can provide different levels of temporal resolution based on their rate of change. Slower evolving nuclear markers can show ancient lineages, although higher taxonomic orders may be less well resolved. Faster evolving mtDNA markers can discern closely related species but are less robust for deeper phylogenetic relationships. The mtDNA cytochrome oxidase subunit I (COI) gene is a reliable identifier due to universal amplification and sufficient genetic variation between species, but limited within species

differences (Hebert et al., 2003). The 650 bp fragment has maternal inheritance and a relatively high nucleotide substitution rate, rare recombination and few indels, ideal for use as a molecular marker (Hebert et al., 2003). Well conserved adjacent sequences also allow for universal primer usage across animal taxa (Taberlet et al., 2012).

Delineation of OTUs is based on differences in COI nucleotide sequence, with values < 1% generally considered to be indicative of the same species (Hebert et al., 2003; Lim et al., 2012). An interchangeable term is the haplotype, originally used to define lineages, whereby 2-3% is the minimum difference defining a distinct species, and distances over 5% indicate divergent species/genera, and those over 10% highly divergent lineages (Stevens et al., 2007; Collins et al., 2019). Most studies use 3% as a threshold to define invertebrate species, as used in rotifers (Fontaneto et al., 2009) and nematodes (Kumari et al., 2010). Comparing results across taxa with broad ranges (e.g. the springtail *Cryptopygus antarcticus*) can further test the applicability of "rule-of-thumb" thresholds across regions. Similarly, cytochrome oxidase-subunit II (COII) is an alternative in species where COI is less informative due to minimal genetic variation in the marker (e.g. cnidarians) and has been sequenced in Antarctic springtails (Stevens et al., 2007; McGaughran et al., 2011).

An increasing number of studies use nuclear markers to support species classification, identification of ancient speciation events (Reitzel et al., 2013), and isolation-by-distance across the landscape (Teske et al., 2018). Such studies commonly target ribosomal DNA (rDNA) genes that encode the small 40S ribosomal subunit including ETS, 18S, ITS1, 5.8S, ITS2 and 28S tandem repeat marker genes (Hwang and Kim, 1999; Adams et al., 2007; Evans and Paulay, 2012). The thresholds for species delineation using nuclear 28S markers H3 and D3 sequences have been shown to be as low as ~0.1–0.5% in comparative studies (Klimov et al., 2019). The 18S rRNA genes have high levels of genetic drift and are frequently used to resolve taxonomic uncertainties

at the species and genus level (Guidetti et al., 2014), as demonstrated in nematodes (Boström et al., 2011), tardigrades (Guil et al., 2019), and springtails (F. Zhang et al., 2019). The 28S rRNA markers have also yielded insights into phylogenies of the marine Straconyxidae tardigrades with a combination of 28S rRNA and COI markers (Fujimoto et al., 2020), whilst COI and Internal Transcribed Spacer 1 (ITS1) were sequenced to determine genetic diversity and theories of dispersal between Victoria Land and other Antarctic regions of *Acutuncus antarcticus* Richters, 1904 (Cesari et al., 2016). Additionally, 18S rRNA and ITS1-2 markers revealed deep lineages in *Scottnema lindsayae* nematode populations across defined geographic areas (Adams et al., 2007). Other single copy nuclear genes such as the Histone-3 (H3) gene that structures the nucleosome, commonly associated with epigenetics, have been used to assess levels of shared ancestry and phylogeographic dispersal among Antarctic mites (Mortimer et al., 2011), whilst the well conserved Wnt signalling pathway genes have been targeted in tardigrades (Hodgson et al., 2010), and the wingless gene (Wg) has been used to identify intergeneric and interspecific mite taxonomies (Czechowski et al., 2012).

2.2.2 Molecular Clocks

Molecular clocks are an essential part of estimating when divergence events occurred. They are often calibrated against fossils or geological events with a verifiable date of origin (Ho and Phillips, 2009), but this can be problematic with Antarctic biota due to the lack of fossil records. Yet, they can still be applied to some degree as the homogenous substitution rate across the mitochondrial genome (mitogenome) simplifies their use in dating (Czechowski et al., 2017b). Secondary calibration that accounts for the uncertainty of phylogenetic based calibration applies corrections to earlier calibrations (Kodandaramaiah, 2011; Schenk, 2016). This has been shown to improve the accuracy of clocks (Hipsley and Müller, 2014), such as that used in dating marine

672 invertebrates (Loeza-Quintana et al., 2019). In the absence of verified ages for indigenous
673 Antarctic taxa, studies commonly use molecular substitution rates derived from non-Antarctic
674 insect phylogenies, with a consensus formed for an evolutionary rate (*R*) for arthropods of *R* =
675 1.5–2.3% Ma^{-1} (Stevens and Hogg, 2006; Brewer et al., 2012; Beet et al., 2016). Basing arthropod
676 evolution on insect-based calibrations lends to overestimation of dating due to potentially faster
677 generation times of insects.

678 More recently, geological events have been used to calibrate molecular clocks. These
679 calibrations have benefitted from computational models used to date warmer periods and ice-sheet
680 collapses for invertebrate analyses (Strugnell et al., 2018; Collins et al., 2020). This has led to a
681 revised rate of *R* = 3.54% Ma^{-1} being used for Antarctic springtails (Papadopoulou et al., 2010).
682 The differences in *R* between non-Antarctic and Antarctic arthropods are considered to be a result
683 of variation in coalescence times and post-separation gene flow (Collins et al., 2020). Additional
684 factors that can improve estimations of divergence times include knowledge of generation time,
685 metabolism and mutation rates and population size. The parthenogenetic, or asexual, nature of
686 many oribatid mites also allows molecular clocks to be used without the complication of sexual
687 recombination (Maraun et al., 2003), yet can yield misleading ages if rates are compared with
688 sexually reproducing taxa. Bayesian relaxed clocks can incorporate variable estimates when
689 setting priors, yet selection bias between competing models, variable mutation rates and generation
690 times in natural systems mean dates are still best estimates and only used as a guide for relative
691 divergence times (Guidetti et al., 2017). Additionally, secondary calibrations of studies relying on
692 single calibration points might compound the unreliability of using geological events in the
693 absence of fossils (Forest, 2009).
694

2.3 Phylogeographic studies of Antarctic soil fauna

Antarctica is an ideal location to study biogeographic history of soil fauna due to the high proportion of endemic species and relatively limited dispersal, both within regions, and from further afield. Phylogeographic analyses is increasingly applied to Antarctic biota and has revealed insights into life history characteristics beyond that which is possible using morphological approaches alone. Here, I synthesise evidence across all phylogeographic studies to form a clearer picture of general patterns of Antarctic soil faunal distribution and evolutionary patterns based on the current literature. All data is based on molecular studies unless otherwise stated.

Table 2.1 Currently known species richness of the main invertebrate groups in continental (C), maritime- (M) and sub-Antarctic (S) regions, alongside the sequenced genes for all species found within each region and estimated date of origin from cited references.

Invertebrate group	# Known species				Target genes	Estimated Antarctic arrival		Phylogeographic Studies
	C	M	S	Total		Ancient (>10 Ma)	Pre-LGM	
Collembola	6	25	55	86	COI, COII, 18S, 28S ITS	~11–21 Ma (C, M)		Beet et al., 2016; Bennett et al., 2016; Carapelli et al., 2017; Collins and Hogg, 2015; Collins et al., 2019 Fanciulli et al., 2001; Frati et al., 2001; Greenslade et al., 2011; Hawes et al., 2010; McGaughran et al., 2008, 2010a, 2010b, 2011, 2019; Myburgh and Chown, 2007; Nolan et al., 2006; Stevens and Hogg, 2003, 2006; Stevens et al., 2006a, 2007; Torricelli et al., 2010a, 2010b;
Acari	5	22	78	105	COI, H3	> 34 Ma (C, M)		McGaughran et al., 2008; Mortimer et al., 2011; Van Vuuren et al., 2018
Nematoda	33	42	12	86	COI 18S	~30 Ma (C, M)		Adams et al., 2014; Kagoshima et al., 2019; Lewis et al., 2009; Maslen and Convey, 2006; Nkem et al., 2006; Velasco-Castrillón and Stevens, 2014
Tardigrada	15	64	22	90	COI, 18S, 28S, Wg	~40 Ma (C)	> 1 Ma (M, S)	Cesari et al., 2016; Czechowski et al., 2012; Convey and McInnes, 2005; Guidetti et al., 2014, 2017; McInnes, 2010;
Rotifera	20	94	81	176	COI		~130 Ka (C, S)	Cromer et al., 2006; Fontaneto et al., 2011; Iakovenko et al., 2015; Velasco-Castrillón et al., 2014b
Chironomidae	0	3	4	7	COI 28S		> 1 Ma (S)	Allegrucci et al., 2006, 2012

2.3.1 Collembola

The arthropod subclass Collembola (springtails) were the first Antarctic invertebrates to be described (Carpenter, 1902). Since their discovery, 17 continental and 25 maritime species representing four families having been recorded (McGaughran et al., 2011), with almost two thirds of all genera endemic (Stevens and Hogg, 2003, 2006; Pugh and Convey, 2008, Torricelli et al., 2010b). Phylogeographic work has mostly focussed on springtails of continental Victoria Land (VL, 71- 78.5 S) in eastern Antarctica and on the peninsula. Six species inhabit northern VL: *Cryptopygus antarcticus; Cryptopygus terranovus* Wise, 1967*; Kaylathalia klovstadi* Carpenter, 1902*; Friesea grisea* Schaffer, 1891 (initially named *F. Antarctica* Willem, 1901)*; F. propria* Greenslade and Fanciulli, 2020 and *F. gretae* Greenslade and Fanciulli, 2020 (Carapelli et al., 2020; Stevens et al., 2021). An additional three species are found in southern VL (sVL); *Antarcticinella monoculata* Salmon, 1965*; Cryptopygus nivicolus* Salmon, 1965 and, *Gomphiocephalus hodgsoni* Carpenter, 1908 (Greenslade, 2018; Collins et al., 2019). Taxonomic and phylogeographic studies have described local distributions and provided some insights into the ancient origins and restricted gene-flow between populations arising from refugial survivors on the continent and other Antarctic regions.

Origins and refugia

Phylogeographic studies have improved theories of springtail origins, with a consensus of most taxa evolving from ancestors arriving on the continent around 20 Ma, prior to the ACC formation (Stevens et al., 2006b; Stevens and D'Haese, 2014). However, several factors also promoting greater number of endemic species have been identified with molecular data, indicating more recently evolved species (Table 2.1) Intermittent dispersal events from

surrounding sub-Antarctic landmasses and inland refugia may have contributed to allopatric speciation of populations separated by glaciers and other dispersal barriers (Convey and Stevens, 2007; McGaughran et al., 2011). Springtails show evidence of complex evolutionary histories, with certain species linked to ancient arrivals and others with more recent dispersal evident from their population structures (Table 2.1). The latter point to a series of post-glacial recolonization events followed by vicariant speciation. Significant divergence and a lack of shared haplotypes between continental and maritime springtail species suggests substantial dispersal barriers and long-term persistence in distinct refugia from which source populations remained separated following dispersal events (Collins et al., 2019, 2020). Most studies found glaciers to be major dispersal barriers, restricting gene flow (Stevens et al., 2006b; Bennett et al., 2016; Collins et al., 2019). This has led to the assumption of multiple refugia in glacial valleys throughout continental VL as the source for distinct haplotypes of *C. terranovus* populations (Carapelli et al., 2017) and *K. klovstadi* (Frati et al., 2001; Fig. 2.4).

Table 2.2 Examples of the main Antarctic faunal types and species that have phylogenetic evidence supporting either ancient origins or more recent dispersal in continental and maritime regions.

	Continental		Maritime	
	Ancient species (pre-LGM)	**Recent dispersers (post-LGM)**	**Ancient species**	**Recent dispersers**
Collembola	*Friesea sp.* *Kaylathalia klovstadi*	*Cryptopygus cisantarcticus*	*Friesea grisea*	*Gomphiocephalus hodgsoni*
Acari	*Maudhemia sp.* *Stereotydeus sp.*	*Halozetes fulvus*	*Halozetes belgicae* *Alaskozetes antarcticus*	*Halozetes marinus* *Halozetes necrophagus*
Nematoda	*Scottnemia lindsayae* *Panagrolaimus davidi*	*Plectus frigophilus*	*Plectus murrayi* *Plectus antarcticus*	*Eudorylaimus coniceps* *Plectus meridianus*
Rotifera	*Adineta grandis* *Philodina gregaria*	*Notholca sp.* *Adineta gracilis*	*Philodena Antarctica* *Brachionus bidentatus*	*Linda torulosa* *Colurella colurus*
Tardigrada	*Acutuncus antarcticus* *Milnesium tardigradum*	*Milnesium antarcticum* *Minibiotus vinciguerrae*	*Testechiniscus meridionalis* *Adropion greveni*	*Mesobiotus aradasi* *Diphascon langhovdense*

Several studies support the refugial theory and the potential for multiple source populations (Marshall and Coetzee, 2000; Fanciulli et al., 2001; Frati et al., 2001; Stevens and Hogg, 2006; McGaughran et al., 2008). Evidence from the *Friesea* genus shows potential colonization by a common ancestor over 20 Ma (Miocene/Oligocene) with a subsequent local speciation with no haplotype sharing between the AP and VL regions (Torricelli et al., 2010b; Collins et al., 2019), and between the AP and South Shetland Islands (Torricelli et al., 2010a).

The northern VL species *K. klovstadi* (formerly *Isotoma klovstadi* and of the *Desoria* genus) show divergences between sub-species that are geographically close to each other, further supporting multi-refugial sources (Stevens et al., 2006a; Stevens et al., 2007; Stevens and D'Haese, 2016), and has been suggested as explanation for several species, including *Friesea grisea,* the only known circumpolar springtail species. The identification of different dominant haplotypes between the peninsular Adelaide, Lagoon and Killingbeck Islands led

768 to theories of separate lineages with multiple refugial populations undergoing rapid
769 evolution throughout the Pleistocene glacial cycles (Fig. 2.5). Another study that supports
770 the idea of multiple refugia maintaining isolated populations found lower than expected
771 genetic distances between *G. hodgsoni* populations on the peninsula, indicating a scenario
772 whereby dispersal from a singular refugial source was followed by minimal genetic
773 admixing (McGaughran et al., 2010a).
774
775 *Dispersal effects on broad-scale and regional distribution*
776 Springtails are motile at short-ranges, with less ability to disperse large distances via wind
777 or water-borne flotation (McGaughran et al., 2010b). The high rate of divergence among
778 haplotypes and patchy distribution of *F. grisea* and *C. antarcticus* sub-species, and *C.*
779 *cisantarcticus* populations, in maritime Antarctica suggests their distribution is strongly
780 associated with a history of repeated dispersal events, as found within the continental Dry
781 Valleys (McGaughran et al., 2011; Table 2.3).
782 The nVL *C. cisantarcticus*, was studied alongside the sVL springtail *G. hodgsoni*
783 where lower genetic diversity between *C. cisantarcticus* haplotypes signalled multiple
784 dispersal from elsewhere in the continent during the Pleistocene (McGaughran et al., 2008).
785 These support an earlier study that also found minimal divergence within *C. cisantarcticus*
786 populations (Hawes et al., 2010), suggesting a Pliocene divergence event ~ 5 Ma, with
787 distributional patterns indicative of recent gene flow from local refugia to the continental
788 foothills.
789 Evidence of refugia as sources of ancient dispersal and broad-scale distribution have
790 also been linked to more recent dispersal events that have contributed to complex regional
791 distribution patterns. Accordingly, *F. grisea* populations were suspected of more recent

dispersal than *C. terranovus* or *G. hodgsoni* populations from unidentified regional refugia. These are suspected of aligning with the shorter intervals between glacial cycles and barriers to dispersal preceding the LGM (Warren et al., 2014; Collins et al., 2019; Table 2.3). Furthermore, Collins et al. (2019) found much higher divergences between *C. cisantarcticus* populations than *K. klovstadi* across a similar geographic range across one glacier. Yet, lower divergences between populations in an adjacent glacier was a result of greater dispersal abilities of *C. cisantarcticus* influencing distribution patterns (Stevens et al., 2006a; McGaughran et al., 2010b; Carapelli et al., 2017).

A study that combined *G. hodgsoni* with two species from sVL (*C. nivicolus* and *A. monoculata)* estimated a speciation event ~4 Ma, followed by glacial isolation that promoted speciation in *G. hodgsoni* and *C. nivicolus* over *A. monoculata* populations. A period of isolation between 3–4 Ma was also found to be consistent with the collapse and reformation of the West Antarctic ice sheet (Beet et al., 2016). The link between populations and access to liquid water was given as a cause for highly divergent *G. hodgsoni* populations (Collins et al., 2019).

Evidence from phylogeographic studies of Antarctic springtails reveals that at the broad-scale interactions with climate and geography have created multi-layered patterns of distributions following waves of speciation and extinction during the Pleistocene (~2.58Ma – 12 Ka), with some events separated by 100 Ka (Huybers, 2006). These findings show that springtails have ancient lineages and recent inter-regional dispersal (Table 2.2), with glaciers forming a strict restriction to gene flow between populations and communities. More widespread sampling will be able to determine pockets of speciation that are still to be fully clarified, whilst identifying further pan-Antarctic species and confirming refugial locations in the peninsula.

Table 2.3 Summary of mean levels of COI and COII sequence divergence and number of haplotypes in springtail species and regions as reported in cited references. *Includes unpublished data from Ross et al., 2022b. Species are ordered alphabetically according to region.

Springtail Species	Region	Mean COI inter-regional divergence (%)	Mean COII inter-population divergence (%)	# Haplotypes	Studies
Cryptopygus cisantarcticus	**nVL**	8.7 (4.3-13)		45	Collins et al., 2019
Cryptopygus terranovus	**nVL**	10.5 (6.8-14.7)		62	Hawes et al., 2010; Collins et al., 2019
Friesea grisea	**nVL**	6 (4.5-10.9)		55	Collins et al., 2019; Torricelli et al., 2010a
Kaylathalia klovstadi	**nVL**	8 (0.7- 27.5)	2.25 (0.1-3.8)	69	Collins et al., 2019; Frati et al., 2001; Stevens et al., 2006a; Stevens et al., 2007
Antarcticinella monoculata	sVL	10.7 (1.25-98.7)		66	Beet et al., 2016; Collins et al., 2019
Cryptopygus nivicolus	sVL	4.5 (1.6-5)	1.6 (0-1.6)	16	Stevens et al., 2006b; Collins et al., 2019; Beet et al., 2016
Gomphiocephalus hodgsoni	sVL	3.63 (1.5-7.3)		289	McGaughran et al., 2008, 2010a Collins et al., 2019; Beet et al., 2016
C. cisantarcticus	**AP**		4.6 (0-9.2)	19	McGaughran et al., 2010a
F. grisea	**AP**		2.7-17.2	80	Torricelli et al., 2010a

2.3.2 Acari

The Acari, or mites, are the most diverse faunal group in terrestrial Antarctica (Pugh, 1993). Current tallies number 105 species with five continental, 22 maritime, and 78 sub-Antarctic species, of which roughly 70 are endemic (Convey, 2011; McGaughran et al., 2011). The biogeography of several mite species have been established on Sub-Antarctic islands (Mortimer et al., 2011) and maritime Antarctica, with more limited evidence of biogeographic patterns on the continent; yet most distributions remain unverified with molecular approaches.

Origins and refugia

First described from the Belgica expedition of 1878-1899 (De Man, 1904), continental mites have some of the earliest links to a pre-Gondwanan lineage (Fig. 2.3). Several species appear

to have ancient origins including members of the oribatid mite genus *Maudhemia* Dalenius and Wilson, 1958 (> 100 Ma; Marshall and Coetzee, 2000) and the prostigmatid mite genus *Stereotydeus* Berlese, 1901 mites (> 10 Ma; Stevens and Hogg, 2006). Whilst some mites have an ancient origin, it is hard to ascribe all as having ancient lineages, with some groups having far more recent histories of colonisation (Table 2.2). Molecular analyses have shown that vicariant speciation was prevalent during interglacial periods in mites (Stevens and Hogg, 2006), similar to observed patterns in springtails (McGaughran et al, 2008). There is evidence of local refugia with oribatid and prostigmatid mites persisting during glacial maxima, and most Antarctic mites appearing to have dispersed from glacial refugia and coastal habitats (Marshall and Coetzee, 2000).

Dispersal effects on broad-scale and regional distribution

Despite their ability for long-distance dispersal, little overlap between continental and maritime populations demonstrates strong geographic barriers limiting dispersal among geographic regions (Convey et al., 2014). However, there is evidence of some dispersal within regions. For example, a study of eight members of the genus *Halozetes* Berlese, 1916 in maritime and sub-Antarctica, found frequent dispersal events between sub-Antarctic islands amongst *H. belgicae* and populations of the mite genus *Alaskozetes* Hammer, 1955 between 6–10 Ma, and a rare case of wind or water-borne dispersal from northern peninsular refugia to surrounding islands (Mortimer et al., 2011; Table 2.1).

Restrictions to dispersal and mixing of populations, evident from a lack of shared haplotypes and gene-flow may also indicate recent colonisation of the sub-Antarctic and continent (Mortimer et al., 2011; Convey, 2014). Limited ranges and high endemism have been shown in peninsular populations of *H. belgicae* and *Alaskozetes antarcticus* Michael,

1903 with further evidence of multiple refugial sources (Van Vuuren et al., 2018). Additionally, contemporary distribution patterns conform with mites' limited short-range dispersal ability, linked to low desiccation tolerance (McGaughran et al., 2010b). Beyond this, studies on the short-range dispersal of mites are limited.

Despite thorough biogeographic investigation (Starý and Block, 1994; Marshall and Coetzee, 2000; Collins et al., 2023), knowledge on the phylogeography of Antarctic mites is limited. However, studies have revealed restricted distributions of several mite species (Van Vuuren et al., 2018), with diverse prostigmatid communities in inland oases (Ross et al., 2022b). Phylogenetic support for their ancient lineages, combined with evidence of recent evolution linked to refugial dispersal can be clarified through targeted sampling. This would overcome the data deficiency for these important decomposers and support their use of refugia and long-range dispersal as explanation of their diverse contemporary distribution.

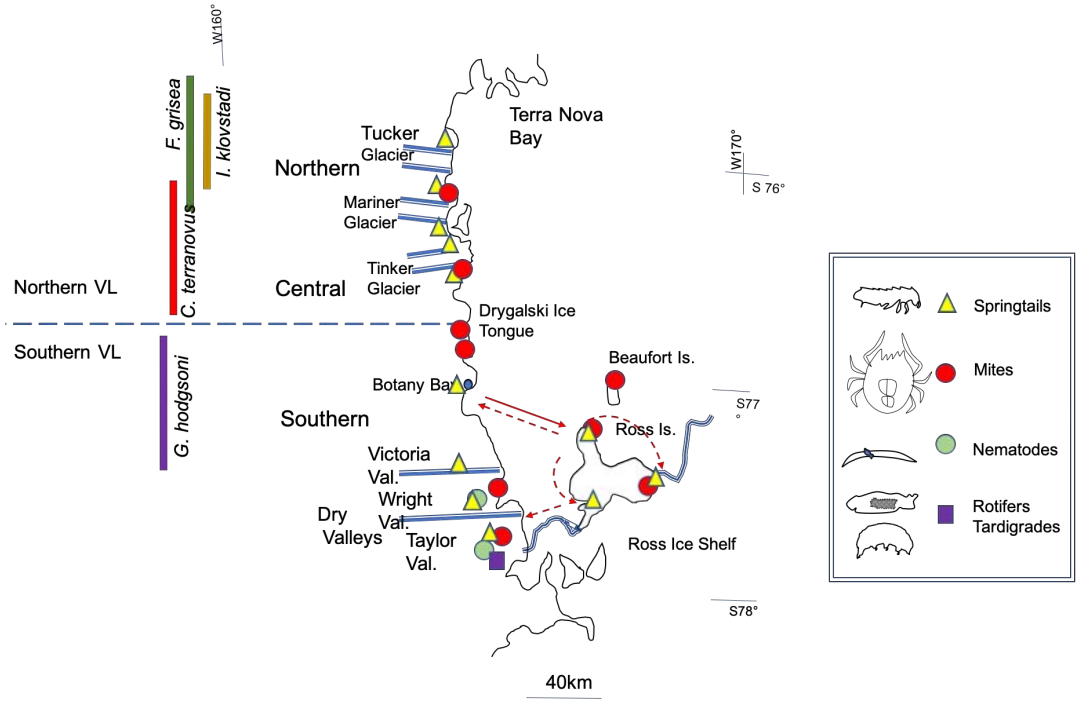

Figure 2.4 Map of Victoria Land with major glacial features and compiled results from phylogeographic studies. Springtail geographic ranges indicated by coloured lines on left panel from McGaughran et al. (2008), with theorised dispersal routes to Ross Island from Stevens and Hogg, 2003 and Collins et al., 2019, 2020. Dashed lines demarcate biotic boundaries used

to define population groups with symbols representing genetically distinct populations of springtails (yellow triangles), mites (red circles), nematodes (green circles) and rotifers (purple squares, sources: Barrett et al., 2006; Torricelli et al., 2010a; McGaughran et al., 2011; Convey et al., 2014: Brunetti et al., 2021).

2.3.3 Nematoda

The first terrestrial nematode to be described from Antarctica was *Plectus antarcticus*, de Man, 1904, and there is now 68 recognised species, including 23 continental and 33 maritime species, with most genera endemic to Antarctica and some shared species across regions (Nielsen et al., 2011a; Maslen and Convey, 2006; Kagoshima et al., 2019). However, the recorded diversity of nematodes is considered a gross underestimate (Nielsen et al., 2011b; Velasco-Castrillón et al., 2014c).

Origins and refugia

Nematodes were well established throughout the Gondwanan continent and molecular data confirms ancient origins (>32 Ma) of most, if not all, indigenous Antarctic taxa (Maslen and Convey, 2006; Adams et al., 2007). High levels of cryptic speciation and low levels of divergence between most nematode genera (< 5%), indicating recent speciation (Adams et al., 2014). This is evidenced by a combination of ancient species, such as *Scottnema lindsayae* (Maslen and Convey, 2006), and those with more recent divergence including *Panagrolaimus davidi* Timm, 1971 (Lewis et al., 2009; Table 2.2). Antarctica's cyclical glaciation events have resulted in a separation of some nematode species between the maritime and continental regions, suggesting separate refugial origins (Andrássy, 1998). Additionally, high nematode endemism on Alexander Island (> 40%) and other sites along the peninsula indicate likely long-term refugial isolation and survival in these areas (Maslen and Convey, 2006).

Dispersal effects on broad-scale and regional distribution

The phylogeographic patterns of nematodes indicate ancient origins, refugial survival and widespread contemporary distribution. Molecular studies of nematodes indicate evolutionary histories as ancient as the arthropods, and show them to be equally dependent on refugia for their survival. High levels of cryptic speciation within endemic taxa warrant further investigation to ascertain the true level of *in situ* diversity, speciation, and evolutionary processes such as convergence or stasis (Struck et al., 2018).

General patterns of nematode distribution are influenced at multiple scales. Minimal genetic variation in southern maritime island *S. lindsayae* populations despite large distances between populations, could indicate a population bottleneck or gene-flow with limited genetic drift of these highly adapted ancient taxa following isolation (Maslen and Convey, 2006). This limited ability for individual motility (Adams et al., 2007), may also support a wind-borne theory of infrequent dispersal (Nkem et al., 2006; Ptatscheck et al., 2018), combined with environmental filtering. However, less evolutionary selection of ITS genes may mask variation compared to mtDNA markers (Evans and Paulay, 2012).

Nematode COI sequences from Larsemann and Vestfold Hills in continental eastern Antarctica showed limited divergence among populations of *Plectus murrayi* Yeates, 1970 (< 0.5%), suggesting a high degree of connectivity. In contrast, the higher rates amongst *Plectus frigophilus* Kiryanova, 1958 populations (8.4%), suggest the presence of cryptic species and taxonomic division. The contrast between *P. murrayi,* and *P. frigophilus* indicate more restricted gene-flow in the latter species, likely because it requires more persistent bodies of water that are few and far between limiting dispersal success (Velasco-Castrillón et al., 2014a, b; Table 2.1).

A more detailed understanding of the distributional ranges of these ubiquitous taxa would improve dating of nematode phylogenies. Our ability to construct robust phylogeographic models are currently constrained by suspected differences in substitution rates due to differences in reproductive mode and other biotic factors that exist between species. Further investigation may reveal more widespread continental nematode species and help explain the high levels of endemism despite their ability for long-range dispersal.

2.3.4 Rotifera

Antarctic rotifers were among the earliest reports of terrestrial fauna from Scott's 1901–1904 Discovery Expedition, where an ancient origin was initially hypothesised (Murray, 1907). Rotifers are semi-aquatic and generally parthenogenetic organisms capable of wind and water-borne dispersal and anhydrobiosis. There are presently 94 recorded rotifer species, of which 66 are continental with the remainder distributed throughout the maritime and sub-Antarctic islands (Velasco-Castrillón and Stevens, 2014); however, rotifer richness is likely underestimated. Most of these species have links to corresponding species from other southern-hemisphere continents, whilst only five species are endemic to the continent (Velasco-Castrillón et al., 2014b) and one species is shared across maritime and continental Antarctica (Segers, 2008; Iakovenko et al., 2015; Table 2.1).

Origins and refugia

The relatively limited genetic variation between Antarctic and non-Antarctic species, points to a far more recent arrival of rotifers than other taxa (Iakovenko et al., 2015; Cakil et al, 2021). The most likely scenario is that arrivals may have commenced from around 130 Ka based on sedimentary evidence of *Notholca* Gosse, 1886 from the Larsemann hills in East

Antarctica (Cromer et al., 2006) from an as-yet unknown location requiring long-distance dispersal. This could be a result of recent dispersal from nearby landmasses or island groups. Their widespread distribution has been aided by their ability for long-distance wind dispersal and the presence of multiple refugial lakes within the harsh glacial environment as found in northern hemisphere systems (Shain et al., 2016). The continental ranges of some *Adineta* Hudson and Gosse, 1886 bdelloid species of up to 2,000 km (Velasco-Castrillón et al., 2014a) remain to be assessed using phylogenetic records. Antarctic Bdelloid rotifers show strong patterns of increased genetic divergence with greater isolation than other non-Antarctic specimens, indicating greater periods of isolation and separation beyond what can be concluded from molecular evidence alone (Cakil et al., 2021).

Dispersal effects on broad-scale and regional distribution

A thorough study of COI sequences from 514 rotifer specimens collected across all Antarctic regions, revealed a total of 92 species, with 63 found throughout continental and maritime regions (Velasco-Castrillón et al., 2014c), whilst seven continental lineages were distributed across 13 lakes (Velasco-Castrillón et al., 2014a). The presence of three pan-Antarctic rotifer species indicates prolonged isolation (*Adineta grandis* Murray, 1910, *Philodina antarctica* Murray, 1910 and *Philodina gregaria* Murray, 1910; Table 2.2) but does not fully explain the high numbers of shared haplotypes with non-Antarctic regions (Pugh and Convey, 2008). Further studies of the rotifers on the Antarctic Peninsula, Signy Island and Tierra del Fuego in South America also indicate that rotifer diversity is currently underestimated. Sequences from 210 individuals from multiple Antarctic and non-Antarctic locations revealed 36 taxa with up to 3.6% between-species divergence for four *Adineta* species, whilst species divergence ranged from 0.5–10.3% between 55 populations (Fontaneto et al., 2011), clearly

raising the issue of cryptic species. With a high level of cryptic speciation and undescribed species, the parthenogenetic nature of rotifers may complicate discerning their evolutionary histories.

Regional dispersal of rotifers has been described as random and often co-occurring with tardigrades that share cold-adaptive traits (Zawierucha et al., 2015). Furthermore, fossil evidence of rotifers present in lakes in the Eastern continental Vestfold hills could not distinguish between the refugial or dispersal histories (Swadling et al., 2001). More sequence information is required to better assess patterns of regional dispersal in rotifer communities (Sohlenius and Boström, 2005).

In summary, the association of rotifers with refugia may demonstrate greater dispersal ability and motility and may explain their widespread and seemingly random distributions. Further analysis to reveal their evolutionary history and to verify the hypothesis of recent colonization followed by rapid post-LGM speciation. Targeted sampling to locate refugia in the eastern continental hills could also equate their ability for long-range dispersal. Future genomic studies may reveal more information about the complex gene-pools, potentially re-evaluating theories of a more ancient origin for rotifers.

2.3.5 Tardigrada

A total of 64 species of limno-terrestrial tardigrades, or waterbears, has been recorded throughout continental and maritime Antarctica, with approximately half of all genera endemic to the region (McInnes and Pugh, 2007). First described in Antarctica by Richters (1904), these ‘slow-steppers’ range from 0.05–1.2 mm in length and are renowned for their survival ability. They tolerate the extreme cold utilising cryptobiosis (Wright, 2001), and are capable of long-distance dispersal in a dormant state (Fontaneto, 2019). Tardigrades are

probably under-sampled throughout Antarctica, but the most broadly distributed continental species are *Hypsibius antarcticus* Richters, 1904 and *Milnesium tardigradum* Doyère, 1840 with no discernable species overlap between the continent and the maritime Antarctic (Cesari et al., 2016; Table 2.1).

Origins and refugia

Phylogeographic analyses of the continental *Echiniscus canadensis* Murray, 1910 using nuclear markers revealed a Gondwanan arrival with speciation event of the ancient lineage in the Eocene (32–48 Ma.; Guidetti et al., 2017, 2019), despite the inherent difficulties in dating tardigrade speciation (Table 2.2). There is a lack of continental-wide surveys, but analysis of specimens from populations in Ellsworth Land in the west of the continent showed low level genotypic endemism and divergence imply that no local refugia were present, with subsequent re-colonisation occurring in the Quaternary period by both endemic and cosmopolitan taxa (McInnes, 2010). The likely ancient origins of tardigrades is supported by their well-known tolerance of harsh conditions.

Dispersal effects on broad-scale and regional distribution

Considering the relatively ancient origins of Tardigrades, combined with their renowned ability for refugial survival, patterns of widespread distribution throughout Antarctic regions would be expected. A study including 343 Antarctic and non-Antarctic specimens identified 70 unique Antarctic haplotypes using 18S rRNA, of which 25 were shared between the sub-Antarctic and Antarctic Peninsula and four between Marion and South Georgia Islands. Minimal haplotype sharing between divergent populations may suggest the presence of cryptic species if divergences are comparable to differences between *Milnesium antarcticum*

Tumanov, 2006 and *M. tardigradum* (Sands et al., 2008).

An analysis of 126 tardigrades from two nunataks around 30 km apart in the SØr Rondane Mountains in Queen Maud Land identified 89 COI, 67 18S rRNA and 22 Wg haplotypes within the Macrobiotidea and Hypsibiodea groups (Czechowski et al., 2012). More recent genetic studies using COI and nuclear 18S markers of the Mesobiotus genus has revealed deep geographic distinctions across continental populations with potential species complexes from distinct tardigrade lineages (Short et al., 2022).

Unexpectedly high levels of divergence indicate strong barriers to gene flow between communities based on 42 specimens collected from the base of the Antarctic Peninsula despite geographic proximity (McInnes, 2010). As tardigrade phylogenies include the presence of high levels of cryptic speciation, a long-term presence, especially in the mountainous nunataks, has been suggested (Altmaier et al., 2010). More detailed phylogeographic studies of Antarctic tardigrades may shed light on the balance between long-range dispersal and refugial survival for these hardy invertebrates.

2.3.6 Insecta

The only known Antarctic insects are wingless species of the globally diverse Chironomidae family of the Diptera order. Two midge species of the genus *Belgica* Jacobs, 1900 are present with *B. Antarctica* Jacobs, 1900 endemic to maritime Antarctica and *B. albipes* Seguy, 1965 endemic to are present the Îles Crozet sub-Antarctic islands. Earlier studies have found evidence for post-LGM vicariance between sub-Antarctic and maritime regions (Allegrucci et al., 2006). However, phylogenetic knowledge of the indigenous Diptera is limited. *Eretmoptera murphyi* Schaeffer 1914 that was paleoendemic to South Georgia was introduced to Signy Island in the 1960's (Allegrucci et al., 2012; Hughes et al., 2013).

Evidence for an ancient origin in the resident midges have been found from multiple sources. Divergence rates of 28S rRNA sequences found a single ancestral midge as the ancestor of the three known Antarctic species, estimated at a colonization event some 49 Ma (Allegrucci et al., 2006). Additionally, four distinct *B. antarctica* and two *B. albipes* haplotypes are closely related to the *Eretmoptera murphyi* lineages (Allegrucci et al., 2012; Fig. 2.5; Table 2.1). This supports an earlier study that found *B. antarctica* and *E. murphyi* distributions result from vicariance with an ancient origin (Allegrucci et al., 2006).

Representatives of the *Belgica* and *Eretmoptera* Kellogg, 1900 genera show minimal gene flow between peninsular and sub-Antarctic populations likely due to their limited ability of long-distance dispersal (Convey, 1992). The limited long-distance dispersal ability of the indigenous wingless chironomids reinforces vicariance as a principal driver of genetic diversity, with a wave of colonisation moving south through the peninsula following the LGM (Pugh and Convey, 2008). Getting a firm grasp of the genetic diversity of chironomids and other potential invasive insect should be central to conservation plans for vulnerable Antarctic ecosystems.

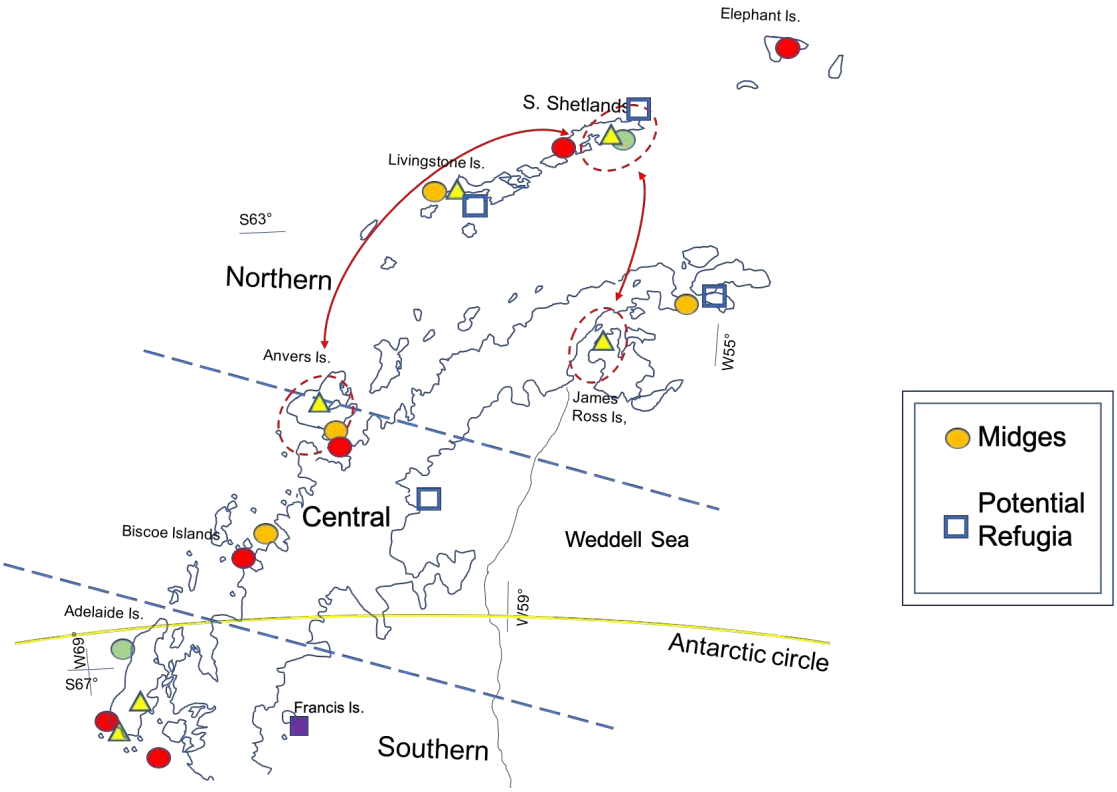

Figure 2.5 Map of the Antarctic Peninsula (Graham Land) with theorised dispersal routes between springtail populations indicated by red arrows from McGaughran et al., 2010. Symbols represent genetically distinct populations of midges (orange circles) and potential refugial locations (empty squares, sources: Torricelli et al., 2010a; Allegrucci et al., 2012; Velasco-Castrillón et al., 2014b; Convey et al., 2014).

2.4 Synthesis

Origins and refugia

There is increasing evidence that most of the terrestrial soil fauna of maritime and continental Antarctica have ancient lineages, although origins differ among taxa. Strong evidence of an ancient origins in mites stems from a lack of genetic admixture between Antarctic and non-Antarctic species dating as far back as 10–100 Ma (Marshall and Coetzee, 2000; Stevens and Hogg, 2006). Yet this large range is indicative of the discrepancies when estimating the origins of Antarctic taxa, with further molecular evidence of other southern

hemisphere taxa to act as much-needed outgroups required to define the ancestry of species. The younger origins of rotifers may be a result of difficulties establishing an evolutionary timeline with current genetic markers, despite extensive comparison with non-Antarctic specimens (Cakil et al., 2021). The parthenogenetic mode of reproduction in rotifers may also mask ancient lineages, but sequencing sister lineages may clarify their ancestry.

Certain species of nematode that are parthenogenetic (e.g. *P. davidi*) are also difficult to accurately date, with estimates of their origins ranging from 1.35–8.5 Ma depending on the generation times used in calculation (Lewis et al., 2009; Schiffer et al., 2019). Similar ages of Antarctic nematodes (~30 Ma), tardigrades (~40 Ma) and chironomids (~49 Ma) indicate parallel evolutionary timescales. By contrast, evidence indicate repeated recent colonisation events for Antarctic rotifers preceding the LGM (~130 Ka). Parthenogenetic reproduction in some species of oribatid mite and springtail also complicate evolutionary analysis with assumptions of consistent rates of genetic drift and background mutation.

Sub-Antarctic and maritime mite and springtail populations have some degree of shared genealogy that points to repeated mixing of ancient survivors with motile dispersers during interglacial periods, and rare trans-oceanic migrations. Founder populations subsequently underwent varying degrees of *in-situ* speciation with localised extinction events. Molecular taxonomy has indicated the presence of refugia as evidenced by: i) single sites that include multiple haplotypes; ii) singular haplotypes at individual sites; or, iii) shared haplotypes between sites that have been geographically separated. As such, a lack of limitations to sample sizes and site replicates may hamper the ability to accurately identify refugia. Despite this, the lack of genetic divergence and motility of mite populations along the peninsula supports a refugial ancestry (Pugh and Convey, 2008). Further evidence comes from localised diversity and reinforces the narrative of multiple dispersal events.

Refugial survival has a defining role in nematode distributions, with interglacial dispersal and fast adaptation to abiotic pressures being key to the establishment of new colonies. For example, nematodes can only actively move short distances and require passive long-distance dispersal to seek out suitable habitats. Mites and springtails are susceptible to desiccation, yet their capacity for passive long-distance dispersal depended on the minimal chance of arrival on vegetated ice-free land during periods of glacial maxima (Hawes et al., 2008; Fig. 2.5).

Dispersal on broad and regional-scale distribution patterns

Phylogeographic studies across all main faunal types have shown a range of divergences (Table 2.1). Strong biotic barriers and biogeographic divisions between the continent and peninsular populations form observable divergence patterns in springtails (McGaughran et al., 2011; Collins et al., 2019), mites (Pugh, 1993), nematodes (Smith, 1984; Maslen and Convey, 2006), and tardigrades (Cesari et al., 2016), yet is lacking for rotifers. At smaller scales, similar dispersal barriers were evident in multiple Antarctic regions within the continental Dry Valleys of VL (Fig. 2.4; Stevens and Hogg, 2003; Barrett et al., 2006), the Antarctic peninsula (Fig. 2.5; Chown and Convey, 2007; McGaughran et al., 2010a), and eastern continental Antarctica (Fig. 2.4; Velasco-Castrillón et al., 2014a). Localised distributions are even present in the circumpolar *Cryptopygus* springtails which has phylogeographic support for an ancient origin for the genus (~24 Ma; Stevens et al., 2006b; McGaughran et al., 2008, 2011), with complex interactions with dispersal ability (McGaughran et al., 2010b). These contrast with the histories of *G. hodgsoni* and *C. cisantarcticus* that diverged over 1 Ma (Stevens and Hogg, 2006; Stevens et al., 2006b). This may also indicate the prominence of recolonization and dispersal events in shaping

distributions. Long-distance dispersal among maritime and sub-Antarctic islands is also clear from mite (Mortimer et al., 2011) and springtail populations (Stevens et al., 2006b). These dispersal events have often been linked with refugial survival and long periods of inter-glacial isolation. This is apparent from molecular evidence for mites and springtails with further investigation required to confirm refugial origins for the non-arthropod nematodes, rotifers and tardigrades that have generally more limited dispersal abilities.

The pre-LGM dispersal events might be masked by more frequent post-LGM dispersal events as evidenced in springtails (Hawes et al., 2010; Carapelli et al., 2017) and potentially occurring in mites (Van Vuuren et al., 2018). Recent speciation (<20 Ka) in the prostigmatid *Stereotydeus mollis* Womersley & Strandtmann, 1963 and the sub-Antarctic oribatid *H. fulvus* mites supports a post-LGM theory of continental mite dispersal and speciation. These findings align with an earlier, morphological-based study (Pugh and Convey, 2008). Further comparisons of mites with *Brachionus plicatilis* Müller, 1786 rotifers showed marked similarities in divergences over similar ranges, whereby patterns of long-distance dispersal were constrained by local geography with evidence of unsubstantiated refugia (Fontaneto et al., 2009).

A study of rotifers using molecular data from a defined transect within the Transantarctic Mountains found three species with limited cryptic speciation that was suggested as indicating limited starting diversity and restricted gene-flow (Hodgson et al., 2010). Additionally, the widespread presence throughout the peninsula and sub-Antarctic islands of tardigrade distribution is similar to rotifers and southern hemisphere nematodes (McInnes and Pugh, 1998, 2007). Reversal of a data deficiency in southern hemisphere species may yet reveal more recent divergence events separate Antarctic taxa from species with shared ancestors on nearby landmasses. Sequences from contents with a Gondwanan

history (e.g. South America, Sri Lanka, Australia and New Zealand) can establish genetic distances and identify recent arrivals as found in other arachnids (Baker et al., 2020).

