The Ecology and Evolution of New Zealand's Endemic Alpine Grasshoppers

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Abstract

Anthropogenic climate change has stimulated interest in how species distributions are influenced by climate and how they might respond to global warming. The alpine zone is a harsh ecosystem to inhabit, as it experiences climate extremes daily and seasonally, whilst also exhibiting a steep environmental gradient linked to elevation. This makes it a particularly vulnerable environment in light of anthropogenic climate change, but also an informative situation to explore evolutionary ecology. Flora and fauna inhabiting alpine environments are predicted to be most strongly impacted by future climatic changes, when compared with other global ecosystems. The alpine zone of New Zealand stretches the length of the South Island, and is represented in south and central North Island. It is the result of mountain building since the late Miocene, and is home to a diverse array of endemic flora and fauna. As a relatively large component of native New Zealand habitats that has so far been preserved in higher proportion to other systems, the alpine zone will not be exempt from the effects of global anthropogenic climate change. To date, little research has directly investigated the impact worldwide climate shifts might have on this ecosystem.

I investigated the impact that climate change has had, and will have, on New Zealand's endemic alpine short-horn grasshoppers (Orthoptera: Acrididae), as representatives of New Zealand's alpine fauna. Four endemic genera contain 13 endemic species that are all freeze-tolerant, open ground specialists. To assess how these species will be affected by future climate change, it was necessary to examine how they have likely responded to past climatic cycles. Phylogenetic relationships were investigated using high-throughput Next Generation Sequencing. The current classification of 13 species into four monophyletic genera was not supported by this study, and the re-classification of members within this group would be appropriate. The mitochondrial (mtDNA) genomes assembled in this analysis, were combined with mitochondrial genome data of Acrididae species from around the world. This allowed identification of species most closely related to the New Zealand alpine taxa and to estimate the timing of divergence using fossil calibration. New Zealand's alpine grasshoppers form a monophyletic group and share a common ancestor with alpine species from Tasmania. Molecular phylogenetic analysis established that the split of the New Zealand clade from their

Tasmanian ancestor probably occurred ~17–19Mya, which is about 12My prior to alpine habitat being available in New Zealand. Thus, the radiation of New Zealand grasshoppers predates the availability of alpine habitat, suggesting they retained shared ancestral cold tolerance for 12 million years or they independently converged on their alpine adaptations.

Using the current distributions of New Zealand's alpine grasshoppers, potential niche spaces were projected for three times periods (Last Glacial Maximum (LGM), current and future). Ecological niche models predict that suitable niche space has been reduced for most grasshopper species since the LGM, and that with future climate change, the suitable niche spaces of these species will be reduced further still. Fine-scale niche partitioning and population dynamics of species inhabiting five mountains were investigated using field sampling data and population genetics. On a fine scale the presence of particular species of grasshopper was found to correlate with habitat patches (e.g. rock or tussock) and elevation. Each species contained high levels of mtDNA genetic diversity, as expected of large stable populations, but the most common grasshopper species collected had the lowest genetic diversity of the three examined (*Sigaus australis*, *Paprides nitidus*, *Brachaspis nivalis)*, suggesting it may have been more restricted in the past (perhaps due to microhabitat preferences) compared to the other species. The sampled mountain populations of each species have apparently existed in isolation long enough to accumulate unique clusters of mtDNA haplotypes, suggesting very low gene flow between populations. The fact that regional populations are already segregated could make them vulnerable to future climate warming, but might have the opportunity to adapt independently of one another.

The loss of alpine habitat due to climate change is likely to be more pronounced on islands than continental systems. I modelled the expected alpine area lost due to anthropogenic climate warming for eight island systems around the world. In each case the size of the alpine habitat is predicted to shrink, potentially leading to the extinction of species as a consequence of their insularity. The grasshopper species in New Zealand provide evidence that predictions of range shifts require knowledge of current large scale climatic limitations on species distributions and an understanding of fine scale biotic and habitat interactions as well as species specific potential for dispersal.

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General introduction: species responses to a warming world

Introduction

By the year 2100, anthropogenic climate change is predicted to overtake land-use change, Nitrogen deposition and biotic exchange as the primary cause of biodiversity disturbance and loss (Brooks *et al.* 2002, Pimm & Raven 2000, Sala *et al.* 2000, Thomas *et al.* 2004). Changes in Earth's abiotic constituents have had, and will continue to have, pronounced impacts on the biotic world, at an individual, population, species, community, ecosystem and biome level (Bellard *et al.* 2014). Although most environments will be at some risk, climate change is predicted to have the greatest impact on the species composition and biodiversity of arctic, alpine and boreal biomes, when compared with other ecosystems (Boggs & Murphy 1997, Galbreath *et al.* 2009, Sala *et al.* 2000, Thuiller *et al*. 2005, Walther *et al*. 2002). How species respond to climate change is of ecological, conservation, human health and economic concern (Bellard *et al*. 2014, 2016, Buczkowski & Bertelsmeier 2017, Dawson *et al.* 2011, Fletcher *et al*. 2016, Ihlow *et al*. 2016). There is uncertainty around the ability of organisms to make suitable adaptations and/or range changes that are able to keep pace with the current rates of climatic change (Devictor *et al.* 2008, Menéndez *et al.* 2006, Visser 2008). It is important for us to predict the impacts that climate change will have, so that suitable changes in policy, law, and conservation management may be made for predicted climate trends in future climate (Bellard *et al*. 2014, 2016, Buczkowski & Bertelsmeier 2017, Dawson *et al.* 2011, Fletcher *et al*. 2016, Ihlow *et al*. 2016). Better understanding of both current environmental envelopes that species tolerate and the responses of species distributions to past climate change is likely to help us predict the effect of current global warming.

Anthropogenic climate change

It has been more than 150 years since John Tyndall experimentally discovered that an accumulation of polyatomic gases in the atmosphere would result in the warming of the planet (Tyndal 1861). Tyndall remarked after his discovery that an "…inappreciable admixture of any of the hydrocarbon vapours would produce effects on the terrestrial rays and produce corresponding changes of climate" and that "Such changes in fact may have produced all the mutations of climate which the researches of geologists reveal" (Tyndall 1861). This last comment was directed at the then, very recent and, at the time, controversial discovery of the 'ice age', and not in the modern context of anthropogenic climate change (Agassiz 1837, Agassiz & Bettannier 1840). Tyndall did not make the connection that advancements in the use of combustible fuel made during his time, in the midst of the industrial revolution, would in fact initiate modern climate change and readily demonstrate his findings.

Ice core samples dated back to the mid-late Pleistocene indicate that current concentrations of greenhouse gases in the atmosphere are higher than at any other time during the last 800,000 years of Earth's history (Lüthi *et al.* 2008, Monin *et al.* 2001, Petit *et al.* 1999, Siegenthaler *et al.* 2005). This is a direct result of the anthropogenic combustion of 'fossil fuels', carbon accumulated in past geological epochs as petroleum, coal and natural gas (Callendar 1938). Increases in the human population, economic growth and technological advancements have come with an increase in the use of fossil fuels, and thus the release of stored carbon (Canadell *et al.* 2007, Dietz & Rosa 1997, Raupach *et al.* 2007, Shi 2003). This, alongside changes in land use and in terrestrial and oceanic processes, has resulted in atmospheric Carbon dioxide (CO_2) increasing from ~278ppm to >400ppm (40% increase), Methane (CH_4) increasing from ~722ppm to ~1803ppm (150% increase), and Nitrous oxide (N_2O) increasing from ~ 270ppm to ~324ppm (20% increase) since the beginning of the industrial revolution in 1750 (Andres *et al.* 1999, Etheridge *et al.* 1998, Etheridge *et al.* 1996, Flückiger *et al.* 1999, Hartmann *et al.* 2013, Indermühle *et al.* 1999, Intergovernmental Panel on Climate Change (IPCC) 2014, Marland *et al.* 1998, National Oceanic and Atmospheric Administration (NOAA) 2017).

Observable changes to the world's atmosphere, oceans, hydrological cycle, cryosphere and sea level have all been reported (Hartmann *et al.* 2013, IPCC 2014). According to

the Intergovernmental Panel on Climate Change Fifth Assessment Climate Change Synthesis Report 2014, the average combined surface temperature of Earth's land and oceans has increased by 0.85°C between 1880 and 2012, with the 30 year period from 1983 to 2012 thought to be the warmest that the Northern Hemisphere has experienced in the last 800 years. The same report states that the ocean is responsible for the greatest uptake of stored energy in the climate system, accumulating 90% of energy that has entered earth's atmosphere between 1971 and 2010, resulting in its upper waters warming by 0.11°C every decade from 1971 to 2010 (IPCC 2014).

Increasing surface temperatures have resulted in the Greenland and Antarctic ice sheets losing mass, with greatest losses seen from 2002 to 2011; the Arctic sea ice has decreased in every successive season since 1979 by approximately 3.5-4.1% per decade. Additionally, snow cover has decreased in the Northern Hemisphere since the 1950's and permafrost temperatures have increased, reducing their thickness and extent in the Northern Hemisphere. Furthermore, between 1901 and 2010 the average global sea level rose by 0.17m at a rate of approximately 1.56mm/year from 1901-2010 and 3.2mm/year between 1993 and 2010 as a result of glacier loss, sea ice melt, and thermal expansion, from increased surface and oceanic temperatures (IPCC 2014). Oceanic uptake of CO_2 has also seen a 26% increase in oceanic acidity since the industrial revolution (IPCC 2014).

Despite these observations, it should not be forgotten that anthropogenic climate change is not the first major climatic event to have had an impact on planet Earth, and that there have been previous climatic events that have shaped the geography, biodiversity, biogeography and climate that we see today. Earth's climate is not static, and like the organisms that inhabit it, it is forever changing. Past climatic cycles can help us to make predictions of what may be expected with current and future climate change.

The great ice ages and the Last Glacial Maximum

During the same time period that Tyndall made his discoveries concerning the climate and global warming, research into glaciation and the possibility of ice ages was well underway (Agassiz 1837, Agassiz & Bettannier 1840). There have been five major ice ages in the last 2.5 billion years of Earth's history, each of which consists of its own

periods of glaciation (Eyles 2008). The first, the Huronian Ice Age occurred ~2.4-2.1 billion years ago during the early Paleoproterozoic Era, and is suggested to have been as a result of the Great Oxygenation Event (Coleman 1908, Pavlov *et al.* 2000). The second, and most severe ice age followed from 850-630 million years ago (Mya), and is known as the Cryogenian Period. It has been hypothesised that during the Huronian and Cryogenian ice ages, ice sheets reached the equator forming what is known as 'snowball Earth', although this continues to be debated (Hoffman & Schrag 2002, Kennedy *et al.* 1998, Kirschvink 1992, MacDonald *et al.* 2010).

The third major ice age is known as the Andean-Saharan glaciation, and occurred 450-420Mya during the late Ordovician and Silurian Periods, the Karoo Ice Age followed 360-260 Mya (Isbell *et al.* 2008, Smith 1990, Tucker & Reid 1973, Visser 1982). The fifth and current ice age is known as the Pliocene-Quaternary glaciation, and began 2.58 Mya. Periods of glacial expansion and contraction during this time initially followed a 40,000 and then 100,000 year cycle (Mudelsee & Schulz 1997, Pisias & Moore 1981, Ruddiman *et al.* 1988). The Earth is currently undergoing an inter-glacial period of relative warmth within the Pliocene-Quaternary glaciation. The last glacial period of the Pliocene-Quaternary ice age, when global ice sheets were at their maximum integrated volume, ended approximately 26-19,000 years ago, this is known as the Last Glacial Maximum (LGM) (Clark *et al.* 2009).

Contractions and expansions

The LGM is of great interest to biologists as it is the most recent major climatic event to have impacted and partially determined the biogeography of life forms on Earth as we see them today. The Pliocene-Quaternary ice age itself is thought to have been partly responsible for one of the largest megafaunal extinctions in Earth's history, the Quarternary extinction event (Iwase *et al.* 2012, Koch & Barnosky 2006, Shapiro *et al.* 2004, Wroe *et al.* 2013). Species that did survive away from the equator would have done so in environmental refugia that would have expanded and contracted with cycles of glaciation (Jackson & Overpeck 2000, Milá *et al.* 2007). A global reduction in polar ice since the LGM has released habitat for the reoccupation of terrestrial species that were unable to survive the harsh and extreme conditions that the glaciers and alpine habitat would have provided.

Global warming after the LGM resulted in contraction of glaciers and the expansion of many species' ranges, along with possible bursts of speciation (Jackson & Overpeck 2000, Milá *et al.* 2007). In contrast, periods of cooling and expansion of glaciers would have been met with reductions in species' ranges and extinctions (Jackson & Overpeck 2000, Milá *et al.* 2007). However, for species that are adapted to cool alpine habitats, this trend is likely to have been in reverse (Galbreath *et al* 2009). Non-alpine terrestrial species have been in an 'expansion' phase since the LGM, whilst alpine species ranges have been contracting over this time, with trends of species ranges moving towards higher latitudes and upwards in elevation (Galbreath *et al* 2009). With anthropogenic climate change, these trends are expected to accelerate.

A definition of 'alpine'

The term 'alpine' is used by botanists and zoologists to refer to organisms that live above the treeline in high elevation areas, although the upper treeline was originally used by geologists to define the beginning of the 'high mountain belt' (Philippson 1924, Troll 1973). For the purpose of this thesis, the alpine 'zone' I refer to is the environmental band that occurs between the natural treeline and the permanent snowline. The position of the treeline and snowline on a mountain varies throughout the world, and is dependent on steepness of the slope, rate of erosion, substrate, aspect, temperature, atmospheric pressure, availability of CO_2, UV and UV-B radiation, precipitation, wind velocity, tree species present and the past geological history of the concerned landscape (Holtmeier & Broll 2007, Körner 2003, 2007). The major variable controlling treeline continues to be debated but may be region specific (Körner 2007).

The elevation of treelines around the world varies with latitude; however, this trend is not symmetrical across the equator with slightly higher treelines found in the Northern Hemisphere than in the Southern Hemisphere. In New Zealand (40˚S), the treeline sits at ~1200m above sea level (m.a.s.l.), while at 40˚N, the treeline is on average slightly higher, at ~1500m.a.s.l. At the equator (0˚), the alpine treeline may be found at ~3500m.a.s.l., and at ~4000m.a.s.l. at its highest point (10˚N-20˚N), but as low as ~100m.a.s.l. near the North and South Poles (70˚N&S) (Körner 1998, 2003). The Northern Hemisphere treeline also exhibits more variance about the mean, and this is

likely due to a higher abundance of both continental and oceanic island landmasses. Islands tend to have a lower treelines than their continental counterparts, giving a wide range of values at Northern latitudes (Irl *et al.* 2015, Leuschner 1996).

A harsh and vulnerable environment

The alpine zone, taking into account the upper and lower elevation limits, is estimated to cover ~3% of Earth's total land area (Körner 2003, Körner *et al.* 2011). It is a harsh environment that experiences temperature extremes seasonally and daily, with a tendency to be cold and dry year round. The gradient provided by mountainous areas results in abrupt changes in ecosystems, often with a minimal transition zone between. It is estimated that for every 100m advance in elevation, temperature decreases by ~0.6˚C (Halloy & Mark 2003, Körner 2003, Mark *et al.* 2000, Wardle 1998). Global temperatures are expected to continue rising, with forecasted temperature predictions ranging from ~2-6˚C under different climate change scenarios (IPCC 2014). Warming temperatures have and will continue to result in rising treelines and snowlines; however mountains are a finite environment, with their height and shape limiting the ability for such environments to persist (Boggs & Murphy 1997). This makes the alpine zone a vulnerable environment to inhabit.

General species trends

A population might respond to climatic/environmental change via *in situ* adaptation (change in allele frequency due to selection), or tolerance via physical or behavioural plasticity (Jackson & Overpeck, 2000). Alternatively, where the habitat envelope shifts spatially at a suitable rate, populations may track available conditions over generations. Rare long-distance dispersal could also result in colonisation if suitable habitat still remains- towards the poles and/or upwards in elevation (downwards in marine species). Dispersal to new environments might involve niche switching. Alternatively, lineage extinction might be the outcome (Bellard *et al.* 2014; Cheung *et al.* 2009; Hickling *et al.* 2006; Jackson & Overpeck, 2000; Parmesan, 2006; Root *et al.* 2003; Thomas *et al.* 2001; Thomas *et al.* 2004).