Local-scale distribution patterns

Fragmented habitats divided by glaciers and ice-sheets are often associated with restricted gene flow and high levels of genetic differentiation, as observed in mites (Mortimer et al., 2011), springtails (Fanciulli et al., 2001; Frati et al., 2001; Stevens et al., 2007; McGaughran et al., 2011) and Chironomids (Allegrucci et al., 2012). The western Antarctic icesheet was shown to be a strong barrier to dispersal. Marked increases in speciation followed its collapse 5 Ma creating large disparities in diversity among populations found on either side (Pollard and DeConto, 2009; Hawes et al., 2010; McGaughran et al., 2010c; Bennett et al., 2016).

At local scales, biotic distributions are influenced by both local barriers and an organism's ability for passive wind or water-borne long-term dispersal and active local motility. This is most apparent around glaciers and other dispersal barriers, where communities often have limited gene flow despite being geographically close to each other. These speciation events are balanced by extinctions that often follow waves of dispersal and colonisation, ultimately contributing to the complex gene pools that are found in mite (Marshall and Coetzee, 2000; Maraun et al., 2003) and springtail populations (McGaughran et al., 2008, 2011; Collins et al., 2019).

Nematodes, tardigrades and rotifers tend to be more locally endemic with limited divergence and gene flow between populations, even within the ancient members of the nematode genus *Scottnema* Timm, 1971 (Adams et al., 2007; Boström et al., 2011). Evidence of recent dispersal for mites and springtails contrasts with the less motile taxa, which tend to have a higher proportion of ancient lineages by enduring glaciation *in situ.* As

such, they are associated with more cryptic speciation, as observed in persistent rotifer (Fontaneto et al., 2011) and tardigrade populations (Czechowski et al., 2012).

Future research priorities

Molecular approaches have revealed the taxonomic diversity, origins and evolutionary history of Antarctic soil invertebrates, yet some details remain unresolved. Advances in molecular techniques and greater sampling depth can help resolve biogeographical patterns in greater detail, provide new insights into unresolved dispersal and refugial locations (e.g. peninsular montane regions) and reveal population dynamics (Convey et al., 2014). In-depth molecular analysis has greatly improved the accuracy of phylogeographic analysis that can resolve spatial and temporal resolutions to within a single glacier below a kilometre, or within a glacial cycle of 10 Ka (McGaughran et al., 2008; Collins et al., 2020). Such resolution can confirm repeated re-colonisation events (Rogers, 2007) and improve models of soil community responses to ecosystem fragmentation (Struck et al., 2018).

The similar morphological traits and life histories of the terrestrial arthropods, mites and springtails, make them ideal candidates for direct comparative analysis. However, comparison with other semi-aquatic, non-arthropod taxa such as tardigrades and nematodes, may also reveal insights into general factors influencing soil faunal distribution, yet may be complicated by certain species that possess parthenogenetic modes of replication. Meanwhile, comparisons with Gondwanan sister lineages can improve dating estimates, that may assist resolving the origins of rotifers. Future studies that apply high-throughput metabarcoding and genomic datasets will benefit from rigorous process standardization and replicability (Avise et al., 2016). Metabarcoding has provided rapid assessments of nematodes, rotifers and tardigrades from VL and maritime islands (Zawierucha, 2021), from

diverse oribatid mite assemblages (Ross et al., 2020). Divergences within *C. cisantarcticus* that have low levels of cryptic speciation, but higher mutation rates could help define OTU delimitation threshold limits and levels of genetic drift. Detailed genomic datasets that are processed using automated-learning algorithms have detailed evolutionary histories of the *Plectus* Laporte, 1840 nematodes (Xue et al., 2020) and sub-Antarctic springtail sub-species *C. cisantarcticus travei* Deharveng, 1981 (Monsanto et al., 2019). Parallel computing can also enable rigorous evaluation of model outputs to assess population genetic analysis using high-throughput and genomic datasets (Panchal and Beaumont, 2010; Yang and Rannala, 2012).

At a broader scale, springtail community insights have been generated from Reduced Representation Sequencing (RRS) approaches such as Restriction site Associated DNA sequencing (RADseq or ddRAD; (McGaughran et al., 2019). These have been shown to provide more marker regions by which to identify polymorphisms and resolve recent gene-flow within populations (Davey et al., 2013). Phylogenetic inferences are clearer using reference genomes (Shafer et al., 2017), however, *de novo* assembly approaches without reference genomes (Catchen et al., 2011; Wright et al., 2019) and alignment of phylogenies with trait databases can give insight into functional speciation (Ross et al., 2022a). This is suited to soil invertebrates that are mostly non-model taxa. Single Nucleotide Polymorphisms (SNPs), microsatellites and DArTseq are other approaches used to extract meaningful ecological information from genomic datasets (Collins et al., 2020), whilst assemblage patterns have been inferred from metagenomics in mites, springtails and beetles (Arribas et al., 2016, 2020a, b). It is hoped that combining molecular approaches with morphological taxonomy can highlight the importance of biodiversity for fragile ecosystems to adapt to both rapid and dramatic shifts in climate and to ensure their continued existence.

Chapter 3 - Phylogenies of traits and functions in soil invertebrate assemblages

Abstract

Soil invertebrates are members of terrestrial ecosystems, contributing to the processing of organic matter, resulting in carbon and nutrient cycling that is essential for sustainable productivity. By linking species delineations, morphological traits and measured or inferred functional roles, we demonstrate a method for functional identification of soil faunal assemblages based on molecular information. Here I align the genetic inter-relatedness and functional trait expression in nematodes and springtails. Nematodes were assigned feeding guild, plant parasitic feeding type and coloniser-persister (c-p) value, with springtail sequences assigned to soil vertical stratification level, soil moisture preference and a selection of morphological traits.

It was found that both nematode and springtail feeding preferences show significant phylogenetic clustering. In contrast, greater dispersal was found amongst nematode coloniser-persister (c-p) values and springtail soil vertical stratification level and moisture preferences. Patristic distances are defined as the number of apomorphic step changes separating two taxa on a cladogram. Minimum patristic (p) distances between species supported the clustering amongst nematode feeding guilds, with plant feeders being separated from all other guilds by at least p = 0.99. Distances between endoparasitic, ectoparasitic and sedentary plant parasitic nematodes were also distinct with minimal distances of p = 0.35–0.72 between parasitic types. Springtail stratification level and soil

moisture preferences showed greater dispersion across phylogenies, with negligible between-group minimum patristic distances. However, nematode c-p values and springtail moisture preference alignments indicate some genetic conservation at the genus level.

These results indicate how ecosystems can direct trait conservation beyond that of environmental stimuli. Being able to assign functional traits to novel sequences will allow individual species' likely contribution to ecosystem functioning to be inferred without the need for exact taxonomic identification. More broadly, such information can advance our understanding of the evolution of soil faunal traits and the contribution of diverse soil assemblages to functional soil systems, particularly those with a high proportion of undescribed species.

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3.1 Introduction

Soil invertebrates are renowned for their diversity and contribution to ecosystem processes (Hunt, 1987; Brussaard, 1998). They are very abundant in the litter layer and upper soil horizons, with particularly high biological diversity and activity in the organic slough that comprises the rhizosphere (Rovira, 1956). The high densities of organisms in soils foster complex trophic interactions among bacteria, fungi, protozoans, nematodes and larger invertebrates (Wall and Moore, 1999; Nielsen, 2019). The contributions of soil fauna to ecosystem functioning are substantial as a result of their routine living, feeding and reproduction in soils. A range of processes are influenced including soil organic matter (SOM) decomposition, nutrient and moisture retention, and primary productivity. Soil fauna work alongside other organisms across the trophic web in the act of comminution, or litter breakdown, that increases litter and SOM surface area upon which microbial-driven decomposition mineralizes nitrogen (N) and phosphorus (P) (Petersen, 1994; Brussaard, 1998). Moreover, soil invertebrates can suppress pathogens and pests, moderate microbial respiration and influence soil aggregation and formation, also known as pedogenesis, through their interaction with soil microbes (Daily, 1997; Nielsen, 2019; Table 3.1).

Springtails and nematodes enhance ecosystem functioning through their actions of nutrient turnover and productivity (Wall and Moore, 1999), that is partly due to feeding and altering bacterial and fungal communities. This imparts an indirect effect on decomposition, nutrient cycling and organic matter turnover rates (Table 3.1). Altering belowground faunal densities and diversity has been shown to have measurable impacts to nutrient turnover (Ineson et al., 1982), moisture retention, soil turnover (Rusek, 1998), and carbon cycling (Nielsen et al., 2011). These direct and indirect actions have significant ecosystem-wide

implications, yet the inclusion of soil fauna in ecosystem-level studies has been held back by the challenges to their identification and a lack of taxonomic expertise. This has limited our knowledge of the correspondence between taxonomic delineations and contributions to ecosystem processes in soil invertebrates (Guerra et al., 2020). Therefore, integrating trait-based and phylogenetic approaches can provide a high-resolution framework linking soil biodiversity and ecosystem functioning (Pey et al., 2014a).

The link between traits and ecosystem processes is based on the assumption that the actions of soil fauna are a product of their trophic web position, environment and other factors (*sensu* Violle et al., 2007). Functional traits can be considered either response or effect traits, although some traits can be considered both response and effect traits depending on the circumstances. Traits such as springtail body size and stratification level are linked to a given organisms' response to, for example, environmental conditions. By contrast, traits such as mouthparts provide insight into the potential effect of a given individual on ecosystem processes based on previously quantified relationships (Berg et al., 2004). Similarly, springtail soil moisture preferences that are based on ecological measures (e.g. micro-habitat) can be considered an ecological rather than functional trait. Yet, further tests linking functional traits and their contribution to ecological processes can confirm springtail functional status.

The increasing availability of DNA sequences provides a robust framework to assess the level of trait conservation within soil invertebrates. These comparisons of genetic distance can be aligned with previously described species with documented roles in ecosystem functioning based on morphological trait and ecological information. Functionally important traits of soil invertebrates that are essential for ecosystem functioning such as decomposition and nutrient turnover are expected to mirror principles outlined in the

niche conservatism theory (Wiens, 2004; Losos, 2008), also observed in insects and other closely related invertebrates (Potapov et al., 2016). This proposes that closely related species have a greater share of commonalities due toa range of potential environmental or competetive factors.

Our aim was to assess whether molecular tools can assist in evaluating the degree to which functional attributes influence clustering of springtails and nematode species across trophic levels. These two groups are ideal study organisms for this purpose, with broad functionality and influence across a range of ecosystem processes, with indirect control of microbial populations. Additionally, detailed trait-based descriptions exist for a number of species. Sufficient genetic data are also available to perform the bioinformatic approach linking the phylogenetic signal with functional traits (Bird et al., 2015; Potapov et al., 2020). Being able to infer functionality from (meta-)barcoding of unknown specimens based on phylogenetic clustering of morphologically important traits can assist in evaluating overall ecosystem functionality from a snapshot of assemblage biodiversity, assisting monitoring and conservation efforts. Having a firm understanding how soil fauna have evolved may also inform how they may functionally respond to future environmental change. Next, we introduce these two soil invertebrate groups as model organisms before aligning their phylogenies with established traits.

Table 3.1 Main nematode and springtail functional and morphological traits that are indicative of involvement in ecosystem processes. Derived from: Yeates et al., 1993; Hopkin, 1997; Ponge and Salmon, 2013; Nielsen, 2019.

Nematode feeding guild	Identifying traits	Direct and indirect actions	Main ecosystem processes
Herbivore	Stylet of various thickness, oesophageal knobs	Root sap feeding N-fixing bacteria grazing Excess nutrient excretion	Primary productivity Nutrient cycling
Bacterivore	Funnel or tubular shaped stoma, no teeth or spear, large basal bulb	Microbial grazing Excess nutrient excretion	Pathogen control Decomposition Mineralisation of nutrients
Fungivore	Thin spear, small basal bulb	Fungal grazing Excess nutrient excretion	Decomposition Nutrient turnover
Predator	Large tooth, muscular body	Nematode predation Excess nutrient excretion	Biological pest control Nutrient cycling
Omnivore	Hollow stylet, no knobs	Microbial grazing and nematode predation Excess nutrient excretion	Decomposition Community multifunctionality
Entomopathogenic Nematodes (EPN)	6 hexaradiate lips—protruding into probolae, parallel basal bulb and excretory pole	Insect parasitism Excess nutrient excretion	Pathogen control Primary productivity
Springtail Stratification Level			
Epigeic Plant/Soil surface	Well-developed legs, ocelli and furcula, pigmented, varied body forms.	Algae, plant and pollen feeding Plant exudates Excess nutrient excretion	Primary productivity Decomposition Nutrient immobilization Soil respiration
Hemiedaphic Soil sub-surface	Round, Less ocelli, and leg development, dispersed pigmentation	Litter and root feeding Microbial grazing Excess nutrient excretion	Primary productivity Decomposition Nutrient turnover Soil respiration
Euedaphic - Soil	Elongated, colourless, Lacking furcula, ocelli, possess pseudocelli, PAO. Common globular body form	Soil organic matter feeding Root feeding Microbial grazing Excess nutrient excretion	Primary productivity Decomposition Soil turnover Soil respiration

3.1.1 Nematodes

Nematodes, or roundworms, are semi-aquatic microfauna that make up a substantial proportion of the soil faunal community. They are ubiquitous to most terrestrial ecosystems,

with an estimated million species globally (Lambshead, 2004). Nematode communities can have a profound impact on ecosystems, particularly as plant parasites and microbial grazers (Brussaard, 1998; Wall and Moore, 1999; Nielsen, 2019). These activities result in a number of trophic interactions, thereby connecting nematodes with several ecosystem processes (Bongers, 1990). The nematode feeding guild is indicative of its trophic position with assumptions of related processes (Ferris et al., 2001). Differences in diet between species within the same guild are minimal, as expected, with challenges discerning some species that have multiple, undefined or overlapping trophic positions. Their contribution to ecosystem processes are mediated by directly feeding on their preferred substrate combined with indirect impacts to microbes and plant communities more broadly (van den Hoogen et al., 2019). Clear links have been established between morphological traits (Griffiths et al., 2006) and their contribution to plant-soil feedbacks via their direct influence on root exudates (Gebremikael et al., 2016; Kuťáková et al., 2018).

Nematode feeding guilds are well-characterised as: i) herbivorous root feeders or plant parasites; ii) fungal feeders; iii) bacterial feeders; iv) predators; v) omnivores; and, vi) entomopathogenic nematodes, EPN (Yeates et al., 1993; Table 3.1). The hollow stylet or spear (odontostyle) is a prominent feature by which plant and fungal feeders are identified (Yeates et al., 1993). Herbivorous nematodes are also classified as plant parasites and indirectly influence biogeochemical properties via their excreta that contains valuable bioavailable amino acids, NH_4^+, and PO_4^- (Ingham and Detling, 1985; Zwart et al., 1994). Their effects are focussed in the rhizosphere where root feeding has been found to increase root exudates (Yeates, 1998; Poll et al., 2007), whilst also reducing exudates in other settings (Van der Puttern, 2003; Maboreke et al., 2017). Plant parasites can be further classified by feeding strategy: i) sedentary endoparasites, enter around the root tip and remain protected

by a hard cyst composed of the female's necromass, e.g. *Heterodera* and *Globodera*, and the cyst-less "root-knot" *Meloidogyne* nematodes that have a broader host range; ii) migratory endoparasites, traverse the rhizosphere consuming plant sap through root walls, e.g. *Pratylenchus* and *Rotylenchus;* and, iii) ectoparasites, feed on the broadest host range of external plant matter, e.g. *Xiphinema* and *Helicotylenchus* sequence (Yeates et al., 1993; Lambert and Bekal, 2002).

Fungal feeders (or fungivores) consume hyphal filaments of mycorrhizal forming fungi, saprophytic free-living fungi and plant pathogens. Bacterial feeders, or bacterivores, have direct impacts on bacterial communities, that in turn increase N and P cycling (Trap et al., 2016). They feed on bacteria through tubular mouth openings, which can be aided by external appendages. Omnivores are often larger species with slower reproductive cycles (Ferris et al., 2001), feeding mainly on algal filaments, protists and other nematodes using a cuticular extension of the oesophageal spear (odontophore). Omnivores, by definition, contribute to processes at multiple trophic levels (Brussaard, 1998) which complicates linking their actions to an appropriate ecosystem processes. The entomopathogenic nematodes (EPN) spend the majority of their lives inside host larvae but are found in soil as free-living infective juveniles (Kaya and Gaugler, 1993). Their net inputs to ecosystem systems via necromass and excreta have yet to be quantified (Stuart et al., 2015). Finally, the less abundant predatory nematodes consume other nematodes as well as other soil faunal groups, and are generally distinguished by a large mouth cavity with one or more large teeth (onchium) and well developed oesophageal muscles. As predators consume nematodes and other invertebrates, they have a significant indirect influence on the size of bacterial and fungal populations, ultimately effecting rates of decomposition, nutrient cycling and pathogen control (Bongers et al., 1997; Wall and Moore, 1999; van den Hoogen et al., 2019).

Nematode community composition also provides a useful ecological indicator of ecosystem functioning. Nematodes are assigned coloniser-persister (*c-p*) values used as a proxy for differences in life strategies (from fast to slow) accounting for population carrying capacity, reproductive rates, generation time and associations with resource availability. Communities with many species of high *c-p* values indicate stable ecosystems with complex food webs. Contrastingly, communities dominated by rapidly reproducing 'r-selected' (low *c-p* values) species reflect colonisers of nutrient enriched, disturbed soils (Bongers,1990; Bongers et al., 1997). Nematode feeding guilds and *c-p* values are therefore useful traits for assessing whether functionally important traits are phylogenetically clustered.

3.1.2 Springtails

Up to 9000 springtail species have been identified globally, constituting around 28 families of four main orders: i) Entomobryomorpha; ii) Symphypleona; iii) Poduromorpha; and, iv) Neelipleona (Bellinger et al., 1996-2021; Hopkin, 1997; Leo at al., 2019). These represent less than a fifth of the fifty thousand species that are expected to exist globally (Potapov et al., 2020). While springtails show approximately the same breadth in feeding guilds as nematodes, e.g. from herbivores to microbial grazers to predators (Berg et al., 2004; Holterman et al., 2006), most are generalist consumers of fungi, roots and decomposing plant material (Malcicka et al., 2017). However, some springtails do show feeding preferences (Jørgensen et al., 2005), as well as predators. It is clear that differences in feeding preference and life-strategy influences their involvement in ecosystem processes (Larink, 1997; Wall and Moore, 1999). As fungal feeders, springtails impact fungal-fungal, plant-mycorrhizal and plant-fungal interactions (Ngosong et al., 2011), with knock-on effects to decomposition, nutrient cycling and plant growth (Addison et al., 2003; De Vries et al.,

2013). Fungal grazing springtails have been shown to disrupt carbon flows via reductions in saprophytic fungi and arbuscular mycorrhizal respiration (Johnson et al., 2005), whilst reducing gross litter decomposition (Kampichler and Bruckner, 2009) and fungal regrowth following intensive grazing (Steinaker and Wilson, 2008). However, low-to-moderate grazing has been shown to increase plant growth (Kaneda and Kaneko, 2004), as well as plant biomass and productivity (Wall and Moore, 1999). Hence, springtails have multiple direct and indirect effects on ecosystem processes, reflecting their diverse life strategies, similar to nematodes.

Springtail soil vertical stratification level is defined as the vertical position in soil at which an adult springtail is found for the majority of its life, and ranges from the upper tree canopy to true organo-mineral soil living species (Bellinger et al., 1996-2021). Springtail stratification levels can be divided into three main classes: i) epigeic, living above or on the litter and soil surface, including aboveground species often living on vegetation, 0.5–10 mm in length; ii) hemiedaphic springtails from 0.5–3 mm in length, living in the litter and upper soil horizons (1–2 cm in depth); and, iii) euedaphic, living belowground throughout all adult stages, 0.5–2 mm in length (Larsen et al., 2004). A shift in species composition may be associated with responses to soil conditions including soil pore space, whilst soil depth is related to decreased SOM quality and altered soil microclimate. This indicates that springtail species have different roles in ecosystem processes dependent on their position within the soil horizon (Faber, 1991; Berg et al. 1998). For example, upper litter layers will primarily involve early-stage decomposition and physical breakdown of complex C-containing compounds such as tannins (gallic and elagic acids) and polyphenolic flavonoids (Coulis et al., 2008). Latter-stage decomposition generally takes place in the deeper litter layers and the soil itself, involving chemical degradation in concert with bacterial and fungal microbes.

These interactions influence soil respiration and nutrient turnover, in addition to the nutrient-immobilization during early decomposition where soil fauna break down larger organic particles before shifting to the mobilisation of nutrients by microbes in the latter stages of decomposition (Faber, 1991; Faber and Verhoef, 1991).

Springtail moisture preferences can be classified into: i) hygrophilic; ii) meso-hygrophilic; iii) mesophilic; iv) xero-mesophilic; and, v) xerophilic species. Preference classes are inferred from the soil moisture content at which the species is most commonly associated (Hopkin, 1997; Kuznetsova, 2003). Soil moisture preference was determined using a combination of factors to determine their tolerance to desiccation. As soil moisture class assignment is primarily based on micro-habitat data, it is defined as an ecological trait because it is based on population or ecologically-based species data. Due to the important biological functions associated with water availability, it is expected that this trait will be highly conserved within closely related species.

Additionally, springtail morphological features that have important functional implications are selected based on their known links and previous use to infer ecosystem functionality: i) mean body length, three classes: < 1 mm; 1 to 2 mm; and, > 2mm; ii) body shape, three classes – spherical, stocky and cylindrical; iii) number of ocelli, three classes — 0, 1 to 7 and 8 ocelli; and the presence/absence of iv) pigmentation; v) furcula; vi); trichobothrium; and, vii) chemosensory postantennal organ, PAO.

An additional well-described morphological feature of springtails are their mouthparts. It can be posited that differences in mouthpart structure reflect their diet and therefore the ecosystem processes in which the springtail is involved (Berg et al., 2004). Springtail mouthparts consist of mandibles and maxillae that can grasp, shear, grind and filter organic matter, with modifications reflecting their principal resource use. The

mandibles are a symmetrical pair of incisal parts (teeth), that can be moved and rotated along both axes to perform a range of feeding actions. These can be accompanied with a molar plate that acts as a grinding surface assisting comminution of recalcitrant particles (Malcicka et al., 2017). Morphological traits have been previously used to relate springtails with their adaptation to environmental conditions (Potapov et al., 2016; Steibl and Laforsch, 2021), including insights into soil habitat vertical stratification level (Salmon et al., 2014; Widenfalk et al., 2016; Raymond-Léonard et al., 2019) and with habitat type being found to shape certain springtail taxonomic structures (Ponge and Salmon, 2013).

Our main objective was to determine the level of trait conservation across springtail and nematode phylogenies and establish the correspondence between clustering and functional traits. By doing so we hope to address whether springtails and nematodes conform to the niche conservation theory (Wiens, 2004; Losos, 2008) whereby species separated by less genetic distance are more likely to share similar trait values. We expect to find traits to be conserved for both nematode feeding guilds and springtail mouthparts, owing to the importance for each in their trophic position and ecosystem functional roles. Alignments for nematode *c-p* values, springtail moisture and functional traits are expected to also align within their taxonomic orders but with greater variability across the phylogenies as species have adapted to local environments. Having a clear determination of functional traits and genetic distance can assist in evaluation of ecosystem functionality from a species and assemblage perspective, assisting biodiversity monitoring and conservation efforts that must consider the adaptability of species to environmental change.

3.2 Methods

Molecular taxonomy can be used to assess phylogenetic clustering in invertebrates but requires the robust reconstruction of phylogenetic trees (Emerson et al., 2011). Multiloci alignments of nuclear (e.g. 28S rRNA) and mitochondrial DNA (mtDNA, e.g. cytochrome oxidase subunit I, COI) markers can distinguish between both ancient and recent phylogenetic relationships. Partial fragments of the nuclear 28S rRNA genes are a commonly used marker for invertebrate phylogenies, and benefit from a regular rate of slowly evolving domains (Subbotin et al., 2008). The COI gene of ~650 basepairs (bp) in length, has a relatively high nucleotide substitution rate, undergoing recombination only rarely, making it ideal for intra- and inter-species sequence comparisons (Hebert et al., 2003a, Yu et al., 2012), and has been shown as a robust identifier of nematodes and springtails (Emerson et al., 2011; Porco et al., 2012).

Sequence alignments

Target nematode and springtail gene sequences were downloaded from the National Centre for Biotechnology Information (NCBI) GenBank® nucleotide sequence database (National Institute for Health, USA; Benson et al., 2012; www.ncbi.nlm.nih.gov/genbank/). We tried to capture as globally diverse set of springtail species as possible; however, the northern hemisphere is over-represented accounting for over 7,700 of the almost 9,000 species described globally (Deharveng et al., 2007). The reliability of the data and search functionality have been shown as statistically rigorous for lower taxonomic ranks (Leray et al., 2019). The GenBank nucleotide BLASTn (non-redundant) general search terms of “Collembola”, “Nematoda”, “COI”, “28S rRNA”, manually selecting sequences available

from both markers for each species, excluding non-terrestrial or animal parasites. All sequences were imported as FASTA files into the Geneious™ v. 2020.2 program, (www.geneious.com, Kearse et al., 2012) before being checked for labelling errors and aligned using the CLUSTALX (Thompson et al., 1994) with IUB cost matrix at 15-point gap open cost, and 6.66-point gap extension cost, before 8 iterations of a Muscle alignment (Edgar, 2004). All sequences were then combined into concatenations with *Eosentomidae sp*. as the outgroup, a Protura that are evolutionary close to both Collembola and Nematoda.

Phylogenetic trees

Phylogenies were merged from trees constructed using Bayesian analysis performed using the Bayesian Evolutionary Analysis Sampling Trees program (BEAST v.2.5.2; Drummond et al., 2012), with best-fit partition model in PartitionFinder v2.1.1 (Lafear et al., 2017) and substitution models using ModelTest-NG (Darriba et al., 2020; Bouckaert and Drummond, 2017) in MEGA X v.10.1.0 (Kumar et al., 2018) using Bayesian information Criterion (BIC) scores. These indicated the optimal substitution model for 28S as the General Time Reversible with Gamma distributed and invariant sites, GTR+G+I, with partition model specifying the use of all codon positions. The most optimal substitution model for COI sequences was deemed GTR+G without invariant sites, and with the third codon excluded in the partition model. A Relaxed Log Normal molecular clock was used with rates set to 0.0168 (3.54% Ma^{-1}; Papadopoulou et al., 2010) for springtails, and 2% Ma^{-1} for nematodes (Denver et al., 2000; Cutter, 2008). The Yule method was used for construction of tree priors and monophyletic constraint prior on all in-group taxa. A minimum of four full simultaneous Markov Chain Monte Carlo (MCMC) sampling runs were performed for each concatenation with 1.1M chains per run, with default heated chain settings and a burn-in of 100,000

iterations. The algorithm outputs were processed using the Tracer program (Rambaut et al., 2018) to establish appropriate burn-in value, visualise and check for convergence, effective sample size (>200) or other stress constraints. All trees were rooted with the Proturan *Eosentomidae* sequence. The output was assigned node positions and tree branch lengths indicating posterior probability, with a 0.5 minimum probability limit, 10 % burn-in, formulating the maximum clade credibility tree formed using Tree Annotator v2.5.2 (Rambaut and Drummond, 2013). The tree was then drawn and colourised in Geneious.

The calculation of uncorrected patristic distances (p) are the sum of the branch lengths separating any two species within the tree. Sufficient sample sizes were achieved so that a Bayesian analysis could be performed for both springtails and nematodes. If insufficient sample sizes were used, Bayesian computation would lead to erroneous outputs. These distances were calculated and visualised as a heatmap matrix in Geneious. Distances were given for the minimum distance between representatives of each major group. This approach provides a more accurate metric of distance between species due to the incorporation of tree topology and Bayesian approaches as applied to springtail phylogenies (Zhang et al., 2019).

Nematode functional traits

Nematode tree outputs were compared and noted for their similarities to previously published phylogenies that also displayed some inconsistencies and incomplete clade resolution (Mallatt et al., 2004; Bik et al., 2010; Bird et al., 2015; Smythe et al., 2019). Nematode species were classified into primary adult feeding guilds as listed in section 3.1.1 (Table 3.1). Guild assignations were derived from well-established published lists (Bongers, 1990; Yeates et al., 1993) and multiple online sources. Animal parasitic nematodes were

omitted from the study as their primary adult phase occurs aboveground. A subset of species were aligned with coloniser-persister *c-p* classes. The *c-p* values range from 1 to 5 with lower values indicating species considered colonisers with fast life cycles, whilst slower species have higher *c-p* values. Uncorrected patristic distance matrices for nematodes were based on distances from trees including all feeding guilds.

Springtail functional traits

Springtail phylogenetic outputs were initially sorted into taxonomic orders with similarities noted to previous published trees (D'Haese, 2002; Holterman et al., 2006; Zhang and Deharveng, 2015; Leo et al., 2019). Springtail phylogenies were aligned with soil vertical stratification levels that were divided into three classes (epigeic, hemiedaphic, euedaphic) as described in section 3.1.2. (Table 3.1). Stratification levels and functional traits were compiled from multiple sources, including the COLTRAIT database (http://www.bdd-inee.cnrs.fr/spip.php?article51&lang=en) that has been used for earlier studies linking traits and environmental factors (Ponge, 2000; Salmon and Ponge, 2012; Salmon et al., 2014). The classification of vertical stratification level, i.e. preferred microhabitat, was derived from a combination of detailed field-based measurements of adult position within the soil profile, alongside corroborating morphological and functional traits (Gisin, 1960; Berg et al., 1998; Ellers et al., 2018). Datasets were compiled in addition to the stated sources including: literature; photographs; figures, and illustrations. Combining datasets constructed using alternate methodologies minimises climatic or seasonal bias (e.g. higher water table depth in winter months). Using only adult data reduces individual variation in level that are found in juveniles that are generally found deeper in soils. All springtail species were also aligned with mouthpart types that were either absent or present in three forms: i) scratching, ii)

piercing, and, iii) biting mandible forms, either with or without a molar plate under the tip of the mandible (Malcicka et al., 2017).

Potential circularities of using data that has been inferred from other sources within the same dataset were avoided. In this case, springtail soil vertical stratification levels based on functional traits in the absence of field-based measurements were omitted from the alignments. Uncorrected patristic distance matrices were generated for all springtails based on tree distances.

3.3 Results

Nematode feeding guild, parasitic feeding type and c-p values

There are clear separations between the major nematode trophic groups as indicated by genetic distances between members of the different guilds (Table 3.2a). Herbivores were generally distinct from other guilds with a minimum patristic distance to fungal feeders (e.g. *Deladenus* to *Aphelenchoides*, p = 0.99). Maximum distances between most distant species across whole datasets was >2.0. Separation between the other guilds were less marked with fungivores separated from bacterivores (*Bursaphelenchus* to *Panagrolaimus*, p = 0.7; Appendix A; Fig. 3.1), and the least separation between predators and bacterivores (*Mononchoides* to *Teratorhabditis*, p = 0.23). Clustering of EPN, omnivores and predators within bacterial feeders is reflected by lower distances from the bacterivore *Teratorhabditis* to EPN (*Steinernema*, p = 0.71) than with fungivores (*Bursaphelenchus*, p = 0.9) and herbivores (*Deladenus*, p = 0.99). The omnivores formed separate sub-clusters, although were distinct from bacterivores (*P. obtusus* to *Plectus*, p = 0.72) and more so from herbivores (*P. obtusus* to *Criconema*, p = 1.1).

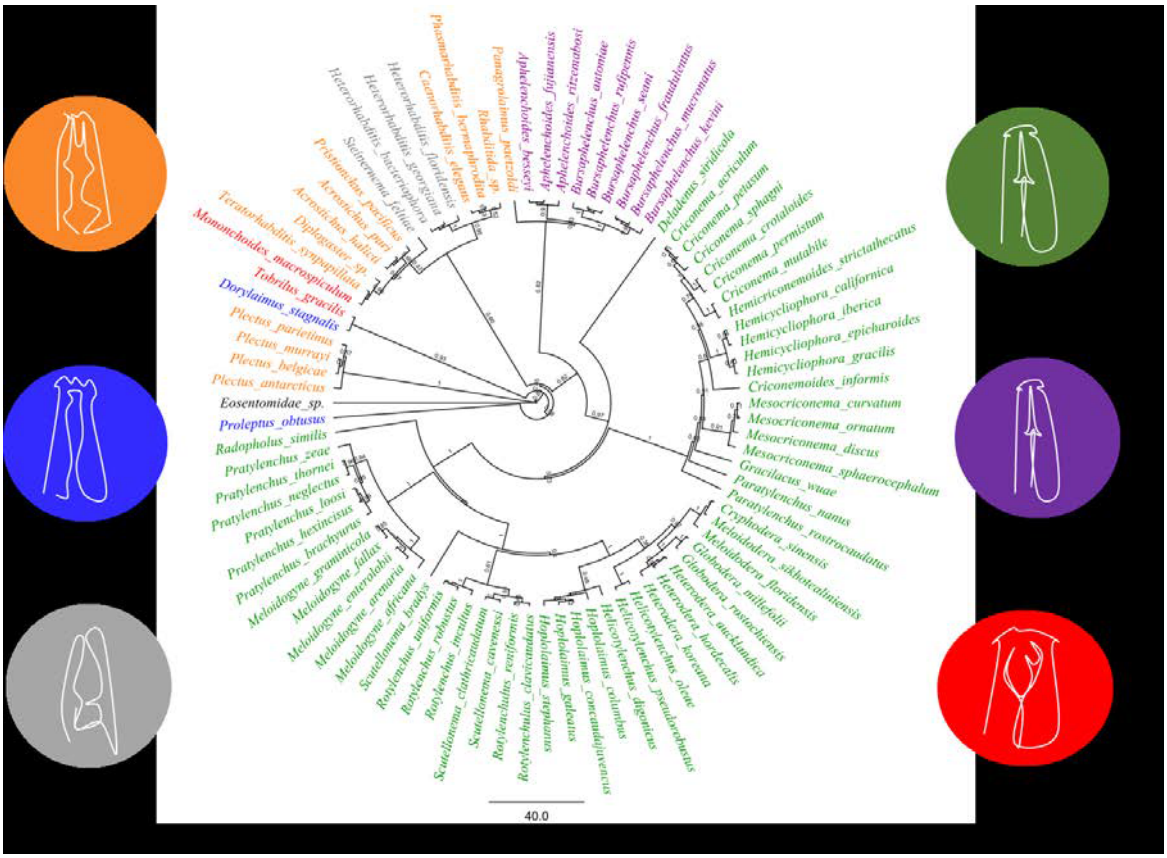


Figure 3.1 Phylogenetic tree based on posterior output of Bayesian reconstruction of nematode COI and 28S rRNA sequence alignments, with feeding guilds: i) Herbivores – green; ii) Bacterivores – orange; iii) Fungivores– purple; iv) Predators - red; v) Omnivores – blue, vi) Entomopathogenic nematodes (EPN) – grey. Branch labels indicating substitutions per site. Scale bar indicates individual branch length. Rooted with *Eosentomidae* sp. (Protura: Hexapoda).

Distances between plant parasitic feeding types were generally lower than those between the broader feeding guilds, with minimal genetic distances between ectoparasites from endoparasitic types (*Helicotylenchus* to *Hoplolaimus*, p = 0.35). The genetic distances were slightly larger between endoparasites and sedentary species (*Pratylenchus* to *Meloidogyne*, p = 0.61) and between ectoparasites and sedentary species (*Helicotylenchus* to *Meloidogyne*, p = 0.72).

Nematode species showed greater phylogenetic separation when c-p values were further apart. Lower patristic distances were observed between nematodes with c-p values

of 1 and 2 (*Panagrolaimus* to *Aphelenchoides*, p = 0.7) than between species with c-p values of 1 and 4 (*Panagrolaimus* to *Plectus*, p = 0.9; Fig. 3.2).

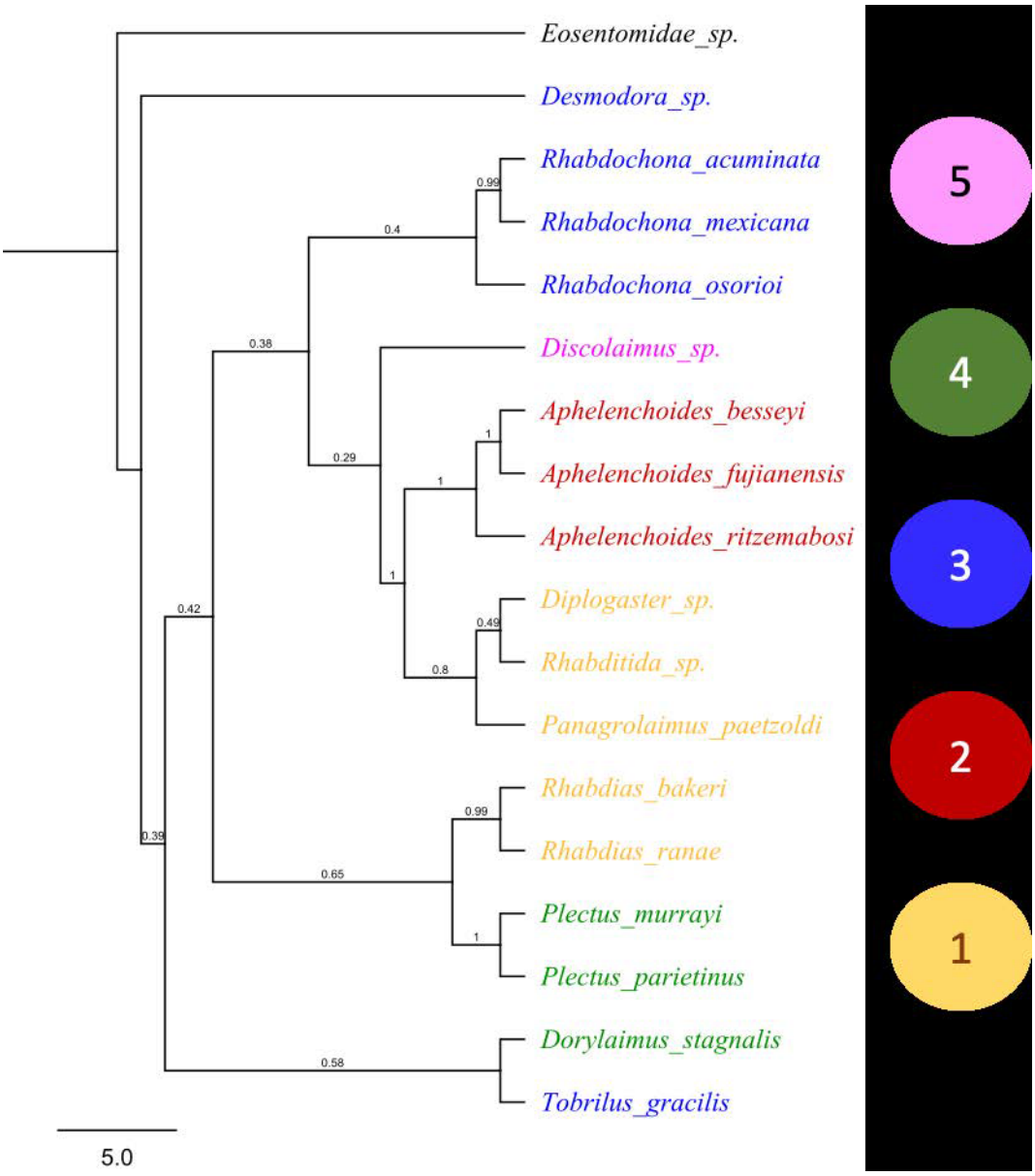


Figure 3.2 Phylogenetic tree based on posterior output of Bayesian reconstruction of nematode COI and 28S rRNA sequence alignments, with c-p value classes: 1–5: 1) pink, 2) green, 3), blue, 4) brown, 5) beige. Rooted with *Eosentomidae* sp. (Protura: Hexapoda).

Springtail stratification, moisture preference and functional traits

The springtail phylogenies also revealed clear separation by genetic distances of epigeic, hemiedaphic and euedaphic species. Patristic distances were slightly lower between epigeic and hemiedaphic species (*Entomobrya* to *Willowsia*, $p = 0.17$) than epigeic and euedaphic species (*Orchesella* to *Sinella*, respectively, $p = 0.25$) and hemiedaphic and euedaphic species (*Neanura* to *Onychirus*, respectively, $p = 0.18$).

mean genetic distances indicate genetic divergence between the groups (Fig. 3.3).

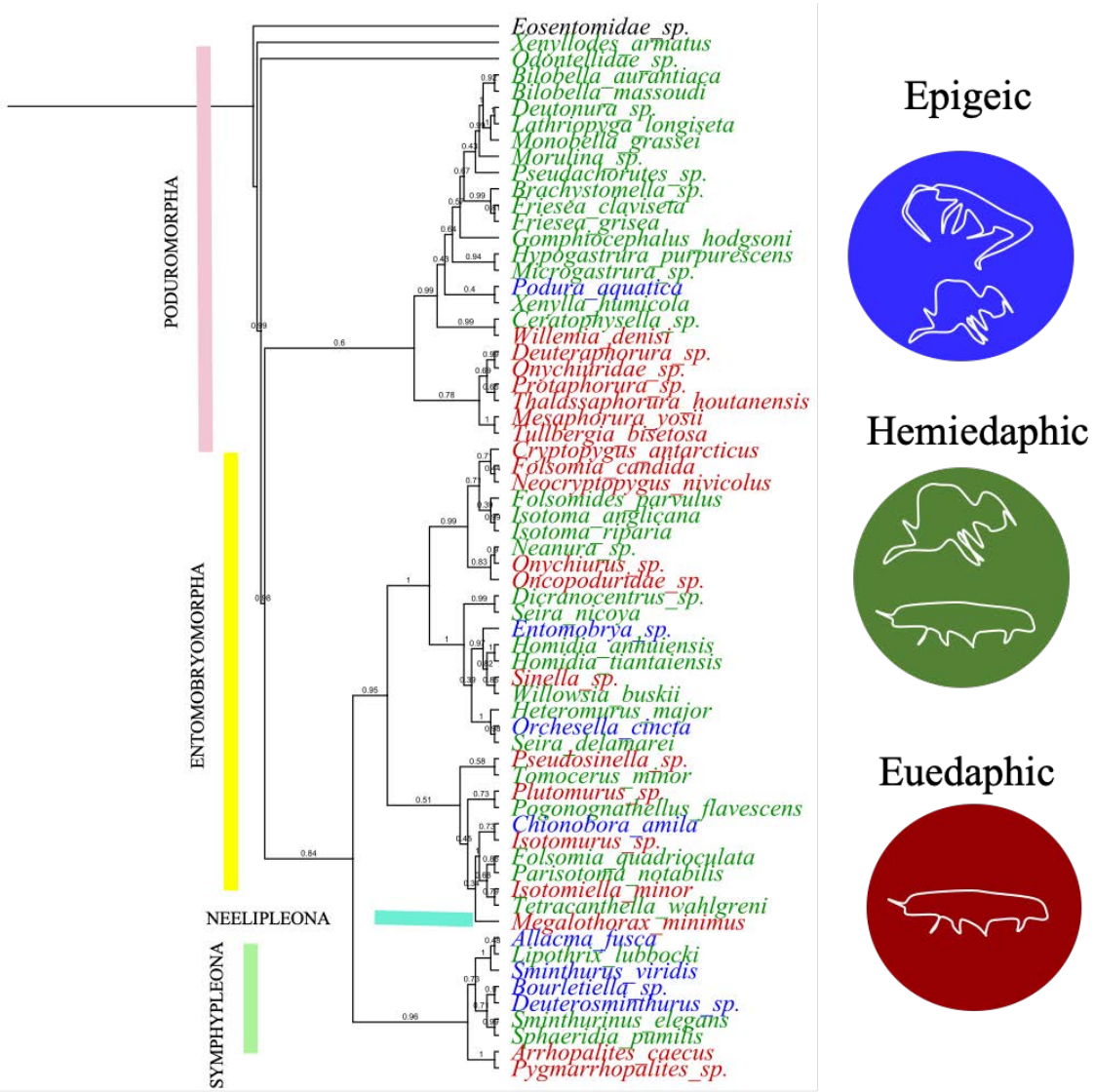


Figure 3.3 Phylogenetic tree based on posterior output of Bayesian reconstruction of springtail COI and 28S rRNA sequence alignments, with stratification level: i) epigeic – blue, ii) hemiedaphic - green, iii) euedaphic - brown. Springtail species taxonomic order indicated in left panel. Rooted with *Eosentomidae* sp. (Protura: Hexapoda).

Springtail mouthparts were found to be highly conserved, with strict alignment to taxonomic orders, with the majority of Entomobryomorpha species possessing scratching mandibles and a molar plate. All other species with complex mouthparts were clustered within the Poduromorpha (Fig. 3.4).

Table 3.2 Minimum uncorrected patristic distances between species at different soil vertical stratification levels and soil moisture preference types.

Springtail moisture preference	Hygro	Meso-hygro	Meso	Xero-meso
Hygro	-			
Meso-hygro	0.29	-		
Meso	0.29	0.32	-	
Xero-meso	0.33	0.42	0.35	-
Xero	0.28	0.29	0.19	0.17

Species of the *Brachystomella* genus that have no mandible was separated from species that have greater mouthpart development, with patristic distances of i) p = 0.29 for biting species with no plate (e.g. *Friesea sp.*), ii) p = 0.30 for scratching species with no plate (e.g. *Gomphiocephalus sp.*), and, iii) p = 0.33 for scratching species with plate (e.g. Microgastrura).

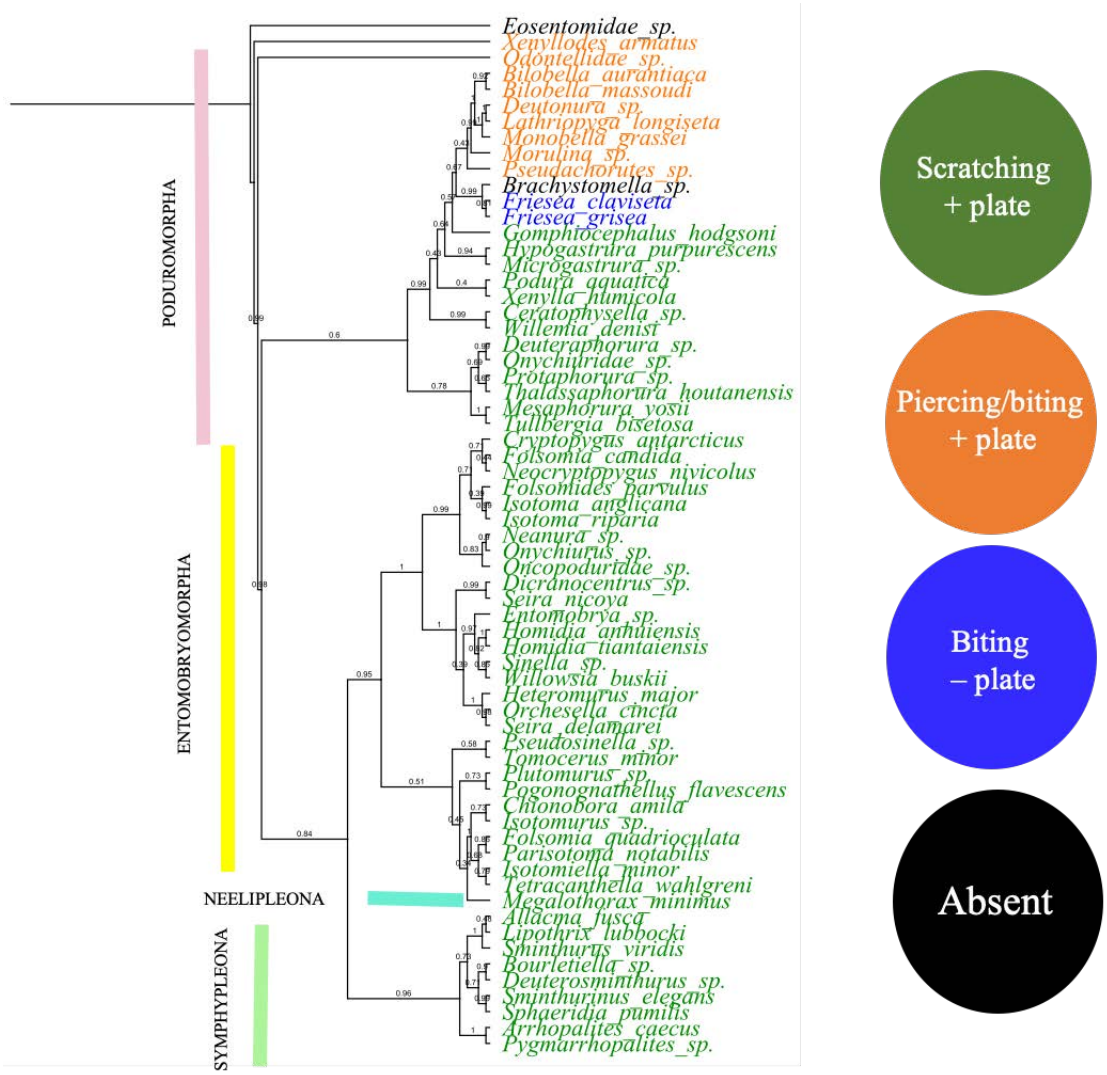


Figure 3.4 Phylogenetic tree based on posterior output of Bayesian reconstruction of springtail COI and 28S rRNA sequence alignments, with springtail mouthpart types: i) scratching with molar plate – green, iii) piercing/biting with molar plate – orange, iii) biting with no molar plate – blue, iv) absent mandible/plate – black. Springtail species taxonomic order indicated in left panel. Rooted with *Eosentomidae* sp. (Protura: Hexapoda).

Soil moisture preferences of Collembola showed more dispersion across the phylogenetic tree (Fig. 3.5). Patristic distances were larger between the most to least dry-tolerant species. The xerophilic (*Willowsia*) species were separated from the xero-mesophilic (*Entombrya*, p = 0.17), the mesophilic (*Heteromurus*, p = 0.22) to meso-

hygrophilic (*F. candida*, p = 0.35), and hygrophilic (*Isotomiella*, p = 0.45) species.

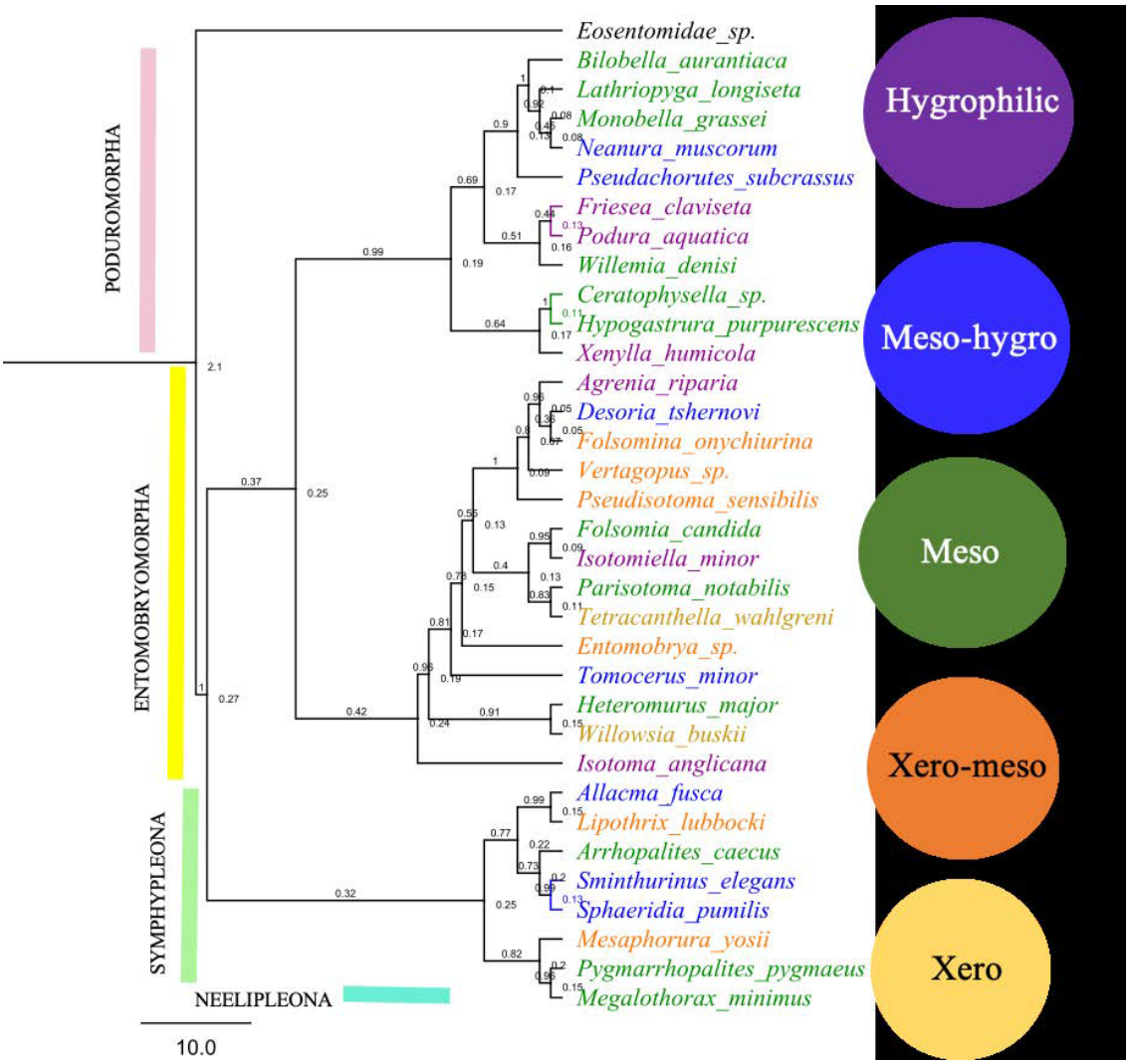


Figure 3.5 Phylogenetic tree based on posterior output of Bayesian reconstruction of springtail COI and 28S rRNA sequence alignments, with springtail soil moisture preference: i) hygrophilic – purple; ii) meso-hygrophilic – blue, iii) mesophilic – green; iv) xero-mesophilic – orange; v) xerophilic – beige. Springtail species taxonomic order indicated in left panel. Rooted with *Eosentomidae* sp. (Protura: Hexapoda).

However, minimal distances between the xerophilic *Tetracanthella* showed less consistency towards each category (Table 3.2). The seven morphological traits showed different levels of alignment. Body shape showed the strongest phylogenetic clustering, whilst more dispersion was observed for body length, pigmentation and furcula. Trichobothria and PAO had mixed dispersion across the phylogeny, with the former being more frequent in the

Symphypleona species and totally absent from Poduramorpha (Fig. 3.6). Furcula was often absent from Poduromorpha and present in all Symphypleona and Entomobryomorpha, except for one species.

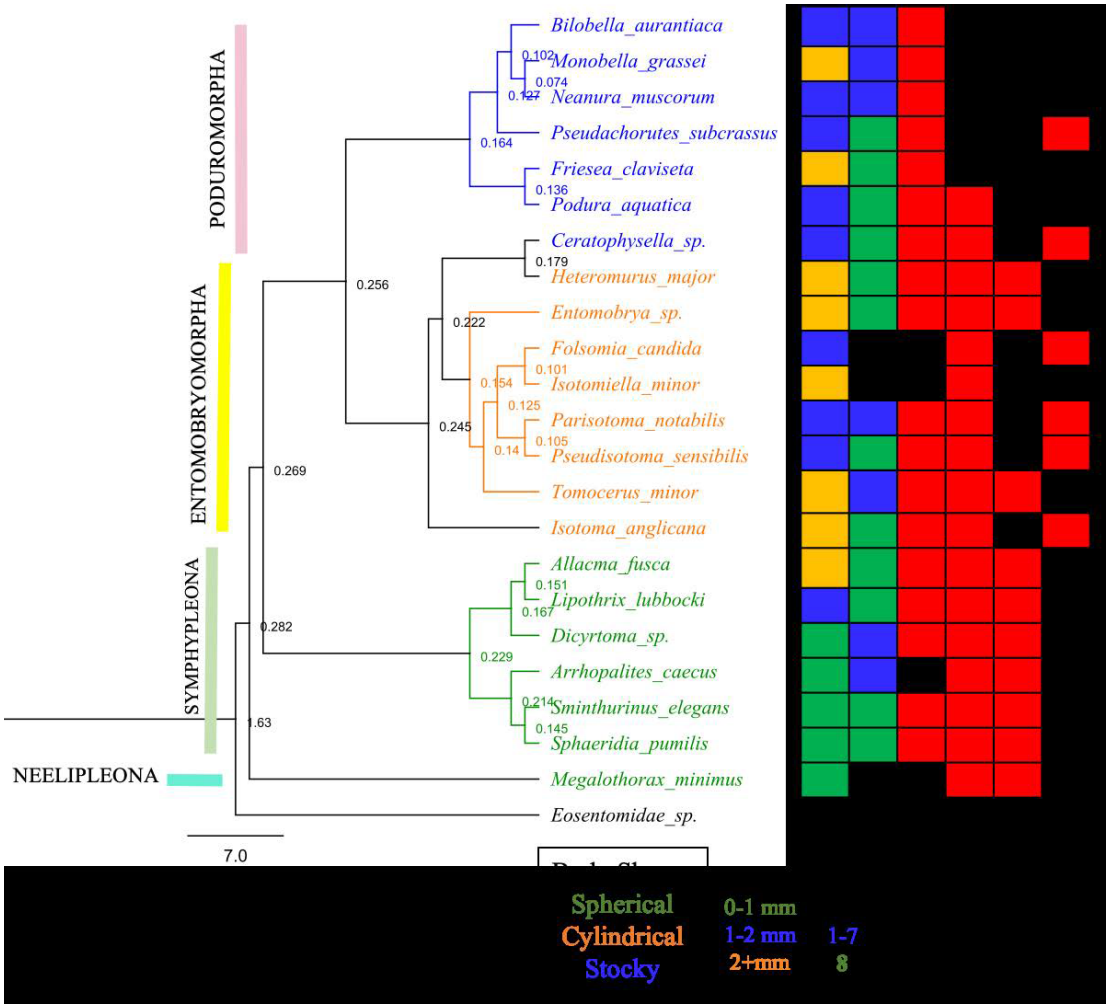


Figure 3.6 Phylogenetic tree based on posterior output of Bayesian reconstruction of springtail COI and 28S rRNA sequence alignments, with springtail morphological traits; i) body shape: spherical – green, stocky – orange, and cylindrical – blue; ii) mean body length: 0 to 1 mm – green, 1 to 2 mm – blue, > 2mm – orange; iii) number of ocelli: 0 – white, 1 to 7 – blue, 8 – green; and presence – red, or absence – white, of iv) pigmentation; v) furcula; vi); trichobothrium; and, vii) postantennal organ (PAO). Springtail species taxonomic order indicated in left panel. Rooted with *Eosentomidae* sp. (Protura: Hexapoda).

3.4 Discussion

The objective of this study was to determine the degree of phylogenetic clustering of functional and morphological traits in two soil invertebrate groups known to influence ecosystem functioning. In doing so, we hoped to establish whether genetic relatedness can robustly infer functional traits of species or assemblages via sequencing information alone. This can aid monitoring and experimental approaches, particularly where there is a high proportion of unknown or undescribed species, and by overcoming shortages of taxonomic expertise.

Our phylogenetic trees show clear clustering of nematode feeding guilds, including the plant parasitic feeding types, with some species in separate clades, as observed in other nematode phylogenies based on barcodes (Blaxter et al., 1998; Holterman et al., 2006; van Megen et al., 2009). Deviations amongst certain nematode clades and taxonomic inconsistencies has also been found in genomic studies of terrestrial nematodes (Bird et al., 2015; Smythe et al., 2019). The plant parasite feeding types showed clear separation between the three main classes. This is expected due to the significant evolutionary adaptations required for sedentary cyst formation. Currently, parasitic feeder type are distinguished by morphological differences in spear shape and life stages, but our findings suggest phylogenetic positioning as a reliable alternative. Additionally, the increasing patristic distances between species that have larger differences in *c-p* values gives confidence in using molecular data to estimate *c-p* value classifications.

Similarly, springtail mouthparts were found to be a phylogenetically well-conserved trait, with taxonomic groupings similar to previous phylogenies (D'Haese, 2002; Holterman et al., 2006; Zhang and Deharveng, 2015; Leo et al., 2019). Species that typically inhabit the

lower litter layers and soil include the majority of Poduromorpha, including Hypogastruridae and Onychiuridae that were clearly separated from those lacking a molar plate. However, the phylogenetic clustering of springtail soil vertical stratification levels was less well-defined, and may reflect the ability of species to occupy and move between stratifications levels, and the flexibility of hemiedaphic members to shift levels in response to limited resource availability and extreme conditions (Ponge, 2020).

The ecological trait that was recorded as soil moisture preference amongst springtails was also phylogenetically dispersed across orders, indicating that adaptation to water availability may be relatively recent in most genera. The capacity to accumulate trehalose sugar and protein secretion (Sjursen et al., 2001) allows species to persist despite limited water availability. The lack of clustering for springtail moisture preferences may also indicate the need for more accurate reference moisture preference input data. This may also shed light on the undefined role of the ventral collophore suspected of being involved with osmoregulation and excretion (Hopkin, 1997).

The greater phylogenetic dispersion amongst ecological traits such as springtail moisture preferences compared to functional mouthpart morphology may reflect a greater role that ecosystems may have in regulating speciation in soil invertebrates. Adaptability to variation in moisture may also be a more frequent necessity rather than changes in feeding resources, especially when migrating to new habitats. This may contribute to a mismatch between the geographic locations from which sequenced specimens and trait reference specimens are sourced. For example, for genera that contain species that exist in both tropical and temperate ecosystems, litter depth and moisture levels would be dependent on local climate and ecosystem type. As such, tropical soils with warmer and wetter conditions have faster litter decomposition and mineralisation leading to a thin or absent litter layer and a

dominance of species with edaphic specialisations such as PAO and trichobothria. By contrast, temperate forests have perennial litter cover and slower litter decomposition with species more likely to show aboveground litter-inhabiting traits, such as number of ocelli and furcular development. In order to clarify these differences, analyses should include multiple species of the same genus or family, as well as incorporating geolocation data. Greater species coverage would also aid comparison with factors such as habitat type, which has been shown as a strongly correlate to springtail taxonomic structure (Ponge and Salmon, 2013).

The phylogenetic signal from springtail body shape corresponded strongly with taxonomic orders as expected, whilst body length was less uniformly expressed. The observation that trichobothria and PAO were not co-occurring in the majority of sampled species, supports the hypothesis raised by Salmon and Ponge (2014), that PAO could compensate the absence of trichobothria, especially in euedaphic species. Trichobothria and PAO are known to play a role in thermo-, hygro- or chemosensory functions (Altner and Thies, 1976; Hopkin, 1997). This suggests the body shape/length may be more indicative of the species functionality, and to its trophic positioning. Phylogenetic dispersion was found across orders for the morphological traits that were less directly linked with functionality, such as ocelli and trichobothria. Further investigation of the alignment with other morphological traits may include scales, parapseudocelli, setae/bristle arrangements, anal spines, integumentary granulation, structure and location of the unguiculus and microsensilla, although their ecological importance is less well-defined.

The findings from both nematodes and springtail phylogenies reveals that more defined phylogenetic clustering was evident in traits most closely associated with trophic positioning. This is a strong signal of resource consumption and feeding type being

1761 intimately related with processes that contribute to rates of ecosystem-wide functioning. This
1762 further indicates that molecular techniques can be used to classify important functional traits
1763 of both nematodes and springtails where sequences can be aligned with reliable voucher
1764 specimens at high phylogenetic resolution (i.e. family to genus in most cases). However, the
1765 more pronounced separation between nematode feeding guilds, as opposed to other traits
1766 such as nematode *c-p* values and springtail moisture preference, suggests a requirement for
1767 higher taxonomic resolution for parity to a known delineated sequence in order to assign
1768 novel sequences to a specific class.

1769 Our results indicate that certain soil invertebrate traits do conform with the niche
1770 conservatism theory (Wiens, 2004; Losos, 2008) that posits closely related species tending
1771 to share similar trait values. Phylogenetic clustering was linked to trophic position, as
1772 hypothesised. This was more pronounced in nematodes, indicating that resource type and
1773 functionality are intimately linked and are perhaps stronger drivers of speciation compared
1774 to habitat, resource or environmental factors. Other insights that may be drawn include the
1775 convergence of certain traits such as nematode stylet evolution from simple tubular mouth
1776 openings of bacterivores to the structurally complex hollow stylets adapted for fungal
1777 feeding. Alternatively, it is unclear if the loss of functional traits in springtails, such as ocelli
1778 and furcula when aboveground springtails regressed belowground can be regained in
1779 response to future environmental change or increased resource competition. As such, these
1780 findings indicate a potential role of ecosystems and the functional requirements it has in the
1781 moderation of speciation in soil invertebrates. For replicability, access to all sequences can
1782 be obtained upon request and personally downloaded from the NCBI database. For
1783 conservation implications, further insights may inform consideration of autecological
1784 processes of evolution in individual species and ecophysiological forces that may influence

speciation (Decaëns et al., 2006). Such correlation between the traits and function also support the use of resource type as indicative of ecosystem functionality by proxy. Further analyses on other soil faunal types are encouraged to determine if the theoretical underpinnings of trait evolution can be applied to all soil faunal types.

Rapid and standardized monitoring of soil fauna using molecular tools will benefit from routine integration of imaging, barcoding and trait information. Sharing of taxonomic data without the need for prior taxonomic identification or exhaustive sampling will enhance efforts to establish the role of soil faunal biodiversity (Potapov et al., 2020), especially the ability to sequence whole assemblages. Such representative sequences will still require morphological identification to avoid blind sequencing (Pey et al., 2014a; Cowart et al., 2015). Longer, more accurate metabarcoding and genomic readouts will also expand our knowledge beyond the model organisms of *C. elegans* and *Pristionchus pacificus*, and overcome the potential under-estimation of genetic distance that is the potential when based on marker genes. High-throughput sequencing can illustrate belowground assemblages in greater resolution (Ross et al., 2020), with correction factors to quantify sequencing read-outs (Lamb et al., 2019). These would allow for the rendering ecological information directly from genetic signatures with local reference libraries that incorporate alternate rates of genetic drift and generation time (Collins et al., 2019), with small geographic distributions or factors influencing speciation rates and genetic drift (Lim et al., 2012).

3.5 Conclusion

Combining soil invertebrate DNA sequences with morphological data and life-history traits suggest that functional traits with ecological roles may be selectively conserved over morphological traits in soil invertebrates. The findings also demonstrate how phylogenetic

information might be used to imply certain traits if these are not known for a particular species. Greater parity was evident for feeding and mouthpart traits that are directly linked to trophic positioning. Limited phylogenetic clustering within certain traits suggests higher resolution taxonomic data may improve our ability to assign these traits to novel sequences. Integrating sequence libraries with functional traits will assist ecosystem monitoring and highlight the value of soil biodiversity to ecosystem functioning. The combined application of molecular data and trait-based knowledge on soil faunal groups can highlight their actions that are a vital component of functional ecosystems.

Chapter 4 - Maritime Antarctic oribatid mite and springtail biodiversity linked to climate, isolation and vegetation cover

Abstract

Soil fauna inhabit all terrestrial environments, with the majority of studies focussing on northern hemisphere taxa. Despite the harsh conditions, relatively diverse communities of soil invertebrates inhabit the seasonally snow and ice-free terrestrial ecosystems of maritime Antarctica, but limited access and challenges to their taxonomic identification leave unresolved questions relating to their distribution and biodiversity at both broad and fine spatial scales. I assessed mite and springtail distribution and diversity at 12 locations along a climatic and environmental gradient throughout maritime Antarctica from Signy Island in the north to inland Ares and Mars Oasis in the south using molecular and morphological approaches. Samples were collected from common vegetation types and bare ground at all sites using a standardised approach, with five replicates per cover type from each site, to assess diversity and distribution at large spatial scales (i.e. landscapes). At five sites, additional replicates were collected for three common cover types (grass, moss, bare ground) to provide greater insight into the drivers of distribution and diversity at fine scales. A total of seven oribatid and five springtail species were collected across all sites, with minimal variation among sites across the latitudinal gradient, except for more diverse assemblages in coastal sites and no shared species with southerly inland sites. Both oribatid mite and springtail densities and species richness were significantly related to climatic factors resulting in lower abundances and diversity at colder and drier sites. Assemblage structures of mites and springtails differed across sites with both mites and springtails being more influenced by soil water content, soil pH and soil nitrogen content compared to

biogeographic factors and measures of climatic variation. Furthermore, within site differences involved springtail species richness being more influenced by vegetation cover type than mites. This study provides a baseline of biodiversity for maritime Antarctic mite and springtail communities.

Acknowledgements: Environmental data including vegetation cover and soil chemistry collected during an US NSF-funded Antarctic expedition 2014-2016 was provided by Dr. Becky Ball and collaborators. The research and environmental data is described in Ball et al. (2022) and (2023).

4.1 Introduction

Antarctic terrestrial ecosystems are subject to extreme climatic conditions, yet despite these contraints, unexpectedly diverse soil invertebrate communities have been found in areas considered lifeless as recently as the late 1980's (Freckman and Virginia, 1990). Mites and springtails, alongside semi-aquatic rotifers, tardigrades and nematodes, occupy the highest trophic levels in simple yet functional terrestrial ecosystems (Chown and Convey, 2007). Previous studies detailing the indigenous soil fauna in maritime Antarctica (Wallwork, 1967; Dastych, 1990; Marshall and Convey, 2002) have not applied molecular approaches to multiple soil faunal types. However, the study of indigenous taxa is challenged by their minute size, mixing of populations and fragmented distributions combined with a lack of access (Nielsen and Wall, 2013). Now molecular tools can support morphological approaches to better map the distribution and diversity of soil invertebrate populations (Orgiazzi et al., 2015).

Maritime Antarctica is defined by gentler climate than continental Antarctica, encompassing the west coast of the Antarctic Peninsula and coastal islands stretching north to South-Shetland and South-Orkney Island groups. It is an environmentally and biologically diverse region, with a high endemicity of soil fauna (Chown and Coney, 2007). There is a significant climatic gradient with colder conditions and reduced vegetative biodiversity closer to the pole (Convey et al., 2014). There is a distinguishing partition in biodiversity above and below 72º S. South of this latitude, in the "microenvironmental" zone, biodiversity is more influenced by microclimatic conditions than latitudinal gradients. North of 72º, the "macroenvironmental" zone's biota are more influenced by precipitation and latitudinal gradients (Green et al., 2011). The distribution of soil fauna in maritime

Antarctica is governed by a combination of both biotic (e.g. dispersal ability, desiccation tolerance) and abiotic factors including temperature, rainfall, and soil parameters that act at both regional and local scales (Usher and Booth, 1987; Danis et al. 2020; Ball et al., 2022). Considering that accelerated greening of islands in the Scotia Arc have been linked to increased invasive vegetation and plant diversity (Cannone et al., 2022), it would be important to know the baseline distribution and diversity of indigenous organisms to assist in future monitoring of impacts to invertebrate biodiversity. I predict a strong link between dominant vegetation cover type and soil faunal diversity and community composition.

Broad-scale distribution gradients

It is clear from previous work that the diversity of soil fauna differs substantially among habitable areas with high levels of endemism throughout the maritime Antarctic region (Chown and Convey, 2007, 2016). This is driven in part by findings from multiple independent studies that focus on either individual species or faunal groups, often over limited spatial scales. These have found that invertebrate biodiversity is directly linked to energy-related diversity gradients that are caused by levels of photosynthetically active radiation, soil moisture content, vegetation growth and ecosystem harshness (Chown and Convey, 2016; Ball et al., 2022). These variables change with increasing latitude (Gaston, 2000), as observed across the sub-Antarctic, Maritime and continental Antarctic regions (Convey, 2013). There are corresponding shifts in soil faunal assemblages, but the trends are less clear.

The spatial distribution of plants influences soil food web structure (De Deyn et al., 2004; Keith et al., 2009); hence, it is expected that the distribution of Antarctic soil invertebrates follows patterns of vegetation cover type. Detailed faunal studies in the region

have found distinct communities with minimal numbers of shared species inhabiting Byers Peninsula on Livingston Island in the South Shetlands (Goddard, 1979), and from Signy Island in the South Orkneys (Block, 1982). These sites are all assumed to share a similar ecosystem type, with insufficient detail of vegetation coverage to propose links between soil fauna and other biota, which is the main aim of this study. Other comprehensive surveys of the region (Pugh, 1993) have also demonstrated distinct bioregions that contributed to the demarcation of Antarctica based on species distributions (Terauds et al., 2012).

Earlier use of the nuclear 28S rRNA marker in Antarctic springtails (Frati et al., 2000), and nematodes (Raymond et al., 2014), alongside use the H3 domain markers in sub-Antarctic oribatids (Mortimer et al., 2011) support their use for the purpose of verification. Molecular analysis of eukaryotic diversity established biogeographic patterns demarking the maritime and continental region (Lawley et al., 2004). In Antarctica, even minimal temperature gradients can be observed to impact the indigenous faunal populations, where colder temperatures can significantly slow generation times and dispersal ability (Convey et al., 2002: Yergeau et al., 2007a, Bokhorst et al., 2008). Assessing broad-scale distribution of soil fauna in maritime Antarctica will determine if soil faunal distribution conforms to latitudinal gradients as found in vegetation that consist mostly of mosses and lichens (Casanovas et al., 2013; Peat et al., 2007).

Local-scale environmental conditions

The presence of soil fauna at a given site is largely governed by processes that operate at broader scales as outlined in the previous section. However, within sites, the influence of environmental variables and biotic interactions become more important. Previous work has shown that barriers to dispersal have prevented the spreading of species across the region,

creating a patchwork of highly endemic distributions. The interaction between species, biogeography and environmental conditions is especially important for oribatid mites and the bryophyte moss vegetation (Booth and Usher, 1986; Glime, 2017) that are common to Antarctic ecosystems. Changes in vegetation composition is well known to influence belowground communities and are likely to also impart a strong influence on Antarctic soil fauna that are already constrained by the harsh environmental conditions (Aerts, 1997; Nielsen, 2019). As microbial populations are important to complete nutrient cycling, their interaction with soil fauna and vegetation cover type can have important impacts to ecosystem functioning. As grazers of bacteria and fungi, soil fauna can contribute to a broader quantification of nutrient flows across the trophic web that is made possible in simple ecosystems as found in Antarctica. Aboveground climatic elements such as mean annual temperature and rainfall that moderate broad-scale distributions may also have direct and indirect effects on local-scale distribution by influencing vegetation productivity and belowground parameters such as soil water content and nutrient availability. As such, they are therefore suspected of driving soil invertebrate communities. Soil parameters including soil organic matter (SOM) content (Bölter et al., 1997; Yergeau et al., 2007a) and soil moisture (Convey et al., 2002; Lawley et al., 2004) are essential sources of nutrients and water for Antarctic microarthropod communities.

Soil communities include the mites that are predominantly detritivore Oribatida, the predatory Mesostigmata, and the highly diverse but less studied Prostigmata and Astigmatina mites. However, the oribatids are the most diverse and ecologically important of the mite orders. As such, oribatids are the most informative bioindicator group within the mites.

Recent molecular evidence support Astigmatina as being a derived clade (Desmonomatides) within the Sarcoptiformes order (Krantz and Walter, 2009; Arribas et al., 2020b). Whilst these are a diverse and ecologically relevant group, astigmatids are enumerated but not further identified due to the limited number of reference sequences and species identifications. Assemblage structure of oribatid mites has been shown to follow similar patterns on a global scale, with predominantly northern hemisphere species informing the influence from environmental or biotic interactions (Lindo and, Winchester, 2009; Caruso et al., 2012; Nielsen, 2019). Determining whether these principles are also applicable in Antarctic fauna would help reveal the factors that influence soil faunal assemblages. Individual studies have shown that both mites and springtails have strong association with soil age and plant species richness (Ingimarsdóttir et al., 2012), although assemblage structures associated with spatial and environmental variables indicate different drivers of assemblages in mites and springtails (Nielsen et al., 2012). Additionally, evidence linking vegetation-cover and soil faunal structures has also been found elsewhere in the maritime region (Caruso et al., 2013). It is therefore imperative to establish a firm assessment of biodiversity across broad spatial scales and to establish how soil fauna respond to shifting climatic and vegetative properties.

Objectives

The main objective of this study was to assess biodiversity and distribution of soil invertebrates, specifically mites and springtails, at broad and fine scales in maritime Antarctica. I capitalised on preserved soil fauna samples collected at twelve sites along the Antarctic Peninsula from Signy Island (60 °S) in the north to sites on Alexander Island (67°S) in the south (Ball et al., 2022). Samples were collected from distinct vegetation cover

types at all sites, with greater sample numbers from five sites allowing more detailed analyses at these sites. Hence, the dataset allowed me to assess large-scale distribution of soil fauna in relation to island latitude, island size and environmental factors. More detailed assessments of community structure related to vegetation cover was conducted at high-intensively sampled sites. I used molecular approaches to identify oribatid mites and springtails to better understand patterns of species richness and diversity along the latitudinal gradient and provide a baseline of maritime Antarctic biodiversity.

My main hypothesis was that soil faunal species richness and diversity of both mites and springtails would both follow the energy-related diversity gradient (lower diversity at colder, less productive sites) described in other terrestrial groups across the Southern Ocean (Terauds et al., 2011). Species assemblages of oribatid and springtails are expected to show more significant relationships with vegetation cover type than with climatic variability or soil microhabitat. Furthermore, as mites and springtails are primarily grazers of fungi and bacteria, oribatid mite and springtail species assemblage structures may have specific relationships with shifts in vegetation cover type and the microbial populations that they support.

4.2 Methods

Study sites and survey design

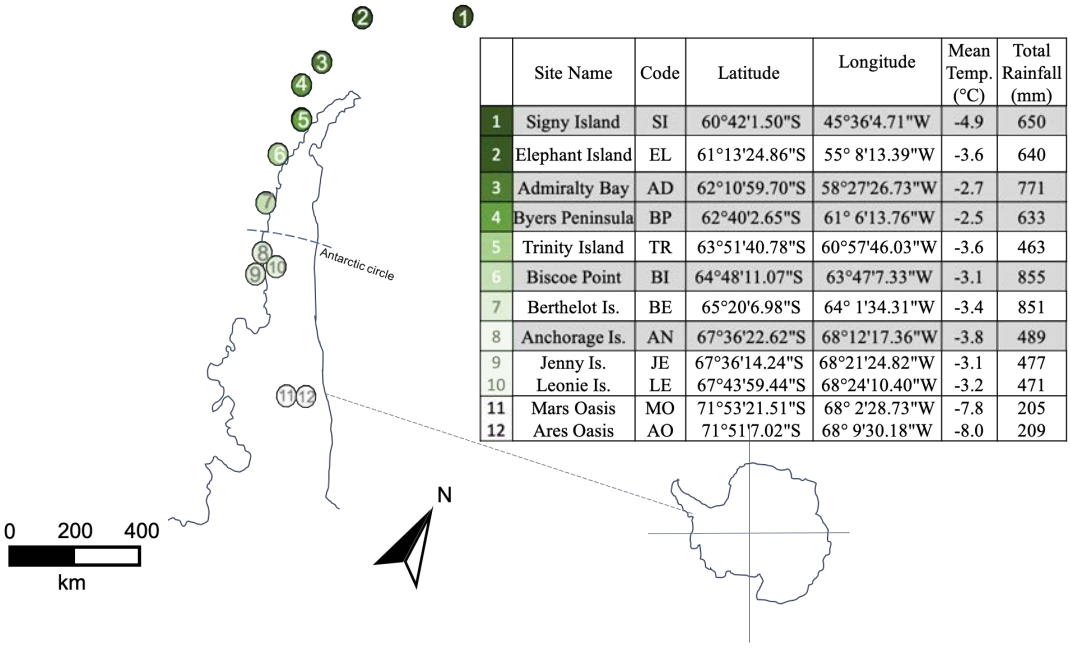
Samples were collected at 12 sites across a latitudinal gradient (60º-71.5º) throughout maritime Antarctica during the summer months from October 2014 to February 2016 (Fig. 13; Ball et al., 2022). Historical climatic data (temperature and rainfall) was extrapolated using WorldClim2 (1971-2000; Fick and Hijmans, 2017) for each site (Tables S1, S2; Fig.13). To establish population densities and diversities based on habitable area, island sizes were measured using the polygon tool in Google Earth v.7.3.2 (Google Inc., Mountain View, USA). The two inland oases, Mars and Ares Oases, situated 4 km apart (along a NW – SE axis) were estimated for size based on historic records of snow- and ice-free area (Convey and Smith, 1997).

At each site, samples were collected from five common vegetation cover types: grass, moss, lichen, algae and bare ground, with five replicates of each cover type where possible. Additional samples were collected for grass, moss and bare ground (n=20) at five sites (Admiralty Bay, Anchorage Island, Anvers Island, Byers Peninsula on Livingston Island and Signy Island) to assess the effect of vegetation cover on soil properties and belowground communities in more detail. Byers Peninsula and Biscoe Point are designated Antarctic Specially Protected Areas (ASPA) with special conservation status.

Vegetation survey and soil sampling

Sampling was conducted within a relatively homogeneous area where the cover types of interest were present. Firstly, vegetation was surveyed within a 25 cm x 25 cm quadrat over the sampling point, with visual estimates of percent cover recorded for each cover type of

grass (*Deschampsia antarctica*), moss, lichen, *Colobanthus quitensis* (Antarctic pearlwort), rock and bare earth. A detailed vegetation survey protocol can be found in Ball et al., (2022, 2023). A sterilised metal collar with 5 cm diameter and 5 cm depth was then used to collect soil samples for invertebrate extraction. The substrate below the removed vegetation/soil core was then homogenised using a sterile metal spatula, after which a soil sample was collected for soil moisture, pH, and total carbon and nitrogen contents. Soil water content (SWC) was estimated from the decrease in weight of a 25g soil sample, following 24hr drying at 105ºC, while soil pH was measured using a 1:2 deionised soil:water solution. Total and organic carbon and nitrogen contents were measured using elemental analysis as described in Ball et al. (2022). All vegetation and environmental data can be found in the supplemental data of published articles from the same expedition (Ball et al., 2022; 2023).



	Site Name	Code	Latitude	Longitude	Mean Temp. (°C)	Total Rainfall (mm)
1	Signy Island	SI	60°42'1.50"S	45°36'4.71"W	-4.9	650
2	Elephant Island	EL	61°13'24.86"S	55° 8'13.39"W	-3.6	640
3	Admiralty Bay	AD	62°10'59.70"S	58°27'26.73"W	-2.7	771
4	Byers Peninsula	BP	62°40'2.65"S	61° 6'13.76"W	-2.5	633
5	Trinity Island	TR	63°51'40.78"S	60°57'46.03"W	-3.6	463
6	Biscoe Point	BI	64°48'11.07"S	63°47'7.33"W	-3.1	855
7	Berthelot Is.	BE	65°20'6.98"S	64° 1'34.31"W	-3.4	851
8	Anchorage Is.	AN	67°36'22.62"S	68°12'17.36"W	-3.8	489
9	Jenny Is.	JE	67°36'14.24"S	68°21'24.82"W	-3.1	477
10	Leonie Is.	LE	67°43'59.44"S	68°24'10.40"W	-3.2	471
11	Mars Oasis	MO	71°53'21.51"S	68° 2'28.73"W	-7.8	205
12	Ares Oasis	AO	71°51'7.02"S	68° 9'30.18"W	-8.0	209

Figure 4.1 Map of maritime Antarctica and Scotia Arc and position in Antarctica with sample site locations, longitude and latitude, and mean annual temperature and rainfall (1971-2000; WorldClim2; Fick and Hijmans, 2017). Sites that underwent high intensity sampling in grey.

Soil faunal extraction

Soil cores were weighed before being placed in modified Tullgren funnels for heat extraction of microarthropods (Ball et al., 2022), and moisture content was calculated as described after drying. Mites and springtails were extracted by incremental temperature increases over 5 days directing migrating fauna into 70% ethanol, prior to being shipped to Western Sydney University for processing. Mites (Acari) were separated from residual soil particles using a binocular light microscope at 40X magnification. All mites were observed and sorted into orders (Oribatida, Mesostigmata, Prostigmata and the derived clade of Astigmatina), before adult oribatid mites were sorted into morphotypes (MT) based on morphology following bibliographies by Block (1992) and earlier surveys (Wallwork, 1967; Usher and Edwards, 1986) and enumerated. Springtails were also sorted into morphotypes based on relevant literature and enumerated (Wallwork, 1967; Dastych, 1990). Abundances were converted from raw counts to number of specimens per m^2.

Sequence generation and analysis

Representative specimens of each MT across sites were photographed and putative species identities were sought using Sanger sequencing targeting mitochondrial COI and 28S rDNA nuclear markers from the D3 expansion segment, and Histone-3 (H3) regions. These markers were selected given their prior use to identify Antarctic specimens and compatibility of providing suitable alignments (Frati et al., 2000; Frati and Dell'Ampio, 2000; Pentinsaari et al., 2016). In order to ascertain biodiversity measures of all species, multiple markers were used to overcome the incomplete records of certain species in sequence archives. As such, separate trees were produced for each of the markers to ensure all species could be identified with a higher chance of matching a reference sequence. The combination of mitochondrial

and nuclear markers ensures a broad approach to species verification using the National Centre for Biotechnology Information (NCBI) GenBank nucleotide sequence database (National Institute for Health, USA; Benson et al., 2012; www.ncbi.nlm.nih.gov/genbank/).

Total gDNA was extracted from mite and springtail specimens by freezing their whole bodies in liquid N_2 before grinding with a micropestle. Recovery rates for specimens stored in 70% EtOH have been found to be high, even for older samples (Marquina et al., 2021). Further lysis was performed using a sterile 1mm diameter steel ball-bearing in a Qiagen tissue-lyser II bead-mill, followed by a typical 'salting out' protocol with the addition of Chelex 100 beads (Sunnucks and Hales, 1996). Upon extraction, gDNA concentrations were checked and then amplified using PCR primers for: i) a ~680bp section of the COI gene; F' 5'GGTCAACAAATCATAAAGATATTGG3' R' 5'TAAACTTCAGGGTGACCAAAAATCA3' (Vrijenhoek et al., 1994; Bennett et al., 2016); ii) A 320 bp region in the D3 region of 28srRNA F' 5-GACCCGTCTTGAAACACGGA-3), R' 5- TCGGAAGGAACCAGCTACTA-3; (Litvaitis et al., 1994); and, iii) a 320bp fragment from the nuclear H3 gene F' CGTAAGTCGGCGCCCAGC, R' GACCCGTTTGGCGTGAATTGC (Mortimer et al., 2011) optimised for sequencing in oribatid mites and springtails.

Once amplified, fragments were run on 1.5% agarose gels in 1 x TBE buffer and the bands separated using 100 V electrophoresis against a standard 1Kb/100bp Hyperladder IV (Mobio). Amplifications were purified using the Promega Wizard PCR Clean-up kit as per the manufacturer's instructions. Elution concentrations were adjusted and prepared for submission to be Sanger sequenced in both forward and reverse directions. All sequences were checked by eye and manually edited for errors, aligned using CLUSTALX v.2.0

(Larkin et al., 2007) with an opening gap of 10.0 and extending gap of 0.1 using the Geneious™ v. 2020.2 program, (www.geneious.com, Kearse et al., 2012).

All sequences were manually trimmed and aligned with the reverse complement before BLAST searches. The threshold used for confidence in matching with species was >99.0% for COI and >98.0% for 18S rRNA. Sequences for which suitable matches with reference species were not found were not used in analysis or identified to family level or designated morphotype. Discerning haplotypes and genetic distances were calculated in MEGA with in-group selection based on the 0.5% OTU delimitation threshold for arthropods using 28S D3 marker. This was based on earlier studies of Antarctic arthropods using nuclear markers (Mortimer et al., 2011). Phylogenetic trees were constructed using a fast Maximum Likelihood method using RaxML (Randomised Axelerate Maximum Likelihood) in the Geneious program with the GTR+G model. Gamma parameters were estimated from Log Likelihood units and bootstrap support values calculated for the best-scoring ML tree (Stamatakis, 2014).

Abundance and diversity measurements

All diversity metrices were produced from five randomly selected samples from each cover type from high intensity sites, so that an equal number of sample plots were used to calculate indices for each site. Species richness was calculated from morphological counts. There were no microarthropods at the most southerly site at Sky Blu (74 ºS), which was excluded from further analyses. In addition, I calculated measures of biotic diversity using Simpson's Diversity (D; Simpson, 1949). This index generates a value based on the number of species and relative abundance of species across sites. This is preferred over Shannon's index owing to the relatively low number of taxa found within any given site (DeJong, 1975).

Additionally, nucleotide diversity (n) was used as a measure of polymorphism within a population. It specifically measures the mean number of differences per site between two DNA sequences in all pairs of the sample population (Nei and Li, 1979). P-distances are the sum of branch lengths representing individual taxa and calculated in Geneious. All available specimen sequences were used at each site, with weighted averages formed from abundances and a minimum of three sequences of each putative species to form trees from which distances were based. As with all measures of diversity, phylogenetic diversity increases monotonically and asymptotically with increasing sample size (Nipperess, 2016). Sites with high nucleotide distance may indicate recent speciation or proximity to refugial locations and can be a result of many closely related species or equally with few species that are genetically distant from each other (McGaughran et al., 2011; Chown et al., 2017). Each p-distance score is the distance between two morphotypes/putative species at the site but mean values can give an idea of the representative level of divergence between all species at a single site.

Statistical analysis

All analyses were performed on R software v. 3.4.3 (R Core Team, 2017) with variables checked for homogenous variance and normality and transformation with natural logs for continuous variables or square roots for abundances when required (Crawley, 2012). Soil mite abundance and diversity with heatmaps using the *ggplot2* package (Wickham, 2016). All environmental datasets were rarefied to the lowest number of plots for low intensity sampling sites (n=20), before biotic relationships were explored. Links between soil faunal abundance, species richness and diversity with latitude, climate (temperature and rainfall) and environmental variables (including soil pH, moisture, total C and N contents) were

assessed across all sites using linear mixed-effects models (LMM) in the *lme4* package (Bates et al., 2014). These were performed with latitude, climate and environmental variables were modelled as fixed effects with sites, plots and cover-type nested as random effects. Repeated measures of analysis of variances (ANOVA) tests were performed to determine the effect size and significance of relationships, with associated R^2 and P-values generated in *vegan 2.0* packages (Oksanen et al., 2013) in R (R Core Team, 2020). These are the equivalent of one-way ANOVA but are used for related, non-independent groups as an extension of the dependent t-test. Significance levels of $p < 0.05$ were used to reject null hypotheses.

Broad-scale diversity patterns were further analysed against geothermal sites that may act as refugia based on geological records (LeMasurier et al., 1990; Fraser et al., 2018). Euclidean distances were mapped and measured from each sample site to the nearest refugia.

Relationships between oribatid mite and springtail assemblages and environmental variables within the five high intensity sites were assessed using Non-Metric Dimensional Scaling (NMDS) using the "envfit" function in the R package *vegan*, with species abundance data log-transformed abundance prior to analyses. These were constructed using the rarified datasets of five randomly selected sites per vegetation cover type. To control for experimental error rate Tukey's post-hoc tests for one-way ANOVA were used to account for inflation of type-1 error (false positive) when multiple variables are included in the analysis.

4.3 Results

Morphologically based mite and springtail abundance, richness and diversity

A total of 29,365 mites across the four main orders were retrieved. Of these, 9,994 were oribatid mites representing seven distinct morphotypes (MTs; Table 4.1, Fig. 4.2). Additionally, a total of 1564 mesostigmatid mites identified as two main species namely, *Gamasellus racovitzai,* Trouessart, and *Hydrogamasellus. r. neorcadensis,* Trouessart were counted. Other non-oribatid mites included 18,741 prostigmatid and astigmatid mites. Estimated numbers of species for astigmatid and prostigmatid mites from all sites across the transect are in the tens and the low hundreds. The majority of sites had diverse mite communities from all three orders (Sarcoptiformes; oribatids, Trombidiformes; prostigmatids, and Mesostigmata; mesostigmatids), with the most southerly Oases sites dominated by prostigmatid mites. Distribution was spread across the transect with the majority of species in sites above 67°S (Table 4.1).

Comparison with known morphological records resulted in putative species names for the genotypes of the oribatid mites: MT1: *Liochthonius mollis* Hammer, 1958; MT2: *Membranoppia loxolineata* Wallwork, 1965; MT3: *Halozetes belgicae* Michael, 1903; MT4: *Halozetes impeditus* Niedbala, 1986; MT5: *Alaskozetes antarcticus* Michael, 1903; MT6: *Ameronothrus sp.* Berlese, 1896; and, MT7: *Podacarus auberti* Grandjean, 1955.