Changes in species' physiology, morphology, ecology, phenology, genetic frequency, distribution and population size, as a result of climate change, have been observed in a

variety of life forms (Bulgarella *et al.* 2014, Chen *et al.* 2011, Hickling *et al.* 2006, Levinsky *et al.* 2007, Moritz *et al.* 2008, Parmesan 2006, Rodríguez-Trelles & Rodríguez 1998, Root *et al.* 2003). Terrestrial and marine mammals, birds, insects, amphibians, fish, marine invertebrates, lichens, woody and herbaceous plants are just some such examples (Chen *et al.* 2011, Cheung *et al.* 2009, Fitter & Fitter 2002, Franks *et al.* 2007, Hickling *et al.* 2006, Moritz *et al.* 2008, Parmesan & Yohe 2003, Pounds *et al.* 1999, Rodríguez-Trelles & Rodríguez 1998, Root *et al.* 2003, Sinervo *et al.* 2010, Thomas *et al.* 2001).

Chen *et al.* (2011) found that the distributions of 764 species examined had moved on average 16.9 km per decade from the equator, and of 1367 species studied, ranges had shifted an average of 11m upwards in elevation per decade. Parmesan and Yohe (2003) found that on average, the phenology's of 172 species studied had shifted by 2.3 days towards earlier spring timing, per decade. Taking into consideration different anthropogenic climate change scenarios, Thomas *et al*. (2004) estimated rates of extinction for 1103 taxonomically and globally diverse species, with ~18% of species expected to go extinct under a minimum climate change scenario, ~24% for a mid-range climate change scenario, and ~35% of species expected to go extinct under a maximum climate change scenario.

Despite these predictions of expected trends and the evidence to support species responding to climate change, some studies have also found the opposite to be true. Of the species investigated ~19-25% have demonstrated range shifts towards the equator, ~25% have shifted their distributions downwards in elevation, and approximately 13% have displayed later phenological timing (Chen *et al.* 2011, Lenoir *et al.* 2010, Parmesan & Yohe 2003). Furthermore, it is not uncommon for related species to display large variation in their reaction to climate change, or for species to demonstrate no change altogether, with no change being observed in the mid-range position of ~10% of species in one study (Chen *et al.* 2011, Lenoir *et al.* 2010).

Alpine species responses to climate change

These trends in responses that have been observed and predicted with climate change also apply to alpine organisms. However, due to their distribution on mountains and

mountain ranges, the ability of alpine organisms to disperse with climate change is limited by the height, size, form and isolation of the mountain they inhabit, along with the orientation of the mountain (range) they may be present on (i.e. if the range goes east-west, or north-south) (Boggs & Murphy 1997). Species adapted to high elevation areas are often surrounded by uninhabitable lowland environments, on their own alpine islands - referred to as 'islands in the sky' or 'sky islands' (Galbreath *et al.* 2009, Gifford & Kozak 2012, Heald 1951). Often, dispersing to suitable habitat means traversing down into unsuitable territories before going back up in to alpine areas, depending on the species involved, this may be too much of an obstacle for some species to survive.

Literature examining the responses of alpine life to climate change is dominated by studies on plants (Dullinger *et al*. 2012, Gottfried *et al*. 2012, Halloy & Mark 2003, Lenoir *et al*. 2008, Pauli *et al*. 2007, Theurillat & Guissan 2001, Walther *et al*. 2005, Wipf *et al*. 2009). The most common responses encountered in these studies are upward shifts in the lower and upper limits (if possible) of species ranges, increases in species richness at higher elevations (and reductions in richness at lower elevations), and shifts in phenological timing due to changes in snow pack thickness and longevity. Here, I will explore some examples of how alpine fauna are responding to climate change.

Changes in species ranges are the most commonly recorded response by species to climate change, globally (Chen *et al*. 2011). For alpine species, shifts in ranges can occur at the lower and upper limits of their distributions, however, shifts in their upper limit become more restricted the higher their range moves up mountains. Eventually, only their lower limit can shift (usually upwards) and their range becomes more restricted with time (Moritz *et al* 2008, Wilson *et al*. 2005). When this occurs to multiple species, it can alter the community composition of high elevation environments. Data collected over 35 years in the Sierra Nevada Mountains along a 2775m elevational gradient, has revealed a decline in butterfly species richness at half of the sites examined (Forister *et al*. 2010). Richness was found to have decreased at the lowest elevations, whilst it had increased at the highest elevation site. The pooling of species at high elevations will increase competition for resources, adding additional stress to alpine species. In central Spain, a clear upward shift of species ranges to higher elevations was observed (Wilson *et al*. 2005). A mean temperature increase of 1.3˚C has

resulted in the ranges of 16 butterfly species rising in elevation by an average of 212m in 30 years; species ranges have consequently been reduced by one-third (Wilson *et al*. 2005).

Mammal and bird communities inhabiting alpine tundra in Sweden have undergone range contractions in northern areas since the 19th century (Elmhagen *et al*. 2015). Moritz *et al*. (2008) repeated a 20th century survey across a 3000m elevational gradient in Yosemite National Park. Of the 28 species monitored, species that were formerly lowland in distribution had expanded their upper limit on average by ~500m, whilst high elevation species had contracted theirs and demonstrated little upper limit range shifting. Two of the mammals studied by Moritz *et al.* (2008), the alpine chipmunk *Tamias alpinus* and the lodgepole chipmunk *Tamias speciosus* have undergone contrasting responses to climate change, with *T. alpinus* experiencing an upslope range reduction of 500m, whilst *T. speciosus* has remained stable. A separate study of these two species revealed that *T. alpinus* has undergone genetic erosion and increased genetic subdivision within the Yosemite National Park (Rubidge *et al.* 2012). In the same period, *T. speciosus* has not undergone any significant genetic change; these two species demonstrate the impact that climate change can have on the genetic diversity of contracted and fragmented populations (Rubidge *et al.* 2012).

Some species, such as the Glaucous gull (*Larus hyperboreus*), have experienced range expansions, as summer sea ice in the Northern Hemisphere has retreated (Winker *et al*. 2002). Additionally, breeding ranges of birds occupying the Sierra Nevada Range compared over a century have demonstrated both upslope and downslope shifts; species range shifts could be predicted by changes in temperature and precipitation. However, of 84% of birds that displayed shifts in their distributions, 51% shifted their upper or lower limit towards higher elevations (Tingley *et al*. 2012).

The larch budmoth, a species recorded to have outbreaks every 8 – 10 years for the last 800 years, has experienced a decline in outbreaks and an elevational shift in its outbreak centre since the 1980's (Johnson *et al*. 2010). Population growth rates are also in decline and are attributed to shifts in the elevation of its optimal growth rates (Johnson *et al*. 2010). This shift brings it to within the distributional limits of its host plant - larch (Johnson *et al*. 2010). Survival of the moths larvae are dependent on them hatching in

synchronicity with the spring flush of the larch's foliage; there is the potential for phenological mismatch to occur if timing of one or the other of these species is to be altered (Johnson *et al*. 2010).

Changes to winter weather and snow conditions in Norway are having a negative effect on the outbreak dynamics of the snow lemming (*Lemmus lemmus*), with population peak years becoming markedly absent; this has been attributed to hardened snow over winter months (Kausrud *et al*. 2008). Lemmings are an important part of the tundra food web, and reductions in their numbers will have a detrimental effect on species within this system; it is likely responsible for declines in arctic foxes and snowy owls, which rely on the rodents as a food source (Kausrud *et al*. 2008, Swedish–Finnish–Norwegian Arctic Fox Project 2004). Conversely, records of the cyclic geometrid moths *Operophtera brumata* and *Epirrita autumna* demonstrate outbreak range expansions for both species beginning over 20 years ago (Jepson *et al*. 2007). *Operophtera brumata* now inhabits an area previously only inhabited by *Epirrita autumna*, putting increased pressure on the mountain birch forest in which they inhabit. Additionally, *Epirrita autumna* has expanded into areas considered colder and less suitable than where it currently inhabits.

A study in the Colorado Rocky Mountains recorded the summer arrival of the migratory American robin (*Turdis migratorius*) over 26 years, in which time it began arriving 14 days earlier (Inouye *et al*. 2000). At the same site and over a period of 23 years, hibernating yellow bellied marmots (*Marmota flaviventus*) were recorded to emerge above ground 38 days earlier (Inouye *et al*. 2000). The Colorado Rocky Mountains are experiencing lengthened periods of winter snow pack and increases in air temperature. By arriving early to the mountains, and by emerging earlier from hibernation, both animals are spending prolonged amounts of time on the mountains unable to feed, as snow pack is still present, whilst also having shortened feeding seasons (Inouye *et al*. 2000). Consequently, decreases in marmot litter size and frequency of reproduction have been observed (Inouye *et al*. 2000, Vuren & Armitage 1991). Alternatively, decreases in the body mass of yellow-bellied marmots (*Marmota alpinus*) in the French Alps has led to smaller litters since 1990; this has been attributed to decreasing snow pack (Tafani *et al*. 2013). Thinner snow cover in the winter reduces insulation for

species hibernating beneath its surface, depleting the animals' fat stores more quickly (Tafani *et al*. 2013).

Laying dates of black kite (*Milvus migrans*) populations in Italian mountains have been recorded 10-11 days earlier; this correlates with warmer spring air temperatures (Sergio 2003). Green (2010), studied the phenological responses to climatic shifts of five different animals from the Snowy Mountains in Australia, and returned three different results. One of two bird species studied, Richard's pipit, returned earlier to the mountains when there was an early snow melt, whereas the return of flame robins correlated with warming temperatures in the valley below (Green 2010). Of three insect species examined - bogong moth, Macleays swallowtail and March flies, none demonstrated a significant response to shifts in climate in the mountains over a period of 33 years. However, for insects that overwinter, increasing temperatures have the potential to reduce insulating winter snow cover (Bale & Hayward 2010). This increases their exposure to severe air temperatures, increasing the frequency of freeze-thaw cycles and risks of encasement in ice (Bale & Hayward 2010). Higher temperatures can also alter development and cause an asynchrony between diapause-sensitive life stages and critical periods for diapause induction (Bale & Hayward 2010).

An experimental study by Brown *et al*. (2007) revealed that a decrease in meltwater of mountain streams would increase alpha diversity at particular sites; however, beta diversity (across sites) will decrease as habitat heterogeneity is reduced with a decline in glacial and snowpack meltwater. In another experiment, mayflies that were exposed to warmer water emerged earlier than those in ambient water temperatures; there are fitness costs to early emerging mayflies (and other stream invertebrates), and this could have a detrimental effect on other members in the stream community such as trout (Harper & Peckarsky 2006).

The reasons behind how and why a species may react to climatic changes are poorly understood. However, it is apparent that species inhabiting alpine zones around the world are responding to changes in their environment. Many factors likely influence species responses, including: whether a species plays a specialist or generalist role in its ecosystem; biotic interactions with other species; life histories and rates of reproduction; a species dispersal ability; the physiological, morphological and behavioural capabilities

of the species; the habitat type a species occupies and the natural abundance of that habitat; population size and the genetic diversity within populations (Chen *et al.* 2011, Lenoir *et al.* 2010). Observations, as well as being able to predict how species will respond to future climate change are essential in supporting policy changes and for making conservation management decisions.

In this thesis I examine the influence of past and future climate change on New Zealand's endemic alpine grasshoppers, as representatives of New Zealand's alpine fauna. Understanding the ancestry, and past responses of this group to climate change during the Last Glacial Maximum, can combine with population genetics and modelling of their current and future ecological niches, to infer how they will respond to future climate change.

Thesis Outline

I take the New Zealand alpine grasshoppers as an exemplar of the alpine fauna that is likely to be affected by global warming. I begin (Chapter Two) by examining the systematics of the group using molecular phylogenetics. High-throughput Next Generation Sequencing was used to assemble the complete mitochondrial genomes, 45S Ribosomal cassettes and H3 sequences of New Zealand's four endemic alpine genera: *Alpinacris, Brachaspis, Paprides* and *Sigaus*. The many re-classifications of species within this group and the presence of species complexes highlight the uncertainty that surrounds relationships within and between these genera.

The four endemic genera of grasshoppers in New Zealand are considered alpine, but is this a trait shared by their common ancestor or the result of convergence? In Chapter Three, I examine whether the New Zealand grasshoppers are monophyletic. I estimate divergences of the lineage using the mitochondrial genomes of 56 grasshopper species from around the world and 16 New Zealand sequences. Comprehensive divergence dating analyses using five fossil calibrations were used to investigate the split of the New Zealand alpine species from their most recent common ancestor, as well as splits within the group itself, after monophyly of New Zealand's alpine species was established. Knowing which species the grasshoppers are most closely related to can reveal if their ability to inhabit the alpine zone is a recent adaptation or an ancestral trait. Additionally, estimating divergence dates of this group can inform us about how New Zealand grasshopper species may have responded to past climatic events.

In Chapter Four, current distributions of New Zealand's alpine grasshoppers are used to model the niche space each species currently occupies. These niche models are applied to the inferred climate experienced by New Zealand in the past (Last Glacial Maximum), and for two future climate change scenarios (RCP 2.6 & 8.5), using Ecological Niche Modelling. Predicted range change and degree of habitat fragmentation as a result of future climate change scenarios are compared among species.

In Chapter Five, the population dynamics of New Zealand's alpine grasshopper species are examined. An extensive field survey of five mountains is used to explore fine scale niche partitioning of eight species. The population genetic structure of three species was further investigated using mitochondrial sequence data (COI and ND2). The effects of past climate change can be revealed through population genetics, whilst also informing the overall genetic diversity of the groups being examined. When combined with the Ecological Niche Models from Chapter Four, predictions of future responses become better informed.

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Alpinacris crassicauda, Rainbow Ski Area, South Island

Chapter Two

Analysis of genes and phylogenetic signal from NGS generated mitochondrial genomes, 45S Ribosomal cassettes and H3 sequences of 16 New Zealand and Australian grasshoppers (Orthoptera: Acrididae)

Introduction

More than a hundred years ago Captain Frederick Wollaston Hutton published his accounts of New Zealand grasshoppers, seeking to describe the taxonomy of Acrididae in New Zealand "...before they vanish" (Hutton 1897). Here, I follow in his footsteps with the aim to establish how well the current division of New Zealand species into genera represents their evolutionary relationships. I will use large DNA sequence datasets obtained through high-throughput DNA sequencing to resolve evolutionary relationships and robust phylogenies of the New Zealand taxa.

New Zealand's Acrididae

There are six genera of short-horned grasshopper (Orthoptera: Acrididae (MacLeay 1821)) currently recognised in New Zealand: *Alpinacris* (Bigelow 1967), *Brachaspis* (Hutton 1897), *Locusta* (Linnaeus 1758), *Paprides* (Hutton 1897), *Phaulacridium* (Brunner von Wattenwyl 1893) and *Sigaus* (Hutton 1897). Four of these genera are endemic to New Zealand: *Alpinacris* (two species), *Brachaspis* (three species), *Paprides* (two species) and *Sigaus* (six species) (Figure 2.1). The species within these four genera are generally referred to as alpine grasshoppers, being predominantly found above the treeline, although some have ranges that extend below this and three species are found only in lowland habitats. Twelve of the alpine species are found in the South

Figure 2.1 Clockwise from top left: *Sigaus australis, Alpinacris tumidicauda, Brachaspis collinus, Sigaus villosus, Brachaspis collinus, Sigaus australis, Alpinacris crassicauda* and *Sigaus minutus.*

Island of New Zealand, whilst one species of Sigaus is found occupying alpine environments of the North Island. The distribution of related species is used by biologists to infer evolutionary processes and is fundamental to the study of biogeography and speciation (Warren *et al.* 2014). Inferences have been made from species' distributions using the current taxonomy (Heads 1998, Wallis *et al.* 2016). These relationships have not been explicitly tested. Using molecular phylogenetics these 13 species of alpine grasshopper are expected to resolve into four monophyletic clades, representing the four genera currently recognised in their taxonomic classification.

How do New Zealand grasshoppers fit into global Acrididae diversity?