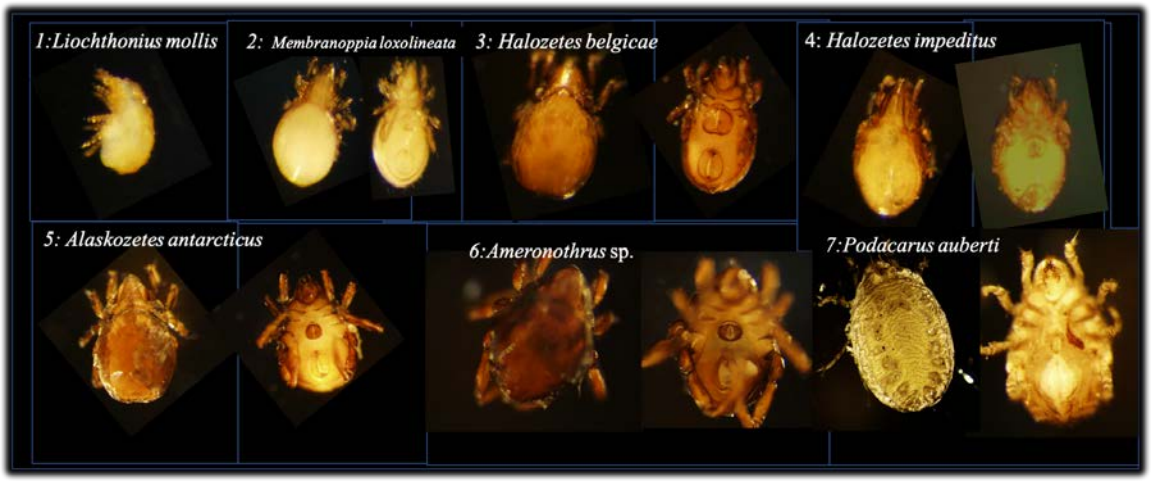


Figure 4.2 Compiled focal plane images of oribatid MT's taken under 40X magnification.

Confusion over the naming of mite MT2 may arise due to multiple synonyms for the same species — this is a good example of how taxonomic identifications can develop over time, alongside technological improvements. These include the specimen that will be referred to as: *Membranoppia loxolineata* Wallwork, 1965, with synonyms*: Oppia loxolineata* Wallwork, 1965; *Globoppia loxolineata longipilosa* Covar, 1968; incl. *O. loxolineata longipilosa* Covarrubias 1968, and, *Membranoppia (Pravoppia) loxolineata longiseta* Wallwork, 1965.

Springtail counting revealed more than 150,000 specimens with five MTs from across all sites. Springtail species were verified as: MT1*: Cryptopygus antarcticus,* Willem, 1903; MT2: *Cryptopygus sverdrupi*, Lawrence, 1972; MT3: *Friesea grisea*, Schaffer, 1891; that could not be distinguished from novel species *F. gretae* or *F. propria,* Carapelli et al., 2020; MT4: *Kaylathalia klovstadi*, Carpenter, 1902; and, MT5: *Folsomotoma octooculata* Willem, 1901. The majority of springtails were found in sites co-occurring with oribatid mites, with an absence in sites below 67°S (Table 4.1).

Table 4.1 Summary of species verified by sequencing and observation presence/absence of oribatid, mesostigmatid and prostigmatid mites and springtail species.

	Signy Is.	Elephant Is.	Admiralty	Byers Pen.	Trinity Is.	Biscoe Pt.	Berthelot	Anchorage	Jennie Is.	Léonie Is.	Ares Oasis	Mars Oasis
Oribatid mites												
Liochthonius mollis		x	x	x	x	x	x	x				
Membranoppia loxolineata		x	x	x	x	x	x	x	x			
Alaskozetes antarcticus					x			x	x	x		x
Halozetes belgicae	x	x		x	x		x	x	x	x		
Halozetes impeditus	x	x		x					x	x		
Ameronothrus sp.	x	x										
Podacarus auberti		x	x	x		x			x	x	x	
Mesostigmatid mites												
Gamasellus racovitzai	x	x		x		x	x	x	x	x	x	
G. r. neorcadensis		x		x		x	x	x	x	x		
Springtails												
Cryptopygus antarcticus	x	x	x	x	x	x	x	x	x	x	x	
Cryptopygus sverdrupi	x	x	x	x	x		x	x	x	x		
Friesea grisea	x	x	x	x	x	x	x	x	x			
Kaylathalia klovstadi					x		x					
Folsomotoma octooculata				x		x						

Figure 4.3 Compiled focal plane images of springtail MT's, with putative species names, taken under 40X magnification.

Phylogenetic taxonomy

Morphological identification was verified using sequencing with species delineations using a 3% threshold for COI, and ~0.1–0.5% for H3 and D3 sequences (Klimov et al., 2019). Phylogenetic trees constructed using Bayesian coalescent approaches supported the division of oribatid mites into seven morphotypes across the transect (Fig. 4.4A).

Due to the incomplete sequencing archives for Antarctic taxa, the nearest matches using the 28S rRNA D3 marker were often non-Antarctic taxa, e.g. the closest match of the mite MT2 was a genotype for *M. loxolineata* while the closest match for springtail MT4 was *Desoria tschernovi* (97.2%) likely because no sequences were available for *K. klovstadi*. Similarly, the closest match for MT7 for the 28S rRNA H3 markers was *Pergalumna cf. nervosa* (89.4%). This species name is derived from BLAST search matching that identified the sequence as the closest match. This is the alternative to slide mounting that would render the specimen unable to be sequenced.

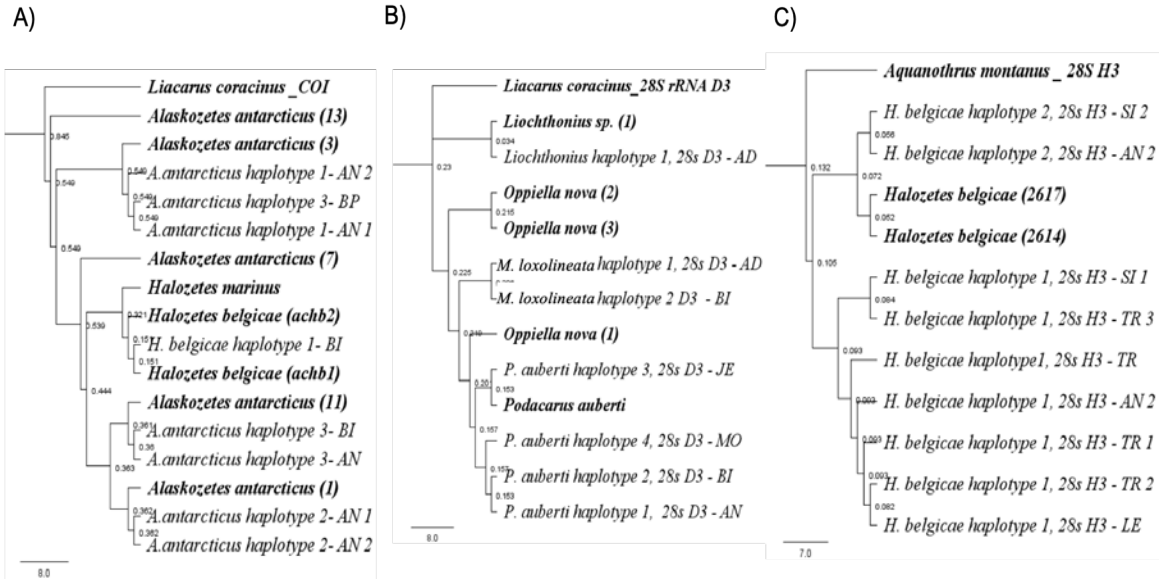


Figure 4.4 Phylogenetic tree based on Maximum likelihood estimation of voucher oribatid mites from across the maritime Antarctic transect. Site codes and BLAST result reference species using A) COI; B), 28s rRNA D3; and C) 28s rRNA H3. Reference species indicated in

bold. All trees were rooted with distantly related mites of the Liacarus genus for COI and 28S rRNA D3 and an Aquanothrus mite for the H3 gene.

Springtail phylogenies

Springtail phylogenies constructed using 28S rRNA marker sequences supported the putative species names of the five morphotypes based on BLAST matches that were above 98%. Phylogenies based on the COI marker with matches above 99% focused on the *C. antarcticus* springtail showed significant clustering of sequences indicated a number of haplotypes for the species. If each node were considered a putative species, this suggests the presence of some polymorphic cryptic speciation (Fig. 4.5).

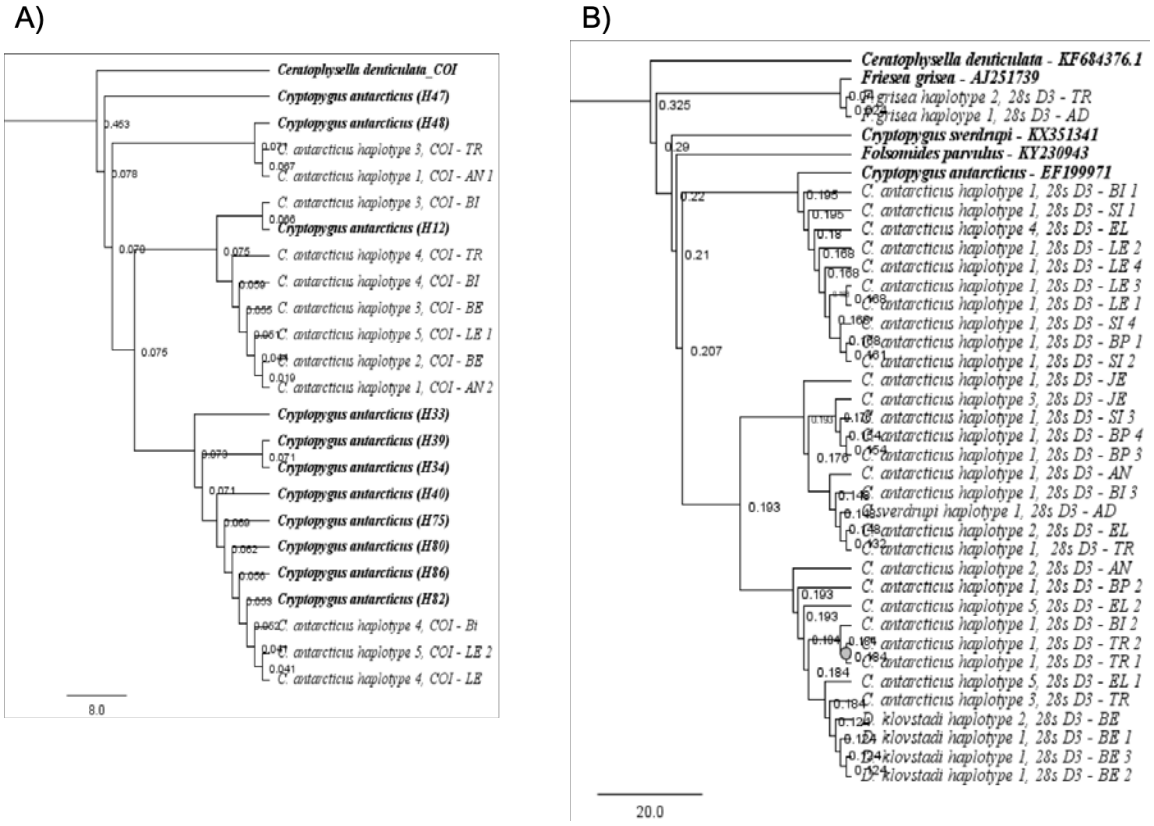
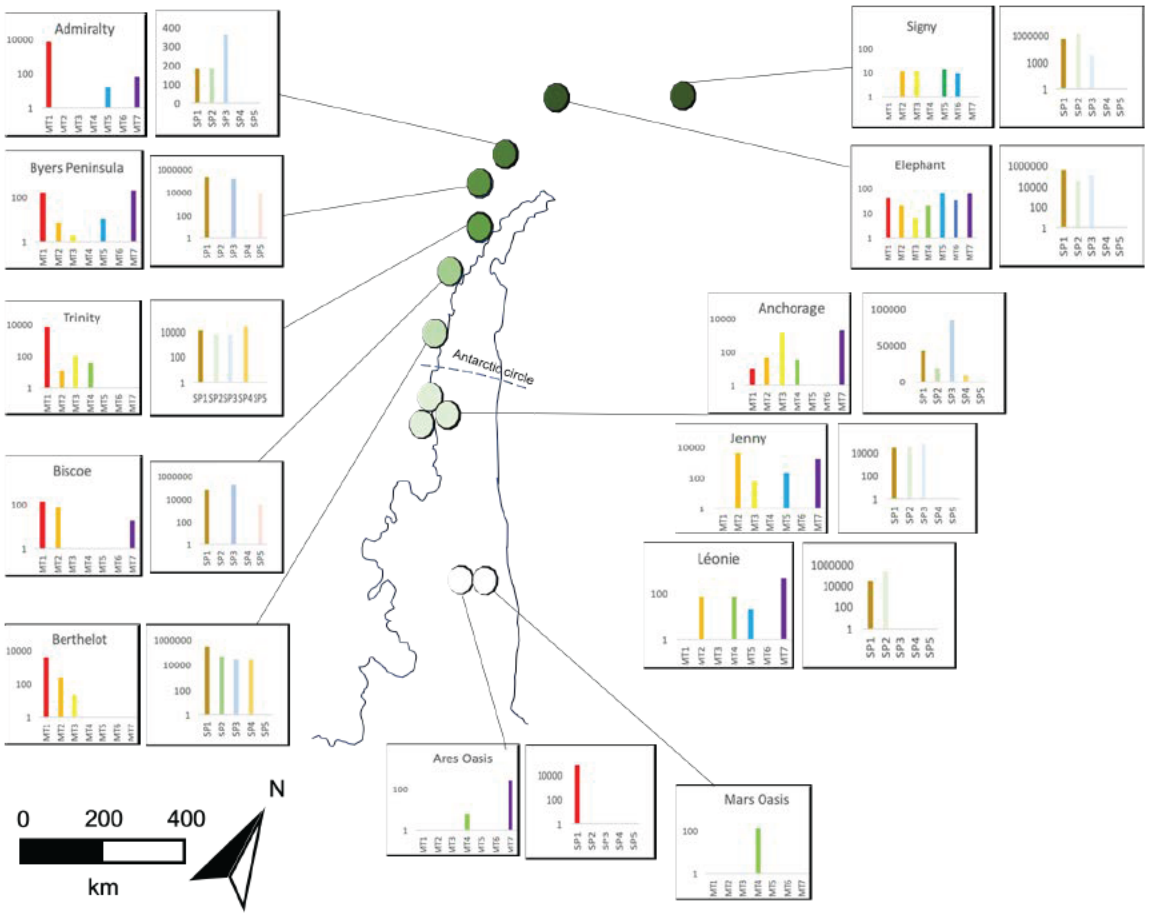


Figure 4.5 Phylogenetic tree based on Maximum likelihood estimation of voucher springtail genotypes using A) COI and B) 28S rRNA D3 region markers from across the maritime Antarctic transect. Trees were rooted with a distantly related *Ceratophysella denticulata* springtail sequences for each marker. Reference sequences in bold.



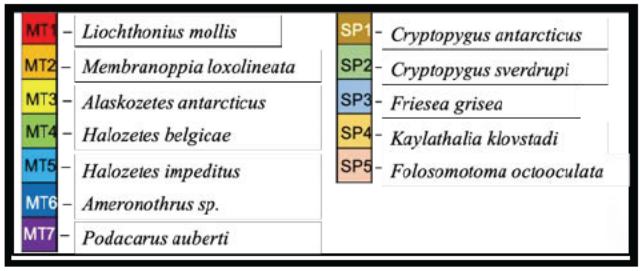


Figure 4.6 Map of maritime Antarctic transect with inset graphs of oribatid species and springtail species abundances, y-axis denotes number of individuals per m^2.

Table 4.2 Mean (± s.d.) oribatid mite and springtail abundance (ind. m^{-2}), species richness and Simpson's diversity across sites. There were too few springtail species at most sites for robust diversity measurements.

SITE	Mean oribatid abundance	Mean springtail abundance	Oribatid diversity	Oribatid species richness	Springtail species richness	Springtail diversity
Signy Is.	14981 ±14626	585923 ±807597	0.31	4	2	3
Elephant Is.	30853 ±31460	46400 ±47830	0.723	5	3	3
Admiralty	61050 ± 57496	22773 ±41568	0.021	3	4	3
Byers Peninsula	104 ±186	534 ±805	0.166	4	3	3
Trinity Is.	42190 ±58887	931831 ±50003	0.1	4	4	4
Biscoe Point	67322 ±97670	124137 ±181716	0.23	3	3	3
Berthelot	77540 ±160648	73899 ±144826	0.362	3	3	4
Anchorage	43878 ±75191	253469 ±388647	0.712	6	2	4
Jenny Is.	81813 ±184836	147879 ±278766	0.521	7	2	3
Leonie Is.	6697 ±7911	376511 ±359920	0.715	6	2	2
Ares Oasis	2334 ±2452	1195 ±1835	0	3	1	1
Mars Oasis	2620 ±2866	23856 ±43929	0	1	0	0

Broad scale patterns of distribution

Relationships between biotic response variables and geographical, climatic and environmental variables were visualised using heatmaps (Fig. 4.7). These indicated that mites and springtail density and diversity were strongly negatively correlated with the latitudinal gradient. Additionally, mite densities and diversity were found to correlate with warmer temperatures, whilst some negative correlations were found with increased rainfall. Consistent positive correlations were found with increased nutrient contents with both mite and springtail densities and diversities, whilst soil pH showed consistent negative correlations to biotic measures. Of the vegetation cover types, grass showed the most consistent correlation to increased faunal densities and diversity.

Mixed-effects models confirmed that soil invertebrate species diversity was lower in the high-latitude sites (nearer the pole), with significant negative relationships between increasing latitude and springtail species richness ($p < 0.001$, $F_{1,10} = 3.45$ $R^2 = 0.19$). No significant relationships were found between oribatid mites and latitude. Oribatid mites and springtails had similar patterns of species richness parameters between most sites, except for a substantial decrease in abundance and diversity below ~68-70ºS.

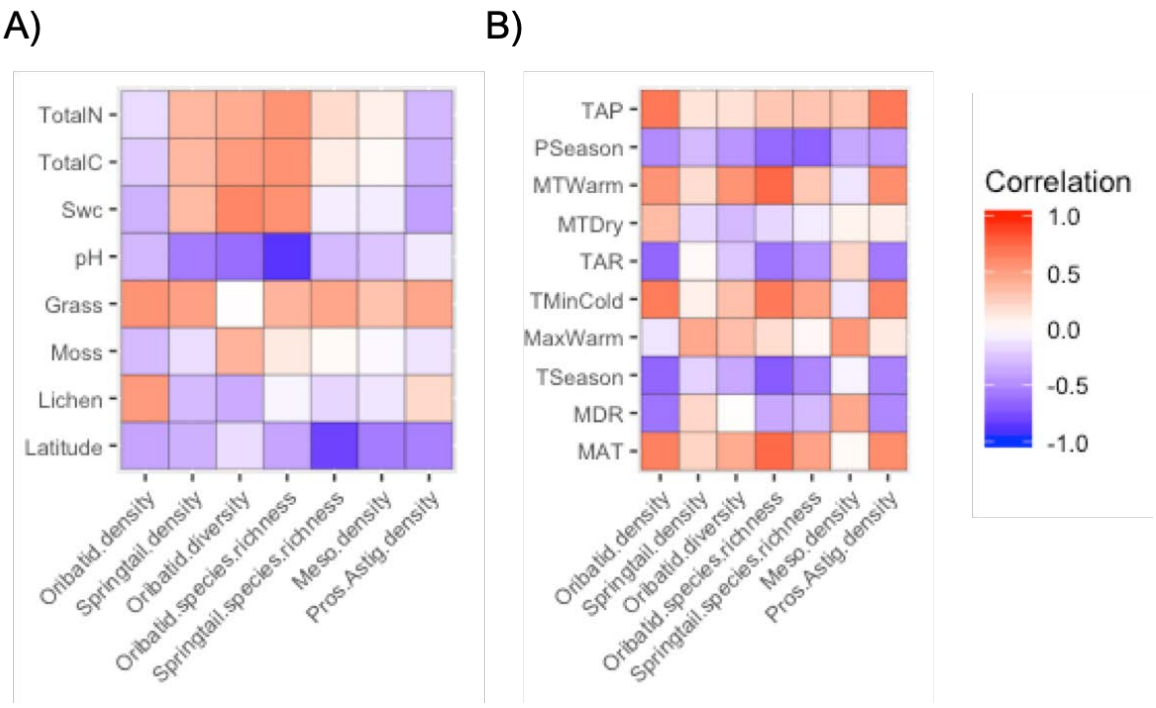


Figure 4.7 Heatmaps of correlation relationships between values of broad-scale biotic density and diversity and abiotic measurements across all sites with A) Vegetation cover and soil parameters: Total Carbon content (TotalC), Total Nitrogen content (TotalN), Soil water content (SWC), soil pH, Vegetation cover types and latitude, and, B) Climatic variables: Total annual precipitation (TAP), Precipitation seasonality (PSeason), Mean Temperature of Warmest quarter (MTWarm), Mean Temperature of Driest quarter (MTDry), Temperature Annual Range (TAR), Minimum Temperature of Coldest month (TMinCold), Maximum Temperature of Warmest month (TMaxWarm), Temperature Seasonality (TSeason), Mean Diurnal Range (MDR), Mean Annual Temperature (MAT).

Significant relationships between biotic measurements and geographic factors found total island area to be positively related to springtail density ($p < 0.01$, $F_{1,10} = 53.8$, $R^2 = 0.29$).

Mite and springtail density, richness and diversity were related to multiple climatic variables across all sites. In particular, oribatid mite densities were negatively related to rainfall in the coldest quarter ($p = 0.03$, $F_{1,10} = 6.1$, $R^2 = 0.05$). Springtail densities were also positively related to maximum temperature of the warmest month ($p = 0.05$, $F_{1,142} = 3.9$, $R^2 = 0.1$). Oribatid species richness was related to several precipitation variables including a positive relationship with wettest month ($p = 0.04$, $F_{1,10} = 5.7$, $R^2 = 0.1$).

Oribatid species richness was most significantly related to mean annual temperature of the warmest quarter ($p < 0.001$, $F_{1,10} = 10.43$, $R^2 = 0.15$). Additionally, springtail species richness was also found to correlate to climatic measurements including a positive relationship with both mean temperature over the warmest quarter ($p = 0.04$, $F_{1,10} = 4.09$, $R^2 = 0.22$) and mean rainfall over the wettest season ($p = 0.006$, $F_{1,10} = 7.7$, $R^2 = 0.33$).

Soil pH levels were found to have significant relationships with higher densities and abundance of oribatids and springtails with more acidic soils (lower pH) correlating to greater oribatid mite density ($p = 0.01$, $F_{1,142} = 6.2$, $R^2 = 0.02$) and species richness ($p < 0.001$, $F_{1,142} = 42.19$, $R^2 = 0.14$) and springtail richness ($p < 0.001$, $F_{1,142} = 213.8$, $R^2 = 0.43$). Oribatid species richness also had significant positive relationships with total C ($p = 0.01$, $F_{1, 142} = 5.9$, $R^2 = 0.02$) and total N contents ($p = 0.002$, $F_{1,142} = 9.4$, $R^2 = 0.03$). A significant positive correlation was also found between oribatid richness and soil water content (SWC) ($p = 0.05$, $F_{1, 142} = 3.6$, $R^2 = 0.05$).

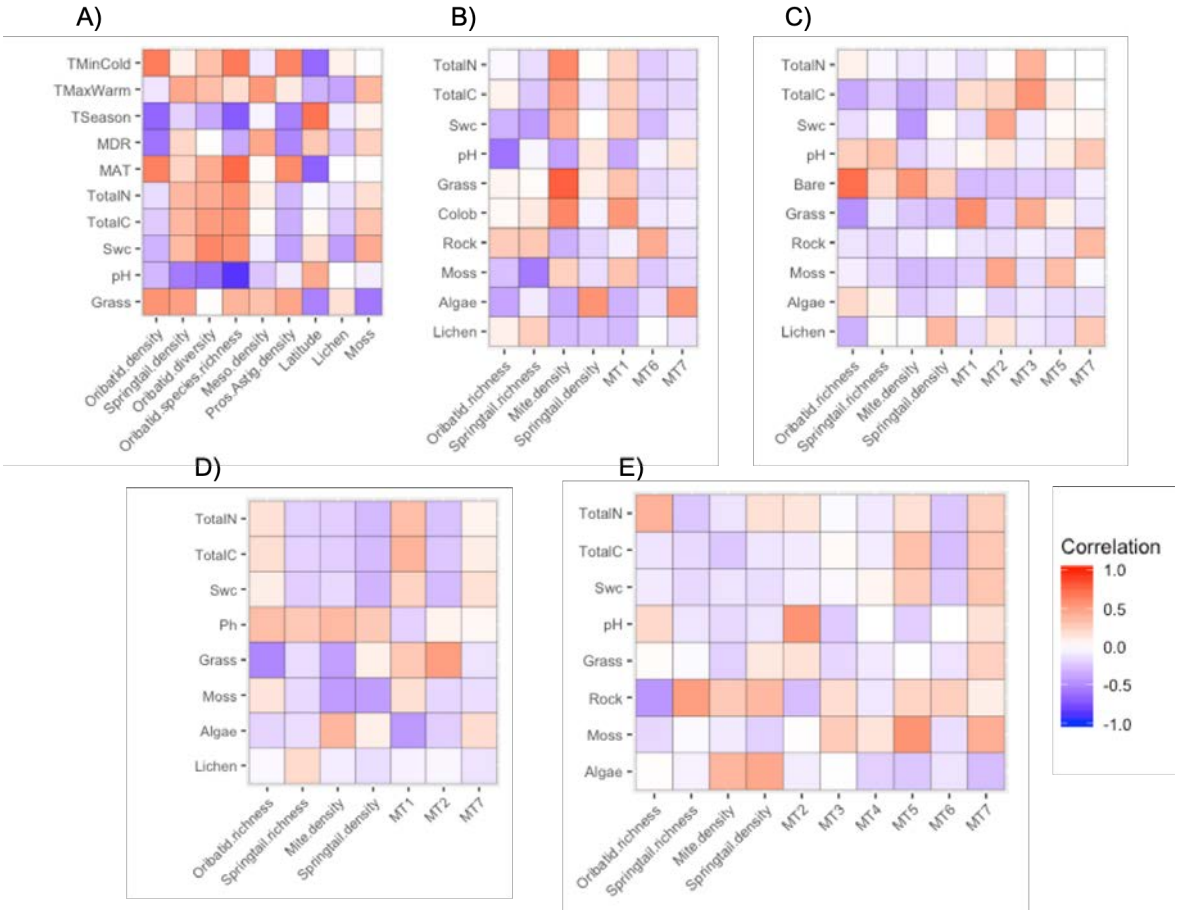


Figure 4.8 Heatmaps of correlation relationships between fine-scale morphotype level abundance and soil parameters and vegetation cover types from high intensity sampled sites in latitudinal order: A) Signy; B) Admiralty; C) Byers Peninsula; D) Biscoe Point; and, E) Anchorage.

To explore these relationships, regression analyses were performed and found oribatid richness to be positively related to vegetation cover type with most significance between grass cover ($p < 0.001$ $F_{1,142} = 13.01$, $R^2=0.05$) species. Additionally, springtail nucleotide diversities were positively related linked with lichen cover ($p = 0.05$, $F_{1,142}= 3.3$, $R^2=0.08$) and Colobanthus cover ($p < 0.05$, $F_{1,142} = 4.3$, $R^2=0.07$).

The heatmap correlations were supported by the NMDS plots that indicate the strength of relationship between oribatid and springtail assemblages from all sites with climatic drivers (Fig. 4.9). When assemblage structures of high-intensively sampled sites

were paired with environmental microhabitat parameters and vegetation cover types, oribatid assemblages had similar realtionships with soil pH and moss cover (Fig. 4.10). Plots for springtail assemblages were shown to have more variability between sites with SP1 as the sole morphotype that displayed any consistent bias associated with vegetation cover type that included moss and algae.

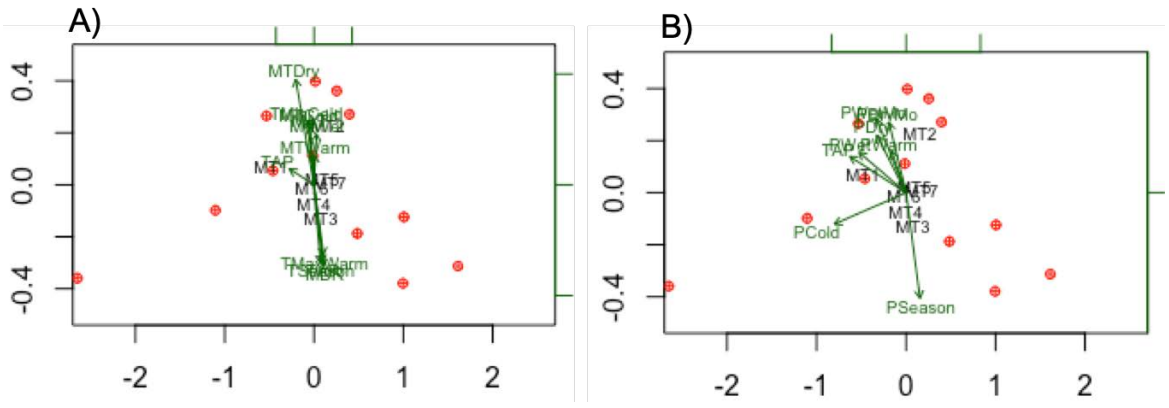
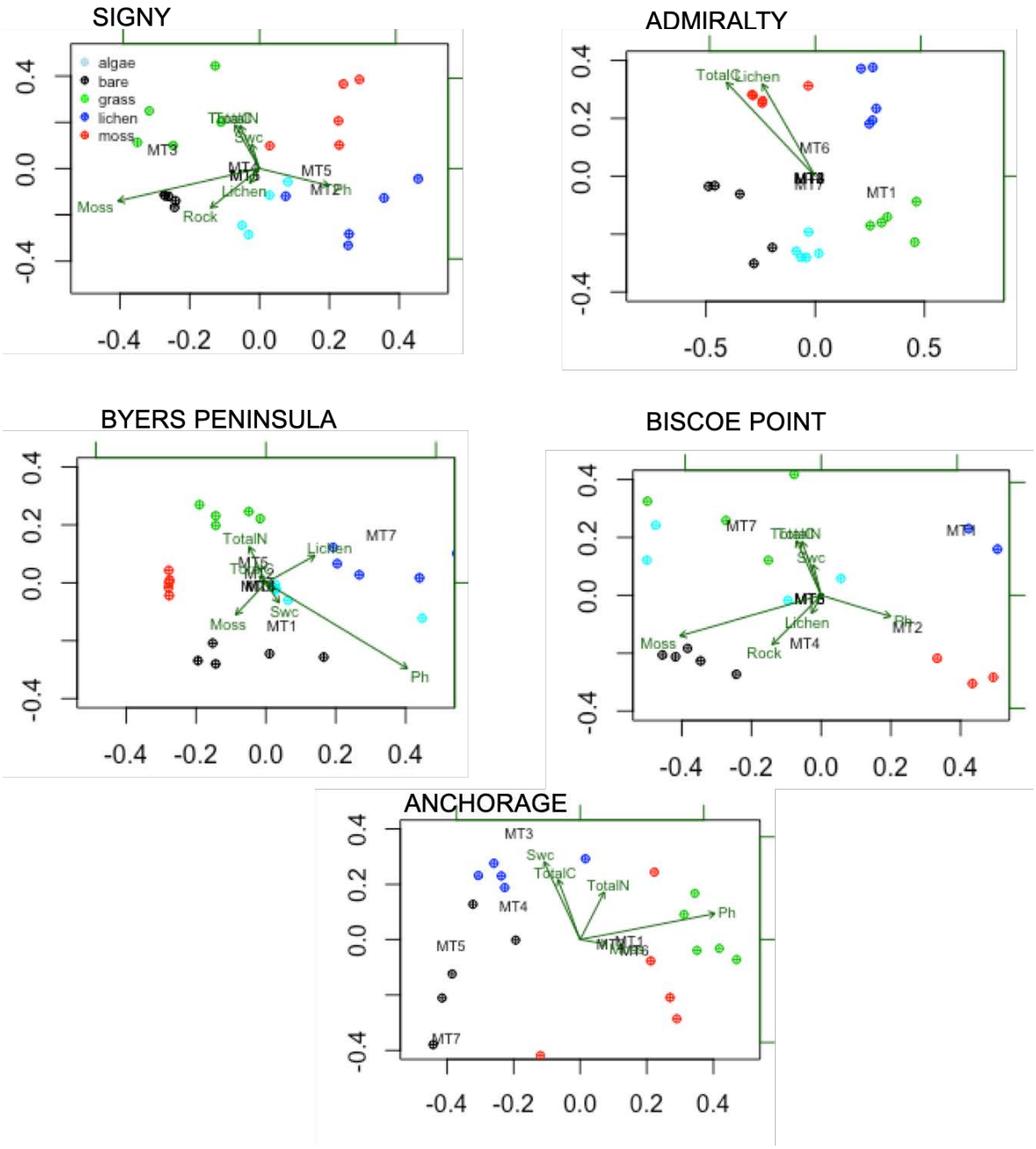


Figure 4.9 NMDS plots of oribatid assemblage structures with morphotypes (MT) in black, plots from across all sites with and green vectors indicating climatic variables: A) temperature variables, MTDry, Mean Temp. Driest month; MTWarm, Mean Temp. Warmest month; ; MTWet, Mean Temp. wettest month; TMCold; Max. Temp. Coldest Month; TMDry, Max Temp. Driest month and B) Rainfall variables; TAP, Total Annual Precipitation; PSeason, Seasonal precipitation; PCold, Precipitations coldest month; PDry, Precipitaion Driest month; PWet, Precipitation wettest month. All plots with stress < 0.2.

The NMDS plots visualise the differences in communities of oribatid mites with statistically significant relationships with environmental variables. The outputs illustrate clustering of sites based on predominant vegetative cover type, and quantitative relationships with oribatid mite communities. The vectors also indicate the variability inherent in the heterogenous composition of sites, with other vegetation categories present.



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2323 **Figure 4.10 NMDS plots of oribatid assemblage structures with morphotypes (MT) in black,**
2324 **plots from different dominant vegetation cover types: Grass (green), Lichen (blue), Bare**
2325 **(black), Moss (black) Algae (light blue), and green vectors indicating soil parameters across**
2326 **high-intensity sites. All plots with stress < 0.2.**

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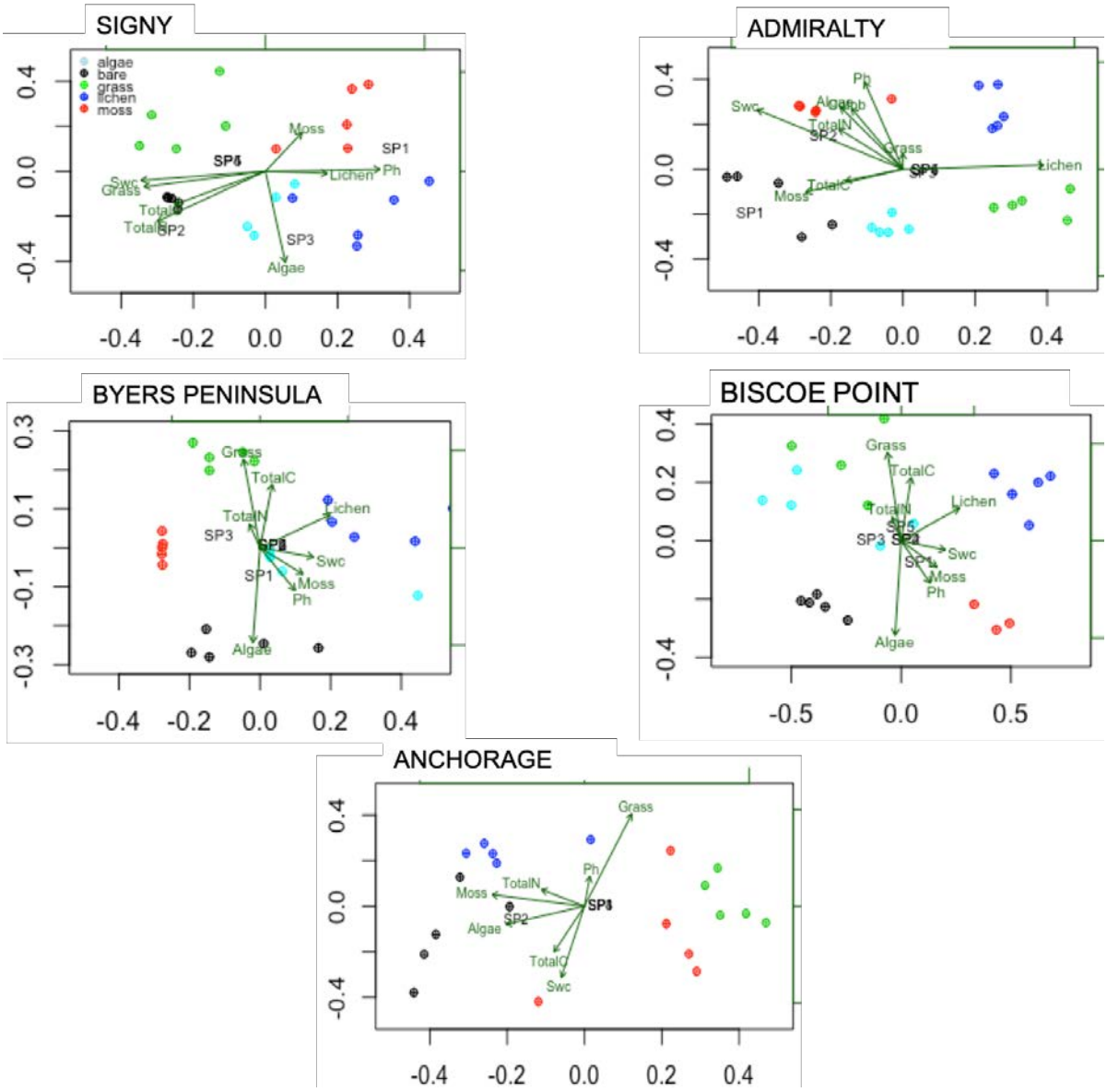

2329

2330 **Figure 4.11 NMDS plots of springtail communities with springtail morphotypes (BLACK),**
2331 **with sites coloured according to dominant vegetation cover types: Grass (green), Lichen (blue),**
2332 **Bare (black), Moss (black) Algae (light blue), green vectors indicating vegetation cover and soil**
2333 **parameters across high intensity sites. All plots with stress < 0.2.**

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4.4 Discussion

This study aimed to survey the distribution and diversity of soil fauna across a broad latitudinal transect of maritime Antarctica. Combining morphological and molecular approaches enabled the verification of species identities but indicate that morphological surveys of the mites and springtails under-estimate the true diversity. Aligning biotic metrics with climatic and environmental factors found mite and springtail abundances and species richness to be significantly related to geographic factors, with more diverse communities on larger islands in more northerly regions. Temperature and rainfall known to influence vegetation were also found to be significantly linked to patterns of mite and springtail distribution and diversity across spatial scales. Furthermore, fine scale micro-habitat parameters, including soil pH and soil nutrient loads, were consistently correlated with assemblage structures across the transect.

Landscape scale diversity of oribatid mites and springtails

A total of seven oribatid mite and five springtail morphotypes were found across all twelve sites. All appear to be species known to the region although sequencing could not confirm identities of certain morphotypes given a lack of appropriate reference sequences. However, our results point toward cryptic species in some taxa, specifically the springtail *Cryptopygus antarcticus*, indicating that the diversity may be greater than previously thought.

Dominant species of mites included members of the Oppiidae and Ameronothroidae families as found in earlier surveys of the region based on morphology (Starý and Block, 1998). The Ameronothroidae were represented by *Alaskozetes antarcticus, Halozetes belgicae,* and *Podacarus auberti.* The smaller *M. loxolineata* oribatids were widespread on

coastal and island sites (BP, EL, JE, LE and SI). *Halozetes impeditus,* Niedbala, 1986 and an *Ameronothrus* sp. were found more sporadically, mostly cooccurring in the northerly Signy and Elephant Islands. Less diverse oribatid communities were found at more southerly latitudes. The resolution of phylogenetic trees for the *A. antarcticus* mites using COI marker may indicate cryptic speciation, or presence of the sub-species: *A. antarcticus antarcticus*, *A. antarcticus grandjeani* Dalenius, 1958 or *A. antarcticus intermedius* Wallwork, 1967. Other oribatid species previously recorded in maritime Antarctica but were not identified in this study includes: *H. capensis* Coetzee and Marshall, 2003; *H. crozetensis* Richters, 1908; *H. edwardensis* Pletzen and Koch, 1971*; H. fulvus* Englebrecht, 1975*; H. impeditus* Niedbala, 1986; *H. intermedius* Wallowrk, 1963; *H. necrophagus* Wallwork, 1967; *Medioppia subpectinata* Oudmans, 1900; and, *Oppiella uliginosa* Willmann, 1919.

The springtail communities were dominated by *Cryptopygus antarcticus, C. sverdrupi* and *Friesea grisea* which were found to be cooccurring across most sites apart from the most southerly sites. Less frequent observations were made for *Kaylathalia klovstadi* and *Folsomotoma octooculata* Willem, 1901 that were found sporadically on islands off the west coast of the peninsula. Other springtail species known to the region that were not identified include *Desoria grisea* Lubbock, 1869, *C. nivocolus*, and the *C. a maximus* Deharveng, 1981, *C. a. reagens* Enderlein, 1909 and *C. a. travei* Deharveng, 1981 subspecies of *Cryptopygus antarcticus*. Future slide-mounting and morphological identification may reveal these previously recorded species

Broad-scale environmental patterns

We found a general decrease in diversity with latitude which align with earlier findings of lower diversity as communities approached the pole (Marshall and Convey, 2002; Van

Vuuren et al., 2018). Mites and springtail densities and diversity gradually decreased with latitude at the northerly sites, but the pattern was mostly driven by a significant reduction in diversity below 67° S conforming with earlier surveys (Convey and Smith, 1997; Maslen and Convey, 2006; Yergeau et al., 2007a; Convey et al., 2012, 2014). Mite and springtail species richness were thus greatest in the most northerly sites with the mildest climatic conditions that also harbour the most diverse vegetation cover types. The most recent meta-analysis of studies on endemic fauna tallied precisely 135 terrestrial invertebrate species from the continent and maritime that could be clustered into two main groups above and below the tip of the Peninsula (Pugh and Convey, 2008). This pattern may be related to changes in temperature given the observed relationships between temperature and mite abundance and diversity which has also been found in other studies of mites (Marshall and Convey, 2002) and microbial diversity (Yergeau et al., 2007b). Temperature is an important factor in metabolic activity, hence greater biotic activity and reproduction must be a contingent of this relationship, which would be more constrained at the more southern, colder sites.

Barriers to dispersal are a major factor often cited as a factor limiting the number of shared species between northern and southern regions that can be demarcated as: i) islands and coastal sites above 65°, with ii) southerly sites that are distant to known refugia and including inland ice-free oases. These were similar to findings based on morphological surveys and the diversity differences between Alexander Island and the inland oases sites (Convey end Smith, 2008; Van Vuuren et al., 2018). We observed high species richness on Signy and Elephant islands which may be a result of favourable currents that would have increased the chances of arrivals of individuals from more northerly islands (Fraser et al., 2011; Mortimer et al., 2011). The ACC have been shown as of greater biogeographic

consequence than island area when genetic diversity of higher invertebrates (Frankham, 1996).

Further implications of dispersal are apparent when considering the numbers of mite and springtail haplotypes which decreased with distance to potential refugia. This relationship was expected to mirror findings on vegetation along the peninsula (Fraser et al., 2015), whilst also adhering to associations with the coastal refugia (Marshall and Convey, 2004; Convey et al., 2014; Chown et al., 2015). Both springtail and mite species richness indicate a strong influence from proximity to geothermal refugia as central to their genetic diversity based on the separation of populations by region. As fewer oribatid mite haplotypes were found in sites further away from refugia, this highlights the importance of dispersal from refugia in soil invertebrate distributions. These are supported by decreasing numbers of springtail haplotypes further from the peninsular, from which they dispersed following the retreating ice-sheets (Davies et al., 2012). The non-climatic environmental factors linked to faunal diversity involve the transition from sites dominated by lichen and grasses in more northerly sites to those that sustain mostly algae and mosses.

Few haplotypes were shared across the regions, in part due to dispersal barriers separating refugial sources in the north of the peninsula with more central island sites as described above. Such disjunct distributions may have formed following repeated dispersal events and shifting species ranges as a response to the Pleistocene glacial cycles. These dispersal events may have coincided with the extirpation of local species and contributed to their current fragmented distributions across islands. Detailing population genetics and gene flow from within individual species will be able to clarify these patterns. The evidence of regional species endemicity support the application for an Antarctic Special Protected Area (ASPA) status as defined by the Antarctic Treaty System (ATS) to preserve Antarctica’s

indigenous fauna and provide further impetus to establish reference sites for future biomonitoring campaigns (Hughes et al., 2016).

The local sequence libraries compiled for mites and springtails indicated haplotypes that were separated by genetic distances that surpass the threshold for new species using the 28S rRNA marker with potential cryptic speciation in both *A. antarcticus* and *H. belgicae* mites. Molecular sequences of *Podacarus auberti* mites were able to distinguish them from the morphologically similar instars of *A. antarcticus* and other Ameronothroids (Ermilov et al., 2012), overcoming the challenges posed by morphology-based surveys. Comparison of *C. antarcticus* springtails indicated far greater cryptic speciation existing within populations. These may indicate similar requirements for species divisions as performed recently to the *Friesea* genus (Carapelli et al., 2020; Stevens et al., 2021).

Soil faunal relationships with environmental variables

Species richness of both mites and springtails were correlated with vegetation cover type, while mite diversity was positively linked to soil pH and and springtail diversity was related to vegetation cover type in springtails. These were reflected in analysis of community structure which showed mite species richness and springtail phylogenetic divergence was related to the cover of Colobanthus, grass and lichen. Soil faunal diversity and abundances were also influenced by soil properties across all twelve sites. Oribatid and springtail densities and species richness were found to be significantly related to local-scale soil parameters. Oribatid mite densities were marginally significant to levels of grass and lichen vegetation cover. Springtail densities were linked with lower soil pH, although mites had no such relationship. More southerly sites had reduced N, soil moisture and organic matter with more acidic soil pH, which might explain the lower microarthropod densities at these sites.

Oribatid species richness was significantly related to soil pH as well as total nitrogen and carbon contents that may be associated with their greater reliance on habitable environments.

These broad scale findings support my hypothesis that biotic factors showed a greater affinity for particular vegetation cover type and other environmental factors, more so than climatic and geographic influences in this region. With increased "greening" of ecosystems across maritime Antarctica, these findings may contribute to informing models aimed at predicting how soil faunal assemblages may respond to shifting vegetation cover types accelerated by climate change and other human-caused activities (Beaugrand et al., 2002; Parmesan and Yohe, 2003).

Assemblage structure

Oribatid mite community composition showed relatively consistent relationships with vegetation cover types and soil parameters within sites across the transect. NMDS outputs of oribatid community structures showed that *P. auberti* (MT7) and *M. loxolineata* (MT2) were more common in soil with higher soil pH and SWC at most sites sampled at high intensity. This demonstrates that soil parameters are influential conditions important to soil fauna at both broad- and fine-scales. The oribatids *M. loxolineata* (MT2) and *A. antarcicus* (MT5) were associated with greater moss and lichen cover, whilst grazers such as *L. mollis* (MT1) were more abundant where cover of grass was higher. Similarly, springtail assemblages were related to both edaphic variables and vegetation cover. Two species, *C. antarcticus* (SP1) and *F. parvulus* (SP3), appeared to be more dominant in soils with lower pH while *C. sverdrupi* (SP2) was more abundant in the grassy vegetation type that is prevalent in the northern Signy and Elephant islands. Springtail phylogenetic divergence

was the sole molecular metric found to be significantly linked to vegetative properties, having greater divergence associated with lichen and *Colobanthus quitensis* (Antarctic Pearlwort) cover, an endemic flowering plant.

Conclusion

In summary, this study found clear links between soil faunal distribution and diversity with climate at the broad-scale and vegetative cover-type and soil parameters at the local-scale. Of the seven oribatid species and five springtail species found across the transect, most were found to be present in sites above 67°S, with minimal diversity in the inland oases at 71.5°S. Mites and springtail densities and species richness showed significant correlations to geographic factors such as latitude and island area, yet climatic factors and soil parameters were also shaped distribution patterns and assemblage composition via shifting vegetation cover types. The molecular approaches indicated that genetic diversity of mites and springtails may be higher in regions with known refugia, although further investigation is required to determine the location of unidentified refugia along the western coast of the peninsula. Moreover, our results provide evidence for cryptic speciation, indicating that the diversity of these communities are greater than currently thought. These findings support the high endemicity of soil fauna within maritime Antarctica and highlight the need for efforts aimed at ensuring the maintenance of the biodiversity in the face of accelerating environmental change.

Chapter 5 - Mites and springtails show contrasting phylogeographic patterns in maritime Antarctica

Abstract

The origins and distributions of soil organisms have long been investigated, but can be hampered by difficulties in determining phylogenetic relationships. Antarctica is known for its pristine environments subject to extreme climatic conditions that provide suitable habitat for a range of vegetation types and associated soil invertebrates; however, we know comparatively little of the endemic Antarctic fauna compared with those of the northern hemisphere. Now, approaches linking genetic and geographic information are revealing many cases of complex ancient histories influenced by dispersal and speciation. In this study, the phylogeography of the oribatid mites, *Podacarus auberti and Membranoppia loxolineata* and the endemic springtail, *Cryptopygus antarcticus* were assessed using the 28S rRNA D3 domain based on sequencing of specimens from populations along a broad transect throughout maritime Antarctica. Phylogenetic reconstruction using evolutionary models and analysis of population genetics indicated a strong influence of geography on nucleotide and haplotype diversity, with greater within population diversity found in oribatid mites in regions with greater dispersal barriers, whilst regional differentiation between populations were more apparent among springtails. The phylogeographic reconstructions also highlighted greater importance of dispersal events in oribatid mites compared to springtails. This study show how high-resolution molecular data can inform phylogeographic analysis of soil invertebrates and improve the capacity to monitor soil invertebrate biodiversity in maritime Antarctica.

5.1 Introduction

Invertebrate phylogeography has been explored in all terrestrial ecosystems, but most studies focus on northern hemisphere taxa. In particular, the distribution, diversity and origins of endemic invertebrates of Antarctic terrestrial ecosystems are understudied. Soil invertebrate communities exist in the seasonally ice- and snow-free habitats of coastal areas and islands along the Antarctic peninsula and Scotia Arc. These faunal assemblages primarily inhabit poorly developed soils beneath grasses, mosses and lichens, but are among the most diverse communities of soil fauna in the Antarctic region (Nielsen et al., 2011). Antarctic ecosystems support a diversity of mites (Acari) and springtails (Collembola) that are active contributors to soil processes underlying ecosystem function (Chown and Convey, 2007; McGaughran et al., 2010a). While they occupy similar habitats, mites and springtails show differences in morphological and physiological traits that affect their distributional patterns, including dispersal ability, life cycles and stress tolerance. Mites are considered more capable of long-range passive dispersal, whilst springtails have greater short-range motility and cold-tolerance (Convey et al., 2012, 2020). Antarctic populations provide an ideal study-system to demonstrate the degree to which biotic and geographic factors interact to influence speciation (McGaughran et al., 2011) across spatial scales along a well-defined climatic gradient.

However, the origins and distribution of Antarctic soil invertebrates have been scarcely examined due to logistical constraints and deficiencies in accurate taxonomic identification. Now, mounting molecular evidence points to the ancient origins of soil invertebrates, having survived in refugia for tens of millions of years (Convey et al., 2008; Fraser et al., 2014). Biogeographic investigation of Antarctic invertebrates has found strong

influences of geographic and historical events on patterns of distribution. During cyclical glaciations, freezing conditions severely limited suitable habitats, constrained dispersal and resulted in fragmented ranges of several maritime and continental species (Chown and Convey, 2007; Nielsen et al., 2011). A synthesis of phylogeographic studies in the Antarctic (Chapter 2) showed that dispersal, rapid speciation and repeated colonisation had profound effects on the region's taxa at geological timescales. However, environmental factors are known to influence broad-scale distributions (Gaston, 2000), whilst biotic factors become more important in structuring communities at the local scale (*sensu* Aerts, 1997), which therefore govern contemporary patterns of distribution. More recent post-LGM dispersal alongside waves of glaciation have created a patchwork distribution that may over-ride earlier signs of migration as found in continental springtails (Carapelli et al., 2017). A recent phylogeographic study of the oribatid mites *Halozetes belgicae* Michael, 1903 and *Alaskozetes antarcticus* Michael, 1903 from maritime Antarctica suggested multiple refugial sources along the peninsula, with implications for contemporary phylogeographical patterns (Van Vuuren et al., 2018). Having a firm grasp of the contrasting responses of soil faunal groups to past ecosystem conditions and fragmentation will help inform ongoing conservation efforts for potential future dispersal amidst a rapidly changing climate and expansion of ice-free areas in the region (Lee et al., 2017).

The small number of non-native terrestrial species that have arrived in Antarctica since the Last Glacial Maximum (LGM, ~12Ka; Barnes et al., 2008) simplifies evolutionary analysis of the taxa present today. This is important as the geography and changing climate have created highly complex patterns of distribution, driven by repeated dispersal events from refugial source populations and recombination within local populations. Maritime Antarctica comprises large areas that have been subject to expanding and contrasting glaciers

and icesheets since the Pliocene (5.3 – 2.6 Ma) through the mid-Pleistocene (~2.6 Ma) until the Last Glacial Maximum (LGM, ~12Ka). Northerly islands in the South Orkney Islands and the Scotia arc are known to have an ancient Gondwanan geological origin (Truow et al., 1997). This has resulted in high species endemicity with restricted genetic exchange in several mites (Van Vuuren et al., 2018) and springtails (Collins et al., 2019). However, past episodes of dispersal and shared ancestry are still evident from detailed analysis of their phylogenetic relatedness. Thus, molecular approaches can overcome the challenges of synonyms and cryptic speciation (Wallwork, 1967) that have limited the number of comparative phylogeographic studies of Antarctic soil fauna.

Mites and springtails are most prominent in Antarctic areas that have highly developed vegetation and regions with milder climates (Chapter 4). Comparing the two faunal groups can highlight the influence of dispersal barriers and refugia on restrictions to gene-flow. These may be a result of differences in dispersal ability whereby mites have lower short-range motility. Other factors involve the ability to resist freezing temperatures, with springtails possessing multiple traits that assist them, and that are less well developed by comparison in mites. These also include desiccation tolerance, dormancy and resource adaptability. Earlier continental studies of the oribatid mite *Liochthonius. mollis* Hammer, 1958 and springtails *Gomphiocephalus hodgsoni* Carpenter, 1908 and *Gressittacantha Terranova* Wise, 1967 found patterns consistent with multiple speciation events that occurred within the last 1 Ma (Widmer and Lexer, 2001; Nolan et al., 2006; Stevens and Hogg, 2006). However, more recent neo-refugial bottleneck events (post-LGM) followed by dispersal are potential mechanisms that drive speciation along the Antarctic peninsula (Allegrucci et al., 2006; McGaughran et al., 2008).

During repeated cycles of glaciation, almost all populations of soil fauna were locally

extirpated. However, refugial survivors are hypothesized to have existed at multiple locations along the peninsula (Fraser et al., 2014; Convey et al., 2020). These were founded during the glacial periods throughout the Pleistocene epoch (~2.6Ma–12Ka) and are considered to have recolonised the entire region in a southerly direction (Stevens and Hogg, 2003). Multiple geothermal sites are suspected of providing refuge for soil fauna during these glacial periods (van Vuurren et al., 2018), however unverified refugial locations are suspected to have existed along the western coast of the Antarctic Peninsula. Further exploration and analysis of biotic populations genetics may clarify these points. Repopulation from multiple refugia and subsequent dispersal and speciation following the end of the LGM has been demonstrated in continental and sub-Antarctic regions (Convey, 2008; Chown et al., 2015). Inferences can thus be drawn from the intraspecific diversity related to the distance between populations or refugia (Avise, 1994; Hewitt, 1996).

Maritime Antarctica has been warming rapidly since the mid-20th Century (Mulvaney et al., 2012), which has resulted in the expansion of vegetation but also increases the risk of non-native species colonizing over uncertain time-frames (Duffy et al., 2017). These scenarios are described as the Antarctic Climate-Dispersal-Invasion (ACDI) hypothesis and have been supported in passive migration of sub-Antarctic springtails (Chown et al., 2022). To ascertain if similar scenarios may also be found in maritime Antarctica, comparison of phylogeographic histories of taxa with biotic differences such as passive and active dispersal ability or survival traits that have shaped their distinct speciation patterns. Whilst tardigrade, nematode and springtail communities have the survival traits of resistance to freezing via anhydrobiosis and cryptobiosis (Sinclair and Sjursen, 2001; Sjursen et al., 2001; Hogg et al., 2014; Velasco-Castrillon et al., 2014), these are unconfirmed in Antarctic springtails and mites. Ingimarsdóttir et al., (2012) have shown they

are useful as paired species in order to determine different survival abilities and therefore colonisation ability in polar ecosystems.

We recently found that the mites *Podacarus auberti* Grandjean, 1955 and *Membranoppia loxolineata* Wallwork, 1965 and the springtail *Cryptopygus a. antarcticus* Willem, 1901 were the dominant species at sites above 67.5º. The mite *M. loxolineata* has multiple synonyms, and is an example of the changing nomenclature common to taxonomy, incorporating novel information based on molecular analyses (Balogh and Balogh, 1992, 2012; Starý and Block, 1997; Colloff and Halliday, 1998; Subias, 2004). However, such discrepencies can be clarified using molecular phylogenies. The high densities and widespread distribution combined with notable differences between the two taxa provide an ideal framework for comparative phylogeographic analyses. Recent work has used interpretation phylogenetic analysis of annelid populations to discern differences between Arctic and Antarctic taxa (Eilertsen et al., 2018). Comparing phylogeographic reconstructions of different soil fauna can therefore reveal the influence of environmental change and influence on dispersal, vicariance and extinction.

Objectives

This main aim of this study was to examine and contrast the population diversity and phylogeography of two mite species and a springtail genotypes that are distributed throughout northern, central and southern regions of maritime Antarctica. Assessing springtail and mite gene-flow among populations throughout maritime Antarctica is expected to highlight the importance of long-range dispersal. Whilst areas with unique and high levels of genetic variation can illustrate the importance of refugia as reservoirs of genetic diversity. A null hypothesis would assume that mites and springtails have similar

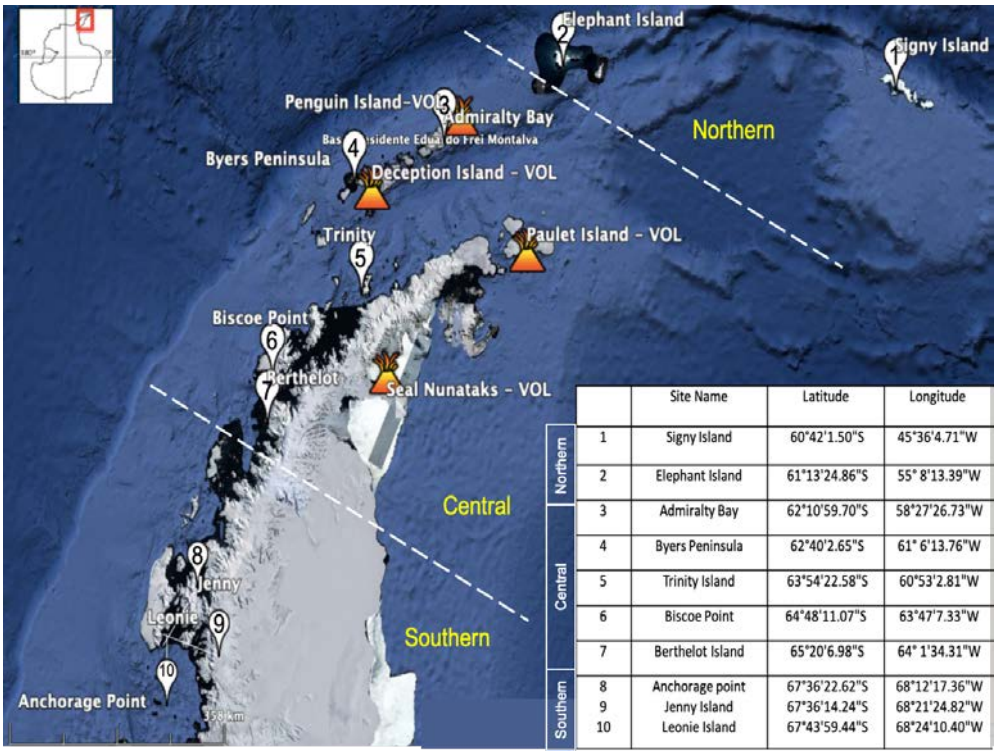
evolutionary histories with no differences in the influence of biogeography on their speciation. However, their biotic differences in dispersal ability and cold-tolerance are expected to be expressed in contrasting phylogeographic reconstructions. Molecular data are expected to demonstrate that oribatid mites have stronger patterns involving multiple dispersal events, rather than singular migratory events for springtails. Islands above the peninsula are expected to have less gene flow and little shared ancestry with other sites closer to refugia. The findings from this investigation are expected to show how phylogeography can improve our understanding of soil faunal evolution and identify unique populations that can advance our conservation strategies in the region

5.2 Methods

Collection of material

Soil samples were collected at 12 sites along a latitudinal gradient (60º-74º) across maritime Antarctica during the southern hemisphere summers in Dec–Feb 2014/15 and 2015/16 (Fig. 2; Ball et al., 2022). Oribatid mites and springtails were only present in sufficient numbers from sites above 67.5º and so the inland oases sites at 74º were excluded. Sampling was conducted within a relatively homogeneous area from a selection of the main vegetation cover types (grass, moss, lichen, algae) and bare ground. A sterilised metal collar with 5 cm diameter was used to collect soil samples to 5 cm in depth for invertebrate extraction. Mites and springtails were extracted by incremental temperature increases over 5 days directing migrating fauna into 70% ethanol, prior to being shipped to Western Sydney University for processing. Mites and springtails were separated from residual soil particles using a binocular light microscope at 40X magnification. Mites were sorted into taxonomic orders (Oribatida, Mesostigmata, Prostigmata and Astigmatina), while adult oribatid mites and springtails were sorted into morphotypes (MT) based on morphology following bibliographies by Block (1992) and earlier surveys (Wallwork, 1967; Usher and Edwards, 1986). These putative species were selected for sequencing to assist in taxonomic identification of the specimens.

Several mites and springtails were common throughout the northern region (i.e. 60–67.5º S; Chapter 4). For this study, I focus on the most common genotypes with the broadest distributions with sufficient densities to test the phylogeographic predictions set forth in the hypotheses. Specifically, genotypes of the oribatid mites, *Podacarus auberti* and *Membranoppia loxolineata*, and the springtail *Cryptopygus a. antarcticus*.



	Site Name	Latitude	Longitude
1	Signy Island	60°42'1.50"S	45°36'4.71"W
2	Elephant Island	61°13'24.86"S	55° 8'13.39"W
3	Admiralty Bay	62°10'59.70"S	58°27'26.73"W
4	Byers Peninsula	62°40'2.65"S	61° 6'13.76"W
5	Trinity Island	63°54'22.58"S	60°53'2.81"W
6	Biscoe Point	64°48'11.07"S	63°47'7.33"W
7	Berthelot Island	65°20'6.98"S	64° 1'34.31"W
8	Anchorage point	67°36'22.62"S	68°12'17.36"W
9	Jenny Island	67°36'14.24"S	68°21'24.82"W
10	Leonie Island	67°43'59.44"S	68°24'10.40"W

Figure 5.1 Map of Antarctic transect and demarcation of main regions (Northern, Central and Southern) from the Scotia Arc along the peninsula and the location of known active geothermal areas. Map excludes sites at 74º as samples from these sites were not included in analysis. Image compiled by Google Earth from satellite data from SIO, NOAA, U.S. Navy, NGA, GEBCO Image U.S. Geological Survey Data LDEO-Columbia, NSF and NOAA.

Sequence generation

In order to investigate the population genetics and phylogeographic patterns of mites and springtails, molecular information was generated from extracted soil faunal samples from maritime Antarctica. To determine levels of gene-flow across the gradient, a nuclear marker was selected to provide a robust indication of divergence as found in earlier oribatid mite analyses (Maraun et al., 2003), that is expected within the timescales associated with Pleistocene glacial cycles. The 28S rRNA D3 marker has been shown as having more efficient sequencing (Zhao et al., 2020) than the D2 region, as well as being more ancient (Gillespie et al. 2005), with fewer complicating indels and retro-transposable elements

(Gillespie et al., 2006) that can complicate assessments of phylogenies in oribatid mites (Lehmitz and Decker, 2017). Earlier use of the 28S rRNA marker in Antarctic springtails (Frati et al., 2000), and nematodes (Raymond et al., 2014), supports its use here.

Three specimens from each site where the species occurred were selected and provided suitable sample numbers when sites grouped into two regions. This provides sufficient sample sizes required to perform Bayesian analysis based on numbers of genetic markers. This overcomes the issue of insufficient numbers of the same species at individual sites. This was to provide sufficient replication of specimens to create phylogenetic trees of populations within species based on Bayesian coalescent theory (Drummond and Bouckaert, 2007). The sites were grouped according to overall biogeographic histories relating to their position along the transect and relative proximity to known refugial locations. The sites were grouped into Northern, Central and Southern regions that were the main regions containing sufficient numbers of *P. auberti* genotypes. However, limited abundance and diversity of *M. loxolineata* oribatid genotypes and *C. antarcticus* springtails restricted their analysis between only Northern and Central regions.

Total gDNA was extracted from mite and springtail specimens by freezing their whole bodies in liquid N_2 before grinding with a micropestle. Further lysis was preformed using a sterile 1mm diameter steel ball-bearings in a Qiagen tissue-lyser II bead-mill, followed by a typical 'salting out' protocol with the addition of Chelex 100 beads (Sunnucks and Hales, 1996). Purified gDNA concentrations were quantified with nanodrop (DeNovix DS-11, Thermo Fischer Scientific, Australia) and then amplified using PCR primers for: i) a 320 bp region in the D3 region of 28srRNA F' 5-GACCCGTCTTGAAACACGGA-3), R' 5- TCGGAAGGAACCAGCTACTA-3; (Litvaitis et al., 1994); and, iii) a 320bp fragment from the nuclear Histone 3 (H3) gene F' CGTAAGTCGGCGCCCAGC, R'

GACCCGTTTGGCGTGAATTGC (Mortimer et al., 2011) were optimised for sequencing in oribatid mites and springtails.

Amplicons were then run on 1.5% agarose gels in 1 x TBE buffer and the bands separated using 100 V electrophoresis against a standard 1Kb/100bp Hyperladder IV (Mobio). Amplicons were purified using the Promega Wizard PCR Clean-up kit as per the manufacturer's instructions. Sample elution concentrations were adjusted and prepared for Sanger sequencing using HiDi – Formamide (Thermo Fisher, Australia) in both forward and reverse directions. Alignments of D3 markers were visually curated and checked for incorrectly labelled bases and other errors prior to alignment. Sequence alignments were performed using the CLUSTALX v.2.0 (Larkin et al., 2007) model with an opening gap of 10.0 and extending gap of 0.1 in the Geneious^{TM} v. 2020.2 program (www.geneious.com). Selection of nucleotide substitution models was performed in MEGA with GTR +G+I = proportion of invariable sites (Proportion invariant = 1.0, default = 0.0), +G = Gamma distributed rate variation among sites (4 = all sites). These can be applied by entering Gamma Category Count - (Set to 4.0 for +G, default=0), HKY – Kappa = Default =2.0. Tree construction was performed with 3-way separation for the optimal partition model with linked clock and substitution models.

Genetic variation across the transect

Only sequences that met the threshold (>99%) were selected as a genotype based on the 28S rRNA marker. Here, sequences that matched to referenced sequences on BLAST were aligned with selection of samples all within < 5% difference in total nucleotide differences. These values are based on supplying the minimum sample size required for stastical analysis and applied in earlier phylogeographic analysis of invertebrate distribution (van Vuuren et

al., 2018). To assess the influence of location on population genetics and phylogenetic variation across the transect, a standardised analysis pipeline was used to process the data from raw sequence output, through to phylogeographic analysis (Table 5.1). Complex algorithms applied to the sequence data were interpreted as haplotype (h) and nucleotide (π) diversity indices using GeneAlEx v. 6.5 (Peakall and Smouse, 2006, 2012). Indels were checked using GBlocks 0.91b (Castresana, 2000) prior to analysis. Trimmed and corrected sequences were imported as FASTA alignments and linked to Longitude and Latitude data using binary worksheets. Pairwise phi-statistics based distance values (PhiPT) based on pairwise distances between populations were calculated and significance tested based on 999 permutations. These are a derivation of Wright's F-statistics and reflect the relative contribution of between-population separation to the overall genetic variation in a sample (Wright, 1965; Weir and Cockerham, 1984). The greater the values are, the greater the differences between populations.

Measures of PhiPT were calculated as: PhiPT = AP / (WP +AP) = AP / TOTAL, where; AP = Estimated variation among populations, and WP = Estimated variation within populations. Spatial autocorrelation analyses were performed in GenAlEx. Regional FST values were calculated using the *DiveRsity* package v1.7.0 in R (Keenan et al., 2013). Sanger sequence readouts were trimmed and aligned in Geneious prior to calculation of phylogenetic distances matrices.

Bayesian framework

Applying Bayesian techniques to infer the most likely biogeographic histories for a given phylogeny and alternatives can be evaluated according to Bayes factors (Dickey, 1971; Verdinelli and Wasserman, 1995; Kass and Raftery, 1995) and Akaike Information Criterion

(AIC) weights, with the lowest AIC score the most likely biogeographical history in most cases (Akaike, 1987; Anderson and Burnham, 2004; Landis et al., 2013). Bayesian phylogenetic trees were constructed using BEAST v1.6.1 (Drummond & Rambaut 2007), with the optimal sequence evolution models determined in jModeltest (Posada, 2009). This model was run for 10,000,000 generations and sampled every 1,000 generations. Plots and diagnostics (standard deviation of split frequencies, effective sample size) from Tracer v1.4 (Drummond and Rambaut, 2007) were visually inspected to ensure that stationarity had been reached. The first 10,000 trees were excluded as burn-in and consensus trees were calculated in TreeAnnotator v1.6.1 (Drummond and Rambaut, 2007). Analyses were repeated three times to check for consistency in topologies. To further test for consistency, phylogenetic trees were also constructed using RAxML v7.2.8 (Stamatakis, 2014). These outputs of Bayesian reconstructions were then applied to evolutionary models to address the hypothesis questioning dispersal histories of the indigenous oribatid mites and springtails.

Reconstructing ancestral histories

In order to deduce whether singular or multiple dispersal events from refugia for the different soil faunal types, measures of heterozygosity, or degree of genetic similarity can be analysed. These generate phi-statistics that are a product of comparing two binary datasets and are suited for genotypic sequence data. As such, these can show deviations from the Hardy-Weinberg equilibrium model that assumes a constant rate of gene flow and haplotype frequencies over time (Edwards, 2008). It may be considered that the parthenogenetic reproductive mode of some oribatid mite species may complicate the comparison of evolution with other sexually reproducing species (Maraun et al., 2003; 2019). However, this has been shown to not have a significant effect on the calculation of evolutionary rates

as shown in other invertebrates (Heethoff et al., 2009; Laumann et al., 2007). Effects of gene flow, genetic drift and population bottlenecks, and especially founder effects from dispersal events, are identified when F_{ST} values are lower than expected for even distributions of haplotypes between populations. As alternate methods can draw different conclusions from the same dataset (Xiang and Thomas, 2008), optimal model selection was based on AIC values, similar to substitution model selection (Matzke, 2014). For soil invertebrates, phylogeographic model comparisons were performed using the Reconstruct Ancestral State in Phylogenies (RASP) program (Yu et al., 2015), as demonstrated elsewhere in invertebrate reconstructions (Miraldo and Hanski, 2014). As indels are treated as missing data in preliminary Bayesian computations, indels were coded as binary characters and included as a separate binary data partition in the analysis (Huelsenbeck and Ronquist, 2001).

Evolutionary models

The power of the Dispersal-Vicariance analysis (DIVA) and Dispersal-Extinction-Cladogenesis (DEC) models to discern vicariance and dispersal, is countered by their inability to discern long-distance founder events (Ronquist and Sanmartin, 2011; Kodandaramaiah 2010; Goldberg and Trewick. 2011). These are frequent factors important in oceanic island and Antarctic peninsula populations (Gillespie et al., 2012). DEC models are based on the LAGRANGE methodology (Likelihood Analysis of Geographic Range Evolution; Ree and Smith, 2008; Table 5.1). This approach is well-suited for phylogenies that are accompanied by detailed temporal distributions of speciation (Moreau and Bell, 2013), and determines the probability of ancestral range at each node. Both DIVA and DEC methods summarize biogeographic histories across all trees, using stochastic models for discrete geographic areas, with parameters estimated using both ML and Bayesian

frameworks (Tierney, 1994). Unlike the DIVA models, DEC models enable sympatric-subset speciation (Ronquist and Sanmartin, 2011). For incorporation of divergence times, despite estimation of major glacial movements as accurate to 10 Ka (Mulvaney et al., 2012), the margins of error might exceed the limits for optimal use with the DEC model.

Table 5.1 Sequence processing pipeline with process and programs for each stage from raw sequence to phylogeographic outputs.

Step	Process	Outcome	Software	Files type Input-output
Sequence check	Visual check Reverse compliment BLAST	Confirm faunal type ID, GC content	Geneious	.az fasta
Sequence alignment	ClustalW Geneious	Gap fill settings	MEGA	nex mega
Nucleotide Substitution model selection	ModelTest- NG Posada et al.,	24 possible combinations, optimal model settings	MEGA	fasta nex
Tree formation	Bayesian MCMC	Prior opt. Multi-loci model assignment	BEAUti 2.5.2 BEAST 2.0	nex .xml .trees .tre
MCMC check and Tree construction	Plotting graphs of statistical outputs	Sample size check, Node labels	FigTree Tracer TreeAnnotator	.png
Molecular variance	AMOVA Spatial correlation	Probability of divergence	GenAlEx Arlequin 3.5.2 R	.arl .xlsx
Dispersal/Vicariance analysis	DEC	Geographic data (Long, Lat) Trees vs states	RASP (including BioGeoBears in R)	.trees .state
Biogeographic/ Statistical analysis	Mantel tests, PCA, Variation partitioning	Dispersal–vicariance modelling, statistical tests	RASP R	.csv

Phylogeographic outputs were divided into two regions based on defined clustering based on latitude, proximity to refugia and environmental similarities as determined in Chapter 4. These regions were: i) Northern islands, Signy and Elephant, and, ii) Central Refugial region.

Estimation of divergence times and dispersal events

Molecular clock dating of mites and springtails in the absence of fossil or geological calibrations was based upon the molecular clock rate of 1.5–2.3% divergence per million years that is commonly applied to arthropods (Brower, 1994, Juan et al., 1996, Quek et al., 2004). The divergence estimates for the lower bound (1.5%) are based on uncorrected p-

distances which do not account for multiple changes at nucleotide sites and become biased towards underestimating older divergences. Here, a rate based on earlier studies was employed using the CO1 gene, which has an estimated divergence rate of 1.9% per million years as calculated and used in previous studies of Antarctic arthropods (Mortimer et al., 2011; Van Vuuren at el., 2018). These were used to frame the analyses based on the uncalibrated D3 markers that have a total percentage of variable sites in the 28S rRNA gene is 5.6% (Frati and Dell'Ampio, 2000). However, the fragment targeted in the D3 domain has a large proportion of this variation with minimal introns and indels as earlier described (Gillespie et al., 2006; Zhao et al., 2020). As the D3 fragment has not been calibrated for oribatid mites, divergence rates were based on CO1 markers (Van Vuureen et al., 2018; Klimov et al., 2019; Collins et al., 2019).

5.3 Results

Oribatid mites

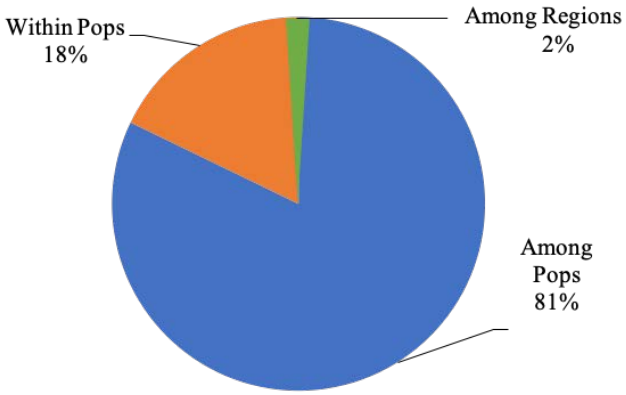
From a total of seven species that were found to populate the transect, the two most wide-spread oribatid mite 28S rRNA sequences were derived from D3 regions for specimens of genotypes of *Podacarus auberti* and *Membranoppia loxolineata.* Sequences of these species were selected and compiled into alignments consisting of two polymorphic loci for 59 individuals for *P. auberti* that had representatives from the northern and central regions.

Table 5.2 Population structure overview of sites across the transect for *P. auberti* mite genotypes. Columns indicate n: number of sequences, x: the number of haplotypes and π is nucleotide diversity. Sites are ordered in descending latitudinal order. Means (±S.E.) of regions in shaded in grey.

SITE	n	x	π
SI	5	1	0.003
EL	6	1	0.009
Northern region	5.5 ±0.5	1 ±0	0.006 ±0.003
AD	3	1	00.04
BP	7	2	0.08
TR	3	2	0
BI	3	2	0.31
BE	3	2	0.04
Central region	3.8 ±3.8	1.8 ±0.2	0.094 ±0.06
AN	15	1	0.04
JE	12	2	0.04
LE	7	1	0.09
Southern region	11.3 ±2.3	1.3 ±0.3	0.06 ±0.017

Optimal substitution models were found to implement HKYI+G for both D3 and H3 nuclear markers. Two haplotypes were found to be present, of which one haplotype was represented

by a single member. This was separated by >3% difference in sequence identity, with the remaining samples within 3% pairwise identity. Analysis of correlation between genetic distance and geographic distance revealed significant spatial autocorrelation. The *P. auberti* alignments based on the D3 domain dsDNA had a total sequence length was 425 bases with 98.1% pairwise identity. AMOVA results showed 82% (7 dF) variation among populations and 18% within populations (48 dF). The PhiPT value of 0.822 had a significant probability of occurrence of 0.001% which demonstrates that sufficient sample size was reached to have confidence in the determination of differences of variance within and between populations. When considering separate geographic regions, AMOVA indicated further significant structure within each of these main clades (northern: ΦST = 0.6, P< 0.001; Central: ΦST= 0.7, P< 0.001).



P. auberti populations

Source	df	SS	MS	Est. Var.	%
Among Regions	2	1213.44	443.54	43.32	2%
Among Pops	7	9942.780	1420.397	213.740	81%
Within Pops	48	2225.010	46.354	46.354	18%
Total	55	12167.790		260.094	100%

Figure 5.2 Pie chart of percentages of molecular variance of *P. auberti* mite populations. Inset table displays within and between populations and between regions. df = Degrees of Freedom, SS = Sum of square, MS = Mean Sum of Squares Est. Var. = Estimated variance.

The *M. loxolineata* genotype D3 domain dsDNA had a total sequence length of 394 bases with 97.5% pairwise identity. For the *M. loxolineata* dataset, specimens for the central and southern regions provided coverage of the whole transect with 442 aligned positions for 20 individuals. A PhiPT value of 0.415 was found to not have a significant value of P = 0.137 (Fig. 5.3). AMOVA indicated further significant structure within each of these main clades (northern: ΦST = 0.3, P= 0.5; Central: ΦST= 0.2, P=0.001).

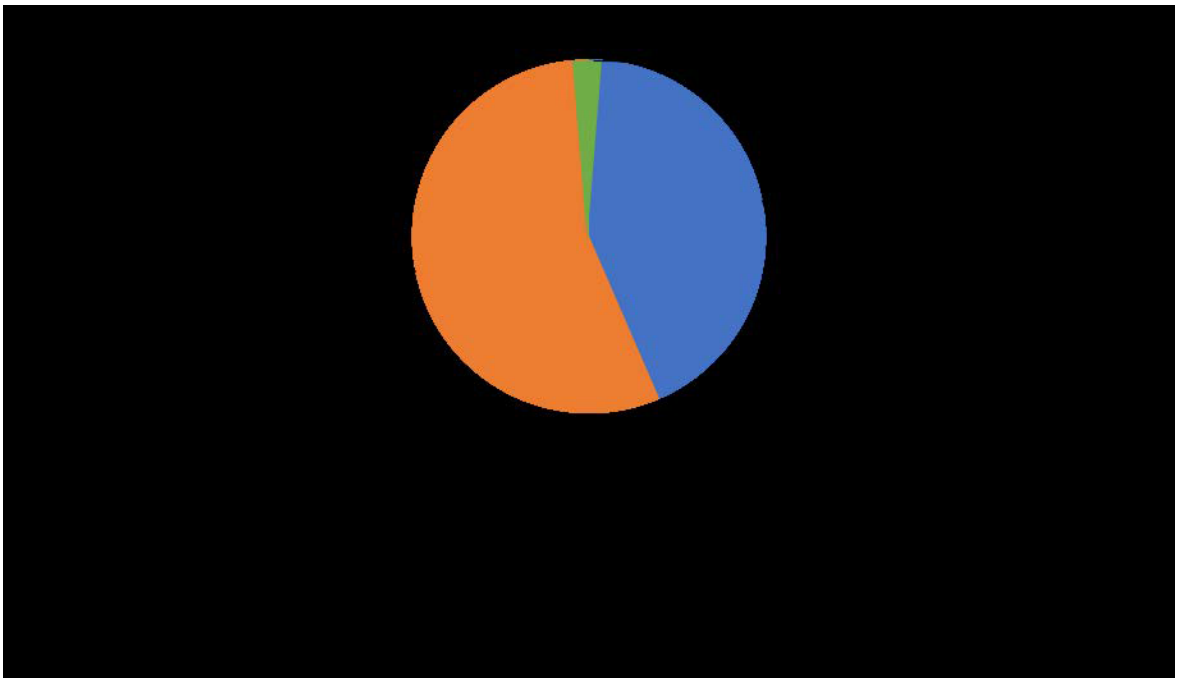
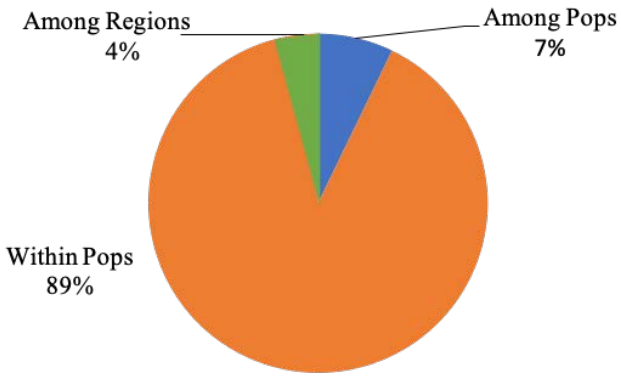


Figure 5.3 Pie chart of percentages of molecular variance of *M. loxolineata* mite populations. Inset table displays within and between populations and between regions. df = Degrees of Freedom, SS = Sum of square, MS = Mean Sum of Squares Est. Var. = Estimated variance.

Springtail

The *Cryptopygus antarcticus* genotype dataset comprised four aligned loci for 44 individuals. Within the dataset, six haplotypes were found across all populations. The D3 domain dsDNA had a total sequence length was 377 bases with 99.4% pairwise identity. The 28S D3 rRNA markers identified structure dividing Northern and Southern Antarctic

Peninsula regions, with Elephant Is. represented by a single locality. When considering separate geographic regions, AMOVA indicated further significant structure within each of these main clades (northern: ΦST = 0.2, P = 0.4; Central: ΦST= 0.5, P = 0.01).



C. antarcticus populations

Source	df	SS	MS	Est. Var.	%
Among Regions	3	544.523	235.689	50.432	4%
Among Pops	2	1027.139	513.569	20.033	7%
Within Pops	41	10602.596	258.600	258.600	89%
Total	43	11629.734		278.633	100%

Figure 5.4 Pie chart of percentages of molecular variance of *C. antarcticus* genotype springtail populations. Inset table displays within and between populations and between regions (defined by regional distinctions). df = Degrees of Freedom, SS = Sum of square, MS = Mean Sum of Squares Est. Var. = Estimated variance. When considering all sampling localities together, AMOVA indicated no significant genetic structure between the regions (ΦST = 0.072, P =0.086).

Table 5.3 Pairwise ΦST values for mite and springtail genotypes and specific values for regions.

Species	ΦST	P
MITES		
P. auberti	0.822	0.001
NORTHERN	0.6	< 0.001
CENTRAL	0.7	< 0.001
M. loxolineata	0.415	0.137
NORTHERN	0.3	0.5
CENTRAL	0.2	0.001
SPRINGTAIL		
C. antarcticus	0.072	0.086
NORTHERN	0.2	0.4
CENTRAL	0.5	0.01

Table 5.4 Population structure overview of sites across the transect for *C. antarcticus* genotype springtails. Columns indicate n: number of sequences, x: number of haplotypes, and π is nucleotide diversity. Sites are ordered in descending latitudinal order. Means (±S.E.) of regions in grey.

SITE	n	x	π
SI	6	2	0.2
EL	7	3	0.2
Northern Region	6.5 ±0.5	2.5 ±0.5	0.2 ±0
AD	3	1	<0.1
BP	4	1	<0.1
TR	5	2	<0.1
BI	3	2	<0.1
BE	4	1	<0.1
Central Region	3.8 ±0.4	1.4 ±0.2	<0.1 ±0
AN	4	1	<0.1
JE	5	2	<0.1
LE	4	2	0.09
Southern Region	4.3 ±0.3	1.7 ±0.3	0.09 ±0.003

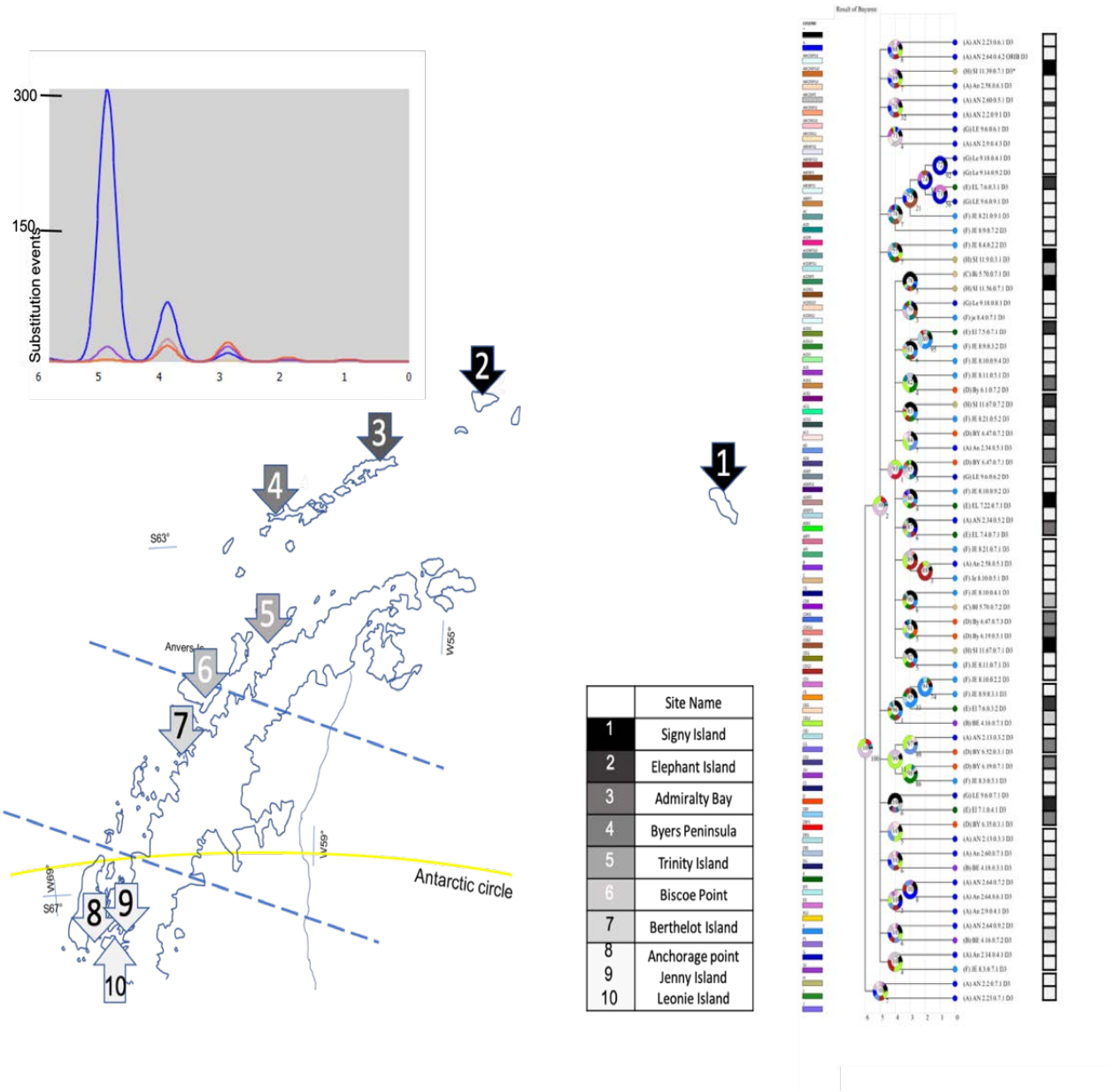
Between-group mean pairwise genetic distances based on regional distinctions of sites indicated *P. auberti* oribatid mites were separated by 0.0079, whilst *C. antarcticus* genotype springtails were separated by 0.0027. Alignment with site coordinates indicated that both mite and springtail genetic distances were spatially auto-correlated.

Bayesian topologies and RASP outputs

The use of the DEC models was most suited based on the partitions found within the phylogenies using the model test function in the RASP program. The Bayesian reconstructions support the presence of two distinct groups within genotypes of *P. auberti* (Fig. 5.5), one for mites from the Signy and Elephant Islands, and one for mites from the

remainder of the islands off the Antarctic Peninsula. Lineage-specific mutation rates using the CO1 marker estimated a dispersal event that split individuals from Elephant and Signy in the northern islands from the Antarctic Peninsula clade at 6.02 Ma ± 0.7 Ma.

Topologies across the transect for *P. auberti* (Fig. 5.5) and *M. loxolineata* (Fig. 5.7) indicates high levels of genetic combination within species across regions. Reconstruction of evolutionary timeline graphs showed a high number of substitution events early in the history of *P. auberti* that indicate an ancient dispersal event ~5 Ma (Fig. 5.6B), with two substitutions, as opposed to singular events in *M. loxolineata* ~3 Ma (Fig. 5.8B) between central and southern sites. The phylogenetic trees of the two mites showed marked distinction in importance of singular early dispersal events shown by blue line for *P. auberti*, whilst *M. loxolineata* appear to have repeated singular dispersal events in the central regions (Fig. 5.8A) associated with refugia. Less influence of vicariance and extinction in southern sites (Fig. 5.8B) also supports the dispersal events indicated by the reconstructions.



	Site Name
1	Signy Island
2	Elephant Island
3	Admiralty Bay
4	Byers Peninsula
5	Trinity Island
6	Biscoe Point
7	Berthelot Island
8	Anchorage point
9	Jenny Island
10	Leonie Island

Figure 5.5 Phylogenetic trees of oribatid mite *P. auberti* genotypes from the D3 marker across transect with latitude. Legend indicates unique ancestral states for each node. Inset graph is of *P. auberti* haplotypes using the DEC model with genetic substitutions attributed to dispersal (blue), vicariance (purple), extinction (beige) and total number of molecular substitution events (red).

Reconstructions of *P. auberti* genotype lineages (Fig. 5.5) shows a dominant influence on dispersal that were likely associated with post-glacial changes in landscape and barriers to dispersal. This also indicates the relative lack of importance of both vicariance and extinction in shaping the *P. auberti* populations. The paired graphs representing the contrasting geographic regions (Fig. 5.6 A, B) indicate the similarities in the overall patterns of dispersal followed by at least one substitution event that was attributed to vicariant speciation. A more pronounced wave of dispersal in the northerly islands (Fig. 5.6A) and is also reflected in the tree reconstructions with three distinct haplotypes in the northerly islands compared to only two in the central regions (Fig. 5.6B).