The orthopteran family of short-horned grasshoppers (Acrididae) contains ~6,677 species worldwide within a complex taxonomic classification system including tribes and subtribes (Table 2.1) (Eades *et al.* 2014a). The four endemic New Zealand genera have been placed within the acridid subfamily Catantopinae (Brunner von Wattenwyl 1893) (Eades *et al.* 2014a, von Wattenwyl & Fea 1893), and with *Sigaus* further diagnosed to the tribe Catantopini (Brunner von Wattenwyl 1893) and subtribe Russalpiina (Key & Colless 1993) (Eades *et al.* 2014b). Members of Catantopinae and Catantopini are found throughout Australia, Asia, Europe and Africa, whilst members of the subtribe Russalpiina are limited to New Zealand and the Australian island of Tasmania (Eades *et al.* 2014a, Eades *et al.* 2014b, Johnston 1956, 1968, Key & Colless 1993, von Wattenwyl & Fea 1893).

This close relationship between Tasmanian Russalpiina and New Zealand *Sigaus* was first suggested by Bigelow (1967), who noted similarities in their ecology and morphology with Tasmanian *Russalpia* species − in particular the internal genitalia of males. Key (1991) also noticed this likeness, and also a similar resemblance in the male genitalia of New Zealand's *Paprides* and *Brachaspis* with Tasmania's *Tasmaniacris*. Key & Colless (1993) established the subtribe Russalpiina in 1993, and *Sigaus* has hence been unofficially placed within Cantantopini and Russalpiina alongside four Tasmanian genera, but not the other New Zealand genera (Eades *et al.* 2014b). Considering the geographical proximity of New Zealand with Tasmania and the close resemblance in internal male genitalia, it is probable that the New Zealand alpine genera

Table 2.1 Approximate number of Orthopteran species at different taxonomic ranks, with a focus on Australasian Acrididae, acquired from the Orthoptera Species File Online Database (December 2017). Genera highlighted in green are endemic to New Zealand, genera highlighted in pink are endemic to New Zealand and Australia, and genera highlighted in orange are endemic to Tasmania.

Order	Suborder	Superfamily	Family	Subfamily	Tribe	Subtribe	Genus	# species
Orthoptera								27,134
	Caelifera							12,114
		Acridoidea						8,106
			Acrididae					6,677
				Catantopinae				1,077
							Alpinacris	2
							Brachaspis	3
							Paprides	2
					Cantantopini			329
						Perbelliina		15
							Phaulacridium	5
						Russalpiina		14
							Russalpia	2
							Sigaus	6
							Tasmaniacris	1

are related to the Tasmanian species, although this has not yet been tested (Bigelow 1967, Key 1991). Species in New Zealand's endemic grasshopper genera and their allies in Tasmania cannot fly due to wing atrophy, nor communicate audibly (Key 1991) (Figure 2.1).

Taxonomic history of NZ grasshoppers

Hutton (1897) described ten New Zealand alpine acridids under four genera: *Paprides*, *Pezotettix* (Burmeister 1840), *Sigaus* and *Trigoniza* (Hutton 1897). In 1898, he transferred New Zealand species from the Northern hemisphere genus *Pezotettix* into the new endemic genus *Brachaspis*. Salmon added *Sigaus villosus* in 1950. Bigelow (1967) reviewed New Zealand Acrididae taxonomy, and made both genus and species additions and re-classifications. The Australasian genus *Trigoniza* was removed from use, with New Zealand species being placed in the endemic genus *Sigaus*. A number of species were merged and/or moved into different genera, highlighting the difficulty of classifying species within these groups. Bigelow's (1967) treatment remains in use today, along with the additions of *Sigaus childi* (Jamieson 1999), *Sigaus takahe* (Morris, 2003) and *Sigaus homerensis* (Morris 2003) (Table 2.2).

Species complexes and discrepancies

The grasshoppers of New Zealand are less neglected now than they were in Hutton's time, leading to the taxonomic status of some species to come under scrutiny in recent years. Specifically the classification of species within *Sigaus* and *Brachaspis* has been examined. With the development of molecular genetic tools the first phylogeographic work on New Zealand alpine Acrididae was Trewick (2001). There are three species of *Brachaspis*, but phylogenetic analysis of mitochondrial (mtDNA) Cytochrome Oxidase One (COI) gene and nuclear Internal Transcribed Spacers (ITS) indicates that the rare lowland *B. robustus* is nested within a southern clade of montane *B. nivalis. Brachaspis collinus* individuals form their own distinct clade but there is genetic evidence of introgression with sympatric *B. nivalis* where the lineages meet (Trewick 2001). Furthermore, lowland populations of the *B. nivalis* 'complex' are more closely related to their nearby montane relatives than to other lowland populations based on mtDNA sequences (Trewick & Morris 2008). Lowland populations, including populations of *B. robustus*, appear to be morphologically and ecologically distinct and deserving of

Table 2.2 Summary of taxonomic, ecological and morphological data for New Zealand's endemic alpine grasshoppers. This includes: the number of genera and species currently recognised; the synonyms previously used to identify these species; the approximate elevational range that each species is found to occupy in meters above sea level (m.a.s.l.); the most common habitat types they are found inhabiting; the known range of body lengths for male and female grasshoppers in millimetres (mm); the colour variants that exist for each species; and how many instars male and female grasshoppers undergo to become adults for each species. * indicates measurements are from one individual.

Genus	Species	Synonyms	Elevational Range (m.a.s.l.)	Habitat type	Male body length (mm)	Female body length (mm)	Colour	Number of male; female instars	References
Alpinacris (Bigelow 1967)	*A. crassicauda* (Bigelow 1967)	-	975–1680	Tussocks, scree	18–22	25–35	Pale brown, olive-grey, reddish brown, dark brown, green, bright green, with pale bands	5;6	Bigelow 1967 Hudson 1970
	A. tumidicauda (Bigelow 1967)	-	600–1830	Tussocks	15.4*	24*	Olive green or pale brown with distinct pale and black bands	5;6	Bigelow 1967 Hudson 1970
Brachaspis (Hutton 1898)	*B. collinus* (Hutton 1897)	*Pezotettix collina* (Hutton 1897)	1000—2000	Tussocks, scree, bare soil, grass	31—32	28—45	Slate grey, grey, grey-brown, green, olive-green, with yellow stripes, and flash-display colours in reddish brown	6;7	Bigelow 1967 Hudson 1970 Hutton 1897
	B. nivalis (Hutton 1897)	*Pezotettix nivalis* (Hutton 1897) *Pezotettix petricola* (Hutton 1897) *Brachaspis petricolus* (Hutton 1898) *Pezotettix terrestris* (Hutton 1897) *Brachaspis terristris* (Hutton 1898)	600–2000	Rocks, scree	15—24	16—40	Grey, grey mottled brown, with flash-display colours scarlet, purple, indigo	6;7	Batcheler 1967 Bigelow 1967 Hudson 1970 Hutton 1897 Hutton 1898
	B. robustus (Bigelow 1967)		500–600	Rocks, scree, bare soil, grass	-	26–29	Grey, grey mottled brown. Flash-display colours scarlet, purple, indigo	-	Bigelow 1967
Paprides (Hutton 1897)	*P. dugdali* (Bigelow 1967)	-	400–1160	Tussocks	15–20	21–27	Green, grey or brown, with yellow-brown longitudinal bands	5;6	Bigelow 1967 Hudson 1970 Hutton 1897

	P. nitidus (Hutton 1897)	*Paprides furcifer* (Hutton 1898)	600–1830	Tussocks, scree,	14–20	22–30	Bright or olive green, bright red hind tibia, pale dorsal bands	5;6	Bigelow 1967 Hudson 1970 Hutton 1897 Hutton 1898
Sigaus (Hutton 1897)	*S. australis* (Hutton, 1897)	*Paprides australis* (Hutton 1897) *Paprides torquatus* (Hutton 1898) *Paprides armillatus* (Hutton 1898) *Sigaus homerensis* (Morris 2003) *Sigaus obelisci* (Bigelow 1967) *Sigaus takahe* (Morris 2003) *Paprides furcifer* (Hutton 1898)	285–2020	Tussocks, scree, bare soil, grass,	12–20 1	17–40	Dark brown, grey, olive-green, light brown, light olive-green.	5;6	Bigelow 1967 Hudson 1970 Hutton 1897 Hutton 1898 Morris 2003
	S. campestris (Hutton 1897)	*Trigoniza campestris* (Hutton 1897) *Trigoniza directa* (Hutton, 1897) *Trigoniza rugosa* (Hutton 1897)	0–1550	Tussocks, grass	13–17	20–32	Light brown, dark brown, green, bright green	-	Bigelow 1967 Hutton 1897 Hutton 1898
	S. childi (Jamieson 1999)	*Sigaus minutus* (Bigelow 1967)	160–420	Grass, bare soil	11–13	21–22	Light brown, dark grey mottled with brown	5;6	Bigelow 1967 Jamieson 1999
	S. minutus (Bigelow 1967)	-	500–1180	Stoney river beds, bare soil, grass	9–10	14–16	Dark grey mottled with brown	-	Bigelow 1967
	S. piliferus (Hutton 1897)	-	725–1400	Tussocks, scree	15–22	25–40	Dark to light brown, reddish brown, grey, olive-green, green with pale bands	-	Bigelow 1967 Hutton 1897
	S. villosus (Salmon 1950)	*Brachaspis villosa* (Salmon 1950)	1370—2130	Tussocks, rocks, scree	24—32	37—48	Brown, dark grey mottled with brown	6;7	Bigelow 1967 Hudson 1970 Salmon 1950

continued conservation protection to maintain diversity (Trewick 2001, Trewick *et al.* 2012). *Sigaus australis* (Hutton 1897) is part of a species complex that includes some geographically restricted populations that have been given species status; the taxonomic standing of five species (*Sigaus childi*, *Sigaus homerensis*, *Sigaus obelisci*, *Sigaus* sp. A, *Sigaus takahe*) has been questioned (Dowle *et al.* 2014, Trewick 2008, Trewick & Morris 2008). Mitochondrial (COI and 12S rDNA) sequences indicate there are four clades consisting of a mix of morphological forms. Although differences in colour, ecology and geographic distribution were found among species within the complex, there was no compelling morphological or genetic evidence to support the species status of some taxa (e.g. *S. obelisci* and *S. homerensis*) (Trewick 2008, Trewick & Morris 2008). While other species (e.g. *S. childi*) have distinctive ecology and morphology despite gene flow with *S. australis* (Dowle *et al*. 2014).

The single North Island species *Sigaus piliferus* (Hutton 1897) was little studied prior to Trewick and Morris (2008). Mitochondrial (COI and 12S) sequences from *S. piliferus* individuals sampled throughout the North Island fell into one of two geographic clades associated with the Tararua Ranges in the southern North Island and central/northern North Island. An average genetic distance among population samples of 5.4% from mtDNA sequences is consistent with the treatment of these clades as distinct species (Trewick & Morris 2008). Such studies further reveal the degree of uncertainty surrounding the current taxonomy and evolutionary history of New Zealand's Acrididae, with further research required to confirm relationships within and between the four endemic genera. For the remainder of the present study, *B. nivalis* will be divided into *B. nivalis* (SA) (St Arnaud (SA), northern clade) and *B. nivalis* (FP) (Fox Peak (FP), southern clade), with all *B. robustus*, *B.* 'lowland', and *B.* 'hunter' individuals included under *B. nivalis* (FP) as suggested by Trewick (2001). Similarly, *Sigaus homerensis*, *S. obelisci*, *S. takahe* and *S.* sp. A. are included within *S. australis* (Trewick 2008). *Sigaus piliferus* is considered as two lineages: *S. piliferus* (LW) (Lake Waikaremoana (LW), central/northern clade) and *S. piliferus* (MH) (Mount Holdsworth (MH), southern clade) (Trewick and Morris 2008).

Phylogenetic markers

Prior to the introduction of high-throughput sequencing (e.g. Next Generation Sequencing (NGS)), individual mtDNA genes (e.g. COI and 12S) amplified via targeted Polymerase Chain Reaction (PCR) were commonly used for phylogenetic investigations (Doddala *et al*. 2015, Goldberg *et al*. 2015, Hills *et al*. 2012, Trewick 2001, Trewick 2008, Trewick & Morris 2008). High-throughput sequencing allows rapid assembly of large tracts of DNA, and it is becoming increasingly common for molecular studies to use complete mtDNA genomes to establish deep evolutionary relationships via robust molecular phylogenies, including in Orthoptera (Gao *et al*. 2017, Song *et al*. 2015, Zhou *et al*. 2017b) and many other organisms (e.g. birds (Garcia-R *et al*. 2014, McCormack *et al*. 2013, Mitchell *et al*. 2014), fungi (Nadimi *et al*. 2016), insects (Song *et al*. 2017, Yong *et al*, 2015, Zhou *et al*. 2017a), mammals (Botero-Castro 2013, Gibb *et al*. 2105, Lindqvist *et al*. 2010, Morin *et al*. 2010, Van Oven 2009), molluscs (Vaux *et al*. 2017), plants (DePriest *et al*. 2014) and reptiles (Kolora *et al*. 2017, Leaché *et al*. 2015)). Numerous copies of the mtDNA genome are found in the mitochondria of eukaryotic cells and it is typically inherited maternally without recombination (Nass & Nass 1963). The mtDNA genomes of Acrididae are known to be 14-16kb in length, and contain 13 protein coding genes, 2 ribosomal RNAs (rDNAs), 22 transfer RNAs (tRNAs) and an A + T rich repeat region (D-loop); an arrangement typical of most insect mtDNA genomes (Cameron 2014, Fenn *et al.* 2008, Flook *et al*. 1995, Ma *et al*. 2009, Song *et al*. 2015, Zhou *et al*. 2010).

The nuclear 45S Ribosomal (rDNA) cassette comprises three rDNAs: 18S, 5.8S and 28S, and two Internal Transcribed Spacers (ITS): ITS1 and ITS2 (Figure 2.2) (Richard *et al*. 2008). As part of the nuclear genome it is inherited from both parents. As the 45S Ribosomal cassette plays a vital role in the formation of ribosomes, it is highly replicated in the genome, with many tandem repeats and copies on multiple chromosomes (Richard *et al*. 2008); this makes it amenable to assembly from NGS data (Vaux *et al*. 2017). Homogeneity of the 45S Ribosomoal cassette is conserved due to concerted evolution (Nei & Rooney 2005). The rDNA regions of the cassette are highly conserved and so show a slow rate of nucleotide substitution. As a result they have been used to study deep phylogenetic relationships (Raué *et al.* 1988). In contrast, the ITS regions are not functionally constrained in the same way, and so have high substitution rates. ITS sequences can vary greatly even within species, and have been used alongside

mtDNA in species and population analyses (Álvarez &Wendel 2003, Richard *et al*. 2008, Trewick 2001). Another nuclear gene assembled from NGS data in the present study was the histone protein H3. H3 is involved in the formation of chromatin. Similarly to rDNA genes, H3 is highly conserved and can readily be attained from short read data, even from large genomes (Maxson *et al*. 1983).

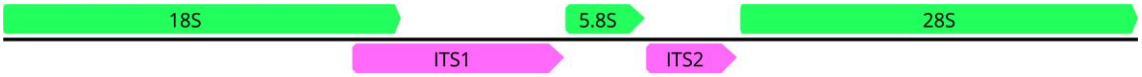

Figure 2.2 Eukaryotic nuclear 45S ribosomal cassette arrangement. The three ribosomal RNA (rDNA) genes are green and the two Internal Transcribed Spacers (ITS) are pink.

Here I used NGS methods to assemble entire mitochondrial genomes, nuclear 45S Ribosomal cassettes and H3 sequences from representatives of the New Zealand alpine genera: *Alpinacris, Brachaspis*, *Paprides* and *Sigaus*, and two Tasmanian alpine genera: *Russalpia* and *Tasmaniacris*. I will test whether the current classification into four endemic genera is consistent with the phylogenetic relationships of New Zealand's alpine grasshopper species.

Materials and Methods

Taxon sampling

Fourteen individuals representing eleven New Zealand endemic grasshopper species were collected by hand from lowland and alpine environments within their natural ranges: *Alpinacris crassicauda*, *Alpinacris crassicauda* (DP), *Alpinacris tumidicauda*, *Brachaspis collinus*, *Brachaspis nivalis* (SA), *Brachaspis nivalis* (FP), *Paprides dugdali*, *Paprides nitidus*, *Sigaus australis*, *Sigaus campestris*, *Sigaus minutus*, *Sigaus piliferus* (LW), *S. piliferus* (MH) and *Sigaus villosus* (Table 2.3, Figure 2.3). Three species were sampled from two populations to encompass potential diversity; *A. crassicauda*, *B. nivalis* and *S. piliferus*. *Alpinacris crassicauda* (DP) represents a distinct lineage of *A. crassicauda* from Denniston Plateau reported here for the first time as it may represent a new species. Species were identified using morphological traits, with the pronotum margin, subgenital plate and epiproct being particularly informative (Bigelow 1967, Jamieson 1999). Additionally specimens of two Australian alpine species: *Russalpia sp.* and *Tasmaniacris tasmaniensis* from Tasmania were obtained. The Tasmanian taxa are considered morphologically and ecologically similar to New Zealand genera (Bigelow 1967, Key 1991) and provide a suitable outgroup (see Chapter Three). All individuals were suspended in 100% ethanol to preserve DNA.

DNA extraction, quantification and sequencing

The middle femora on the right side of each specimen were removed. A sterile scalpel blade was used to finely dice up the leg and its muscle tissue. Whole genomic DNA was extracted from this leg tissue using the solvent-free Proteinase K and salting-out method (Sunnucks & Hales 1996). Leg tissue was incubated at 55^{o}C overnight in a 1.5mL microfuge tube containing 600µL of TNES (2.5ml 1M Tris, 4ml 5M NaCl, 2ml 500mM EDTA and 5% SDS) and 10µL of Proteinase K. Proteins were precipitated from this mix using 170µL of NaCl. The supernatant was transferred to a fresh sample tube and 780µL of ice cold 95% ethanol added. After gentle mixing, tubes were centrifuged for 30 minutes at 14000 rpm and the ethanol discarded. Samples were rinsed with 400µL of 70% ethanol which was discarded after spinning for a further 5 minutes at 14000rpm. Dry DNA was re-suspended in 50µL of Milli-Q water.

Table 2.3 The New Zealand and Tasmanian grasshopper individuals for which mitochondrial genomes, 45S Ribosomal cassettes and H3 sequences were acquired, summarised and used in the phylogenetic analyses of this study. Specimen codes are linked with specimens kept in the Phoenix Collection at Massey University, Palmerston North. Collecting locations shown in Figure 2.3.

Origin	Genus	Species	Specimen code	Location
New Zealand	*Alpinacris*	*A. crassicauda*	AC25	Mt Peel, Tasman, South Island
		A. crassicauda (DP)	GH749	Denniston Plateau, West Coast, South Island
		A. tumidicauda	GH1295	Mt Cardrona, Otago, South Island
	Brachaspis	*B. collinus*	GH1405	St Arnaud Range, Tasman, South Island
		B. nivalis (SA)	GH1404	St Arnaud Range, Tasman, South Island
		B. nivalis (FP)	GH1371	Fox Peak, Canterbury, South Island
	Paprides	*P. dugdali*	GH830	Mt Teviot, Otago, South Island
		P. nitidus	GH1407	St Arnaud Range, Tasman, South Island
	Sigaus	*S. australis*	GH1387	Mt Hutt, Canterbury, South Island
		S. campestris	GH1036	Lake Tekapo, Canterbury, South Island
		S. minutus	GH433	Edward Stream, Canterbury, South Island
		S. piliferus (LW)	GH5	Lake Waikaremoana, Hawke's Bay, North Island
		S. piliferus (MH)	GH60	Mt Holdsworth, Wellington, North Island
		S. villosus	GH1374	Fox Peak, Canterbury, South Island
Tasmania	*Russalpia*	*Russalpia sp.*	TAZ3	Mt Wellington, Hobart
	Tasmaniacris	*T. tasmaniensis*	TAZ4	Mt Wellington, Hobart

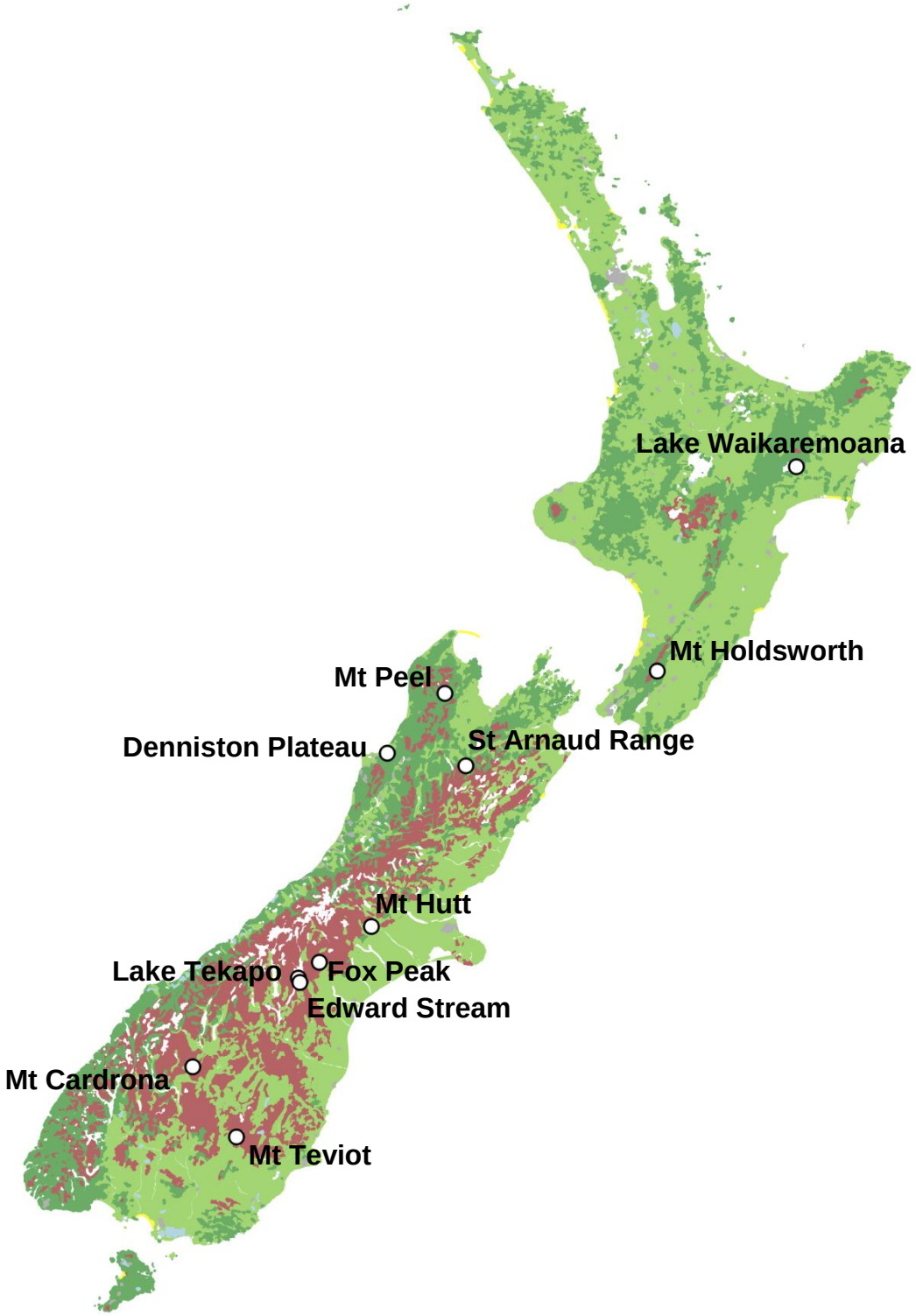

Figure 2.3 Sampling locations for representatives of 14 New Zealand endemic alpine grasshoppers used in this study. From northern to southern locations, *Sigaus piliferus* (LW) Lake Waikaremoana; *S. piliferus* (MH) Mt Holdsworth; *Alpinacris crassicauda* Mt Peel; *Brachaspis collinus*, *B. nivalis* (SA) and *Paprides nitidus* St Arnaud Range; *A. crassicauda* (DP) Denniston Plateau; *S. australis* Mt Hutt; *B. nivalis* (FP) and *S. villosus* Fox Peak; *S. campestris* Lake Tekapo; *S. minutus* Stream; *A. tumidicauda* Mt Cardrona; and *P. dugdali* Mt Teviot.

Next Generation Sequencing (NGS) was used to acquire DNA sequences from the 16 New Zealand and Australian Acrididae for this study. DNA was quantified using the Qubit Fluorometric Quantitation kit (Life Technologies, Thermo Fisher Scientific Inc.). Subsamples of each genomic DNA extract were paired-end sequenced through massive parallel, high-throughput sequencing on an Illumina HiSeq 2500 by Macrogen (http://dna.macrogen.com). The 100bp paired-end reads produced through Illumina sequencing were edited with the Python plugin 'Cutadapt' v 1.11 (Martin 2011) to remove DNA barcodes. Geneious R9 v9.1.4 (Kearse *et al*. 2012) was used to pair sequence reads.

Assembly, alignment and editing of mitochondrial genomes, 45S Ribosomal cassettes and H3

Whole mtDNA genomes were assembled individually by mapping paired reads to a reference sequence. The first of the New Zealand grasshopper genome assemblies used the published annotated mtDNA genome of locust *Locusta migratoria* (Genbank: JN858150) obtained from GenBank (Benson *et al*. 2013), for initial mapping. Paired reads were iteratively mapped to the reference sequence in Geneious generating a novel consensus sequence, which was used as a reference to remap raw sequence reads to in each case. This process was repeated until all alignment gaps were filled by extension with the new sequence data (Sivyer *et al.* 2018, Vaux *et al*. 2017). Sequences were uploaded as raw fasta files to MITOS (Bernt *et al.* 2013) to initially identify protein coding regions, rDNAs and tRNAs. Annotations were transferred and individually checked by comparison of reading frames, amino acid translation and RNA structure.

A similar approach was used to assemble, align and edit 45S Ribosomal cassettes and Histone (H3) sequences for all 16 grasshopper species. Two short horn grasshopper 45S templates: *Locusta migratoria* (Genbank: KM853191) and a species of Gomphocerinae (GenBank: AY859546), were used as reference sequences for the ribosomal cassette and *L. migratoria* (Genbank: AF370817) as the reference for H3.

Phylogenetic analysis

PartitionFinder2 (Lanfear *et al*. 2016) was used to identify the most suitable models of DNA evolution for each gene partition (mtDNA and nuclear), before a number of

analyses investigating the phylogenetic relationship of New Zealand's alpine grasshoppers were performed. Initially, separate phylogenetic analyses using alignments of mtDNA genomes, 45S Ribosomal cassettes and H3 sequences were examined. Alignments comprising only protein coding genes or tRNAs or rDNAs and all genes combined were then also investigated. All alignments were created using the alignment plugin MUSCLE (Edgar 2004) in Geneious, and were edited manually before being run through the Gblocks server (Castresana 2000) to remove any remaining ambiguous characters. All alignments consisted of the fourteen newly sequenced New Zealand individuals. The two Tasmanian taxa, *Russalpia sp.* and *T. tasmaniensis* were used for the outgroup in all phylogenetic analyses.

Prior to tree building, the Neighbour-net network algorithm (Bryant & Moulton 2004) was implemented in SplitsTree4 (Huson & Bryant 2006) to generate networks of each alignment. Alignments were analysed using Maximum Likelihood (ML) (RAxML v 8 (Stamatakis 2014)) and Bayesian Inference (BI) (MrBayes v 3.2.2 (Ronquist & Huelsenbeck 2014)) through the CIPRES Science Gateway Server (Miller *et al*. 2010). Maximum Likelihood analyses were conducted using 1000 random starting trees under a GAMMA model and GTR substitution matrix. Node support was assessed using 1000 non-parametric bootstrap iterations, applying fast bootstrapping, alongside a subsequent ML search. Phylogenies were visualised in FigTree v 1.4.3 (Rambaut 2016). For the BI analyses, each analysis was run using a GTR substitution model combined with the proportion of invariable sites model Invgamma; models were run for 30 million generations with four MCMC chains in each, and a burnin of one million generations. Outputs were analysed in Tracer v 1.6 (Rambaut *et al*. 2015), before trees were produced and visualised in FigTree.

Gene tree analysis

Forty-four unrooted phylogenetic gene trees for each of the 13 protein coding genes, 22 tRNAs, five rDNAs, two ITS regions, H3 and D-loop were inferred using Bayesian Inference. Each alignment analysis was run for 1 million generations using a GTR substitution model and GAMMA rate variation model; no outgroup was enforced. Trees were produced and visualised in FigTree. Gene trees were then loaded into

SplitsTree4 where a Consensus splits algorithm was implemented to generate a splits network (Holland & Moulton 2003).

Results

Mitochondrial sequence data

Complete mtDNA genome sequences were generated for 16 grasshopper individuals. Paired read coverage was sufficient overall, although the number and depth of paired read sequences varied between individuals, ranging from 5,951 (*P. dugdali*) to 67,214 (*T. tasmaniensis*) in number; mean depth of paired reads per sample ranged from 55 (*S. campestris*) to 526.8 (*T. tasmaniensis*) sequences (Table 2.4). Assembled mtDNA genomes ranged in length from 15,468 base pairs (bp) (*S. piliferus* (LW)) to 15,837bp (*S. villosus*), with all genomes comprising the expected mtDNA arrangement of 13 protein coding genes, 22 tRNAs, two rDNAs and a repeat region (D-loop) (Figure 2.4) (Table 2.5) (Cameron 2014, Fenn *et al.* 2008, Flook *et al*. 1995, Ma *et al*. 2009, Zhou *et al*. 2010). Variation in genome length was mainly attributable to differences in length of the D-loop region and intergenic regions. Overall GC content of the mtDNA genomes was low, varying from 25.3% (*S. piliferus* (MH)) to 27% (*S. australis*).

The order and orientation of genes was the same for all 16 individuals and identical to that of other Acrididae for which data are available (Fenn *et al.* 2008, Flook *et al*. 1995, Ma *et al*. 2009, Song *et al*. 2015, Zhou *et al*. 2010). The length of the 13 concatenated protein coding genes varied from 11,139bp (*P. dugdali*) to 11,151bp (*S. campestris*, *S. minutus*, *S. piliferus* (MH) and *Russalpia sp.*), reflecting differences in the number of amino acids found between some individuals. *Sigaus villosus* was found to have two amino acid insertions at ND5 compared to others, while the ND4L gene of *T. tasmaniensis* is two amino acids shorter than all others. *Sigaus campestris*, *S. minutus*, *S. piliferus* (LW), *S. piliferus* (MH), *S. villosus* and *Russalpia* sp. all had an amino acid insertion at ND6, and *S. piliferus* (LW) and *S. villosus* each had one fewer amino acid at CYTB. The concatenated rDNA regions (12S and 16S) ranged in length from 2138bp (*S. villosus*) to 2180bp (*S. piliferus* (LW)), while concatenated tRNA length varied from 1473bp (*A. crassicauda* (DP)) to 1483bp (*T. tasmaniensis*). Intergenic regions were found to account for no more than 0.8% of total genome lengths. Gene overlap of 7bp was evident between ATP6 and ATP8, and between ND4 and ND4L. This is consistent

Table 2.4 Summary of the Next Generation Sequencing (NGS) 100 base pair (bp) paired read data from which the mitochondrial genomes of 14 New Zealand grasshoppers and two Tasmanian grasshoppers were assembled.