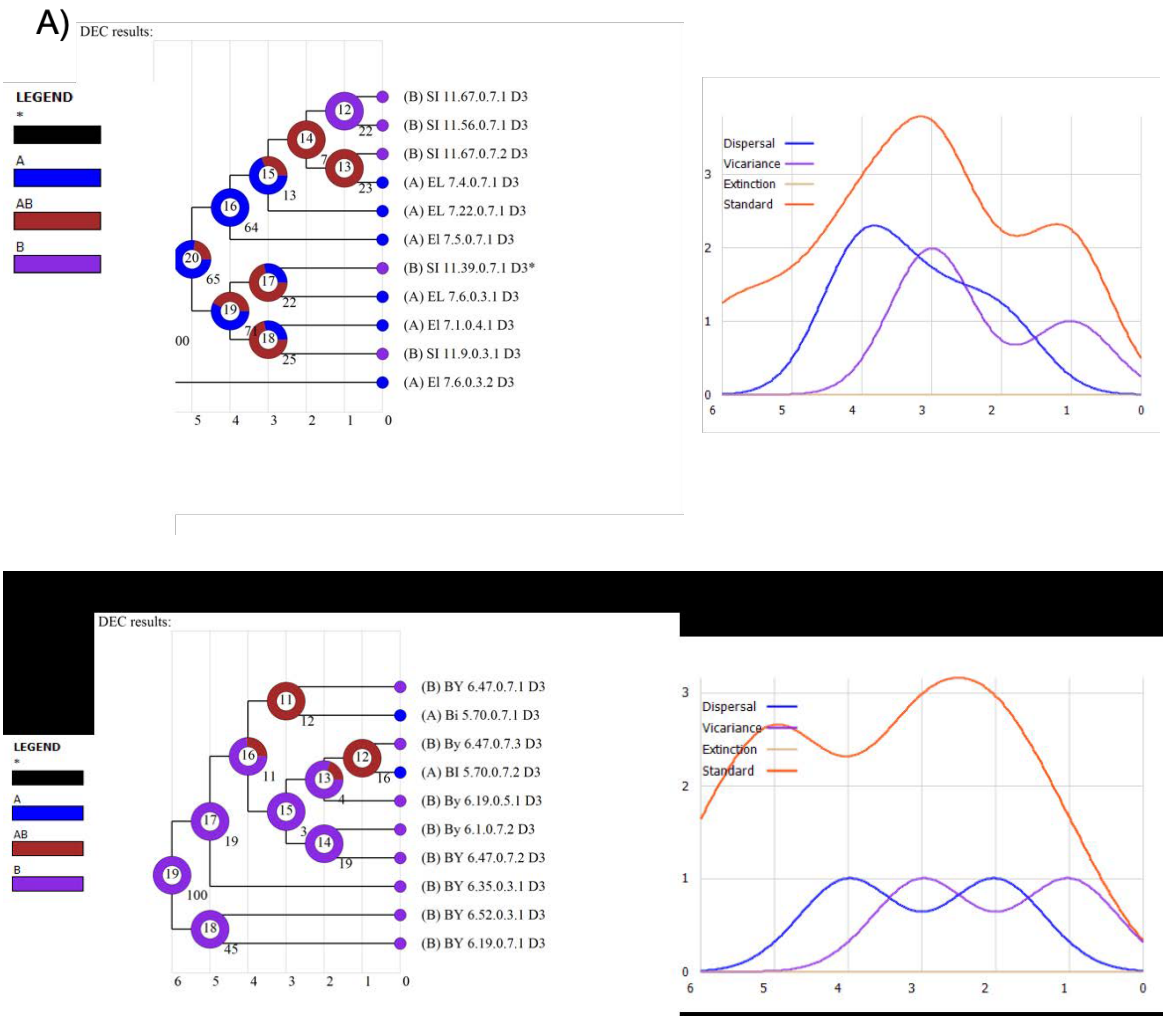


Figure 5.6 Phylogenetic trees of oribatid mite *P. auberti* genotypes from the D3 marker displaying level of shared ancestral character states with each unique state indicated in legend comparing specimens from the A) Northerly islands, and, B) Central refugial sites. Graphs represent the reconstructions of evolutionary histories using the DEC model with genetic substitutions attributed to dispersal (blue), vicariance (purple), extinction (beige) and total number of molecular substitution events (red).

The phylogenetic tree of the *M. loxolineata* oribatid genotypes shows fewer ancestral states (n = 18) than the *P. auberti* lineages (n = 77). However, the sequences indicated a clearer distinction between two main putative species complexes that are not geographically distinct, i.e. occurring at the same sites (Fig. 5.7). Differences in evolutionary histories between northern and central regions were also apparent from the contrasting model outputs Monotonic dispersal events were most likely for central

regions (Fig. 5.8 A), whilst bimodal dispersal events were more likely in the southern regional populations (Fig. 5.8B).

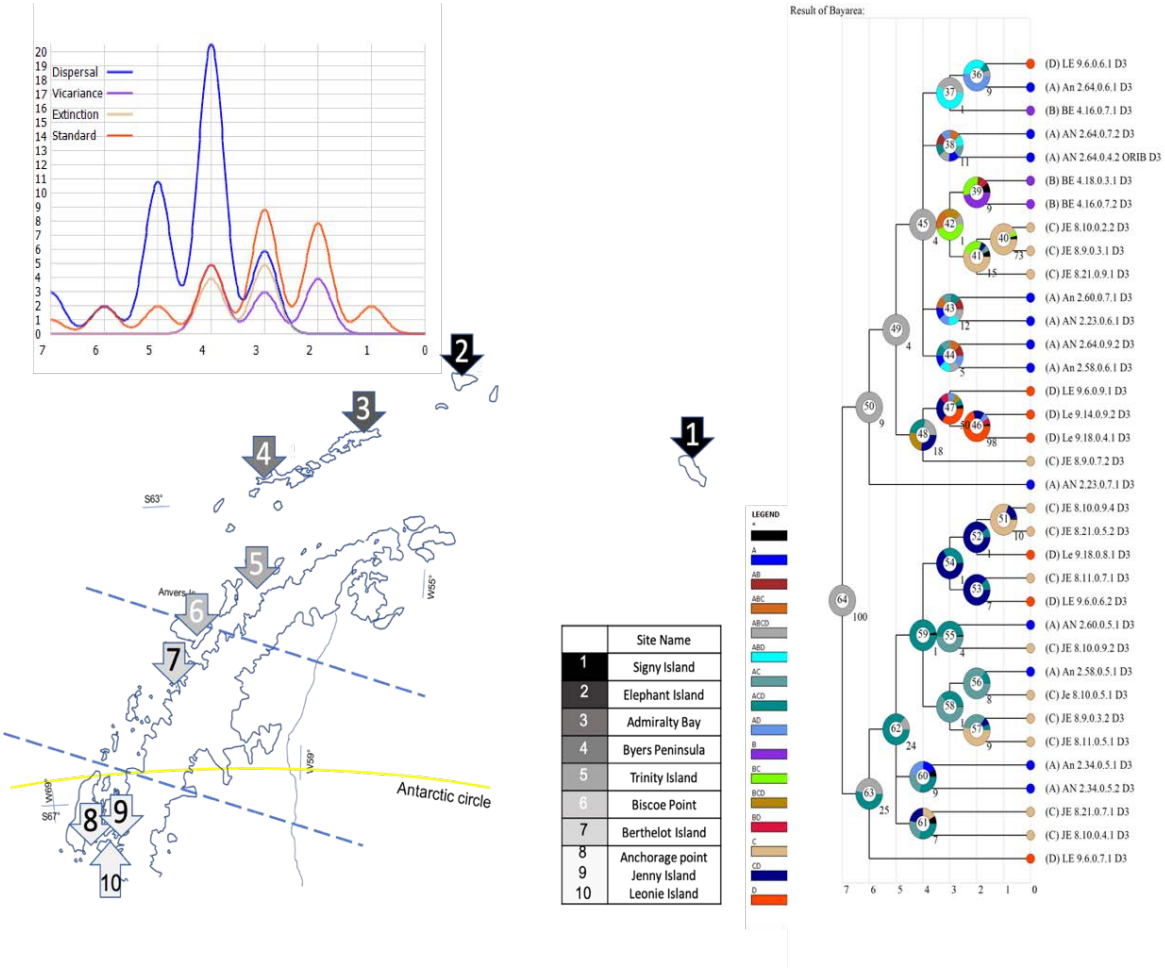
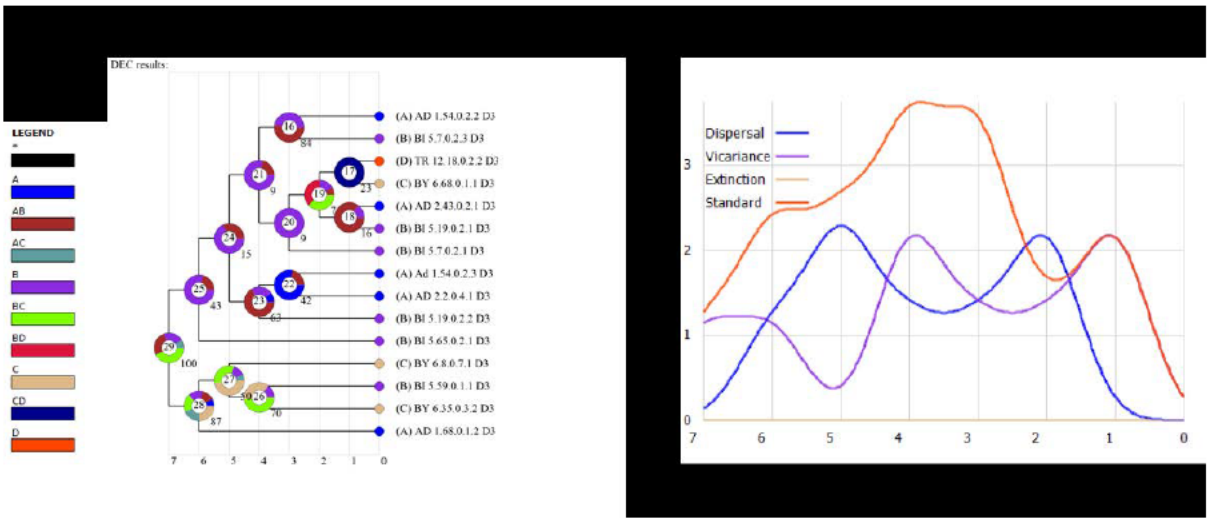


Figure 5.7 Phylogenetic trees of oribatid *M. loxolineata* genotypes from the D3 marker displaying level of shared ancestral character states across whole transect with latitude. Inset graph represents the reconstructions of evolutionary histories of *M. loxolineata* using the DEC model with genetic substitutions attributed to dispersal (blue), vicariance (purple), extinction (beige) and total number of molecular substitution events (red).



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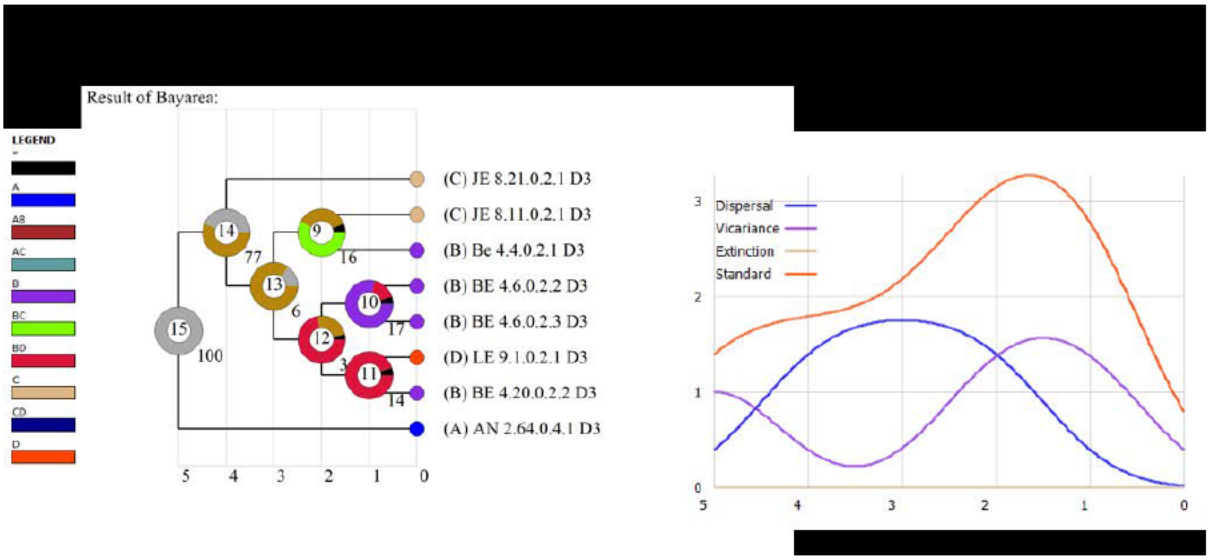


2988

2989 **Figure 5.8 Phylogenetic trees of *M. loxolineata* genotypes from the D3 marker with pie-charts**
2990 **displaying level of shared ancestral character states with each unique state indicated in legend**
2991 **from sites A) central region, proximal to refugial, B) southern region; non-refugial sites.**
2992 **Graphs represent the reconstructions of evolutionary histories using the DEC model with**
2993 **genetic substitutions attributed to dispersal (blue), vicariance (purple), extinction (beige) and**
2994 **total number of molecular substitution events (red).**

2995

Springtail phylogeographic reconstructions

DEC model outputs of the *C. antarcticus* genotype dataset indicated shared ancestral states across the transect and indicated a similar pattern of mass-dispersal events around 5 Ma (Fig. 5.9 inset). This was interspersed with periods of vicariance in northern islands and only two shared states (Fig. 5.10A), that contrasts with more ancient short-distance dispersal and mixing from within the central region (Fig. 5.10 B, C).

The Bayesian topology displayed three distinct genetic lineages along the regional divisions (Fig. 5.9 inset). These were geographically overlapping, with individuals from the three clades present in 5 out of 12 locations. The single site on Elephant Island was represented by 2 haplotypes both of which clustered within the regional clade. Applying the substitution rates to the speciation dates between the two regions using the CO1 markers estimated a dispersal event at 4.33 Ma.

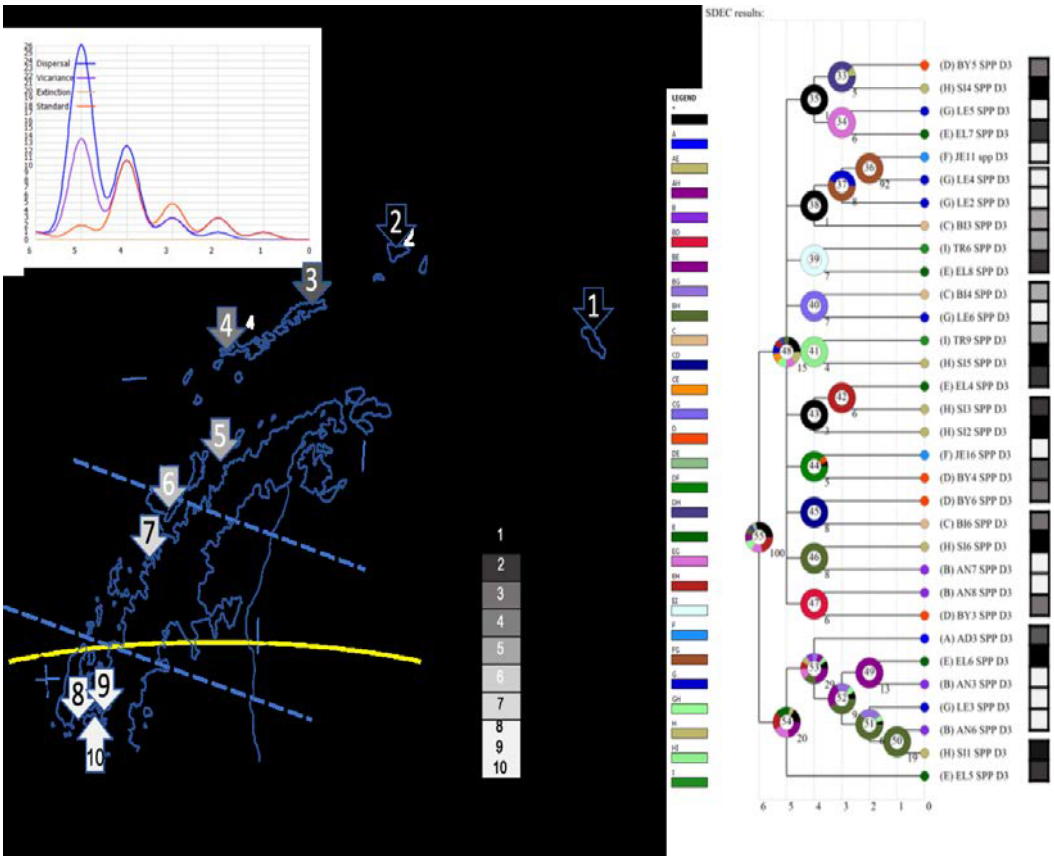


Figure 5.9 Phylogenetic trees of springtail *C. antarcticus* genotypes from the D3 marker displaying level of shared ancestral character states across whole transect with latitude. Inset graph displaying reconstruction of substitution events along a timeline for *C. antarcticus* sequences from D3 markers from all sites. Indicated genetic substitutions attributed to dispersal (blue), vicariance (purple), extinction (beige) and total number of molecular substitution events (red).

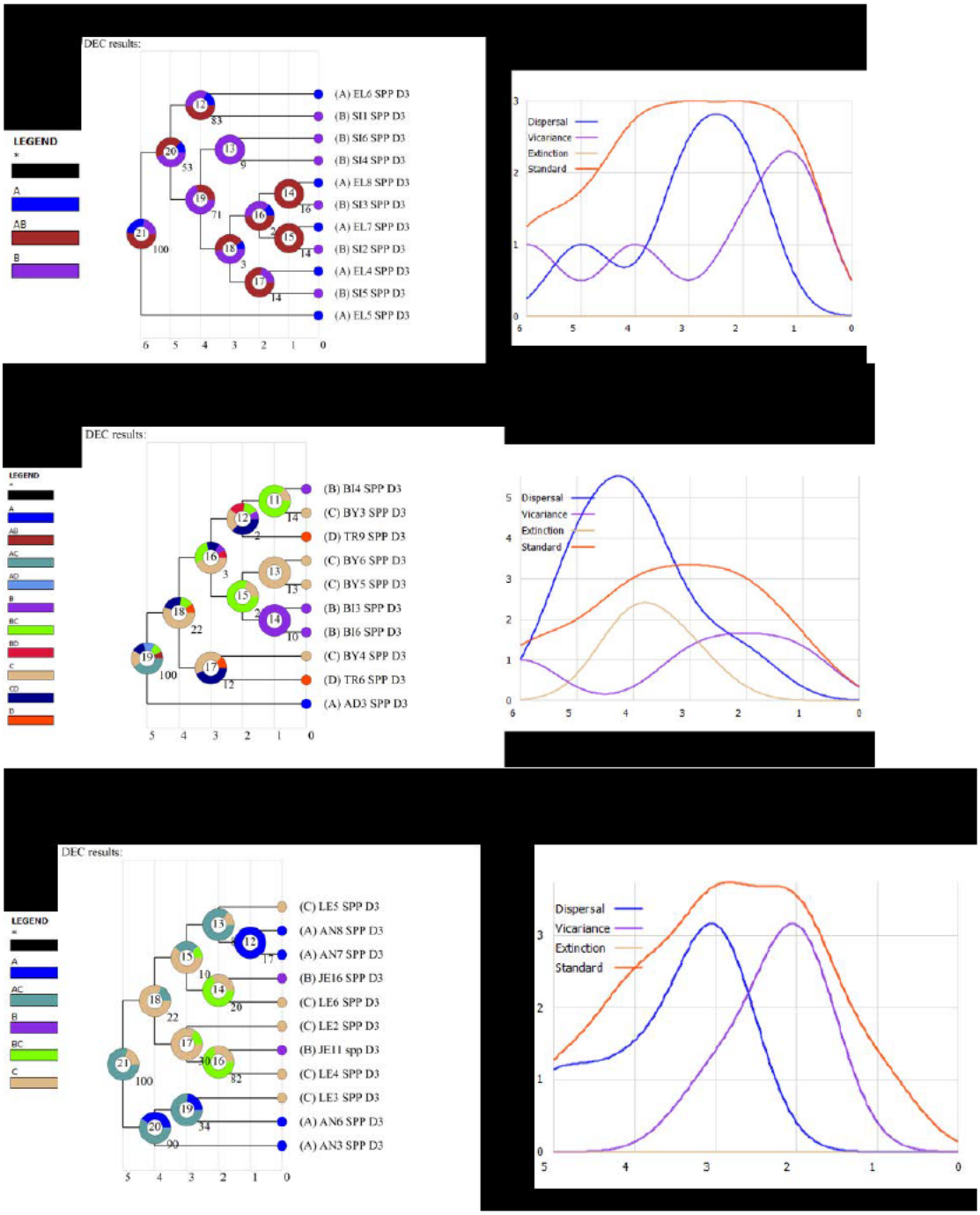


Figure 5.10 Phylogenetic trees of springtail *C. antarcticus* genotypes from the D3 marker displaying level of shared ancestral character states with each unique state indicated in legend from A) Northerly islands, B) Central refugial sites, C) non-refugial sites. Graphs represent the reconstructions of evolutionary histories using the DEC model with genetic differences associated with dispersal (blue), vicariance (purple), extinction (beige) and total number of speciation events (red).

5.4 Discussion

This study compared the genetic variation and evolutionary history of the widespread oribatid mites *Podacarus auberti* and *Membranoppia loxolineata* with the springtail *Cryptopygus antarcticus* in maritime Antarctica. Phylogeographic reconstructions revealed genetic structures that were influenced by biotic differences inherent to the two faunal groups. Oribatid mite genotypes showed greater history of repeated dispersal and episodes of vicariance, whereas springtails generally showed more recent, and often singular dispersal, that may involve local extinction of lineages.

I sampled 10 sites representing two distinct biogeographical regions where the dominant mode of colonisation and speciation was likely to differ. Namely, dispersal and vicariance which are dependent upon factors such as isolation distance and proximity to known geothermal refugia were expected to influence the phylogeography of mites and springtails. Differences in nucleotide and haplotype diversities based on Analysis of Molecular Variance (AMOVA) outputs showed that springtail *C. antarcticus* had larger within-population variation (93%) compared to the mites *M. loxolineata* (43%) and *P. auberti* (57%). The highest levels of variation among populations were detected in *P. auberti* populations (82% with only 18% variance within populations). This may indicate more ancient dispersal events and mixing between *P. auberti* populations, with their ability for local motility improving survival and colony persistence. Such genetic differentiation corresponds with earlier findings in Ameronothroid mites in the region (Mortimer et al., 2011; Van Vuuren et al., 2018).

DEC model outputs

The model outputs revealed more recent dispersal events in the northerly islands which confirm the more periodic glaciation known to have covered the peninsula (Hall, 2002). Ancestral reconstructions of mite and springtail phylogenetic histories show strong association with dispersal, with contrasting abilities between mites and springtails reflected in different patterns expressed between the faunal types. Ancestral histories based on the tree outputs indicated two waves of dispersal in *P. auberti* in Pleistocene-era interglacial periods. A potential third event is evident from reconstructions of the smaller-bodied *M. loxolineata* specimens that may be more capable of airborne transport (Pugh, 1993). Patterns of dispersal and vicariance were mirrored between the mite species, with no discernible extinction events. By contrast, more pronounced waves of extinction were evident in the *C. antarcticus* reconstructions. Considering the co-occurrence and shared environmental conditions that are common between the faunal groups, this suggests that differences in morphological or life history traits between the two groups may have led to such contrasting phylogeographic patterns. These differences include desiccation tolerance and ability for long-distance passive dispersal and short-distance active motility.

Despite earlier theories linking high diversity to recent vicariance following post-LGM arrival, the outputs support the hypothesis of ancient taxa that have undergone multiple episodes of dispersal to sites closer to refugial locations (Marshall and Coetzee, 2000; Maraun et al., 2003, Convey et al., 2020). Similar conclusions linking refugia and genetic variation were found in earlier studies of continental mites (Pugh and Convey, 2008) and springtails (Stevens et al., 2007; McGaughran et al., 2008, 2010b). Long-distance mite dispersal has been observed among maritime and sub-Antarctic islands (Mortimer et al., 2011) and springtail populations (Stevens et al., 2006b). Greater genetic diversity normally

3077 associated with refugial locations compared to recently colonised areas (Stevens and Hogg,
3078 2003), was not consistent for both springtails and mites, suggesting different species having
3079 survived and dispersed from geographically distinct refugia as shown in previous studies
3080 exploring latitudinal variation in mites (Marshall and Convey, 2002).

3081 The reconstructions from across all sites indicated a large influence of ancient
3082 dispersal events (~5 Ma) in *P. auberti;* more so than for *C. antarcticus* springtails (~3 Ma).
3083 Dispersal was also an important speciation factor for *C. antarcticus* springtails. When
3084 grouped with regional populations, level of shared ancestry indicated that gene flow was not
3085 equal across the transect. On average, greater levels of shared ancestry were found in species
3086 far from refugial sites (region B) and oribatid populations had higher genetic admixture
3087 (mixing of once genetically isolated lineages) promoted by their geographic proximity to
3088 each other (Anchorage, Jenny and Leonie Is.). The differences in isolation distance were
3089 found to correlate with genetic divergences when comparing regional populations.
3090 Specifically, northerly islands (region A) with significant barriers to dispersal separating
3091 them the source populations on the peninsula had lower within-group variation than
3092 southerly islands that were contiguous with peninsular sites. There has been speculation that
3093 there are multiple unverified refugial locations on the West coast of the Peninsula that may
3094 also act as source populations for soil fauna (Fraser et al., 2014). The greater physical
3095 barriers of northerly islands highlight the importance of dispersal events via wind or water
3096 in soil invertebrate populations, that were often followed by a period of vicariance.

3097 Clustering of haplotypes can indicate that minimal exchanges have occurred between
3098 regions with no known refugia and may indicate populations based on single founder events.
3099 This supports the earlier findings of minimal gene flow throughout the peninsula
3100 (McGraughan et al., 2012). It also provides further evidence for the demarcation of

Antarctica based on bioregions (Terauds et al., 2012), with refinement of distinct populations along the peninsula.

Levels of genetic divergence and shared ancestry between sites

The restricted distributions of soil invertebrate species were reflected by similar levels of within-region genetic divergence along the transect. This supports the association of genetic distance with proximity to refugia (Fraser et al., 2014; Convey et al., 2020). The excess of similarity between randomly chosen haplotypes within sites closer to refugia, alongside the far greater admixture of sites in the non-refugial region indicates the restricted gene-flow between regions. Following dispersal, localised extinctions of populations may have occurred as communities struggled to establish themselves in new habitats. However, these short-term timeframes may be undetected by the slower-evolving nuclear markers.

Long-distance dispersal from refugia is essential for colonisation of new habitats as they become available. The advancing ice-sheets are known to have reached the South-Orkney (including Signy Island) and South-Shetland (including Elephant Is.) Islands throughout the Pleistocene epoch, and up until the LGM (Davies et al., 2012). Thus, establishing a firm timeline and dating of speciation events in Antarctic taxa would be possible with calibration of the molecular clock using the Australian taxa. Greater periods of isolation may allow more dispersal events; however, more exposure has been linked to more salt deposition and lower water availability (Lyons et al., 2016; Dragone et al., 2021), that are also associated with harsher habitat and ecosystem conditions noted to exist along the latitudinal gradient through maritime Antarctica (Ball et al., 2022). This would therefore balance the period of ice-free and susceptibility to water borne migrants in establishing persistent colonies.

Contrasting phylogeographies of mites and springtails

Clear distinctions between the reconstructions of mite and springtail ancestries suggest dispersal patterns differed between the two groups as expected. The local extinction of springtails in sites closer to refugia (Fig. 5.10C), may indicate difficulties of finding a suitable habitat following dispersal, either in terms of water availability or overly acidic soil conditions, as found in other taxa on the continent (Franco et al., 2022). Local conditions around active geothermal refugia are often highly acidic and could limit the vegetation growth (Chown et al., 2015). However, as the ice-sheets continued to recede and climatic conditions shifted, conditions may have found a new equilibrium that promoted habitat suitability allowing populations to establish, eventually resulting in local vicariance due to genetic drift or adaptation. The differences in modes of reproduction between the mite species may be considered when comparing their evolution. For example, the parthenogenetic mode of *M. loxolineata* contrasts that of *P. auberti* that are capable of both sexual and asexual reproduction based on level of environmental stress. However, these differences have shown to not have a significant effect on calculation of evolutionary rates (Heethoff et al., 2009; Maraun et al., 2020).

Mites are more capable of surviving long distance dispersal aboard flotsam, defined as a random agglomeration of plant matter that form rafts capable of floating (Gillespie et al., 2012; Convey et al., 2014; Mortimer et al., 2011). This ability may explain their more wide-spread distribution and greater level of shared ancestry between distant populations. Mite diversity followed patterns found in reference biogeographic records (Starý and Block, 1998), however, greater resolution of genetic divergence provides unparalleled insights into evolutionary histories and degree of isolation between communities. Mites are more prone to dispersal and mixing and have more ancient origins than springtails (McGaughran et al.,

2011). The parthenogenetic mode of reproduction for certain oribatid mites may exert an advantage for population persistence over sexual species (minimising deleterious mutations through recombination at the expense of mate limitation). However, parthenogenetic species may still reveal rare or spanandric males (Lynch, 1984; Palmer and Norton, 1991; Little and Hebert, 1996), enabling greater gene-pool admixture and survival during ice ages. Additionally, these dispersal events may also contribute to the purging of genetic load, thereby removing deleterious genes and improving overall fitness and survival of populations.

As regional climates continue to warm, dispersal distance and numbers of dispersal events will be promoted (Duffy et al., 2017; Ma et al., 2021), especially via oceanic currents (Bartlett et al., 2021), and greater areas will become suitable habitats for soil invertebrates. However, indirect impacts to soil moisture and primary productivity may shift resource type far faster than mouthparts can adapt (Chapter 3). Understanding how belowground fauna respond to ecosystem fragmentation will help disentangle the complex array of factors that influence invertebrate distributions. The ability for springtails to adapt their stratification level under certain conditions would assist Antarctic species to persist in sub-optimal conditions following dispersal, but such shifts are yet to be confirmed in Antarctic taxa.

Conclusions

This study highlights how the biotic differences between mites and springtails have led to significant differences in their dispersal and speciation in the highly fragmented landscape of maritime Antarctica. The greater long-range dispersal ability of mites was found to align with evidence for multiple dispersal events in their evolutionary history, whilst springtails were more susceptible to extinction events following dispersal. The greater than expected

endemicity of haplotypes supports the increased demarcation of regions along the peninsula to ensure that these populations are not impacted by inadvertant transferal of species between regions. These findings will support the inclusion of biotic factors in dispersal models for soil fauna and enhance our ability to predict potential responses to forecast environmental change. Harnessing this knowledge can assist in mitigating unexpected shifts in biodiversity and enact appropriate policies aimed at ensuring the persistence of faunal groups that are essential for terrestrial ecosystems in maritime Antarctica.

Chapter 6 - Phylogeography of Austral oribatid mite and springtail assemblages: shared influences of isolation and environmental variation

Abstract

Soil fauna are integral members of terrestrial ecosystems from equatorial to polar latitudes. While knowledge of soil fauna diversity and distribution has improved substantially over the past century, the influence of climatic and geographic factors on soil invertebrate diversity at landscape scales is still unresolved, especially in southern hemisphere (Austral) taxa. Accordingly, an assessment of Australia's indigenous soil faunal biodiversity along a continental-scale transect can provide new insights. I collected mites and springtails using a standardised approach from three island groups and adjacent mainland sites across a latitudinal transect (23º-40º) along the Eastern Australian Seaboard. Morphotype identities of oribatid mites and springtails were then assessed using sequencing of the 28S rRNA D3 marker, which also formed the basis for phylogeographic analysis of their distributions and evolution. A total of 21 putative oribatid mite species from 13 families and nine springtail species were found, with species richness across sites strongly influenced by period of isolation, with weaker correlation to either isolation distance or climatic variability. Reconstructed ancestries indicated contrasting levels of influence of recent founder effects and population bottlenecks on mites and springtails, with springtails being more influenced by biogeographic barriers to dispersal. Moreover, remnant populations in Bass strait islands shared similarities to islands subject to longer periods of isolation that were subject to

repeated colonisation and reduced genetic drift. These results highlight how genetic diversity of southern hemisphere fauna are similarly responsive to refugial dispersal over time, and how phylogeographic patterns are affected by biotic differences between faunal groups. Such baseline assessment of biodiversity and distribution patterns of southern hemisphere invertebrates can inform conservation models for these important components of terrestrial ecosystems.

6.1 Introduction

Soil fauna are component members of terrestrial ecosystems at all latitudes (Nielsen, 2019), and play an important role in ecosystem functioning through decomposition of soil organic matter (SOM) and nutrient turnover. Despite their importance, most studies have focused on northern hemisphere taxa (Balogh and Balogh, 2012; Pfinsgtl, 2017). Hence, there is limited knowledge of the drivers of landscape-scale distribution and diversity of soil fauna in the southern hemisphere. As there is a historic lack of rigorous sampling across broad transects, establishing correlations with environmental variability at the landscape-scale has also been limited. Furthermore, there has been little application of molecular taxonomic approaches on soil fauna from temperate ecosystems such as the Eastern Australian Seaboard.

Assessing the climatic, environmental and biotic drivers of Australian soil faunal distribution and genetic diversity can indicate principles that may be relevant to other southern hemisphere, or "Austral", soil fauna. Limited knowledge of the factors that influence soil faunal assemblages at both broad- and fine-scales reduces our ability to predict responses to environmental change. An approach to gain insight into these influences on soil fauna is to compare how these factors influence population structure and gene-flow of different soil faunal groups, thereby determining the relative importance of geography, isolation distance and period of isolation on distribution and diversity.

Australian soil invertebrates

Australia contains a diverse range of ecosystem types, that are known to harbour soil faunal assemblages of similar biodiversity (Greenslade, 1983; Bardgett and Van der Putten, 2014; Nielsen, 2019). Studies on Australian mites and springtails have included taxonomic

morphological surveys (Rainbow, 1906; Lee et al., 1986). The only comprehensive studies using morphology are of singular genera (Colloff and Halliday, 1998; Colloff, 2010, 2011). Overall, more than 2,600 mite and 350 springtail species have been identified in Australia (Halliday, 1998; Colloff and Halliday, 2013; Greenslade, 2014), but many species remain to be discovered or await formal taxonomic descriptions. Biogeographic work on inland artesian springs has focused on invertebrate conservation strategies (Rossini et al., 2018), and could be similarly applied to soil invertebrates.

However, molecular resolution has yet to be broadly applied to the study of Australian soil invertebrates. Limited sequence libraries of Australian species constrain identification efforts, whilst morphological identification of oribatid mites (Colloff and Halliday, 1998) calls for integrative taxonomy with genetic approaches remain largely unfulfilled. Springtails are equally under-represented in the literature, with studies on individual locations in sub-tropical ecosystems (Maunsell et al., 2013), yet high genetic diversity was found in an arid ecosystem (Guzik et al., 2021). Therefore, efforts to expand knowledge of Australian soil fauna will enhance future understanding on distribution and speciation of the indigenous taxa.

Biogeography and dispersal barriers

Restricted species distributions are often a result of geographic features that act as barriers to dispersal. Without such impediments, environmental filtering and biotic differences would be the key factors responsible for any discernible patterns. Differences in distribution patterns stemming from isolation over spatial and temporal gradients are expected to be apparent when comparing soil mite and springtail populations that have undergone contrasting modes of isolation. For example, newly formed islands colonized by species

capable of long-distance dispersal would contrast with populations stemming from remnant species on islands formed following sea-level rises (Fig. 6.1). Determining variation in diversity between islands also requires sampling from the mainland to act as a baseline between which islands can be more uniformly compared in terms of temporal isolation. The dating of speciation events in Australian soil invertebrates can also indicate the relative evolutionary rates. Here, I use nuclear 28S rRNA D3 domain markers for rate reconstructions, using the period of isolation of LHI (7 Ma) to calibrate divergence from mainland sites and rates of speciation.

Objectives

The main objective of this study is to detail the distribution, diversity and phylogeography of mite and springtail populations along a broad Australian transect to reveal potential drivers of landscape-scale diversity and densities. Rather than an exhaustive biodiversity assessment, the study aims to compare oribatid mite and springtail populations across climatic, latitudinal and isolation gradients to determine whether responses to such influences are comparable between faunal groups. Once these are performed, molecular verification of species will aid in the phylogeographic reconstructions of the most widespread mite and springtail species. These will then be aligned with known periods of geographic isolation to gain insight into the dating of speciation rates.

A null hypothesis considers the rate of speciation to be constant with no deviations from simple vicariance, with a linear relationship to isolation distance. Yet, the known importance of biotic differences and dispersal barriers will show contrasting patterns of mite and springtail speciation. Sites with longer periods of isolation are expected to lead to greater differentiation between populations, whereas sites closer together are expected to be more

closely related. On the other hand, remnant populations stemming from larger populations with higher diversity may contrast with populations arising from refugia that may harbour lower initial diversity, with greater potential for genetic drift that leads to more differentiation between populations despite minimal geographic distance separating them. Parameters including source population size and gene-pool diversity and the timing and number of speciation events will further refine estimates of evolution of soil fauna with biogeographic and geological events.

I established a transect along the Eastern Seaboard where a relatively uniform vegetation type dominated by eucalypt woodlands can be sampled along its entire length. Associated mainland sites were included in the experimental design to enable comparison of island ages. Providing an estimated date of temporal isolation based on the age of island formation can help calibrate measures of evolutionary change over time. The use of island size also aims to establish any connections between habitable area and genetic diversity to analyse any spatial effects. These ecosystems contain members from all of the main soil faunal groups, the actions of which are intrinsic to functional soil systems (Lee and Foster, 1991). Associations between Australian vegetation surviving Pleistocene-era climatic shifts in refugia suggest similar patterns may be found in its soil faunal populations (Byrne, 2008). Having a firm grasp of how southern hemisphere populations have adapted to past changes will enhance our knowledge of these under-studied taxa so vital to full ecosystem functioning. Furthermore, the outcomes of this study will inform potential conservation prioritisation and management actions under altered land-use and climate change.

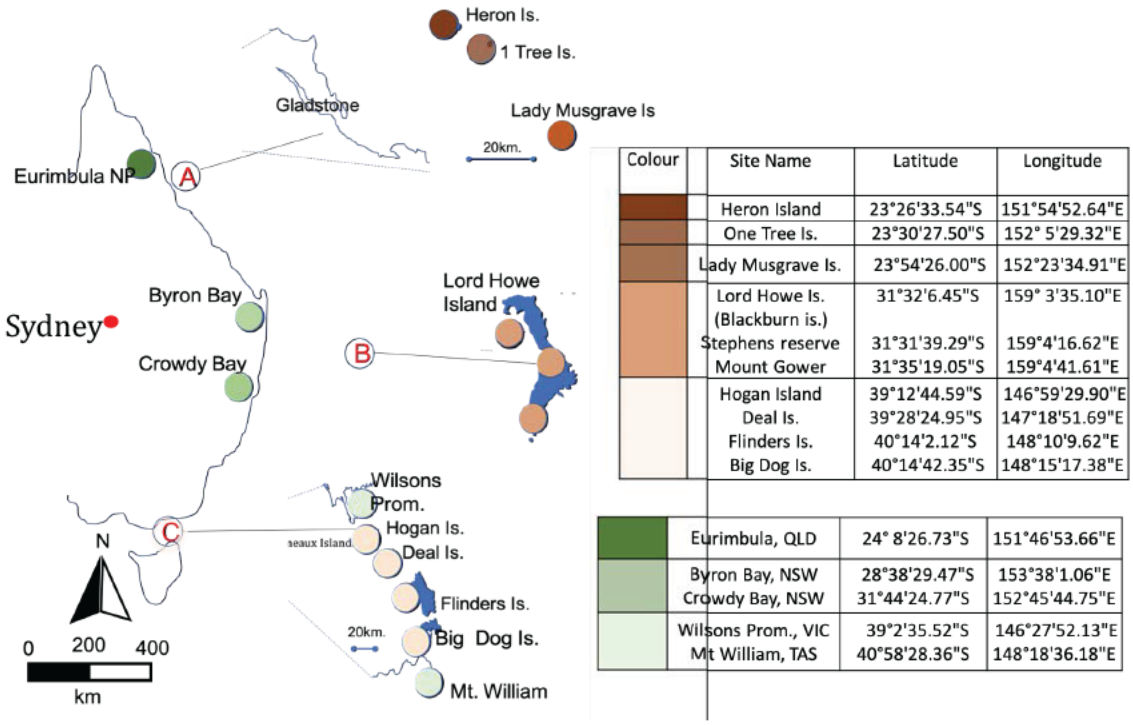
6.2 Methods

Sample collection

Samples were collected along the Australian Eastern Seaboard representing a latitudinal gradient (23º-40º; Fig. 6.1) between Aug 2019 – Dec 2021. Sites included mainland locations, near-shore islands and oceanic islands requiring different modes of dispersal. Vegetation consisted of temperate rainforest ecosystems with canopy cover and tree species on sandy calciferous soils (Clarke, 2009). Island sites were showed significant differences in time of isolation and distance to source populations. The most northerly islands in the Capricornia Cays NP in QLD (23ºS) have been colonised relatively recently (~6 Ka) via short-range biodispersal events (Muldavin et al., 2021). Vegetation on the coral cays was heavily shaded by *Pisonia* canopy had minimal understory vegetation (Walker et al., 1991; Rogers et al., 2015). Soil invertebrate populations subject to ancient, long-distance biodispersal were sampled from Lord Howe Island. This volcanic island is over 580 km from the mainland and formed 6.4–6.9 Ma (McDougall et al., 1981). Points along the NSW coast were sampled, the closest at Crowdy Bay NP and Byron Bay (28ºS), NSW. Sites from LHI were taken from i) the central Steven's Reserve, ii) the lagoon islet, Blackburn (Rabbit) island, and, iii) Mt. Gower summit (elevation: 856m). Contrasting soil sub-structure and "gnarled mossy cloud forest" on the summit of Mt. Gower, harbours a number of endemic species and is a classified a threatened ecosystem (Auld and Leishman, 2015).

The southerly extent of the transect included islands with populations subject to isolation from larger starting population sizes following post-LGM sea-level rise (~12 Ka). Islands in the Bass Strait from the Furneaux Island group (Flinders and Big Dog) and Kent Island group (Deal and Hogan) were sampled. Corresponding samples on the other side of

the Bass strait were collected from Mt William NP in Cape Portland, Tasmania (40ºS) that harbour areas of remnant rainforest on Wilson's promontory in South Gippsland, Victoria (39ºS) with noted similarities in vegetation with the Bass Strait islands (Ladd et al., 1992; Harris and Davis, 1995; Williams and Potts, 1996).



Colour	Site Name	Latitude	Longitude
	Heron Island	23°26'33.54"S	151°54'52.64"E
	One Tree Is.	23°30'27.50"S	152° 5'29.32"E
	Lady Musgrave Is.	23°54'26.00"S	152°23'34.91"E
	Lord Howe Is.	31°32'6.45"S	159° 3'35.10"E
	(Blackburn is.)		
	Stephens reserve	31°31'39.29"S	159°4'16.62"E
	Mount Gower	31°35'19.05"S	159°4'41.61"E
	Hogan Island	39°12'44.59"S	146°59'29.90"E
	Deal Is.	39°28'24.95"S	147°18'51.69"E
	Flinders Is.	40°14'2.12"S	148°10'9.62"E
	Big Dog Is.	40°14'42.35"S	148°15'17.38"E

	Eurimbula, QLD	24° 8'26.73"S	151°46'53.66"E
	Byron Bay, NSW	28°38'29.47"S	153°38'1.06"E
	Crowdy Bay, NSW	31°44'24.77"S	152°45'44.75"E
	Wilsons Prom., VIC	39°2'35.52"S	146°27'52.13"E
	Mt William, TAS	40°58'28.36"S	148°18'36.18"E

Figure 6.1 Map of sample sites along the Australian Eastern Seaboard transect, 23°–40°S. Sites are listed into two inset tables with islands and latitude and longitudinal coordinates for brown coloured sites, with mainland sites indicated by green sites.

Table 6.1 Australian transect sample sites and locations and dominant plant species. Mainland sites in grey.

Site Name	Site Code	Latitude Longitudinal	Dominant tree species / groundcover
Capricornia Cays NP, QLD			
Byron Bay	BY	28°38'35 (S), 153°36'54 (E)	*Pisonia grandis* *Pandanus tectorius*
Crowdy Head NP	CR	31°48'59.9 (S). 152°43'51.4 (E)	*Pisonia grandis*
Eurimbula NP	EU	24°10'48.11 (S), 151°49'59.9 (E)	*Pisonia grandis* *Pandanus tectorius*
Heron Is.	HE	23°36'38.7 (S), 150°44'57.1 (E)	*Pisonia grandis* *Cordia subcordata*
Lady Musgrave Is.	LM	23°54'30.6 (S), 152°23'39 (E)	*Pisonia grandis* *Pandanus tectorius*
One Tree Is.	OT	23°30'30.7 (S), 152°05'30.1 (E)	*Pisonia grandis*
Bass Strait Islands, TAS			
Big Dog Is.	BD	40°14'47.0"(S), 148°15'19.4"(E)	*Crassula tetragona* *Eucalyptus nitida*
Deal Is.	DE	39°20.49.8 (S), 147°11.04.7 (E)	*C. tetragona* *Eucalyptus nitida*
Flinders Is.	FL	39°43.54.1 (S), 147°56.31.0 (E)	Ferns *Eucalyptus globulus*
Hogan Is.	HO	39°12.44.5(S), 146°59.30.7(E)	*Paspalum dilatatum* *Eucalyptus nitida*
Mt. William NP	MW	40°13.58.0 (S), 148°11.00.4 (E)	Ferns *Eucalyptus globulus*
Wilsons Prom	WP	39°02'24.7 (S), 146°27'44.4 (E)	*Eucalyptus globulus*
Lord Howe Island group, NSW			
LHI- Stevens Reserve	LH	31°31'31.8 (S) 159°04'02.7 (E)	*Syzygium fullagarii* *Howeia fosteriana*
LHI-Blackburn Is.	LB	31°32'05.2 (S) 159°02'38.1 (E)	*Commelina cyanea,* *Pisonia grandis*
LHI - Mt. Gower	LG	31°34'48 (S) 159°04'58 (E)	Bryophytes *Casuarina equisetifolia*

At each site, soil substrata, dominant vegetation cover type and canopy cover percentage were determined to be representative of the requisite cover-type, before a plot (10 m x 10 m) was established with relatively uniform vegetation. Within this area, six 0.5 m x 0.5 m quadrats were six randomly assigned from which soil cores were collected using pre-cut PVC piping (5cm in depth and diameter). One soil core was collected for invertebrate extractions and an additional core for soil physical-chemical property measurements. Soil samples were stored in a portable fridge and transported back to the lab where they were stored at 4°C for processing within 48 hours.