Species	# Paired Reads	Max. read depth	Mean read depth	Read depth Std. Dev.
A. crassicauda	24,204	224	157.2	28.4
A. crassicauda (DP)	20,663	198	134.1	28.1
A. tumidicauda	7,336	103	57	19.7
B. collinus	14,108	171	112.4	30.1
B. nivalis (SA)	9,107	121	73.1	16.7
B. nivalis (FP)	21,345	272	169.5	45.1
P. dugdali	5,951	89	47.8	15.2
P. nitidus	9,066	116	72.6	18
S. australis	16,378	203	131.3	30.9
S. campestris	6,891	107	55	15.9
S. minutus	12,611	193	104.5	32.8
S. piliferus (LW)	9,741	123	78.5	18.1
S. piliferus (MH)	25,158	289	199.9	49.3
S. villosus	13,934	179	117.1	32.9
Russalpia sp.	24,291	273	192.5	45.8
T. tasmaniensis	67,214	755	526.8	132.2

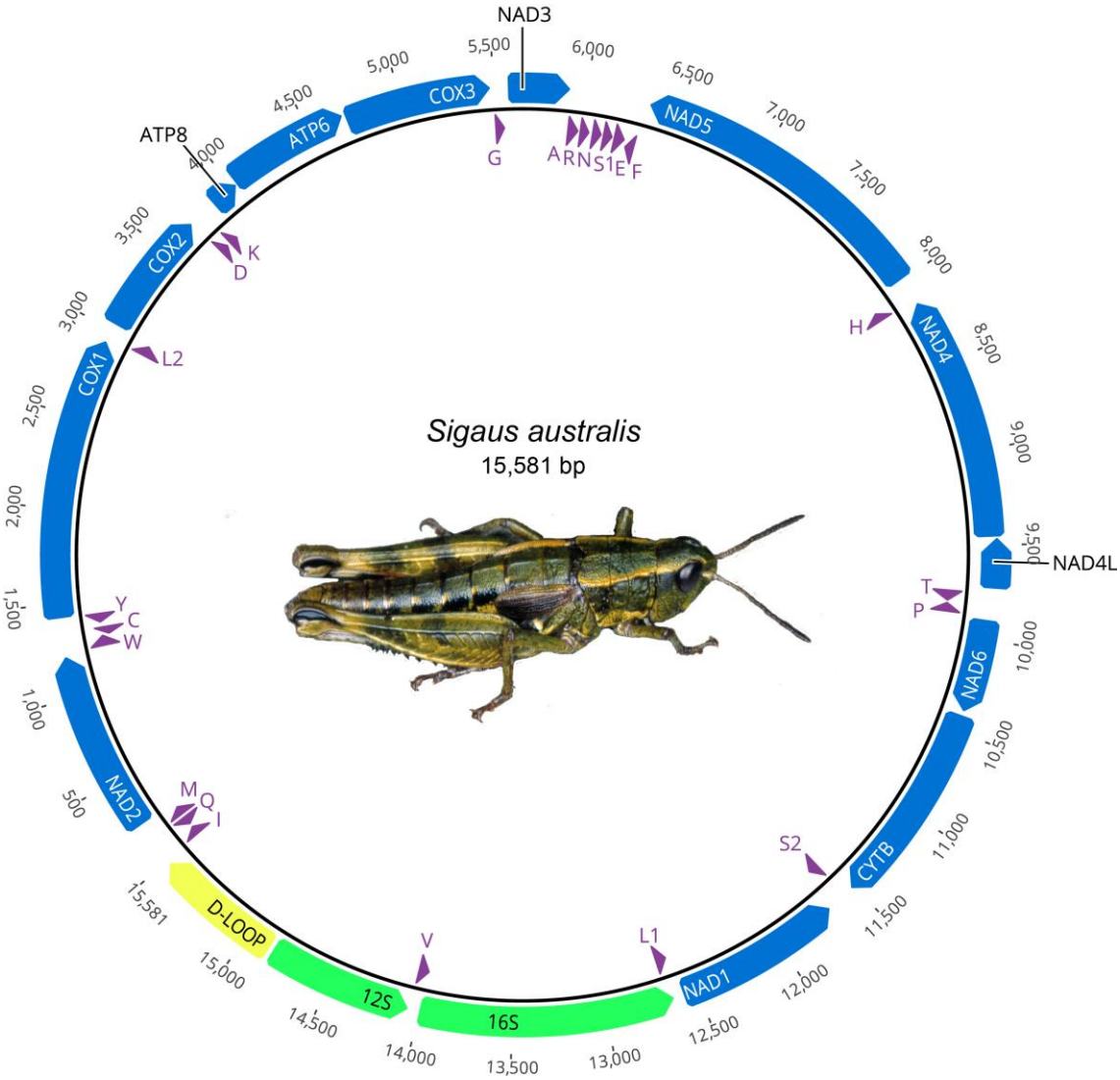

Figure 2.4 The mitochondrial genome composition of *Sigaus australis*, as a representative for all the grasshopper mitochondrial genomes used in this study. Protein coding genes are coloured in blue, tRNA genes are coloured in purple, rDNA genes are coloured in green and the A+T rich repeat region is coloured in yellow. Arrows at the ends of annotation bars indicate the gene direction. Transfer RNA genes are labelled using IUPAC-IUB single letter amino acid codes, full names are listed in Table 2.7.

Table 2.5 Summary of the Next Generation Sequencing (NGS) paired read data from which mitochondrial genomes of 14 New Zealand grasshoppers and two Tasmanian grasshoppers were assembled, alongside summaries of their compositions. bp = base pairs.

Species	mtDNA sequence lengths (bp)	Protein coding regions	rDNAs	tRNAs	D-Loop	Intergenic regions	GC%
A. crassicauda	15,522	11,148	2,149	1,474	656	95	26.6
A. crassicauda (DP)	15,524	11,148	2,162	1,473	650	91	26.8
A. tumidicauda	15,546	11,148	2,161	1,481	696	60	26.2
B. collinus	15,638	11,148	2,160	1,481	765	84	26.2
B. nivalis (SA)	15,546	11,148	2,165	1,478	670	85	26.4
B. nivalis (FP)	15,744	11,148	2,161	1,479	857	99	25.7
P. dugdali	15,609	11,139	2,156	1,480	731	103	26.4
P. nitidus	15,581	11,148	2,165	1,475	695	98	25.9
S. australis	15,581	11,148	2,161	1,476	712	84	27.0
S. campestris	15,673	11,151	2,161	1,481	812	68	26.1
S. minutus	15,781	11,151	2,156	1,479	882	113	26.1
S. piliferus (LW)	15,468	11,148	2,180	1,479	555	106	25.8
S. piliferus (MH)	15,751	11,151	2,165	1,480	888	67	25.3
S. villosus	15,837	11,148	2,138	1,478	952	121	26.0
Russalpia sp.	15,762	11,151	2,151	1,481	855	124	26.2
T. tasmaniensis	15,822	11,145	2,164	1,483	912	118	26.0

with published mtDNA genomes of other Acrididae members (Fenn *et al.* 2008, Flook *et al*. 1995, Ma *et al*. 2009, Song *et al*. 2015, Zhou *et al*. 2010).

Nuclear sequence data

45S Ribosomal Cassettes were assembled for all 16 individuals with good depth of paired reads during assembly. The 45S cassette is repeated many times throughout the nuclear genome, which is reflected in the large number of reads that were available to assemble the cassettes, ranging from 12,510 (*Russalpia sp.*) to 121,737 (*A. crassicauda*) in number of sequences, and 229.8 (*Russalpia sp.*) to 3,974.8 (*S. villosus*) in mean sequence depth (Table 2.6). The cassettes varied in length from 6,858 (*S. australis*) to 6,917 bp (*T. tasmaniensis*). GC content was found to be more than twice that of the mtDNA genomes, ranging from 56.6% *(S. campestris*) to 58% (*Russalpia sp.*). The combined length of the three rDNAs ranged from 6,257 (*S. minutus*) to 6,305 bp (*B. nivalis* (SA)), while overall length of the two ITS regions ranged from 600 (*A. tumidicauda*) to 650 bp (*Russalpia sp.*). The order and orientation of genes within the cassette was as expected. The 16 H3 sequences generated varied in length from 796 to 846 bp, and GC content was found to be almost three times that of the mtDNA genomes, ranging from 65.5% to 68.8%.

Alignment and gene summaries

Prior to phylogenetic analysis, unrooted SplitsTree networks were generated for each partition and summary statistics were generated for each individual gene (Table 2.7) (Supplementary Figure 2.1, 2.2, 2.3, 2.4, 2.5). Mean pairwise percent identity (MPPI) across alpine members and the Tasmanian taxa was found to be 88.8% for complete mtDNA genomes (Table 2.7). The mtDNA genomes of *A. crassicauda* and *A. crassicauda* (DP) were the most similar, with a pairwise variability (PV) of only 4.5%, while the most diverged mtDNA genomes were that of *A. crassicauda* (DP) and *Russalpia sp.* (PV = 13.6%) (Supplementary Table 2.1)(Supplementary Figure 2.1). When D-loop was removed, MPPI increased from 88.8% to 90.4%; *Alpinacris crassicauda* and *A. crassicauda* (DP) remained the most similar sequences (PV = 4.4%) (Supplementary Table2.2)(Supplementary Figure 2.2). Mean pairwise percent identity of individual mtDNA gene alignments varied from 62.8% (D-loop) to 97.9% (Serine (S1)). Overall, tRNAs were slightly more conserved

Table 2.6 Summary of the Next Generation Sequencing (NGS) paired read data from which 45S ribosomal (rDNA) cassettes and H3 sequences of 14 New Zealand grasshoppers and two Tasmanian grasshoppers were assembled, alongside summaries of their compositions.

Species	45S # paired reads	45S max. read depth	45S mean read depth	45S read depth std. dev.	45S sequence lengths	45S rDNAs	45S ITS	45S GC%	H3 sequence lengths	H3 GC%
A. crassicauda	121,737	27,206	1,635	4,315.4	6,863	6,290	610	57.5	815	66.4
A. crassicauda (DP)	32,523	818	337	193.2	6,861	6,290	608	57.4	838	65.5
A. tumidicauda	16,534	400	274.9	70.4	6,863	6,300	600	57.6	828	67.0
B. collinus	27,787	4,374	771	1,190.8	6,870	6,301	606	57.5	813	66.5
B. nivalis (SA)	82,908	26,661	2,552	5,438.8	6,880	6,305	612	57.5	807	66.6
B. nivalis (FP)	82,479	13,176	2,205.6	3,386.8	6,892	6,304	625	57.6	819	66.7
P. dugdali	50,376	1,341	851.4	246.9	6,867	6,293	611	57.5	810	66.3
P. nitidus	20,633	2,033	333.8	339.3	6,862	6,293	606	57.5	796	67.0
S. australis	36,468	5,278	860.1	1,260.9	6,858	6,300	640	57.6	829	66.6
S. campestris	13,642	375	243.8	62.3	6,889	6,291	604	56.6	825	66.3
S. minutus	21,407	1,357	376.3	209.2	6,873	6,257	632	57.5	817	66.5
S. piliferus (LW)	24,494	1,343	440.5	141.5	6,889	6,257	616	57.7	830	66.7
S. piliferus (MH)	22,502	3,710	590.1	892.9	6,893	6,260	629	57.7	826	66.5
S. villosus	114,911	11,257	3,974.8	4,520.5	6,899	6,260	633	57.6	846	65.8
Russalpia sp.	12,510	422	229.8	126.1	6,911	6,261	650	58	818	66.5
T. tasmaniensis	21,139	1,052	377.3	290.9	6,917	6,269	648	57.6	808	67.1

Table 2.7 Summary statistics of gene and partition alignments of 14 New Zealand and two Tasmanian alpine grasshoppers. Transfer RNA (tRNA) genes are highlighted in purple, protein coding genes are highlighted in blue, ribosomal RNA (rDNA) genes are highlighted in green, D-loop is highlighted in yellow, ITS regions are highlighted in pink and Histone 3 is highlighted in orange. Genes are in the order they occur within the mitochondrial genome (mtDNA), beginning at the tRNA Isoleucina (I). Following the mtDNA partitions, the 45S ribosomal cassette genes are also in the order they appear in, beginning with the rDNA 18S. bp= base pairs, AA = Amino Acids.

Genes	Identical % bp	Pairwise % Identity	Identical % AA	AA % Pairwise Identity	GC %	Mean Length	Length Std. Dev	Gene Type	Gene Direction	Partition-finder Scheme
mtDNA										
Isoleucina (I)	78.3	93.1	-	-	31.1	65.9	1.1	tRNA	F	GTR+I+G
Glutamine (Q)	76.8	93.8	-	-	29	69	0	tRNA	R	GTR+I+G
Methionine (M)	84.1	96.2	-	-	32.5	68.8	0.4	tRNA	F	GTR+I+G
ND2	60.3	87.2	62.2	87.7	26.6	1023	0	CDS	F	GTR+I+G
Tryptophane (W)	82.9	95.4	-	-	23.9	68.1	0.5	tRNA	F	GTR+I+G
Cysteine (C)	62.7	87.1	-	-	35.1	64.8	1.3	tRNA	R	GTR+I+G
Tyrosine (Y)	76.1	94	-	-	35.1	65.3	0.5	tRNA	R	GTR+G
COI	70.7	90.1	92.6	98.2	32.7	1503	0	CDS	F	GTR+I+G
Leucine (L2)	81.8	95.9	-	-	29.3	66	0	tRNA	F	GTR+I+G
COII	68.7	90.6	83.7	96.2	30.6	684	0	CDS	F	GTR+I+G
Aspartic (D)	85.1	95.7	-	-	24.3	65.4	0.6	tRNA	F	GTR+I+G
Lysine (K)	88.7	97.4	-	-	30.5	71	0	tRNA	F	GTR+I+G
ATP8	59.3	87.6	60.4	84.2	20.1	162	0	CDS	F	GTR+I+G
ATP6	65.5	89	80.4	94.9	26.2	678	0	CDS	F	GTR+G
COXIII	68.1	89.8	82.5	95.4	32.3	792	0	CDS	F	GTR+I+G
Glycine (G)	73.1	94.3	-	-	23.9	66.8	0.4	tRNA	F	GTR+G

ND3	61	88.1	69.2	91.2	26.6	354	0	CDS	F	GTR+G
Alanine (A)	80.6	93.3	-	-	28.4	67	0	tRNA	F	GTR+G
Arginine (R)	58.9	87.4	-	-	33	66.4	1.3	tRNA	F	GTR+I+G
Asparagine (N)	82.6	82.6	-	-	25.4	66.9	1.1	tRNA	F	GTR+I+G
Serine (S1)	91	97.9	-	-	29.5	67	0	tRNA	F	GTR+I+G
Glutamic (E)	78.6	93.2	-	-	10.3	66.5	1.1	tRNA	F	GTR+I+G
Phenylalanine (F)	79.1	93.8	-	-	25.7	65.8	0.4	tRNA	R	GTR+I+G
ND5	69.9	91.2	76.6	93	24.5	1734.33	1.4	CDS	R	GTR+I+G
Histidine (H)	74.3	91.9	-	-	27	66.5	1.1	tRNA	R	GTR+I+G
ND4	70	91.7	77.5	93.6	25.4	1335	0	CDS	R	GTR+I+G
ND4L	71.4	92.5	73.2	92.3	21.3	293.7	1.4	CDS	R	GTR+I+G
Threonine (T)	75.7	93.3	-	-	33	70.5	0.7	tRNA	F	GTR+I+G
Proline (P)	63.6	88.2	-	-	36.8	64.3	0.8	tRNA	R	GTR+I+G
ND6	59.8	88.7	61.4	88.4	19	520.2	1.5	CDS	F	GTR+I+G
CYTB	69.6	90.4	87.9	96.3	28.1	1139.7	0.9	CDS	F	GTR+G
Serine (S2)	91.4	97.8	-	-	23.8	70	0	tRNA	F	GTR+I+G
ND1	71.9	91.7	82.8	94.9	25	945	0	CDS	R	GTR+I+G
Leucine (L1)	75.8	94	-	-	22	65.7	0.6	tRNA	R	GTR+G
16S	74.2	92.4	-	-	24.6	1378.9	2.7	rDNA	R	GTR+I+G
Valine (V)	73.6	93.1	-	-	28.2	71.1	0.2	tRNA	R	GTR+G
12S	71.6	92.1	-	-	28	780.9	5.5	rDNA	R	GTR+I+G
Control Region (D-loop)	20.6	62.8	-	-	13.9	767.4	111.2	-	F	GTR+I+G
mtDNA CDS Concatenated	67.5	90.1	-	-	26.9	11172.1	4.1	CDS	-	-
mtDNA rDNA Concatenated	73	92.2	-	-	25.9	2159.7	6.7	rDNA	-	-
mtDNA tRNA Concatenated	76.2	92.9	-	-	27.8	1496.3	6.7	tRNA	-	-

All mtDNA (no D-loop)	68.2	90.4	-	-	27.1	14557.5	23.9	-	-	-
All mtDNA	65.2	88.8	-	-	26.5	15335	117.5	-	-	-
45S Cassette										
18S	98.2	99.6	-	-	52.4	1945.4	0.7	rDNA	F	GTR+I+G
ITS1	66.6	87.3	-	-	58	442.2	11.3	ITS	F	GTR+I+G
5.8S	100	100	-	-	57.7	163	0	rDNA	F	GTR+I+G
ITS2	71.5	89.4	-	-	62.4	175.4	5.5	ITS	F	GTR+G
28S	98.2	99.5	-	-	59.4	4249.9	5	rDNA	F	GTR+I+G
45S ITS concatenated	68.0	87.9	-	-	59.3	617.6	14.4	ITS	-	-
45S rDNA concatenated	98.2	99.5	-	-	57.4	6260.3	5.2	rDNA	-	-
45S Cassette Concatentated	95.3	98.4	-	-	57.6	6877.9	16.9	-	-	-
Histone										
H3	87.5	96.9	-	-	67.5	743.5	0.9	Histone	-	GTR+I+G
Total evidence	74.5	91.9	-	-	37.1	22956.3	124.8	-	-	-

(92.9%) when compared to rDNAs (92.2%) and protein coding regions (90.1%). Among protein coding genes, MPPI of translated amino acid sequences ranged from 84.2% (ATP6) to 98.2% (COI).

Mean pairwise percent identity across 45S cassette sequences was higher than that of the mtDNA genomes (98.4%), with *A. crassicauda* and *A. crassicauda* (DP) again being the most similar (PV = 0.3%), while *Russalpia sp.* and *B. collinus*, and *T. tasmaniensis* and *A. crassicauda* had the least similar 45S cassettes (PV = 2.2%) (Supplementary Table 2.3) (Supplementary Figure 2.3). Mean pairwise percent identity of individual 45S cassette gene alignments ranged from 87.3% (ITS1) to 100% (5.8S). When concatenated, the rDNAs were 10% higher in MPPI than the concatenated ITS regions (99.5% and 87.9% respectively). Mean pairwise percent identity was also high among Histone sequences (96.9%), with *B. nivalis* (FP) and *P. dugdali* being the most similar (PV = 0.5%), and *P. dugdali* and *S. campestris* being the most diverged (PV = 5.1%) (Supplementary Table 2.4)(Supplementary Figure 2.4).

Phylogenetic analysis

The following phylogenetic analyses were made with alignments consisting of 14 New Zealand and two Tasmanian alpine species; these alignments contain no gaps or ambiguities. Alignments of entire mtDNA genomes were initially investigated, both with and without repeat regions (D-loop); they were 14,516bp and 14,176bp in length respectively. D-loop is an A+T rich repeat region which can be problematic to assemble. Because it can be difficult to confirm site homology in parts of the D-loop alignment, phylogenetic analyses of mtDNA were carried out with and without D-loop (these datasets are referred to as ‘complete mtDNA+D-loop’ and ‘complete mtDNA’). There was agreement in tree topology between the ML and BI trees generated for the ‘complete mtDNA+D-loop’ alignment (Figure 2.5A). Almost half of the nodes (6/13) among the New Zealand taxa had ML bootstrap (MLB) values of 100 and Bayesian posterior probabilities (BIPP) of 1. Topology between the two alignments was near identical, although MLB and BIPP support values were slightly better in the absence of D-Loop. For example, seven New Zealand nodes had MLB and BIPP values of 1 and 100 (Figure 2.5B).

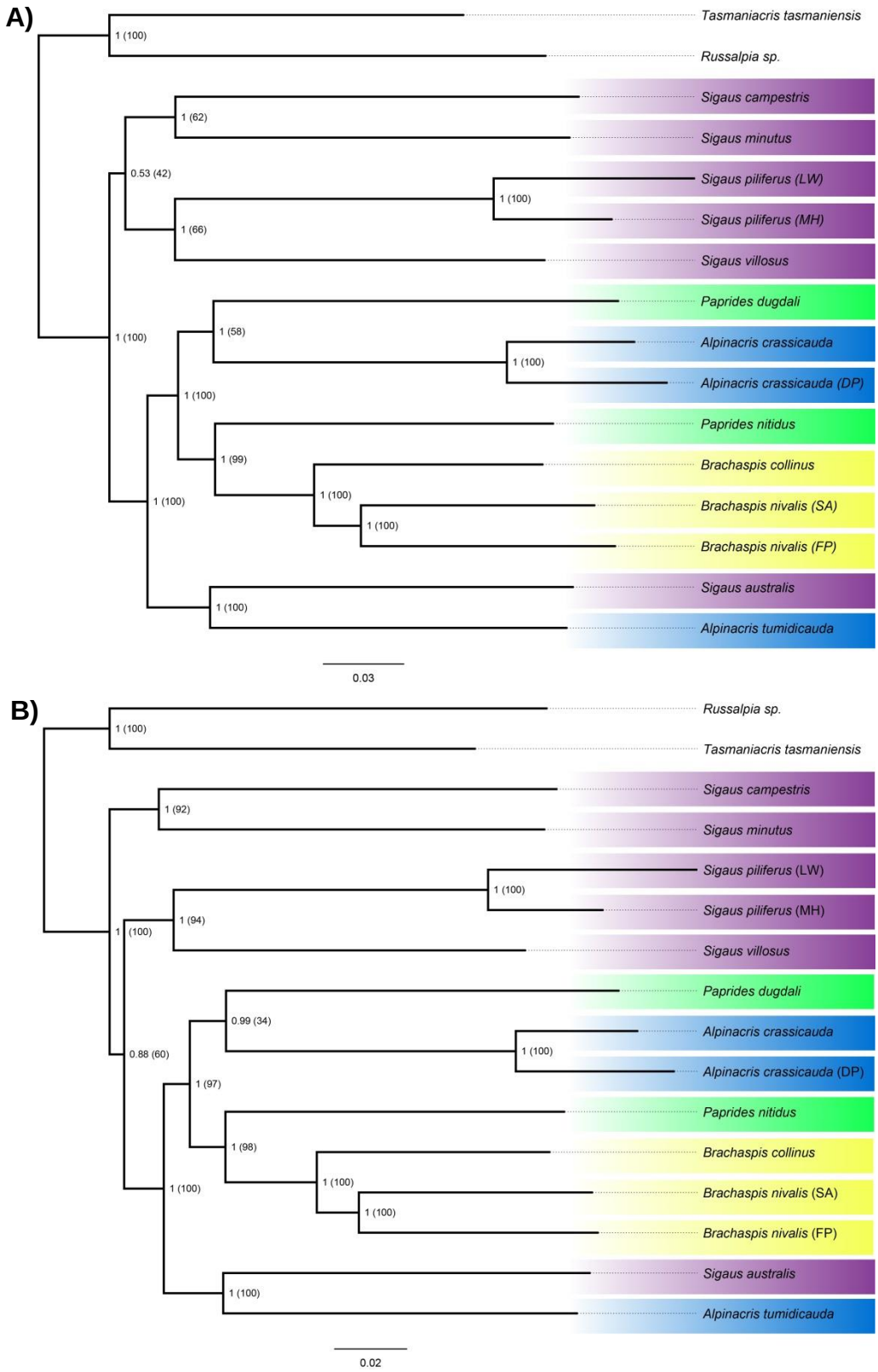

Figure 2.5 Phylogenetic hypotheses for the New Zealand alpine grasshoppers inferred from mitochondrial DNA sequences**.** Combined Bayesian Inference and Maximum Likelihood phylogenetic trees of mitochondrial genome alignments with (**A)** and without (**B)** A+T rich repeat regions (D-loop). These alignments consisted of 16 aligned sequences, and were **A)** 14,516bp and **B)** 14,176bp in length. *Tasmaniacris tasmaniensis* and *Russalpia sp.* were enforced as the outgroup. Posterior probabilities are shown at nodes, with ML support values in brackets. *Alpinacris* are highlighted in blue; *Brachaspis* yellow; *Paprides* green; and *Sigaus* purple.

Neither of the phylogenetic trees generated from these alignments support the current taxonomy of four monophyletic genera, with *Alpinacris*, *Paprides* and *Sigaus* found to be polyphyletic in both instances. The three *Brachaspis* individuals were monophyletic, and sister to *P. nitidus*. The 'complete mtDNA+D-loop' dataset inferred a poorly supported clade with five *Sigaus* taxa (*S. campestris*, *S. minutus*, *S. piliferus* (LW), *S. piliferus* (MH), *S. villosus*), but this was not resolved by the 'complete mtDNA' dataset. However, both datasets supported a clade with seven taxa (*P. dugdali*, *A. crassicauda*, *A. crassicauda* (DP), *P. nitidus*, *B. collinus*, *B. nivalis* (SA), *B. nivalis* (FP)), sister to the clade of *S. australis* and *A. tumidicauda*.

Maximum Likelihood and BI trees generated from the alignment of 45S Ribosomal cassettes provide no support for current taxonomic groupings. The 45S Ribosomal cassette alignments were 6,713bp in length. MLB and BIPP values were much lower than for the mtDNA genome trees, with only four nodes having MLB and BIPP values of 100 and 1 (Figure 2.6). Topology between the ML and BI, and between these and the mtDNA trees is also different, however monophyly of the three *Brachaspis* individuals remains, along with the groupings of *S. campestris* and *S. minutus*, the two *S. piliferus* and the two *A. crassicauda*. The ML and BI trees generated from the H3 alignments (744bp in length) are also incongruent and have poor support, with only two nodes having MLB and BIPP values of 100 and 1 (Figure 2.7). Monophyly of the *Brachaspis* group, the two *S. piliferus* and two *A. crassicauda* can still be found in these trees, however.

Further investigations of partitions within the mtDNA genomes were also carried out, with the separate alignments of 13 protein coding genes, 22 tRNAs, and of the two rDNAs. The lengths of these alignments were 11,183, 1,551 and 2,218bp respectively. The topologies of the ML and BI trees generated from the protein coding gene alignment were congruent, and were also identical to the topology of the 'complete mtDNA' tree (Figure 2.8).

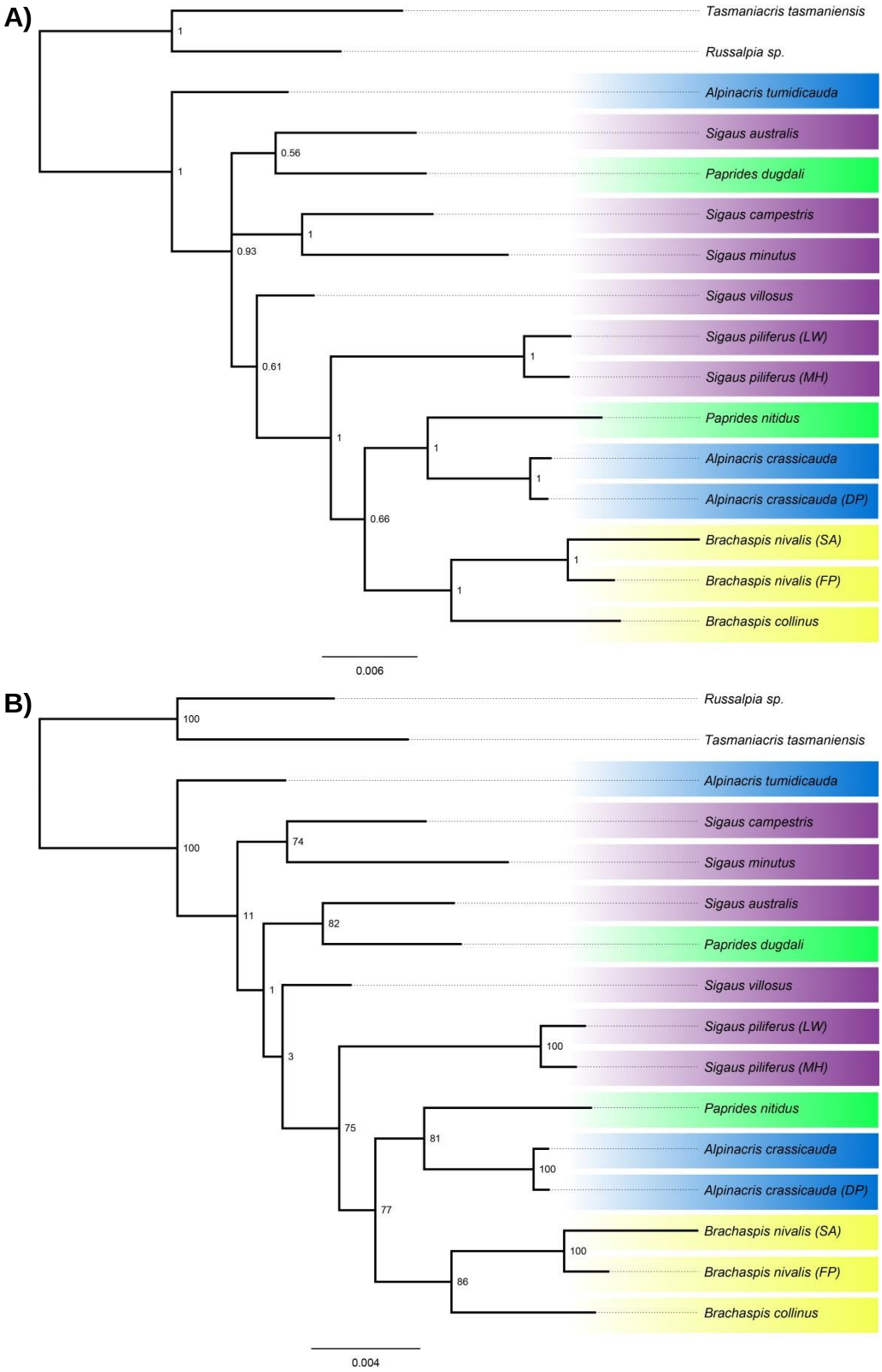

Figure 2.6 Phylogenetic hypotheses for the New Zealand alpine grasshoppers infered from nuclear DNA sequences, **A)** Bayesian Inference and **B)** Maximum Likelihood phylogenetic trees of the 6,713bp 45S Ribosomal cassette alignment. *Tasmaniacris tasmaniensis* and *Russalpia sp.* were enforced as the outgroup. Posterior probabilities are shown at the nodes for the BI and ML analyses analyses, respectively. *Alpinacris* are highlighted in blue; *Brachaspis* yellow; *Paprides* green; and *Sigaus* purple.

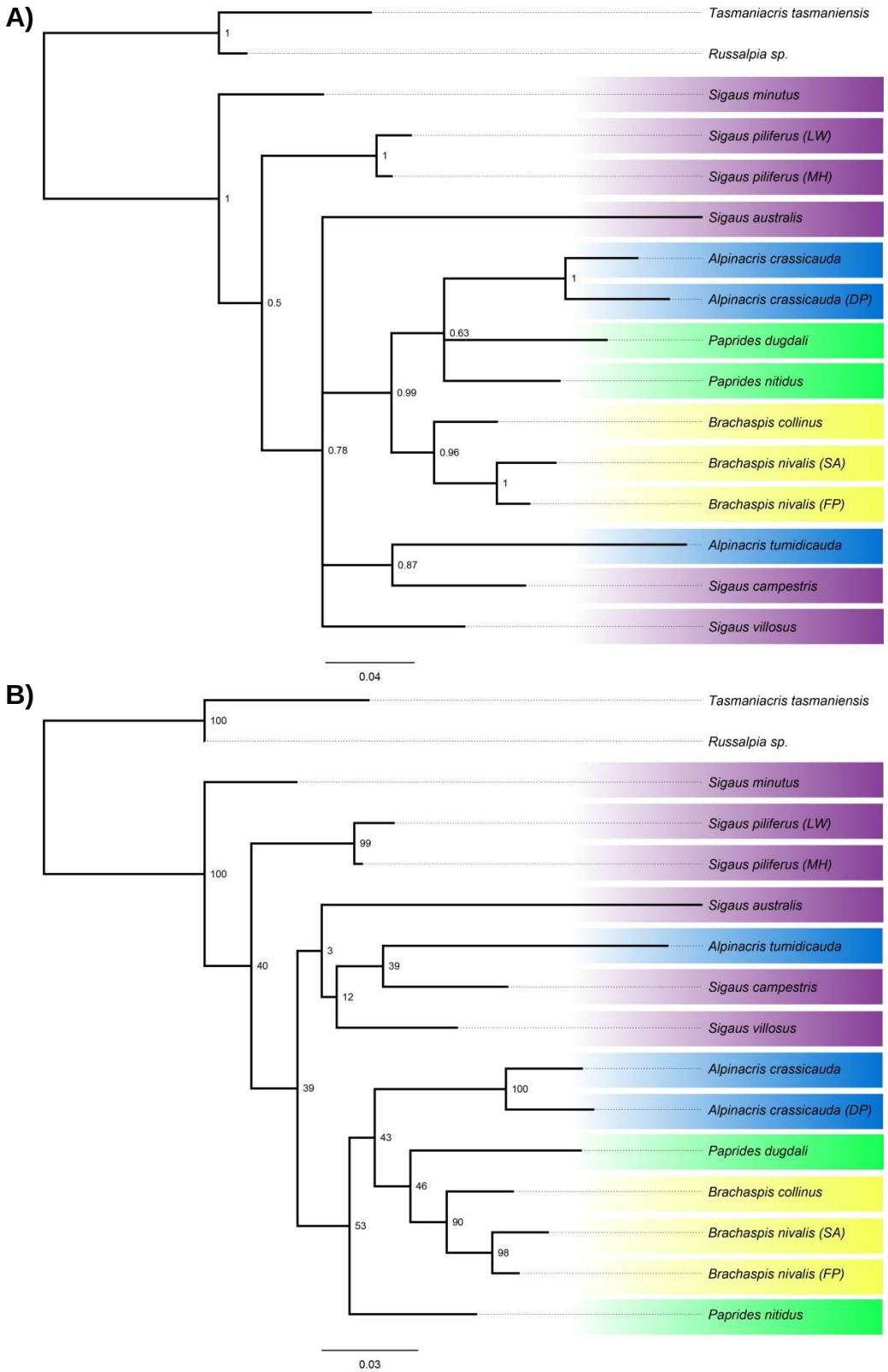

Figure 2.7 Phylogenetic hypotheses for NZ alpine grasshoppers inferred from nuclear DNA sequences **A)** Bayesian Inference and **B)** Maximum Likelihood phylogenetic trees of the 744bp Histone 3 (H3) alignment. *Tasmaniacris tasmaniensis* and *Russalpia sp.* were enforced as the outgroup. Posterior probabilities are shown at the nodes for the BI and ML analyses, respectively. *Alpinacris* are highlighted in blue; *Brachaspis* yellow; *Paprides* green; and *Sigaus* purple.