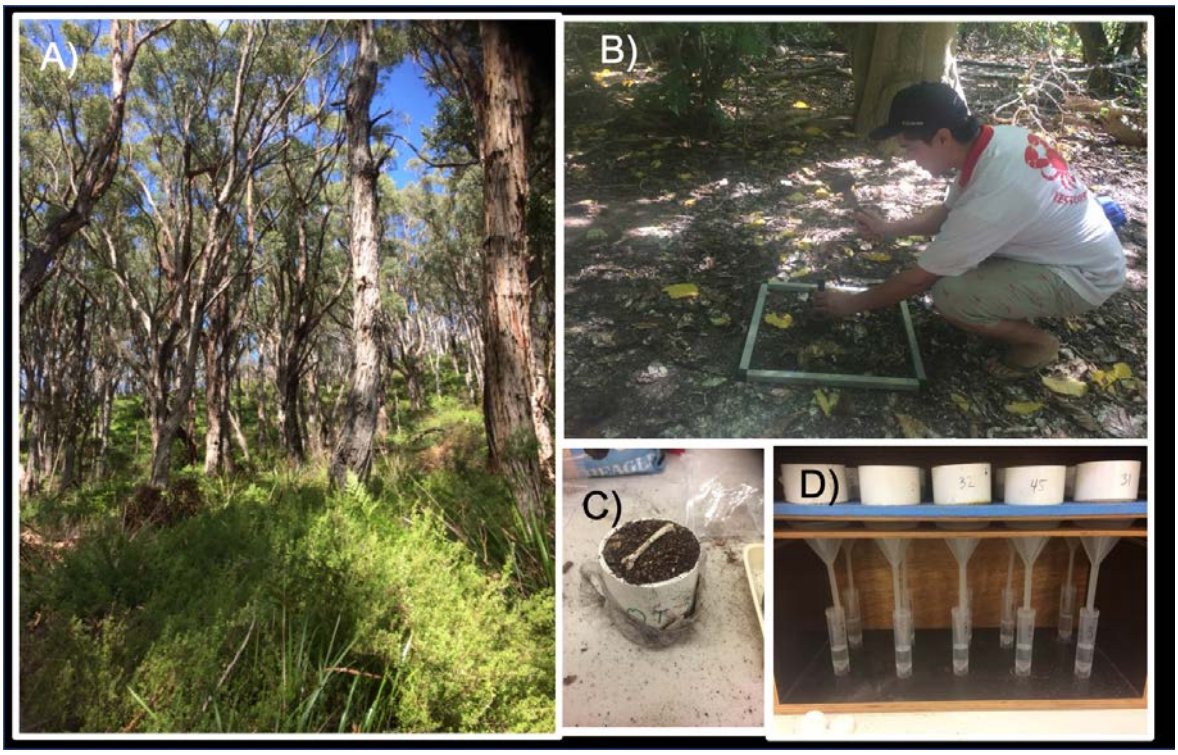


Figure 6.2 Photos of sampling and sample sites: A) Typical Eucalypt vegetation at Refuge Bay, Wilsons Prom site. B) Soil sampling with a 0.5 m X 0.5 m quadrat on One Tree Island, QLD. C) Example pre-cut PVC bevelled 5mm diameter soil corers sealed with parafilm, and, D) and modified Tullgren funnel soil fauna extraction set-up with heatlamps directing migrating biota into 10 ml 70% ethanol.

Soil fauna extraction

Mites and springtails were extracted using modified Tullgren funnels with the temperature increased incrementally over 5 days directing migrating fauna into 70% ethanol (Fig. 6.2D). Mites and springtails were separated from residual soil particles using a binocular light microscope at 40X magnification and counted. Soil mites were sorted into taxonomic orders: Parasitiformes which includes the predatory Mesostigmata, the Trombidiformes which includes the diverse Prostigmata, and the Sarcoptiformes which includes the functionally important detritivores Oribatida and the derived clade of Astigmatina (Krantz and Walter, 2009; Arribas et al., 2020b).

Taxonomic identification

In order to determine biodiversity and examine phylogenetic patterns, adult oribatid mites and springtails were further sorted into morphotypes (MT) based on morphologies described in Colloff and Halliday's (1998) catalogue, Nothridiae descriptions (Colloff, 2010, 2011), morphological descriptions of Australian oribatids (Rainbow, 1906) and supplements to oribatid records (Niedbała, 2006). Springtails were identified with the assistance of morphological keys (Greenslade, 2014). Specimens were not slide-mounted as the same samples were later used for Sanger sequencing. All specimens have a reference specimen that has been stored for future slide-mounting. Due to the destructive nature of acquiring molecular data, oribatid mite identification was based on closest NCBI BLAST match which aided in assigning speciems to ehir appropriate family taxonomic level.

Environmental parameters

Mean annual temperature (MAT) and precipitation (MAP) for each site were obtained from the nearest weather station (Australian Bureau of Meteorology; www.bom.gov.au). Soil pH was measured after mixing sieved samples with deionised water (1:2 w/v) and measuring two technical replicates of each sample using a S20 SevenEasy™ pH Meter (Mettler-Toledo International Inc., USA). Soil moisture content was calculated as the dry mass divided by the fresh mass weight difference as a percentage measured by drying approximately 50 g of wet soil samples at 105°C for 24 h. Total soil C and N content was measured on air dried soil (40°C for 48 h) milled 2 min at 25 vibrations per sec (Mixer Mill MM 400; Retsch GmbH, Germany) by Dumas combustions using a LECO TruMac C/N device (LECO Corporation, USA) with thermal conductivity detection of N_2 and CO_2.

Sequencing of soil invertebrates

A primary objective from the generation of molecular information from 28S rRNA D3 markers was to assign putative taxonomic species/family and provide coverage within the most prevalent species types across the transect. Each 28S rRNA D3 marker region was sequenced from representative specimens of each species from each site, when present. This was to provide sufficient replication of specimens to create phylogenetic trees based on Bayesian coalescent theory (Drummond and Bouckaert, 2015), the principal input for phylogeographic reconstructions.

Secondly, the sequences from the most common species of mite and springtail species were used to explore their population genetics by detailing phylogeographic reconstructions of shared ancestry. These analyses were based on the selection of three specimens of the species that were present. As the speciation involved with the ancient sites may also include recent dispersers, levels of genetic drift will be able to identify the relative age and integration of new arrivals.

Data analysis

All statistical analyses were performed using the R program version 3.4.3 (R Core Team, 2017). All biotic, climatic and environmental response variables were checked for homogenous variance and normality and transformation with natural logs for continuous variables or square roots for abundances when required (Crawley, 2012). Correlative heat-maps were produced using the corrplot package in R (Wei and Simko, 2021), with Oribatid morphotype counts based on totals across all sites. To determine the statistical significance of soil faunal responses to the biogeographic context, four sites per category were used as

the unit of replication. Linear mixed effects models were performed using the *lme4* package (Bates et al., 2014) where latitude, climate and environmental variables were modelled as fixed effects, whilst sub-plots and plots were nested as random effects. Repeated measures of analysis of variances (ANOVA) tests were performed, which are more advanced than one-way analyses, to determine the effect size and significance of relationships, with associated R^2 and p-values generated in *vegan 2.0* packages (Oksanen et al., 2013) in R (R Core Team, 2020). These are the equivalent of one-way ANOVA but are suited for related, non-independent groups as an extension of the dependent t-test. Oribatid assemblage MT counts were converted into species richness as a measure of diversity. Climatic and environmental drivers of soil mite abundance and diversity were explored and modelled as fixed effects with plot and subplot included as nested random effects.

Phylogeographic analysis

To determine changes in population structure across the climatic, latitudinal and biogeographic gradients, the two dominant oribatid mite and springtail genotypes were selected from across the transects. Subplots within each site acted as the unit of replication with 4 sites within each biogeographic class: i) short-range biodispersers to QLD sites, ii) geodispersers to TAS sites, and, iii) long-range biodispersers to LHI sites. Genetic distances calculated from a minimum of three specimens from each site with in-group populations. Owing to the high diversity among oribatid mite and springtail species, insufficient replicates of specimens of the same species restricted the ability to make direct a reliable measure of gene flow between populations along the transect.

Bayesian reconstructions of ancestral lineages were performed, with DEC models were performed using the RASP program. Alignments of D3 markers were visually curated

and checked for genetic distance and selection of those within 1% similarity. Sequence alignments performed with CLUSTALX v.2.0 (Larkin et al., 2007) with an opening gap of 10.0 and extending gap of 0.1in Geneious^{TM} v. 2020.2 program, (www.geneious.com, Kearse et al., 2012). Model selection of nucleotide substitution models were performed in MEGA with GTR +G+I = proportion of invariable sites (Proportion invariant = 1.0, default = 0.0), +G = Gamma distributed rate variation among sites (4 = all sites) based on AIC values (Akaike, 1973). These can be applied by entering Gamma Category Count - (Set to 4.0 for +G, default=0), HKY – Kappa = Default =2.0. Tree construction performed with 3-way separation for optimal partition model with linked clock and substitution models. Optimal MCMC outputs and substitution models were checked in Tracer program, for sufficient effective sample sizes (ESSs) of all parameters (>200). Tree Annotator program was used to process BEAST outputs and condenses multiple raw trees to most optimal single tree. Applies specific burn-in value as defined from multiple runs to determine consistency (default 10%, discarding first 1M trees). Application of Bayesian reconstructions follows the same pipeline as described in Chapter 5.3.

Dispersal dating and speciation rates

The dating of dispersal and speciation events using molecular clocks for mite and springtail genotypes in the absence of fossil or geological calibrations the molecular clock rate of 1.5–2.3% divergence per million years that are commonly applied to arthropods using the CO1 marker (Juan et al., 1996, Quek et al., 2004; Klimov et al., 2019). The divergence calculations based on the uncalibrated 28S rRNA D3 region can be used to estimate relative speciation rate based on uncorrected p-distances which do not correct for multiple changes at nucleotide sites and are prone to underestimating older divergences (Porco et al., 2012).

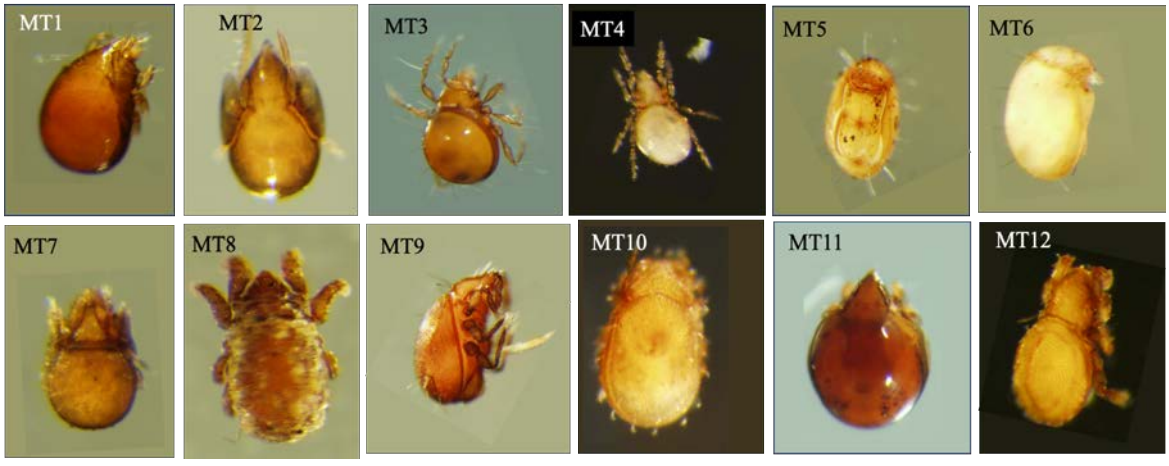
6.3 Results

Distribution and diversity of mites and springtails

A total of 21 oribatid morphotypes were identified from across all sites (Fig 6.3). Oribatid mite genotypes were then verified by DNA sequencing with a minimum of 98% identity to reference sequences. Oribatids were matched to family group based on NCBI BLAST sequence searches of species. Exact species identifications were not available for the majority due to a lack of reference data. Most taxa were distributed across the transect with minimal observed endemicity between island groups (Table 6.2). The dominant predatory mesostigmatid mite species was putatively identified as *Asternolaelaps sp*. Oribatids found throughout the transect included 13 families that are listed in alphabetic order: Ceratozetoidea Jacot, 1925; Compactozetidae Luxton, 1988; Cymbaeremaeidae Sellnick, 1928; Galumnidae Jacot 1925; Haplozetidae Grandjean, 1936; Hermannielloidea Grandjean, 1934; Lohmanniidae Berlese, 1916; Mesoplophoridae Ewing, 1917; Oppiidae Grandjean, 1954; Oribotritiidae Grandjean, 1954; Phthiracaridae Perty, 1841; Rhynchoribatidae Balogh, 1961; Scheloribatidae Grandjean, 1933; Zetorchestidae Michael, 1898 (Table 6.2).

Table 6.2 Presence absence of all oribatid mite taxa along the transect. Sites in latitudinal order with mainland sites shaded in grey.

		Eurimbula NP	Heron Is.	Lady Musgrave Is.	One Tree Is.	Byron Bay	Crowdy Head	Lord Howe Is.	Wilsons Prom	Deal Is.	Hogan Is.	Flinders Is.	Big Dog Is.	Mt. William NP
MT1	Scheloribatidae		X	X		X	X	X	X	X	X	X	X	
MT2	Galumnidae		X	X	X		X	X	X	X			X	
MT3	Oppiidae	X	X	X	X		X	X		X	X	X	X	
MT4	Oppiidae	X		X	X				X			X		
MT5	Phthiracaridae	X						X	X		X		X	
MT6	Oribotritiidae						X		X		X			
MT7	Scheloribatidae	X							X		X	X		
MT8	Scheloribatidae	X					X		X	X				
MT9	Scheloribatidae			X		X						X	X	
MT10	Hermannielloidea		X										X	
MT11	Ceratozetoidea			X				X		X	X			
MT12	Zetorchestidae					X							X	
MT13	Haplozetidae	X						X			X			
MT14	Cymbaeremaeidae							X						
MT15	Rhynchoribatidae										X			
MT16	Galumnidae					X	X	X			X			
MT17	Galumnidae				X			X						
MT18	Mesoplophoridae							X						
MT19	Lohmanniidae			X				X	X					
MT20	Lohmanniidae				X			X		X				
MT21	Brachychthoniidae	X	X	X	X		X		X	X	X			X



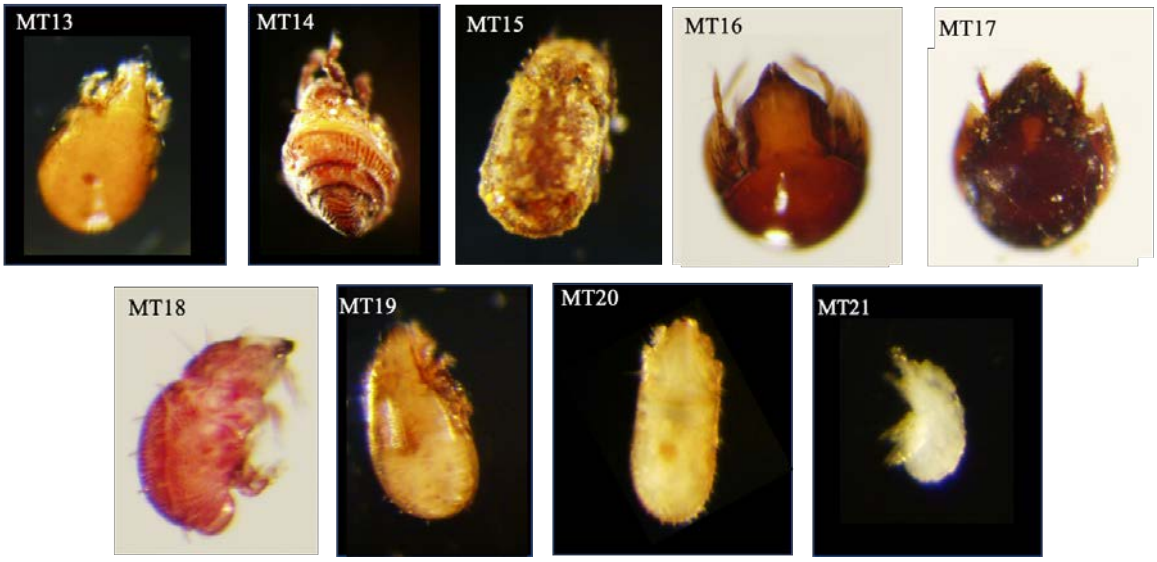


Figure 6.3 Dorsal view photographs taken under 40X magnification of assigned morphotypes and putative species for oribatid mites from all sites.

A total of nine putative springtail spescies were identified from across the transect (Fig. 6 4). These were sequenced and identified as belonging to the following families and genera: MT1: Hypogastrudidae Börner, 1906; MT2: Isotomidae Schäffer, 1896*;* MT3: *Weberacantha* sp. Christiansen, 1951; MT4: *Isotomiella* sp. Bagnall, 1939; MT5: *Tetracanthella* sp. Schött, 1891; MT6: *Dicyrtomina sp.* Börner, 1903 : MT7: *Folsomia* sp. Willem, 1902; MT8: *Brachystomella* sp. Agren, 1903; MT9: *Brachystomella* sp.

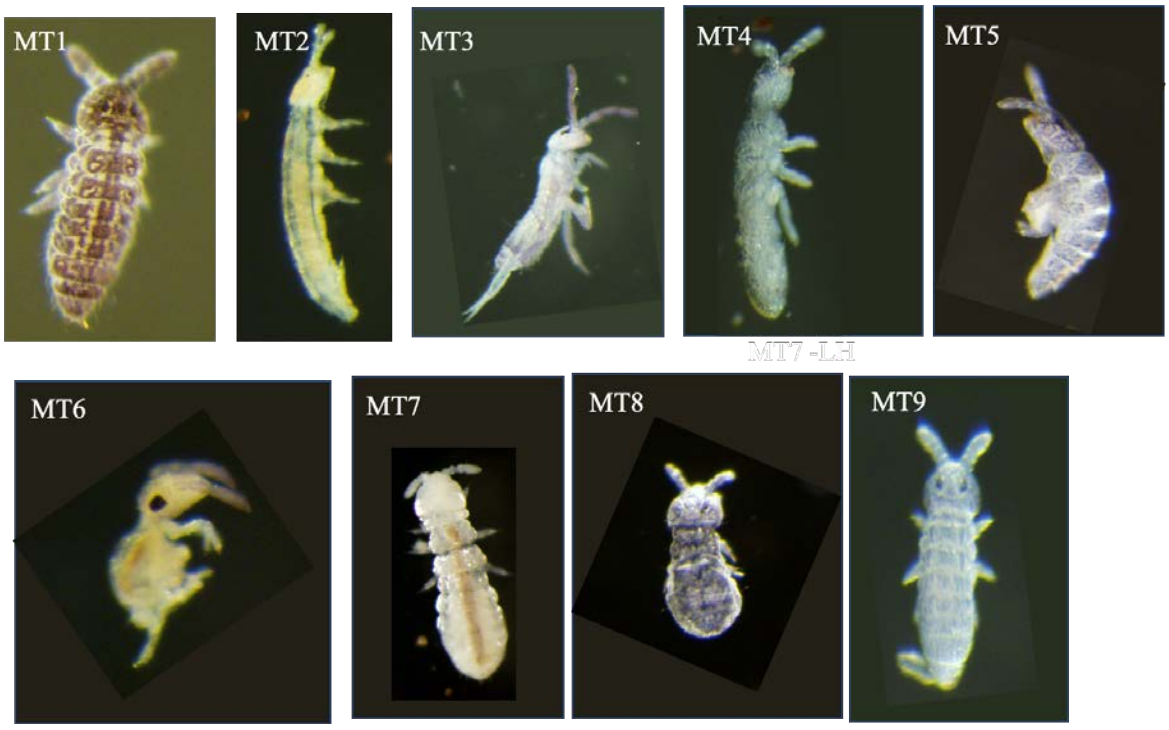


Figure 6.4 Dorsal view photographs taken under 40X magnification of assigned morphotypes and putative species for oribatid mites from all sites.

Phylogenetic verification of mite and springtail species

Maximum likelihood trees of oribatid mites based on 28S rRNA D3 sequences displayed clustering of morphotypes by geographic locations, indicated by suffixed site initials (Fig. 6.5). Tree topology indicates *C. pacificus* as a potential ancestral species of oribatid mites in island groups, especially LHI. Specimens from LHI aligned closest with those from central mainland sites (e.g. Crowdy Bay, Byron Bay), over southerly Bass strait sites, indicating potential source populations from the northerly mainland location at Eurimbula NP.

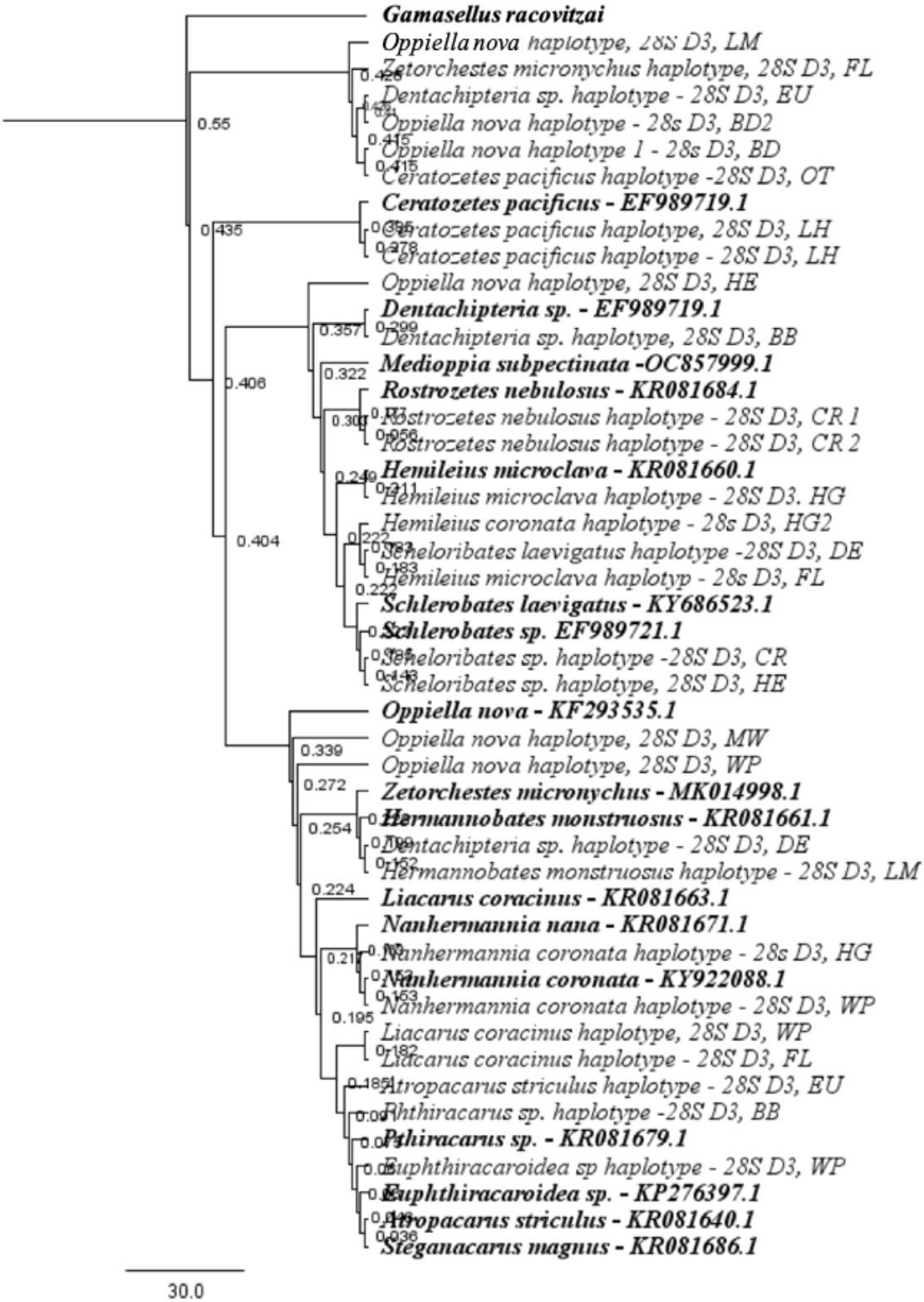


Figure 6.5 Maximum likelihood estimation phylogenetic tree of Australian oribatid mite OTUs and identified sequences. Reference sequences in bold. Site Code indicated at end of sequence name. Tree is rooted with a distantly related mesostigmatid mite, *Gamasellus racovitzai*.

The springtail phylogenies included several members of the *Isotomidae* genus with indications that *Weberacantha* sp. and *Tetracanthella sp* were potential ancestral species. In contrast with oribatid mites, springtail species were more widespread and showed minimal clustering by geographic island groupings (Fig. 6.6).

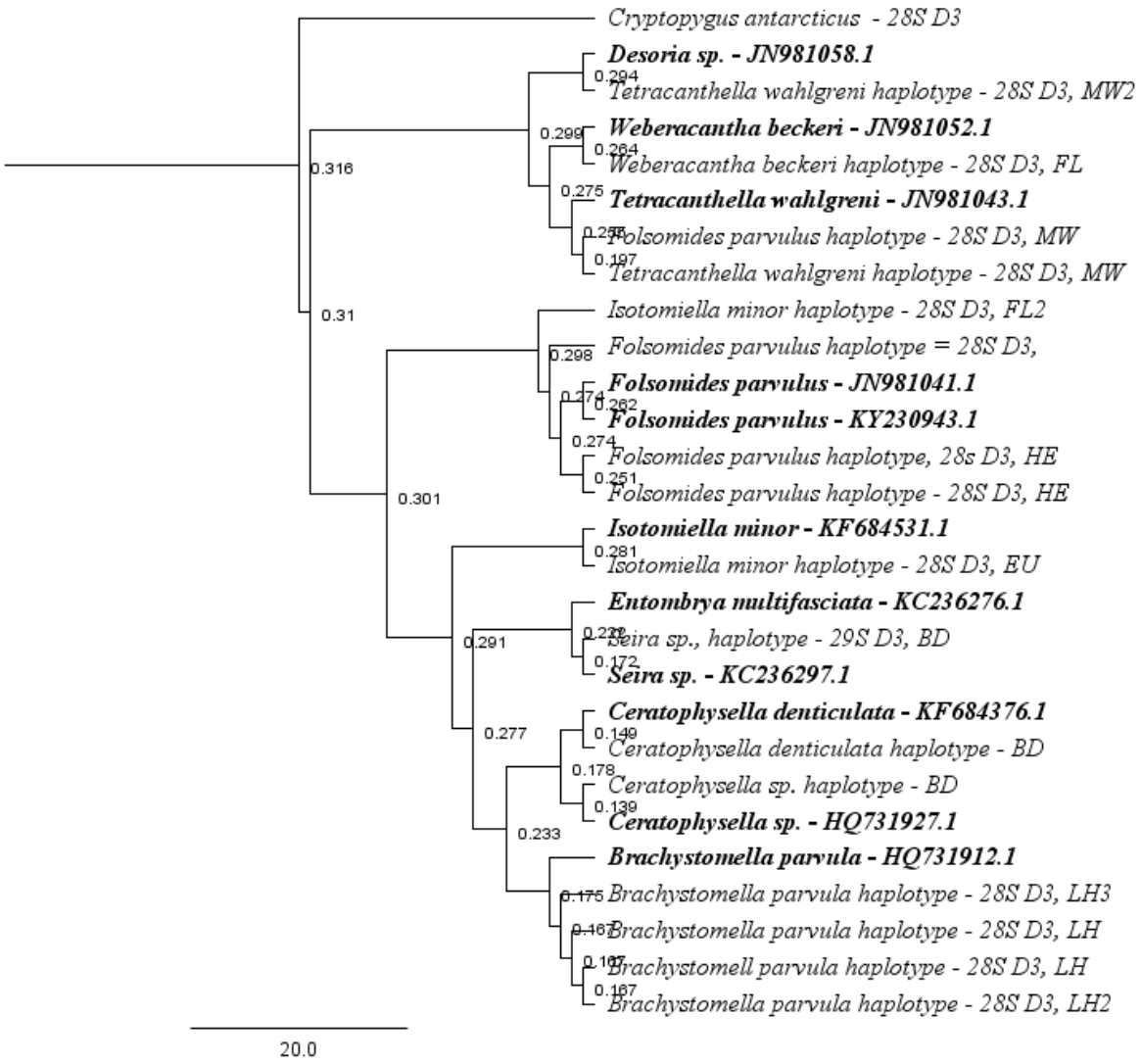


Figure 6.6 Maximum likelihood estimation phylogenetic tree of Australian springtail OTUs and identified sequences. Reference sequences in bold. Site Code indicated at end of sequence name. Tree rooted with a distantly related Antarctic springtail, *Cryptopygus antarcticus*.

Density and diversity across broad- and fine-scales

Mite and springtail density, richness and diversity varied considerably among sites (Table 6.3). Significant relationships with climatic variables were observed with positive links between oribatid mite density and mean annual rainfall ($p = 0.05$, $F_{1,13} = 0.04$, $R^2 = 0.003$), whilst oribatid diversity and springtail density was greater in more acidic soils (i.e. lower pH, $p = 0.05$, $F_{1,13} = 0.4$, $R^2 = 0.03$; Table 6.4).

Table 6.3 Summary of the mean (± s.d.) species densities (ind.m^{-2}), and Simpson's diversity of soil invertebrates across the transect with sites in latitudinal order. Mainland sites indicated in grey rows.

SITE	Oribatid density	Meso density	Prostig density	Springtail density	Oribatid Diversity
Eurimbula NP	20333 ±2533	6000 ±2330	20000 ±1500	2667 ±546	0.62
One Tree Is.	103667 ±4504	12000 ±6490	32667 ±334	107667 ±27688	0.61
Heron Is.	50400 ±4422	21200 ±7660	95200 ±7800	36000 ±2350	0.50
Lady Musgrave Is.	94000 ±8500	17500 ±4390	68667 ±3323	71000 ±23200	0.83
Byron Bay	30667 ±5459	8000 ±3220	35000 ±8990	20000 ±5500	0.77
Crowdy Head NP	20667 ±4334	16333 ±4339	33333 ±4382	20333 ±2803	0.84
Lord Howe Is.	112000 ±5660	25333 ±2212	199333 ±33355	421000 ±22500	0.92
Wilsons Prom	8000 ±3200	2000 ±120	7333 ±332	4000 ±700	0.84
Deal Is.	60800 ±45000	15000 ±7790	135200 ±43340	14000 ±4000	0.70
Hogan Is.	27500 ±12900	18000 ±7670	132000 ±77760	28000 ±7800	0.86
Flinders Is.	74000 ±2390	21000 ±4560	95667 ±2398	31200 ±3340	0.78
Big Dog Is.	96333 ±5497	13333 ±2239	243333 ±9200	66667 ±4438	0.62
Mt. William NP	48000 ±5430	13000 ±5540	89600 ±3200	22400 ±2200	0.58

Table 6.4 Summary of main environmental parameters at across transect with sites in latitudinal order. Mainland sites indicated in grey rows. ANOVA results against latitude at bottom of table (F statistic, p-value). Mean Annual Temperature – MAT; Mean Annual Precipitation – MAP.

SITE		MAT	MAP	SWC	pH	Total N %	Total C %	C:N
Eurimbula NP		25.8	1066.2	9.8	5.2	0.68	11.88	20.03
Heron Is.		26.2	718.4	15.7	6.86	0.97	20.11	16.49
Lady Musgrave Is.		26.2	718.4	41.8	3.4	0.17	5.09	15.46
One Tree Is.		26.2	718.4	24.6	5.14	0.15	4.00	36.60
Byron Bay		23.7	1969.2	46	5.2	0.20	2.43	20.03
Crowdy Head NP		24.2	1690.3	14.1	5.26	0.25	4.83	15.48
Lord Howe Is.		22.5	1628.0	35.6	6.745	0.9711	15.755	16.22
Wilsons Prom		16.8	1352.0	44.6	5.5	0.24661	6.821	30.10
Deal Is.		16.4	687.1	25	7.65	1.45	28.85	22.39
Hogan Is.		16.4	687.1	14.3	7.63	0.18	5.40	17.83
Flinders Is.		16.9	737.6	14.2	4.59	0.57	10.29	13.63
Big Dog Is.		16.9	737.6	15.4	5.84	0.80	20.88	24.33
Mt. William NP		17.2	991.4	26.8	5.16	0.17	4.55	16.11
Latitude	F	0.7	1.9	0.73	1.7	0.38	0.26	0..001
	p	0.4	0.2	0.4	0.2	0.5	0.6	0.9

Biotic relationships with environmental variability

Environmental conditions, climate and soil parameters were found to vary along the transect, with corresponding shifts in measures of soil faunal density and diversity as can be seen in a heatmap of oribatid morphotypes (Fig. 6.7). Patterns of association between soil faunal community composition and microhabitat conditions were revealed potential indicator

species such as MT 9 and 10 with heightened association with total N and C contents (Fig. 6.7). Whilst these heatmaps are useful indicators of overall patterns between the data, they do not account for nestedness and may therefore present different patterns compared to the results of Linear Mixed Effects models. These account for the experimental design in a more appropriate way so as to ensure that pseudoreplication is avoided and is a more informative approach to determine links that incorporate site, plot and vegetation cover type as nested random effects.

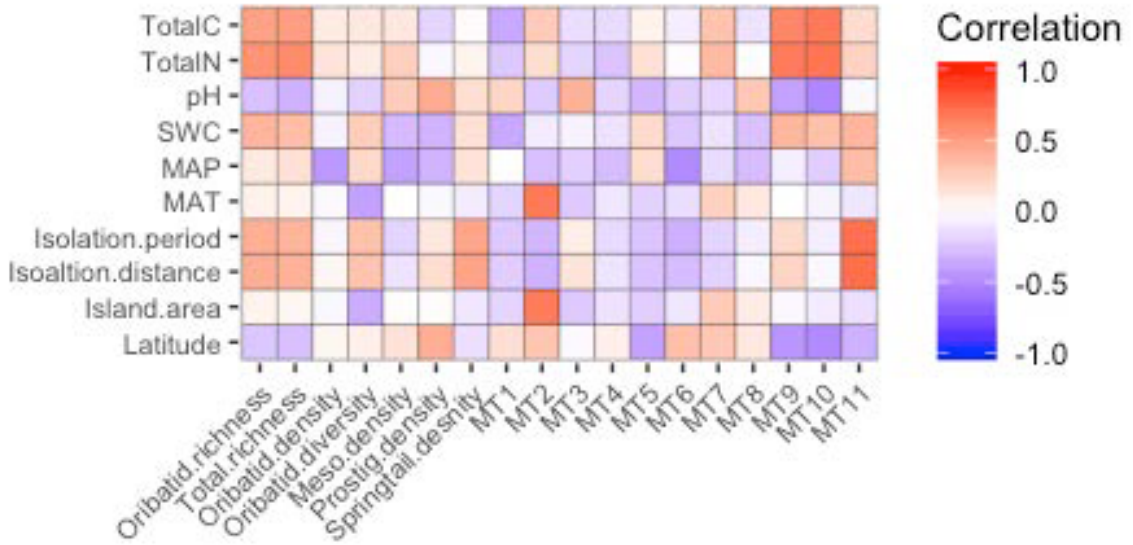


Figure 6.7 Heat map displaying relationships between biotic measures and oribatid morphotypes with broad-scale geographic factors, climatic variables and soil parameters variables (Latitude – LAT, and Isolation distance – ISO, Total Carbon content – TotalC, Total Nitrogen content –Total N, Carbon: Nitrogen ratio – C.N, Soil Water Content – SWC, Soil pH – pH, Mean Annual Temperature – MAT, Mean Annual Precipitation – MAP.

Oribatid mite density was positively related to mean rainfall (p = 0.05, $F_{1,13}$ = 3.2, R^2=0.2). Additionally, more acidic soil pH represented by lower pH values were related to greater oribatid species richness (p = 0.02, $F_{1,13}$ = 8.3, R^2=0.1) and springtail species richness (p = 0.006, $F_{1,13}$ = 12.2, R^2=0.1).

Further exploration of microhabitat conditions and oribatid mite community assemblies indicated strong correlations between MT9, MT11 and Soil Water Content, and between MT2, MT4 and Mean Annual Temperature as indicated in the NMDS plots (Fig. 6.8). Insufficient numbers of replicates for springtail species negated the application of NMDS for springtail species.

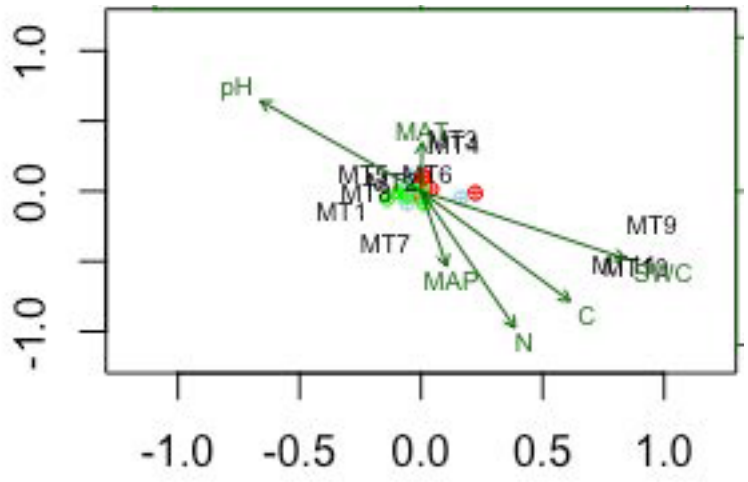


Figure 6.8 NMDS outputs for oribatid mite community structure across all sites with significant soil parameters. All stress levels < 0.2.

Molecular variation between mite and springtail populations

Genetic distances were based on nucleotide differences between populations of the most common mite gentoypes of the *C. pacificus* mite and the *B. parvula* springtail. These alignments were grouped into regions and showed contrasting levels of within-region distances (Table 6.5). Mean genetic distances of mite specimens from the Coral Cays (Heron, One Tree, Lady Musgrave Island, d =0.06) were similar to those between specimens from the Bass Strait islands (Deal, Hogan, Big Dog, Flinders Islands, d = 0.05). These between-island distances were far greater than within group distances in the larger oceanic island (LHI, d = 0.02). By contrast, genetic distances of mites among mainland sites

(Eurimbula NP, EU; Crowdy Bay NP, CR, Byron Bay, BB; Wilson's Promontory, WP, and Mt. William NP in TAS, MW), which covered a much greater area showed much larger distances between specimens compared to island groupings (d=0.36). Between group distances between island groups were negligible with no significant differences between groups for both mite (Table 6.5B) and springtail genotypes (Table 6.5C). Distance between LHI sites and mainland were the largest distances for mites (d = 0.44) and for springtails (d = 0.23).

Table 6.5 Mean pairwise within-group genetic distances between oribatid mites and springtail genotypes based on 28S D3 domain markers for: A) mean within-group mean distance, B) mean oribatid mite between-group distances, and, C) mean springtail between-group distances. CORAL: Coral Cays: HE, LM, OT; BASS: Bass Strait islands: BD, DE, FL; LHI, Lord Howe Island; and, Mainland group: EU, BB, CR MW, WP.

A)

Mean Within group distance	Oribatid mites	Springtails
CORAL	0.13	0.19
BASS	0.12	0.10
LHI	0.02	0.02

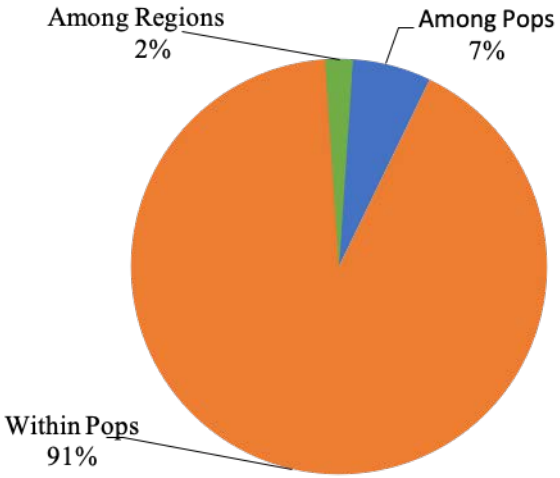
B)

Mean Oribatid Between-group	CORAL	BASS
CORAL	-	-
BASS	0.125	-
LHI	0.122	0.125

C)

Mean Springtail Between-group	CORAL	BASS
CORAL	-	-
BASS	0.143	-
LHI	0.177	0.136

Molecular variation between specimens of the oribatid mite *C. pacificus* across the transect revealed considerable variation within populations compared to among populations (Fig 6.9), indicating that barriers to dispersal limited movement between populations once colonisation of islands occurred. This is more apparent in the lower within site variance in LHI compared to other groups and can be understood considering the distance (580 km) separating the island from the mainland.



C. pacificus populations

Source	df	SS	MS	Est. Var.	%
Among Regions	3	534.998	168.837	5.003	2%
Between Pops	2	1027.139	513.569	20.033	7%
Within Pops	41	10602.596	258.600	258.600	91%
Total	43	11629.734		278.633	100%

Figure 6.9 Pie chart of percentages of molecular variance of *C. pacificus* populations grouped by region. Inset table displays within and between populations (defined by regional distinctions). When considering all sampling localities together, AMOVA indicated no significant genetic structure between the regions (ΦST =0.001, P =0.564).

Genetic distances between members of the same species from different regions indicated greater divergence than within site distances. For *C. pacificus*, the distance between Tasmanian (MW) and Bass Strait (BD) specimens was 90.98%. Meanwhile, the minimum

distance between LHI specimens of *C. pacificus* was 87.6% to mainland specimens from Crowdy and Byron Bay. For *Oppiela nova*, the closest % identity, between the specimens from the coral cays Heron and One Tree Island, was 85.74%. Insufficient replicates for *O. nova* mites precluded further population genetics analysis, as was also the case for the *B. parvula* springtails.

Speciation rates were estimated based on the levels of genetic divergence found between *C. pacificus* mites on LHI specimens and the mainland. As island formation is dated to around 7 Ma, speciation rates would be estimated at between 0.04 –0.06% Ma^{-1} based on 28S rRNA D3 markers.

Spatial population genetics in mites and springtails

The Bayesian topologies of oribatid mites across the transect using the DEC models represented distinct histories for the *C. pacificus* across within the three island groups (Fig. 6.10A-C). As the speciation rates are uncalibrated, the reconstructions indicate three vicariance events occurring at time-point 2 compared to 2–3 dispersal events at an earlier time-point 3 characterises the alignments based on sequences from remnant TAS island (Fig. 6.10A), compared to the QLD coral cays (Fig. 6.10B), that showed a more significant dispersal event. Considering the greater geographical distance between the mainland and LHI sites, it is surprising that the diversity of LHI sites far exceeds that of sites closer to mainland and LHI sites.

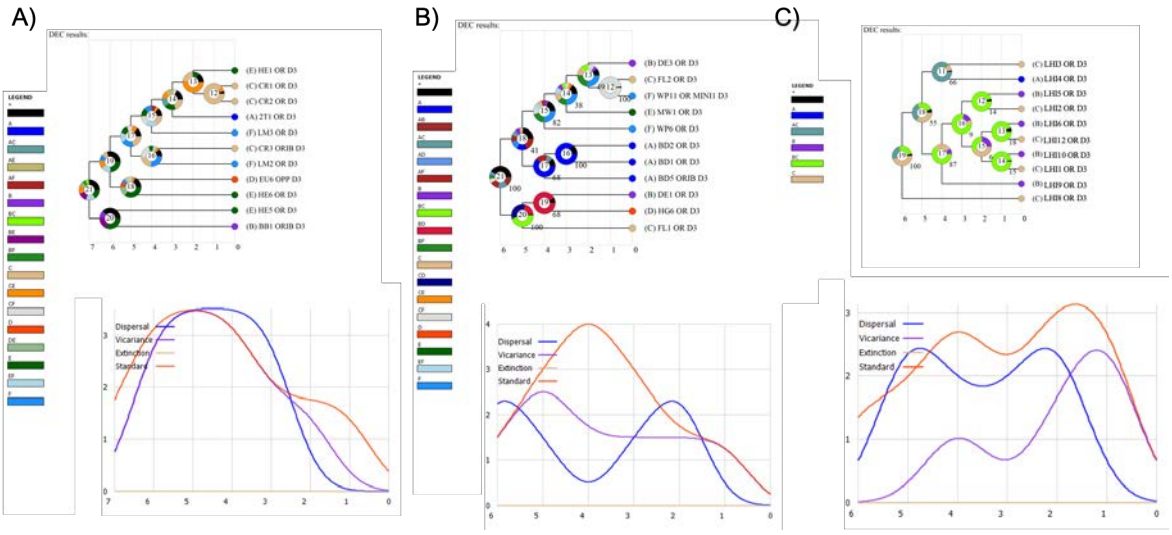


Figure 6.10 Phylogenetic trees of *C. pacificus* genotype sequences from the D3 marker with pie-charts displaying level of shared ancestral character states with each unique state indicated in legend from sites A) QLD B) TAS, and, C) LHI. Graphs represent the reconstructions of evolutionary histories using the DEC model with genetic substitutions attributed to dispersal (blue), vicariance (purple), extinction (beige) and total number of molecular substitution events (red).

Reconstructions using a similar partitioning between the major island groups for the springtails across the transect indicated a similar singular dispersal event that was interspersed with two main episodes of vicariance that was more recent for the TAS island group (6.11B).