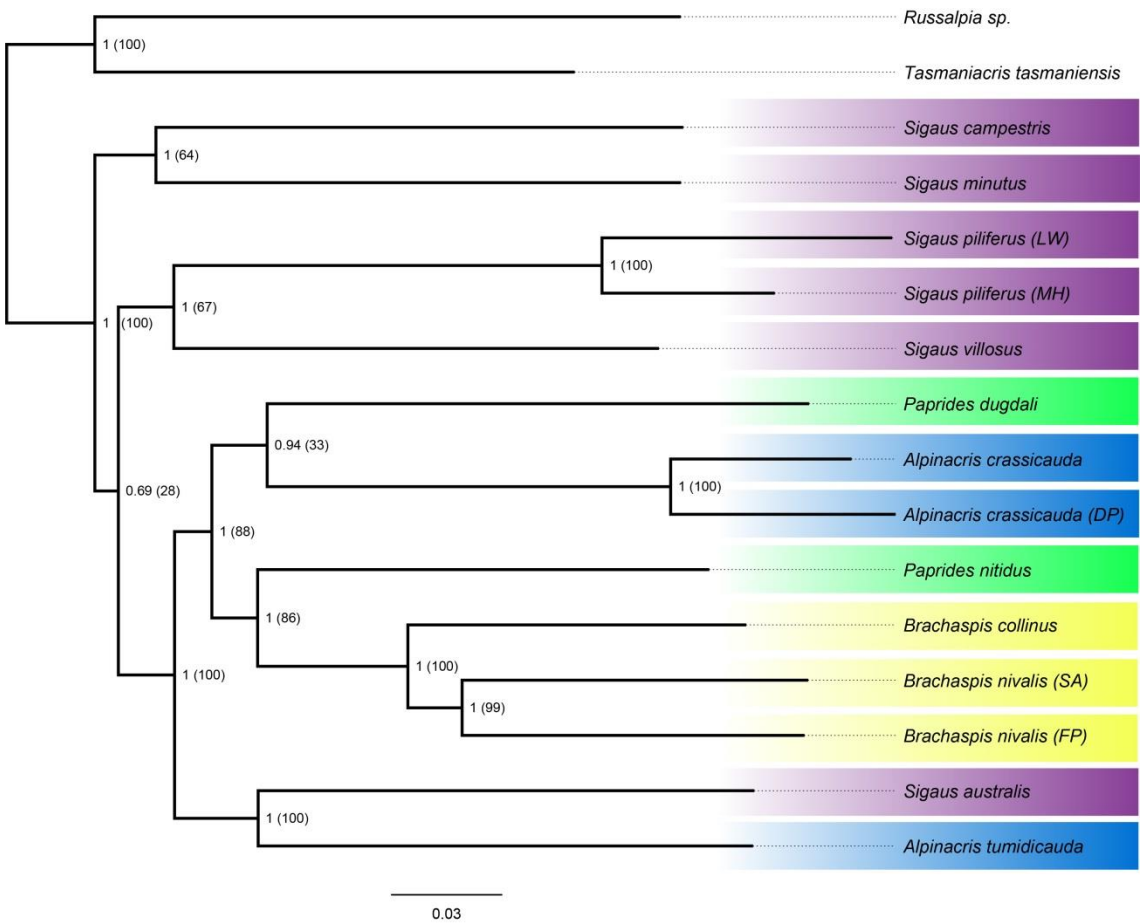

Figure 2.8 Phylogenetic hypothesis for NZ alpine grasshoppers inferred from mitochondrial DNA . A combined Bayesian Inference and Maximum Likelihood phylogenetic trees of the 11,183bp protein coding gene alignment is shown. *Tasmaniacris tasmaniensis* and *Russalpia sp.* were enforced as the outgroup. Posterior probabilities are shown at nodes, with ML support values in brackets. *Alpinacris* individuals are shaded in blue; *Brachaspis* yellow; *Paprides* green; and *Sigaus* purple.

Support values for the protein coding tree were overall lower than those of the 'complete mtDNA' tree, and had one fewer node with MLB and BIPP values of 100 and 1. Topologies of the ML and BI trees obtained with the tRNA alignments were not identical, but monophyly of the *Brachaspis* group, *S. campestris* with *S. minutus,* the two *S. piliferus* and two *A. crassicauda* remained (Figure 2.9). Only two nodes had MLB and BIPP values of 1 and 100. The concatenated rDNA ML and BI trees produced were incongruent, also only had two nodes with MLB and BIPP values of 1 and 100, and monophyly only remained between the *Brachaspis* group, the two *S. piliferus* and two *A. crassicauda* (Figure 2.10).

Gene trees

Forty-four BI phylogenetic gene trees consisting of 13 protein coding genes, 22 tRNAs, five rDNAs, two ITS regions, H3 and D-loop alignments were produced from this data set (Figure 2.11, 2.12, 2.13). The topology of each was compared with that of the 'complete mtDNA' phylogenetic tree. Of these, 42 alternative tree topologies were returned, none of which demonstrated the same phylogenetic relationships as the 'complete mtDNA' tree. Only two gene trees (16s and NAD2) demonstrated purely dichotomous branching, with the remainder displaying some degree of polytomy. The grouping of previously well-supported pairs from the prior phylogenetic analyses (*T. tasmaniensis* and *Russalpia sp.*, *A. crassicauda* and *A. crassicauda* (DP), *B. nivalis* (SA) and *B. nivalis* (FP) and *S. piliferus* (LW) and *S. piliferus* (MH)) was also investigated. In 21 instances at least one of these species pairs was not found together. The difference in phylogenetic signal contained in each gene tree was clear in a SplitsTree Consensus network, and this indicated congruence in signal, but very different levels of resolution (Figure 2.14). Summary statistics for each individual gene alignment are summarised in Table 2.7.

Total evidence analysis

A final alignment of 22,164bp concatenating all 38 mtDNA and 6 nuclear genes provided a total evidence dataset. Mean pairwise percent identity was found to be 91.9%, with *A. crassicauda* and *A. crassicauda* (DP) being predictably the most similar (PV = 3.5%), and *Russalpia sp.* and *A. crassicauda* (DP) the least similar (PV = 10%)

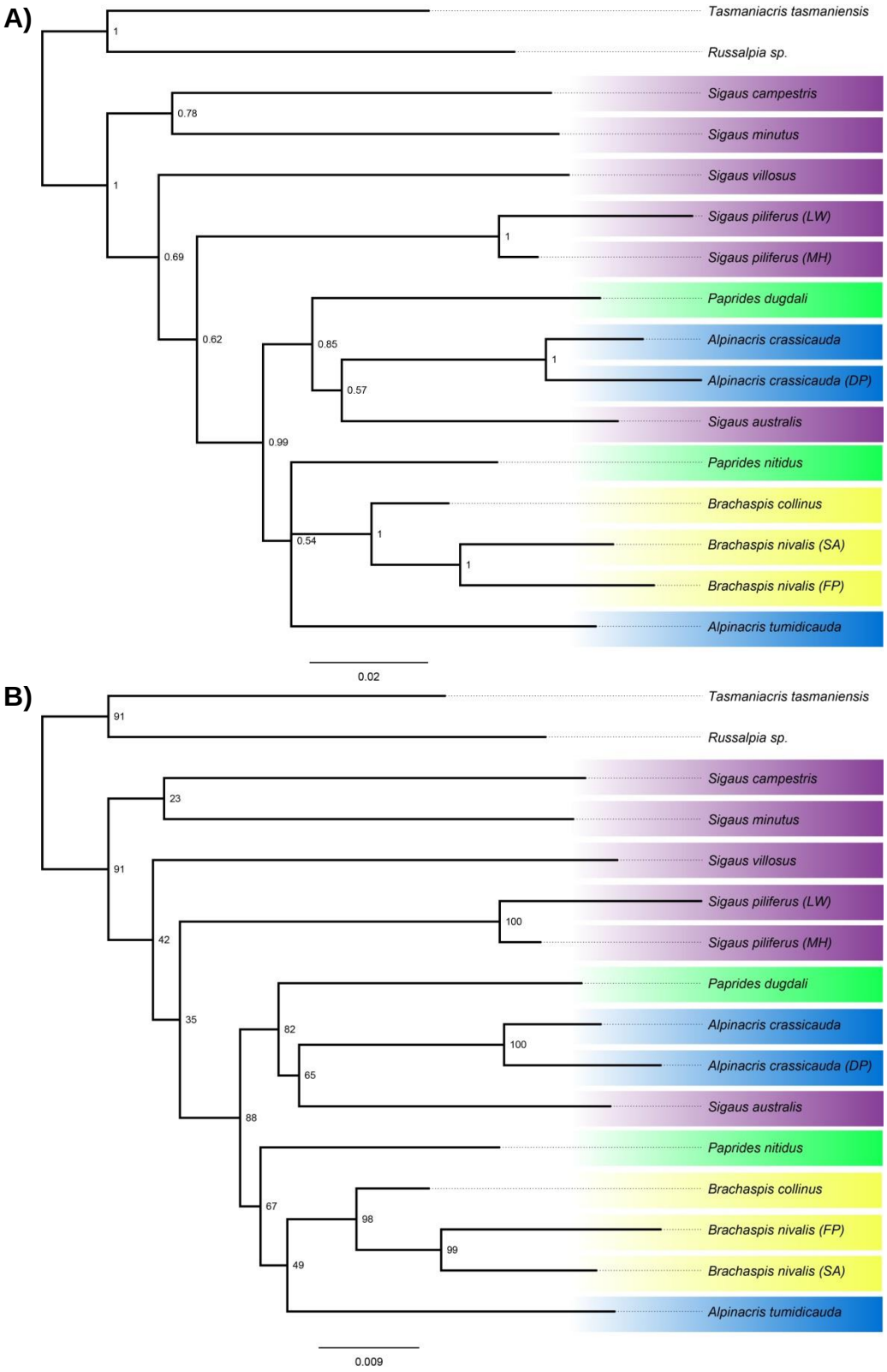

Figure 2.9 Phylogenetic hypotheses for NZ alpine grasshoppers inferred from mitochondrial DNA. **A)** Bayesian Inference and **B)** Maximum Likelihood phylogenetic trees of the 1,551bp tRNA alignment are shown. *Tasmaniacris tasmaniensis* and *Russalpia sp.* were enforced as the outgroup. Posterior probabilities are shown at the nodes for the BI and ML analyses, respectively. *Alpinacris* individuals are shaded in blue; *Brachaspis* yellow; *Paprides* green; and *Sigaus* purple.

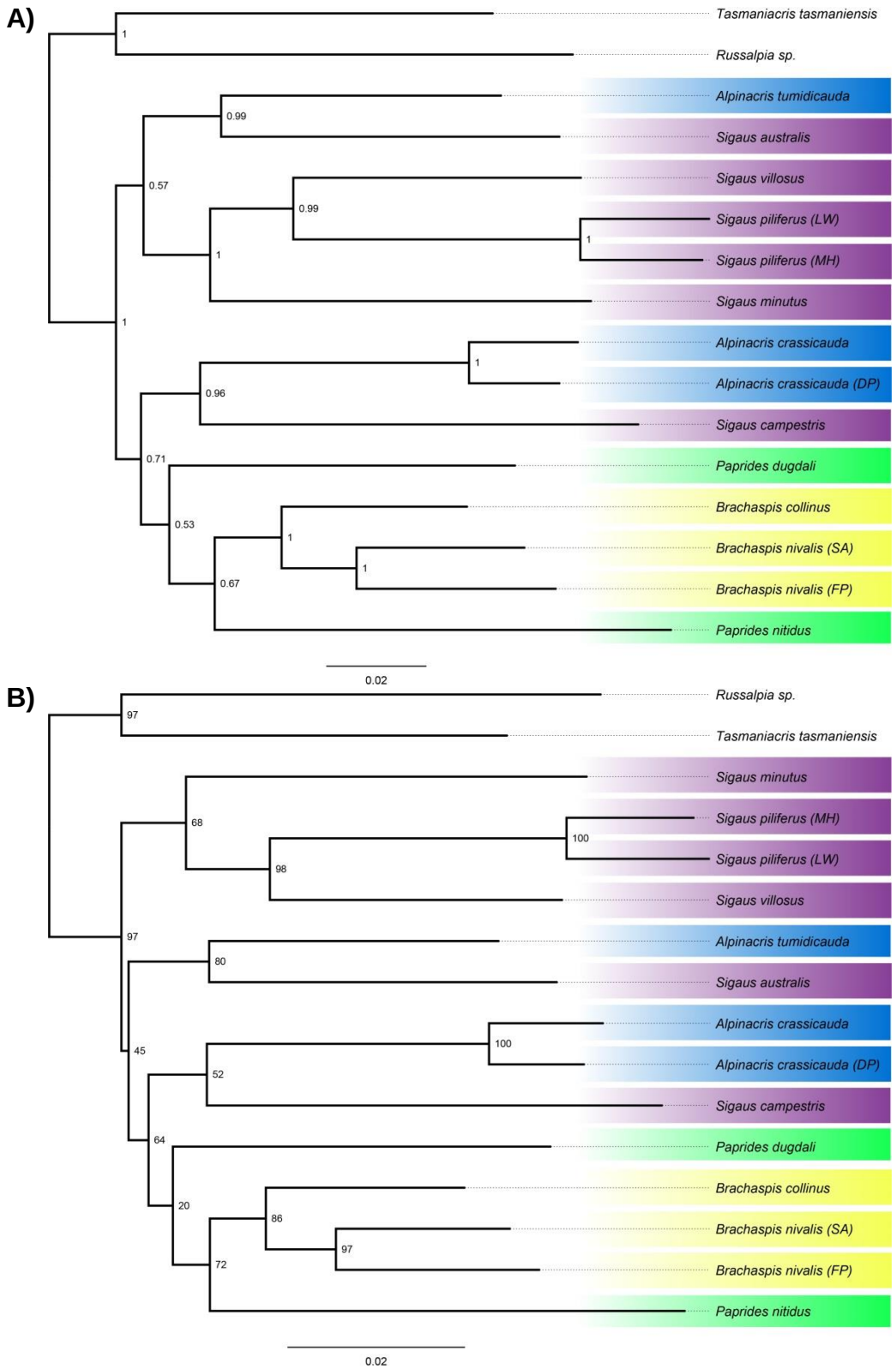

Figure 2.10 Phylogenetic hypotheses for NZ alpine grasshoppers inferred from mitochondrial DNA. **A)** Bayesian Inference and **B)** Maximum Likelihood phylogenetic trees of the 2,218bp rDNA alignment are shown. *Tasmaniacris tasmaniensis* and *Russalpia sp.* were enforced as the outgroup. Posterior probabilities are shown at the nodes for the BI and ML analyses, respectively. *Alpinacris* individuals are shaded in blue; *Brachaspis* yellow; *Paprides* green; and *Sigaus* purple.