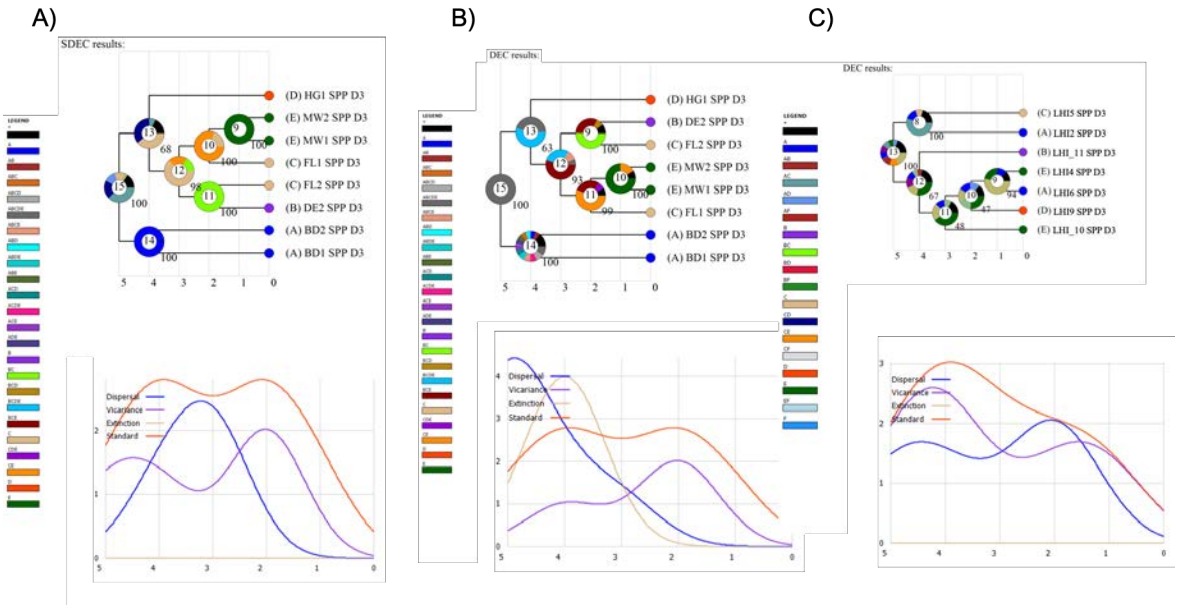


Figure 6.11 Phylogenetic trees of *B. parvula* genotype sequences from the D3 marker with pie-charts displaying level of shared ancestral character states with each unique state indicated in legend from sites A) QLD, B) TAS, and, C) LHI. Graphs represent the reconstructions of evolutionary histories using the DEC model with genetic substitutions attributed to dispersal (blue), vicariance (purple), extinction (beige) and total number of molecular substitution events (red).

The paired dispersal and vicariance events visible from the LHI reconstructions suggest that multiple arrivals of both mites and springtails have contributed to the diversity on the island (Fig. 6.11C), with prolonged periods of vicariance also contributing to marked differentiation from mainland sites considering the AMOVA values.

6.4 Discussion

This study aimed to further our understanding of the distribution and biogeographic patterns in soil invertebrate taxa by strategic sampling along a latitudinal transect in eastern Australia. I found a total of 21 oribatid mites and nine springtail genotypes along the transect belonging to 13 different families, with some overlap in species between sites. Oribatid mite and springtail richness were comparable to values in other regional studies of Australian arthropods in temperate systems (Ross et al., 2020) and morphology-based surveys (Paoletti et al., 2007; Greenslade, 2014). The lower than expected diversity across such an extensive transect may be due to the similarity in ecosystem type that was sampled and the relatively low number of samples collected within a given site. The study was suited for the aims of finding the same species across the transect rather than providing an in-depth assessment of total biodiversity

Overall species diversity might be underestimated owing to the large number of undescribed species in Australia, combined with the high diversity of the tiny *Brachychthoniidae* species that were classified as a single MT despite having up to 12 genera globally of this family alone (Schatz, 2021). Further to oribatid mites, the only other endemic mite known to LHI, the mesostigmatid *Lindothyrus elongatus*, Lehtinen, 1995. was not identified. Species richness varied considerably among sites, but only a small proportion of this variation could be explained by variation in climatic factors. Soil pH was the best predictor of oribatid and total species richness, possibly because pH may be a proxy for a number of other ecosystem characteristics. Springtail abundances were generally lower than those of oribatid mites that contradicts earlier studies of *F. deserticola* springtail and oribatids across an aridity gradient in Australian sites (Wood, 1971).

Mite and springtail diversity and assemblage structure

Oribatid mite assemblage structure was correlated with soil parameters, especially soil pH and total C content. Relationships with these variables were more prominent than climatic effects and reflect similar findings in oribatid assemblages across a tropical elevation gradient (Illig et al., 2010), suggesting a common driver of oribatid mite and springtail assemblages. Oribatid mite assemblages can be considered prone to stochastic events that shift the soil water content and pH in their micro-habitat. This alteration in the community structure may indeed have implications for the functional processes that the oribatid community performs. Such unknown consequences of shifts in biodiversity, without knowing "umbrella" species that are present across a range of ecosystem types, raise questions of ecosystem viability. Total C and N contents are good proxies for nutrient turnover (Wickings and Grandy, 2011), and carbon cycling as performed by oribatid mites (Nielsen et al., 2011), and were also shown to cluster towards a small group of species (Fig. 6.8). Total C and N contents were also the most significant parameters found to be related to springtail assemblages, that indicates their importance to soil faunal structure more generally. As springtails are highly motile, this may explain their ability to seek out preferential habitats with greater resources and associated levels of nutrient levels.

Phylogeographic patterns of common mite and springtail species

The population genetics of the Australian taxa demonstrate the interaction between periods of isolation and dispersal as the main driving forces of species richness and diversity. The results support the hypothesis that speciation of contrasting soil faunal groups is affected differently by isolation barriers to dispersal and biogeography.

Comparing faunal distributions between island groups with alternate biogeographic histories demonstrated how period and distance of isolation can determine levels of dispersal and gene-flow between populations. Taken together, the results from the mite *C. pacificus* and the springtail *B. parvula* indicate that long-distance dispersal plays a key role in soil invertebrate diversity, regardless of biogeographic setting. Oribatid mite and springtail species richness correlated to the biogeographic histories shaped either by vicariance (Bass strait islands), or dispersal (Coral cays and LHI). These biogeographic factors were found to be more influential than soil and vegetation cover parameters. The northern coral cays are very young in terms of geological age (< 8 Ka), and their fauna proved to have no genetic divergence from the mainland communities, with negligible p-distances separating the same species, as would be expected. Whilst there is a greater geographic distance separating LHI from mainland populations, its location is exposed to an eddy from the strong current that flows down the Eastern Seaboard of Australia (Suthers et al., 2011), bringing the potential for substantial amounts of flotsam that have been shown as a main source for its diverse vegetation.

These multiple founder events over LHI's history may explain its greater diversity compared to the coral cays that are much younger and far closer to the mainland. However, such proximity can also greatly increase the impact of human activity and introduction of mainland species (Nakamura et al., 2015). Phylogenetic divergence was also strongly related to species richness although it did not follow the expected gradient from geodispersal to the long-range dispersal sites, mostly due to the unexpected diversity of LHI. Despite the most recent surveys of LHI that have established its unique biodiversity and the importance of conservation, specifically of its Coleoptera (Reid et al., 2018) and Gastropods (Köhler et al., 2018), soil invertebrates were absent from previous studies and highlight the need for greater

attention that will probably lead to the discovery of new species. Further investigation of Norfolk Island which is 1067km from the coast (almost double the 585km to LHI) yet still in the flow of the Eastern Australian current (Ridgeway and Hill, 2009). The minimal morphological distinctions between *Isotomiella minor* and *Brachystomella* springtails found on LHI, closely resemble *C. antarcticus* and *F. grisea* springtails found in the Antarctic, more so than those found on the Australia mainland. This may indicate the pre-Pleistocene arrival of Gondwanan taxa on LHI, that have subsequently undergone minimal evolutionary change.

Alignment of speciation with biogeographic events: molecular clock dating

Dating of soil invertebrate speciation using sequence data requires the calibration of the molecular clock with known standards, often found in fossil records. Genetic distances were used to ascertain speciation rates based on the distances from the LHI island group to the mainland, based on the two main assumptions; i) the current species are direct descendants of the early arrivals 7 Ma, and, ii) there was limited admixture with other dispersers in the intervening period. However, based on the DEC model outputs, this can be disputed because both multiple dispersal events and episodes of vicariance are evident in both the *C. pacificus* mite and *B. parvula* springtail ancestral reconstructions. As such, future investigation may benefit from including other target species, or analysis of both nuclear and mitochondrial markers (such as CO1 or COII) to have a measure of recent admixture in populations. Additionally, further exploration of an extended transect may include the remnant populations of a previously diverse contiguous population that would have stretched up to Papua New Guinea (2ºS; Dougherty et al. 2019).

6. 5 Conclusion

This study of Australian soil fauna highlighted how distribution and diversity of soil mites and springtails can differ depending on different biogeographic contexts. Specifically, the results show contrasting influence of barriers to dispersal and environmental influences on the evolutionary history of mites and springtails. Species richness and genetic distances were found to correlate with period of isolation rather than distance to source populations. More frequent dispersal events and period of evolution meant speciation by vicariance may have contributed to elevated species richness. This baseline biodiversity can hopefully be built upon to further our understanding of Austral soil invertebrates.

Chapter 7 – Synthesis

This thesis set out to advance our knowledge of the biogeography and evolution of southern hemisphere soil invertebrates that are a crucial component of terrestrial ecosystems. Using standardised approaches to generate molecular taxonomic information expanded our knowledge of the distribution, evolution and function of underexplored southern hemisphere taxa and regions. Such analyses provide a robust platform by which measures of species inter-relatedness are compared increases the repeatability of analyses, whilst also contributing biodiversity data that can be incorporated in future analyses. These in turn provided reliable measures of evolutionary processes and mode of speciation in soil fauna, with statistical support for evolutionary theories and dispersal processes across broad spatial and temporal scales. By combining species phylogenies with data along latitudinal, climatic and environmental gradients, the phylogeographic factors influencing soil faunal distribution, the key objectives of this thesis, are clear. The bioinformatic approaches also proved capable of answering hypotheses linking soil invertebrate assemblages and the evolution of functional traits in soil ecosystems. In doing so, I contributed to our understanding of the importance and relevance of southern hemisphere soil fauna, while implications for their conservation and continued investigation are demonstrated.

Chapter 2

A review of the current extent of knowledge pertaining to the phylogeography of Antarctic soil fauna covering all of the Antarctic faunal groups found overwhelming support from molecular and phylogenetic analyses for contemporary populations having ancient origins that involved refugial survival and subsequent dispersal. However, the precise link between

invertebrates and multiple glacial cycles during the Pleistocene (~2Ma–12Ka), was constrained by a lack of comparative studies. This chapter provided a solid context for a survey of soil fauna across a broad Antarctic transect, with uniform use of molecular taxonomic approaches, and presented evidence for the high species and genetic diversity amongst mites and springtails. However, it also demonstrated the drawbacks and difficulties of drawing conclusions from multiple previous studies from disparate soil faunal populations and regions.

Chapter 3

In Chapter three, I revealed the links between soil faunal phylogenies and ecological and functional traits. Phylogenetic clustering was pronounced in traits that were closely related to resource type and functional traits, such as body size or dispersal ability. The phylogenetic analysis of springtails and nematodes demonstrated how functional and morphological traits may be predicted based on sequence barcodes alone. The study demonstrated the capacity for using the phylogenetic data to address ecological and evolutionary questions, but also showed how integrative taxonomy is required. Potential reasons for the spread of soil moisture preferences may be intrinsic to greater source error in recording accurate classifications. Additionally, it emphasised the bias towards northern-hemisphere taxa and the benefits of more studies on southern hemisphere species and sequence generation. This chapter provides a global perspective on the drivers of evolution and distribution of soil invertebrates. Discerning the biotic components that drive soil invertebrate evolution can inform our understanding of the importance of environmental and geographic factors that are explored in the following two data chapters. The biotic element is also evaluated by

comparative analysis between springtail and mite species in both Antarctica and Australian ecosystems to determine general patterns among Austral taxa.

Chapter 4

In Chapter four, I document biodiversity and distribution of mites and springtails from a latitudinal transect (60º–74ºS) in maritime Antarctica. Empirical evidence linking broad and fine-scale patterns of soil invertebrate biodiversity were verified by multilocus phylogenies. Broad scale factors included latitudinal and a range of climatic factors, including temperature and rainfall extremes, having significant impacts on invertebrate diversity. However, local-scale factors such as soil pH and vegetation cover were found to be the most important drivers, with contrasts between mite and springtail populations.

The strong links between species richness and dispersal from known refugia also support suspected unknown refugia on Alexander Island and resolve the previously unexplained high levels of diversity found throughout the Antarctic peninsula.

Detailed species ratios indicated a potential switch from documented dominance of *Alaskozetes antarcticus* over *Halozetes belgicae* oribatid mites along the peninsula. This may be an early indication of shifting species structure in response to accelerated regional "greening" (Cannone et al., 2022). Increased temperature and precipitation predicted along the peninsula (Turner et al., 2005; Cook et al., 2005; Day et al., 2008) have already been linked to larger ice-free areas, and vegetation greening, with associated chances of colonisation from alien taxa (Day et al., 2008; Cannone et al., 2022). Therefore, impacts from both above and belowground forces via altered resource availability and nutrient cycling may lead to unpredictable ecosystem perturbations, with unknown implications to belowground faunal communities (Frenot et al., 2005; Nielsen and Wall, 2013). Thus,

indicator species of *Podacarus auberti* may be suggested as a link with elevated vegetative properties conducive to persistent ecosystems.

Chapter 5

In Chapter five, I built upon the knowledge of distinct distribution patterns and genetic structure within soil faunal populations throughout maritime Antarctica. This was performed using a detailed phylogenetic analysis of target species of mites and springtails to explore phylogeographic and population genetic patterns. Questions involving the biotic influence of invertebrate speciation were addressed by comparing the evolutionary histories of genotypes of two mite species, *Podacarus auberti* and *Membranoppia loxolineata,* and the springtail *Cryptopygus antarcticus*. These were associated with spatial and temporal gradients whilst being grouped into the regions linked to: i) sites requiring dispersal to reach isolated islands; ii) sites < 200km away from known active refugia; and, iii) sites > 200km away from known active refugia inland oases sites. Lack of shared species between the regions demonstrates the importance of biogeographic barriers to dispersal, and the multiple dispersal events from potentially separate refugia.

The different patterns of mite and springtail reconstructions indicated a strong biotic influence on the relative histories of the faunal groups. There were more dispersal events of mites than springtails from refugial regions, and comparatively fewer events to islands might be associated with more infrequent long-distance wind or water-borne biodispersal. The reconstructions recognise specific waves of extinction in springtails, but not mites, following dispersal events. This reverses the trend of under-accounted localised extirpations and extinction events in evolutionary models (Ree et al., 2005; Kodandaramaiah, 2010). Further

investigation could include an assessment of the spatial aspects to complement the temporal aspects were assessed by comparison between species.

Chapter 6

This chapter explored the phylogeographic and population genetic patterns associated with island biogeography of mites and springtails in terrestrial soil ecosystems in the southern hemisphere. Analysis of the distribution of soil mites and springtails along a temperate ecosystem in mainland Australia and offshore islands showed a far greater level of species diversity and trophic complexity compared to Antarctic systems. The Australian transect highlighted the importance of biogeography to contemporary distributions, and the contrasting numbers of dispersal events in mites and springtails. Species richness and genetic distances for mites and springtails were found to correlate with period of isolation rather than distance to the source population, as demonstrated by the very low diversity found within Lord Howe Island, yet high differentiation from mainland sites and other island groups. More frequent dispersal events to LHI meant speciation by vicariance may have contributed to greater contemporary levels of species richness in the intervening 7 Ma since initial colonisation. The location of LHI in the stream of the Eastern Australian oceanic current, was presumed as responsible for its diverse vegetation, and as a likely cause of multiple dispersal events, which complicate calibration of the 28S rRNA marker. Based on assumptions involving contemporary populations being direct descendants of early colonisers, speciation rates were estimated at between 0.04%–0.06% Ma^{-1}.

Contrasting Antarctic and Australian phylogeographic patterns

The similar environmental variation along a broad transect made Australian mites and springtails made an ideal analogue by which to compare the phylogeographic findings from Antarctic taxa. The biodiversity of Australian soil invertebrate communities reflects the highly productive and diverse vegetation that is supported by soils in temperate latitudes. In comparison, species richness and diversity were consistently lower in the maritime Antarctic ecosystems, reflecting the environmental limitations or barriers to dispersal posed by the harsh Antarctic environment.

Phylogeographic reconstructions of the most prevalent oribatid mites from the Australian and Antarctic transect were found to reflect biogeographic differences more readily than differences in climatic or environmental variability. This reflects that barriers to dispersal influence broad scale distribution in mites, in the similar way that species richness in maritime Antarctica were associated with distance from refugia. This supports the use of the evolutionary rates that are slower in the Antarctic as suggested (Kuhner et al., 1998; Schneider and Excoffier, 1999).

Environmental parameters that were linked to greater abundance and richness were soil pH, water content and total C and N contents, as found in both transects. Species richness was significantly related to the biogeographic context, with ancestral states far less diverse in the sites that required biodispersal compared to populations having undergone vicariance such as Australian Bass Strait islands, or populations that had dispersed from refugia in maritime Antarctica. Differences in genetic diversity between mites and springtails was also similar between Antarctic and Australian regions, indicating similar biotic pressures occurring, albeit interacting with different biogeographic settings. In Australia, the long-range dispersal ability of mites would not give them as much an advantage over springtails

as they are both likely to require water-borne raft aboard flotsam. Whereas in Antarctica, the ability for mites to endure wind-borne transport may favour them over springtails and create the observed patterns of distribution.

Dating Austral speciation

The dating of Australian speciation events and estimation of rates of speciation of its indigenous taxa can be applied to assist in determining the relative rate in Antarctic taxa that lack fossil records for accurate calibration of the molecular clock. As Antarctic taxa are known to have longer generation times and lower baseline mutation rates than temperate taxa due to the extreme cold (Fanciulli et al., 2001; Gilooly et al., 2005; Nolan et al., 2006), speciation rates based on non-Antarctic taxa may not be representative of Antarctic taxa (Martin and Palumbi, 1993). However, potential elevated levels of PAR and UV intensity and frequency may also increase mutation rates, especially during periods of minimal ozone coverage (Nielsen, 2019). Whilst these may slightly increase mutation rates in more primitive microbes and prokaryotes (Wynn-Williams, 1994), these would not contribute to significant germline congenital alterations in soil fauna. Therefore, a firm understanding of the evolutionary rates and an alternative means by which to calibrate the molecular clock should improve dating estimates. This can therefore assist aligning phylogenies with events that led to the current biotic distributions found in the region (Graham and Fine, 2008).

The use of biogeography as an alternative method to fossils for molecular clock calibration is well-founded, using sites with contrasting biogeographic histories that can be matched with divergence records (Ho and Duchêne, 2014). This can address the discrepancies that currently exist when including the use of tenebrionid beetles for dating springtail evolution (Collins et al., 2020). Slower generation times and colder temperatures

are considered strong factors that reduce genetic drift as observed in springtails and tardigrades (McGaughran et al., 2010; Guidetti et al., 2017). These can contribute towards better analysis of gene flow and genetic drift whilst accounting for simultaneously occurring evolutionary processes (Andrews, 2010; Edelaar and Bolnick, 2012).

Here, Australian speciation rates were calculated in an attempt to determine the relative rates of speciation found in Antarctic taxa. However, the stated difficulties in calibrating 28S rRNA markers for the oribatid mites *M. loxolineata* and *P. auberti* restricted the ability to align these with known Antarctic glacial cycles that are known to have occurred between ~ 1Ma and 1.5Ma during the Pleistocene. These were expected to be resolved from the contrasting biogeographic conditions present in the Australian dataset, with vicariance playing a stronger role in islands that did not require dispersal, although insufficient levels of divergence and numbers of replicates of the same species limited the effectiveness of this approach. Additionally, differences between Australian and Antarctic taxa must incorporate the faster generation times and baseline rates of mutation associated with higher temperatures and activity, promoting speciation and diversity in temperate taxa.

Synthesis

The separate approaches applied in this thesis aimed to demonstrate the capacity for molecular taxonomy to help address ecological questions. In doing so, the advantages provided by phylogenetic analysis for detailing biodiversity and phylogeography also reinforced the need for continued integration with conventional morphological taxonomy to verify species identities and inform interpretations of results. This will ensure future-proofing of data and allow compilation of sequences to reveal more insights as performed in springtail studies (Collins et al., 2019). Results from both the Australian and Antarctic

transects showed that biogeographic context has a strong influence on the dispersal and ultimate distribution of soil faunal communities. Along the Antarctic peninsula, islands such as Signy and Elephant, with greater ice-free periods and favourable currents, received long-range dispersers to islands eventuating in greater than expected diversity. Considering areas subject to repeated episodes of local extirpation as the advancing glaciers throughout the Pleistocene and up to the LGM, it can be understood that dispersal from refugia would be crucial for re-population of such areas.

As availability of resource type can act as an environmental filter following dispersal, faunal groups that are incapable of rapid adaptation became extinct, leaving more adaptable species to benefit and occupy available niches. However, it has been suggested that Antarctic species may be already burdened by adaptation to local extreme conditions limiting their capacity for further adaptation to new environmental conditions (Convey and Smith, 2005; Nielsen et al., 2011b). These requirements to adapt to the harsh environment may also explain the greater than expected clustering of Antarctic taxa within a singular taxonomic family, despite already divergent mouthpart forms (Chapter 3). These compromises to survive the harsh environment would suggest a limited capacity for Antarctic springtails to make further adaptations to their feeding preferences, especially if predictions of accelerated greening in the Peninsula (Cannone et al., 2022) continue to shift vegetation type from mosses to lichens to more productive grasses. Therefore, discerning the association of soil faunal species richness and assemblage structure with specific vegetation cover type will show that shifting vegetation cover type will undoubtedly lead to disruption of belowground biodiversity. Thus, as Antarctica is as a global indicator for environmental change, determining whether the indigenous soil fauna can adapt to climatic change can inform us about the potential responses of temperate species.

Suspected high levels of cryptic speciation in Antarctic springtails are exacerbated by limited gene-flow between populations following wind or water-borne dispersal events amidst formidable barriers to dispersal. Furthermore, adaptation to specific environmental conditions may supersede the needs of local-scale adaptation further limiting speciation. However, the strong correlation between trophic positioning in nematodes and their trait conservation shows that the contribution to ecosystem function may mediate speciation, especially in environments prone to heightened variation. Other scenarios may involve introduced species following shifting environmental conditions or disruption of the ACC.

The genetic distances between distantly dispersed oribatid mites from the Australian mainland gave a semi-accurate marker by which baseline mutation could be calibrated. This relies on the assumption that there were few colonisation events, with minimal vicariance, in the intervening 7 Ma. Applying these assumptions to resolve the alternative evolutionary rates that are used to reconstruct Antarctic phylogenies would support the use of slower rates of 1.9% Ma^{-1} for the CO1 marker as opposed to the previously ascribed rate of 3.5 % Ma^{-1} (Van Vuuren et al., 2018; Collins et al., 2019). Establishing evolutionary rates that are representative for local taxa are important parameters when analysing phylogeographic histories and can highlight endemic populations that may benefit from increased conservation management and maintain biodiversity and ecosystem resilience despite potential environmental change.

Future directions

This thesis capitalised on the advantages afforded by molecular approaches to the analysis of preserved samples collected from across broad transects in two southern hemisphere regions. Yet, financial and time constraints were still a limiting factor in the number of

specimens that could be sequenced. In-field genetic identification that is now possible in plants (Parker et al., 2017), may soon also be applied to invertebrate taxa. Whilst sufficient representative sequences were acquired to i) confirm species identities, ii) have effective sample sizes for Bayesian analysis, and, iii) enough replicates within sites to explore gene-flow and within site divergence, further sequencing of multiple loci with different evolutionary rates should allow analysis of both deep and shallow nodes within invertebrate phylogenies. Further, multiple loci sequencing would also enable robust measures of phylogenetic diversity, accounting for more recent speciation as is associated with the mitochondrial CO1 marker. In addition, indicator species may be more accurately identified to simplify future diversity analyses, eliminating the requirement for exhaustive surveys. This would also improve the phylogeographic resolution of dispersal events that are known to have strong associations with recent post-LGM dispersal events.

Identifying the factors that are applicable to other non-arthropod groups of belowground taxa (i.e. rotifers, tardigrades, nematodes) would enable a more comprehensive understanding of the factors that influence all terrestrial soil organisms that inhabit Antarctic terrestrial ecosystems and contribute to ecosystem functioning to persist. These will contribute to future studies and promote a greater collaboration between international agencies and maintain large-scale surveys with further advancements of our knowledge, as posited in the most positive forecasts of Antarctic research (Liggett et al, 2017).

The confirmation of refugia as being significant to the diversity and speciation of multiple faunal types raises the prospect that targeted sampling may identify further unconfirmed refugia along the West coast of the Peninsula, as suggested in other studies of the region (Van Vuuren et al., 2018). Detailed analysis of known geothermal refugia with smaller increments of sampling distances around known active refugia would also generate

robust assessments of the spatial gradients that exist around refugia. However, as proximity to refugia has been claimed to be limited to distances within 200km (Fraser et al., 2014), the sites used here (Admiralty Bay, Byers Peninsula and Trinity Island) lie within the range from multiple refugial sources, leading to potential mixing of populations from different refugia. Such complexities may only be resolved with more detailed sampling of sites surrounding known refugia. However, the current findings are sufficient to act as complementary understanding of the endemic soil fauna, in combination with existing research linking vegetation cover, latitude and fungal diversity across maritime Antarctica (Ball et al., 2022). As molecular taxonomy continues to become more readily available, more in-depth sequencing and whole-community analysis will undoubtedly provide more ancestral and phylogeographic information on these overlooked taxa, ensuring their inclusion in future monitoring and conservation efforts. Furthering the “integrated taxonomy” approach by pairing morphological and molecular data has been constrained by the destructive approaches of DNA extraction that limits the preservation of type specimens.

Future approaches to build local sequence libraries and generate complete archival databases include sampling environmental DNA (eDNA; Shokralla et al., 2012), and reversing the morphologically preserving, yet DNA adducting formaldehyde solutions used to preserve nematodes (Karmaker et al., 2015). Methodologies have also been developed to overcome these challenges for high-throughput sequencing (Hykin et al., 2015). Yet as computing power enables ever greater analysis of total biodiversity, turbotaxonomy and metagenomics, sufficient standardisation must be included to aid in the interpretation of information.

By streamlining monitoring capabilities, efforts to recognise shifts in community composition will be enhanced, presenting a more indicative means to measure responses to

ecosystem changes associated with shifting vegetative cover types. The classification of a finite number of cover types may have omitted certain intermediary microhabitats, potentially underestimating diversity and endemic species. As such, future studies may benefit from including such intermediary habitats to ensure complete coverage of all biodiversity. The greater ability for comparison with other studies will also contribute to reversing the historic lack of conservation priority associated with invertebrates.

Furthering our knowledge of gene-flow within Antarctica and northerly sub-Antarctic islands and non-Antarctic taxa from southern hemisphere continental landmasses can also highlight whether the drivers of Antarctic speciation are applicable to temperate and tropical fauna. More accurate parameterisation based on population size and derivation from other former Gondwanan taxa found in South America, Sri Lanka and New Zealand may assist in defining evolutionary rates in Austral soil invertebrates. Such studies may also establish the role of ancient vicariance in contemporary soil faunal populations, as found in other arachnids (Baker et al., 2020).

Extension of the study transects used in this study may include the tropical ecosystems found in the formerly conjoined Papuan landmass and islands off the western coast of Sumatera in Indonesia that have been confirmed as having a pre-Pleistocene origin (Moore et al., 1980), allowing comparison with the biotic barriers found in the Wallace line, and the proposed Antarctic “Gressitt Line” that separates continental and peninsular biota (Wallace, 1863; Terauds et al., 2012).

Thus, investigation of invertebrate communities across latitudes reaching the equator would enable similar comparisons to northern hemisphere ecosystems, encompassing a global outlook for soil fauna. Such studies counter the bias towards northern hemisphere species (Chapter 2). Establishing the links between dispersal and colonisation will also

reveal the potential for insect taxa such as the Chironomid midges that currently inhabit some sub-Antarctic islands to encroach into maritime and continental regions. If temperatures continue to become more favourable for their southerly advance, it would be worth determining any impacts these would have to the local vegetation, and any knock-on impacts to overall ecosystem functioning.

In conclusion, these research directions will culminate in raising the profile of soil invertebrates and their contribution to ecosystem functioning, without which, the existence of terrestrial ecosystems is severely compromised. The observed patterns of genetic differentiation suggest that geographical barriers play a pivotal role in limiting gene flow, leading to distinct genetic clusters within and between islands. Additionally, the influence of island age on genetic diversity underscores the cumulative effect of colonization events and genetic drift over generations. Furthermore, the coexistence of springtails and oribaid mites, despite differences in dispersal capabilities and colonization history, points to the importance of niche differentiation and adaptation in facilitating their persistence on isolated islands. Understanding the drivers of dispersal and adaptation in soil fauna will aid in predicting potential responses to future environmental pressures. The soil invertebrates make soil our most precious commodity upon which whole ecosystems depend. Developing the tools to recognise changes in biodiversity will help mitigate unseen perturbations that may lead to irrevocable ecosystem change, thereby safeguarding our most fragile ecosystems for future generations.

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9. Supplementary Information

Table S1 Mean Annual Temperature (MAT), Mean Diurnal Range (MDR), Temperature Seasonality (TSeason), Maximum Temperature of Warmest month (TMaxWarm), Minimum Temperature of Coldest month (TMinCold), Temperature Annual Range (TAR), mean Temperature of Wettest quarter (MTWet), Mean Temperature of Driest quarter (MTDry), Mean Temperature of Warmest quarter (MTWarm), Mean Temperature of Coldest quarter (MTCold),

SITE	MAT	MDR	TSeason	TMaxWarm	TMinCold	TAR	MTWet	MTDry	MTCold	MTWarm
Signy Is.	-4.9	7.3	405.0	5.0	-14.4	19.4	-4.8	-0.9	-0.1	-10.1
Elephant Is.	-3.6	5.7	361.2	4.3	-10.9	15.2	0.9	-3.5	0.9	-8.0
Admiralty	-2.7	4.4	306.5	4.1	-8.7	12.8	-2.2	1.0	1.0	-6.5
Byers Peninsula	-2.5	3.7	330.4	3.5	-9.1	12.6	-0.2	-6.3	1.2	-7.0
Trinity Is.	-3.1	5.3	363.2	4.6	-10.6	15.2	-6.1	0.4	1.2	-7.7
Biscoe Point	-3.4	4.8	346.0	3.4	-11.0	14.4	-0.7	-0.3	0.6	-7.9
Berthelot	-3.8	5.7	392.8	4.4	-11.9	16.3	-0.9	-8.5	1.0	-8.7
Anchorage	-3.2	4.8	337.7	3.7	-10.6	14.3	-0.8	0.2	1.0	-7.5
Jenny Is.	-3.1	4.8	337.8	3.7	-10.5	14.2	-0.7	0.3	1.1	-7.4
Leonie Is.	-7.8	6.8	526.1	3.7	-17.7	21.4	-8.1	-1.7	-0.8	-13.9
Ares Oasis	-8.0	6.4	529.2	3.3	-17.7	21.0	-8.2	-1.8	-0.9	-14.1
Mars Oasis	-4.9	7.3	405.0	5.0	-14.4	19.4	-4.8	-0.9	-0.1	-10.1

Table S2 Total Annual Precipitation (TAP), Precipitation of Wettest Month (PWetMo), Precipitation of Driest Month (PDryMo), Precipitation seasonality (PSeason), Precipitation of Wettest quarter (PWet), Precipitation of Driest quarter (PDry), Precipitation of Warmest quarter (PWarm), and, Precipitation of Coldest quarter (PCold).

SITE	TAP	PWetMo	PDryMo	PSeason	PWet	PWarm	PCold
Signy Is.	650	77	38	27.0	220	116	138
Elephant Is.	640	64	37	18.5	185	135	185
Admiralty	771	92	47	24.3	259	160	160
Byers Peninsula	633	83	33	27.1	214	113	189
Trinity Is.	855	102	25	38.1	280	113	126
Biscoe Point	851	97	39	24.1	265	142	170
Berthelot	489	73	27	35.3	187	91	121
Anchorage	471	72	25	35.2	176	86	104
Jenny Is.	477	71	23	34.6	178	83	103
Leonie Is.	205	34	0	62.6	93	6	13
Ares Oasis	209	34	0	62.9	96	6	16
Mars Oasis	650	77	38	27.0	220	116	138

Table S3 Pairwise GGD values (Genetic and Geographic distances) for individual haplotypes of *P. auberti* mites used for calculating FST values incorporating latitudinal and longitudinal position of populations.

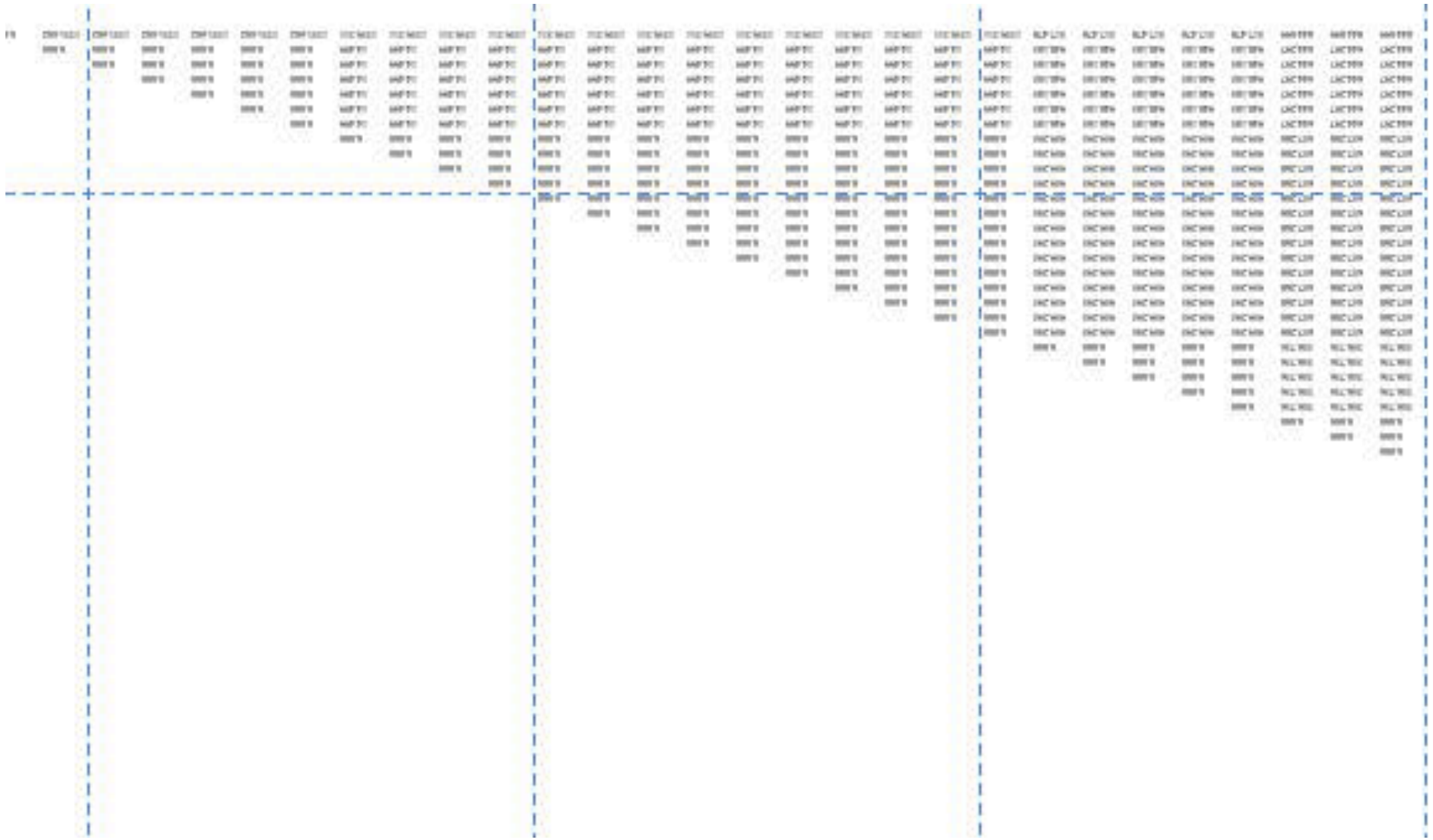

Table S4 Pairwise GGD values (Genetic and Geographic distances) for individual haplotypes of *C. antarcticus* springtails used for calculating FST values incorporating latitudinal and longitudinal position of populations.

springtail vs spp D		Geographic Distance																
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
0.000																		
0.000	0.000																	
0.000	0.000	0.000																
0.000	0.000	0.000	0.000															
0.000	0.000	0.000	0.000	0.000														
863.777	863.777	863.777	863.777	863.777	0.000													
863.777	863.777	863.777	863.777	863.777	0.000	0.000												
863.777	863.777	863.777	863.777	863.777	0.000	0.000	0.000											
863.777	863.777	863.777	863.777	863.777	0.000	0.000	0.000	0.000										
863.777	863.777	863.777	863.777	863.777	0.000	0.000	0.000	0.000	0.000									
863.777	863.777	863.777	863.777	863.777	0.000	0.000	0.000	0.000	0.000	0.000								
533.590	533.590	533.590	533.590	533.590	1331.602	1331.602	1331.602	1331.602	1331.602	1331.602	0.000							
418.840	418.840	418.840	418.840	418.840	517.478	517.478	517.478	517.478	517.478	517.478	948.185	0.000						
533.590	533.590	533.590	533.590	533.590	1331.602	1331.602	1331.602	1331.602	1331.602	1331.602	0.000	948.185	0.000					
533.590	533.590	533.590	533.590	533.590	1331.602	1331.602	1331.602	1331.602	1331.602	1331.602	0.000	948.185	0.000	0.000				
533.590	533.590	533.590	533.590	533.590	1331.602	1331.602	1331.602	1331.602	1331.602	1331.602	0.000	948.185	0.000	0.000	0.000			
533.590	533.590	533.590	533.590	533.590	1331.602	1331.602	1331.602	1331.602	1331.602	1331.602	0.000	948.185	0.000	0.000	0.000	0.000		
545.454	545.454	545.454	545.454	545.454	1339.311	1339.311	1339.311	1339.311	1339.311	1339.311	14.499	959.295	14.499	14.499	14.499	14.499	0.000	
545.454	545.454	545.454	545.454	545.454	1339.311	1339.311	1339.311	1339.311	1339.311	1339.311	14.499	959.295	14.499	14.499	14.499	14.499	0.000	0.000
418.840	418.840	418.840	418.840	418.840	517.478	517.478	517.478	517.478	517.478	517.478	948.185	0.000	948.185	948.185	948.185	948.185	959.295	959.295
418.840	418.840	418.840	418.840	418.840	517.478	517.478	517.478	517.478	517.478	517.478	948.185	0.000	948.185	948.185	948.185	948.185	959.295	959.295
418.840	418.840	418.840	418.840	418.840	517.478	517.478	517.478	517.478	517.478	517.478	948.185	0.000	948.185	948.185	948.185	948.185	959.295	959.295
418.840	418.840	418.840	418.840	418.840	517.478	517.478	517.478	517.478	517.478	517.478	948.185	0.000	948.185	948.185	948.185	948.185	959.295	959.295
418.840	418.840	418.840	418.840	418.840	517.478	517.478	517.478	517.478	517.478	517.478	948.185	0.000	948.185	948.185	948.185	948.185	959.295	959.295
418.840	418.840	418.840	418.840	418.840	517.478	517.478	517.478	517.478	517.478	517.478	948.185	0.000	948.185	948.185	948.185	948.185	959.295	959.295
418.840	418.840	418.840	418.840	418.840	517.478	517.478	517.478	517.478	517.478	517.478	948.185	0.000	948.185	948.185	948.185	948.185	959.295	959.295
418.840	418.840	418.840	418.840	418.840	517.478	517.478	517.478	517.478	517.478	517.478	948.185	0.000	948.185	948.185	948.185	948.185	959.295	959.295
138.193	138.193	138.193	138.193	138.193	844.099	844.099	844.099	844.099	844.099	844.099	644.297	350.736	644.297	644.297	644.297	644.297	657.208	657.208
138.193	138.193	138.193	138.193	138.193	844.099	844.099	844.099	844.099	844.099	844.099	644.297	350.736	644.297	644.297	644.297	644.297	657.208	657.208
138.193	138.193	138.193	138.193	138.193	844.099	844.099	844.099	844.099	844.099	844.099	644.297	350.736	644.297	644.297	644.297	644.297	657.208	657.208
138.193	138.193	138.193	138.193	138.193	844.099	844.099	844.099	844.099	844.099	844.099	644.297	350.736	644.297	644.297	644.297	644.297	657.208	657.208
171.551	171.551	171.551	171.551	171.551	1027.115	1027.115	1027.115	1027.115	1027.115	1027.115	372.705	589.733	372.705	372.705	372.705	372.705	385.591	385.591
171.551	171.551	171.551	171.551	171.551	1027.115	1027.115	1027.115	1027.115	1027.115	1027.115	372.705	589.733	372.705	372.705	372.705	372.705	385.591	385.591
171.551	171.551	171.551	171.551	171.551	1027.115	1027.115	1027.115	1027.115	1027.115	1027.115	372.705	589.733	372.705	372.705	372.705	372.705	385.591	385.591
218.293	218.293	218.293	218.293	218.293	1057.289	1057.289	1057.289	1057.289	1057.289	1057.289	317.022	636.516	317.022	317.022	317.022	317.022	329.455	329.455
218.293	218.293	218.293	218.293	218.293	1057.289	1057.289	1057.289	1057.289	1057.289	1057.289	317.022	636.516	317.022	317.022	317.022	317.022	329.455	329.455
218.293	218.293	218.293	218.293	218.293	1057.289	1057.289	1057.289	1057.289	1057.289	1057.289	317.022	636.516	317.022	317.022	317.022	317.022	329.455	329.455
218.293	218.293	218.293	218.293	218.293	1057.289	1057.289	1057.289	1057.289	1057.289	1057.289	317.022	636.516	317.022	317.022	317.022	317.022	329.455	329.455
529.391	529.391	529.391	529.391	529.391	1325.837	1325.837	1325.837	1325.837	1325.837	1325.837	6.448	943.591	6.448	6.448	6.448	6.448	16.404	16.404
529.391	529.391	529.391	529.391	529.391	1325.837	1325.837	1325.837	1325.837	1325.837	1325.837	6.448	943.591	6.448	6.448	6.448	6.448	16.404	16.404
529.391	529.391	529.391	529.391	529.391	1325.837	1325.837	1325.837	1325.837	1325.837	1325.837	6.448	943.591	6.448	6.448	6.448	6.448	16.404	16.404
529.391	529.391	529.391	529.391	529.391	1325.837	1325.837	1325.837	1325.837	1325.837	1325.837	6.448	943.591	6.448	6.448	6.448	6.448	16.404	16.404
227.271	227.271	227.271	227.271	227.271	701.718	701.718	701.718	701.718	701.718	701.718	760.676	204.948	760.676	760.676	760.676	760.676	772.646	772.646
227.271	227.271	227.271	227.271	227.271	701.718	701.718	701.718	701.718	701.718	701.718	760.676	204.948	760.676	760.676	760.676	760.676	772.646	772.646

20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
0.000																							
0.000	0.000																						
0.000	0.000	0.000																					
0.000	0.000	0.000	0.000																				
0.000	0.000	0.000	0.000	0.000																			
0.000	0.000	0.000	0.000	0.000	0.000																		
0.000	0.000	0.000	0.000	0.000	0.000	0.000																	
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000																
350.736	350.736	350.736	350.736	350.736	350.736	350.736	350.736	0.000															
350.736	350.736	350.736	350.736	350.736	350.736	350.736	350.736	0.000	0.000														
350.736	350.736	350.736	350.736	350.736	350.736	350.736	350.736	0.000	0.000	0.000													
350.736	350.736	350.736	350.736	350.736	350.736	350.736	350.736	0.000	0.000	0.000	0.000												
589.733	589.733	589.733	589.733	589.733	589.733	589.733	589.733	271.617	271.617	271.617	271.617	0.000											
589.733	589.733	589.733	589.733	589.733	589.733	589.733	589.733	271.617	271.617	271.617	271.617	0.000	0.000										
589.733	589.733	589.733	589.733	589.733	589.733	589.733	589.733	271.617	271.617	271.617	271.617	0.000	0.000	0.000									
636.516	636.516	636.516	636.516	636.516	636.516	636.516	636.516	328.998	328.998	328.998	328.998	60.244	60.244	60.244	0.000								
636.516	636.516	636.516	636.516	636.516	636.516	636.516	636.516	328.998	328.998	328.998	328.998	60.244	60.244	60.244	0.000	0.000							
636.516	636.516	636.516	636.516	636.516	636.516	636.516	636.516	328.998	328.998	328.998	328.998	60.244	60.244	60.244	0.000	0.000	0.000						
636.516	636.516	636.516	636.516	636.516	636.516	636.516	636.516	328.998	328.998	328.998	328.998	60.244	60.244	60.244	0.000	0.000	0.000	0.000					
943.591	943.591	943.591	943.591	943.591	943.591	943.591	943.591	640.839	640.839	640.839	640.839	369.223	369.223	369.223	313.190	313.190	313.190	313.190	0.000				
943.591	943.591	943.591	943.591	943.591	943.591	943.591	943.591	640.839	640.839	640.839	640.839	369.223	369.223	369.223	313.190	313.190	313.190	313.190	0.000	0.000			
943.591	943.591	943.591	943.591	943.591	943.591	943.591	943.591	640.839	640.839	640.839	640.839	369.223	369.223	369.223	313.190	313.190	313.190	313.190	0.000	0.000	0.000		
943.591	943.591	943.591	943.591	943.591	943.591	943.591	943.591	640.839	640.839	640.839	640.839	369.223	369.223	369.223	313.190	313.190	313.190	313.190	0.000	0.000	0.000	0.000	
204.948	204.948	204.948	204.948	204.948	204.948	204.948	204.948	146.456	146.456	146.456	146.456	393.160	393.160	393.160	444.410	444.410	444.410	444.410	756.550	756.550	756.550	756.550	0.000
204.948	204.948	204.948	204.948	204.948	204.948	204.948	204.948	146.456	146.456	146.456	146.456	393.160	393.160	393.160	444.410	444.410	444.410	444.410	756.550	756.550	756.550	756.550	0.000