(Supplementary Table 2.5) (Supplementary Figure 2.5). The topologies of the ML and BI trees generated from the total evidence data (Figure 2.15) were identical to that of the mtDNA genome 'complete mtDNA+D-loop' tree (Figure 2.5A). Similar to the 'complete mtDNA' tree (Figure 2.5B), seven nodes had MLB and BIPP values of 100 and 1; however other nodes had very low MLB support. The only difference between these trees is the position of *S. campestris* and *S. minutus* in regards to being positioned as a sister clade to the remaining taxa or falling within, regardless, both topologies confirm that current taxonomic grouping into the four genera (*Alpinacris*, *Brachaspis*, *Paprides* and *Sigaus*) does not adequately reflect the evolutionary history of the taxa.

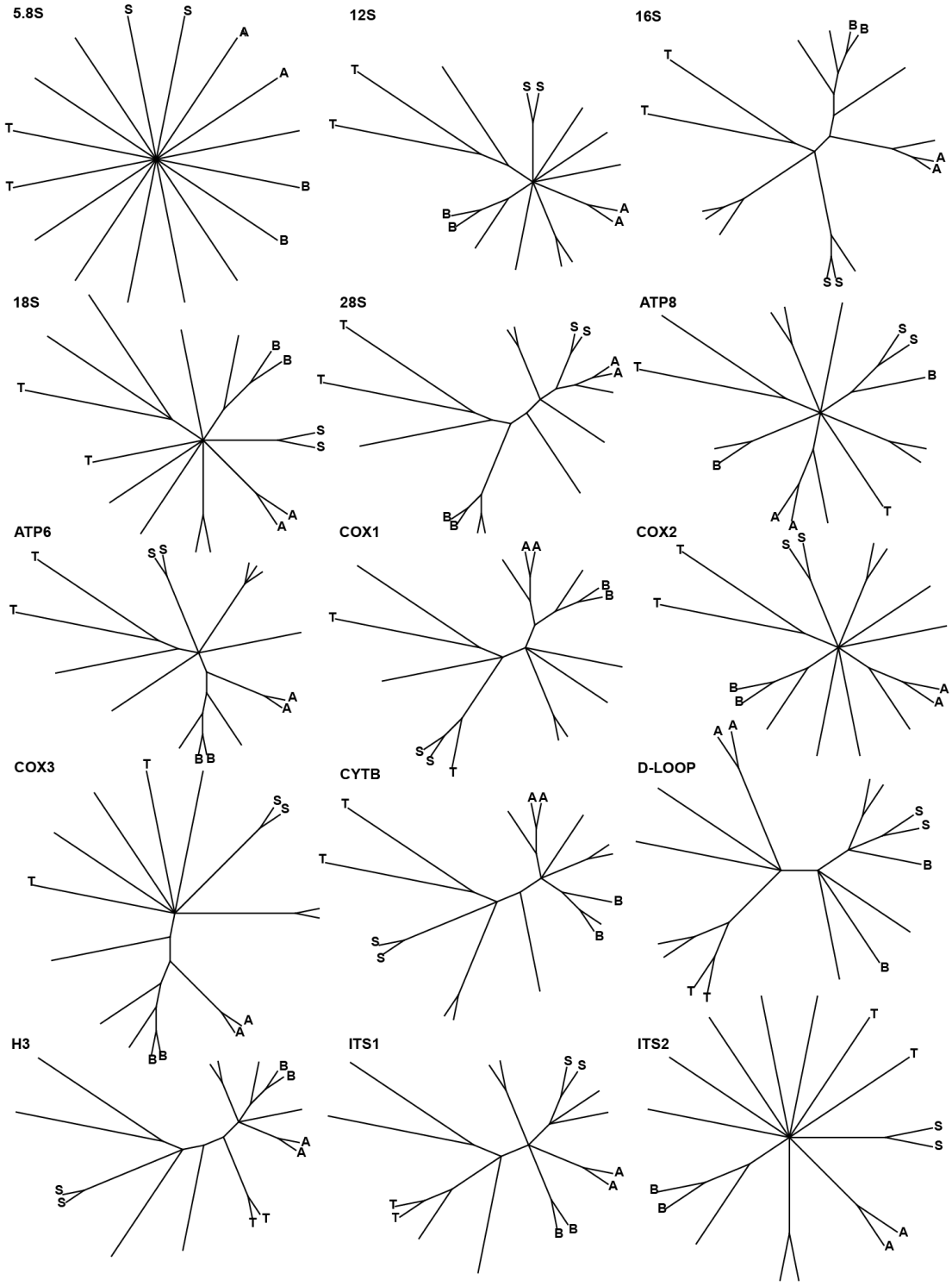

Figure 2.11 Unrooted Bayesian Inference (BI) phylogenetic trees of 15/44 genes. Pairs of letters represent pairs of individuals that clustered together in prior phylogenetic analyses: A = *Alpinacris crassicauda* and *A. crassicauda* (DP); B = *Brachaspis nivalis* (SA) and *B. nivalis* (FP); S = *Sigaus piliferus* (LW) and *S. piliferus* (MH); and T = *Tasmaniacris tasmaniensis* and *Russalpia sp.*.

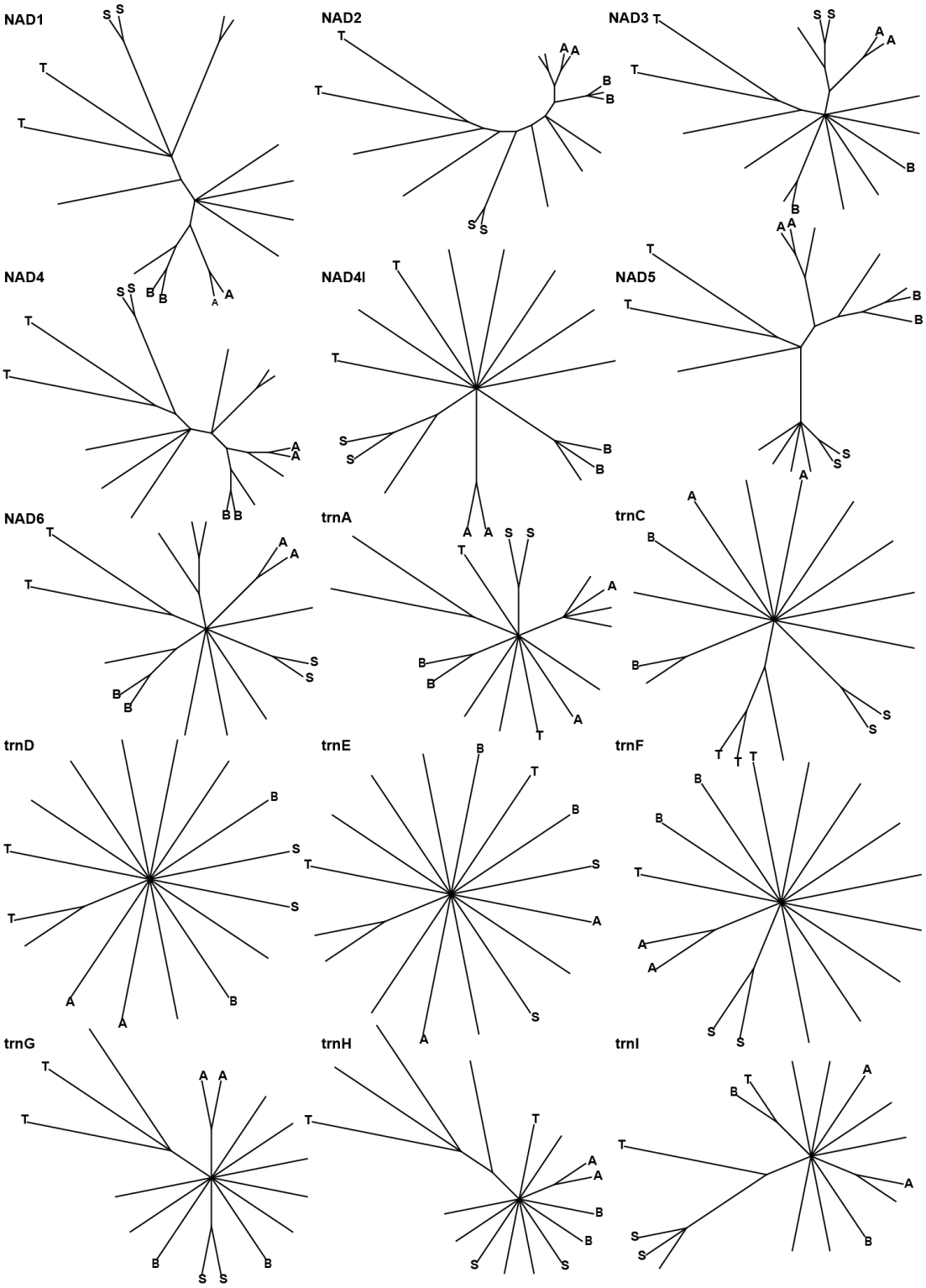

Figure 2.12 Unrooted Bayesian Inference (BI) phylogenetic trees of 15/44 genes. Pairs of letters represent pairs of individuals that clustered together in prior phylogenetic analyses: A = *Alpinacris crassicauda* and *A. crassicauda* (DP); B = *Brachaspis nivalis* (SA) and *B. nivalis* (FP); S = *Sigaus piliferus* (LW) and *S. piliferus* (MH); and T = *Tasmaniacris tasmaniensis* and *Russalpia sp.*.

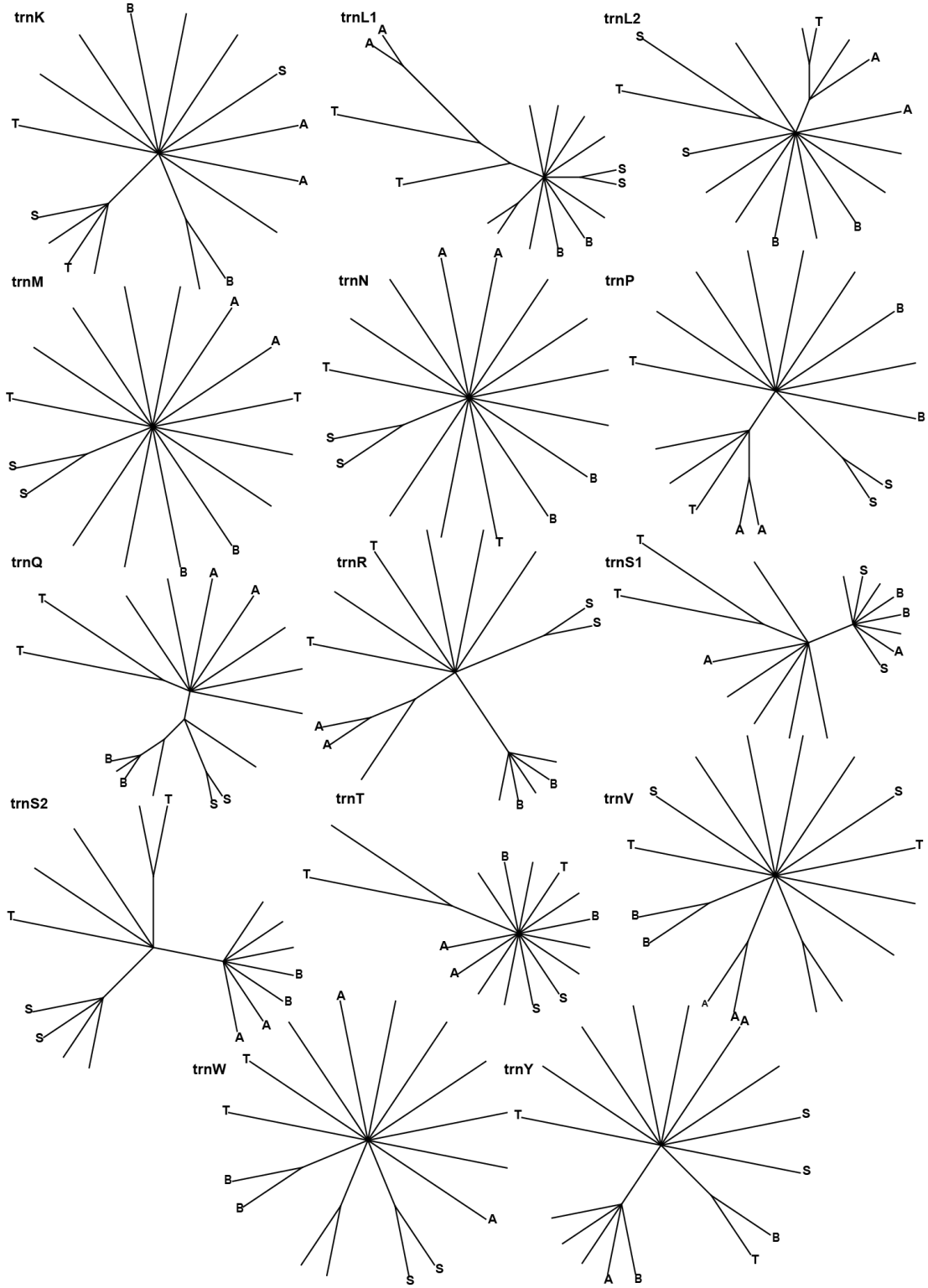

Figure 2.13 Unrooted Bayesian Inference (BI) phylogenetic trees of 14/44 genes. Pairs of letters represent pairs of individuals that clustered together in prior phylogenetic analyses: A = *Alpinacris crassicauda* and *A. crassicauda* (DP); B = *Brachaspis nivalis* (SA) and *B. nivalis* (FP); S = *Sigaus piliferus* (LW) and *S. piliferus* (MH); and T = *Tasmaniacris tasmaniensis* and *Russalpia sp.*.

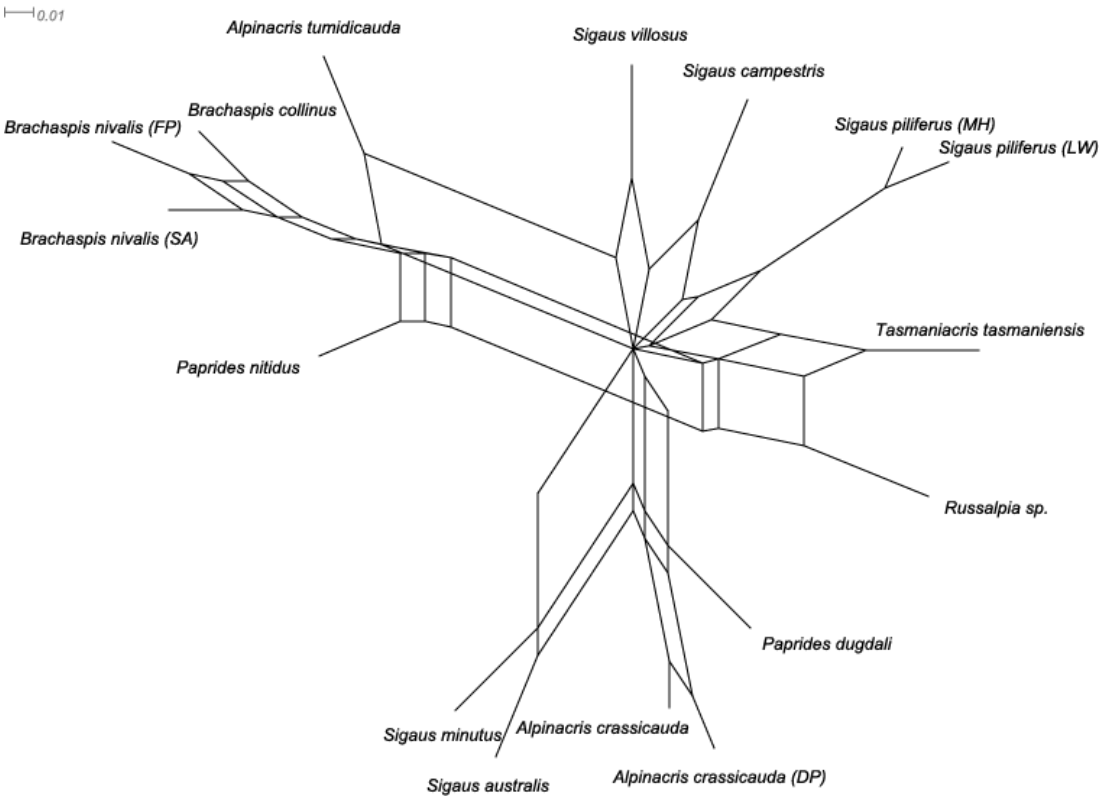

Figure 2.14 SplitsTree network of 44 Bayesian Inference gene trees. Splits were generated using the Consensus network algorithm in SplitsTree4.

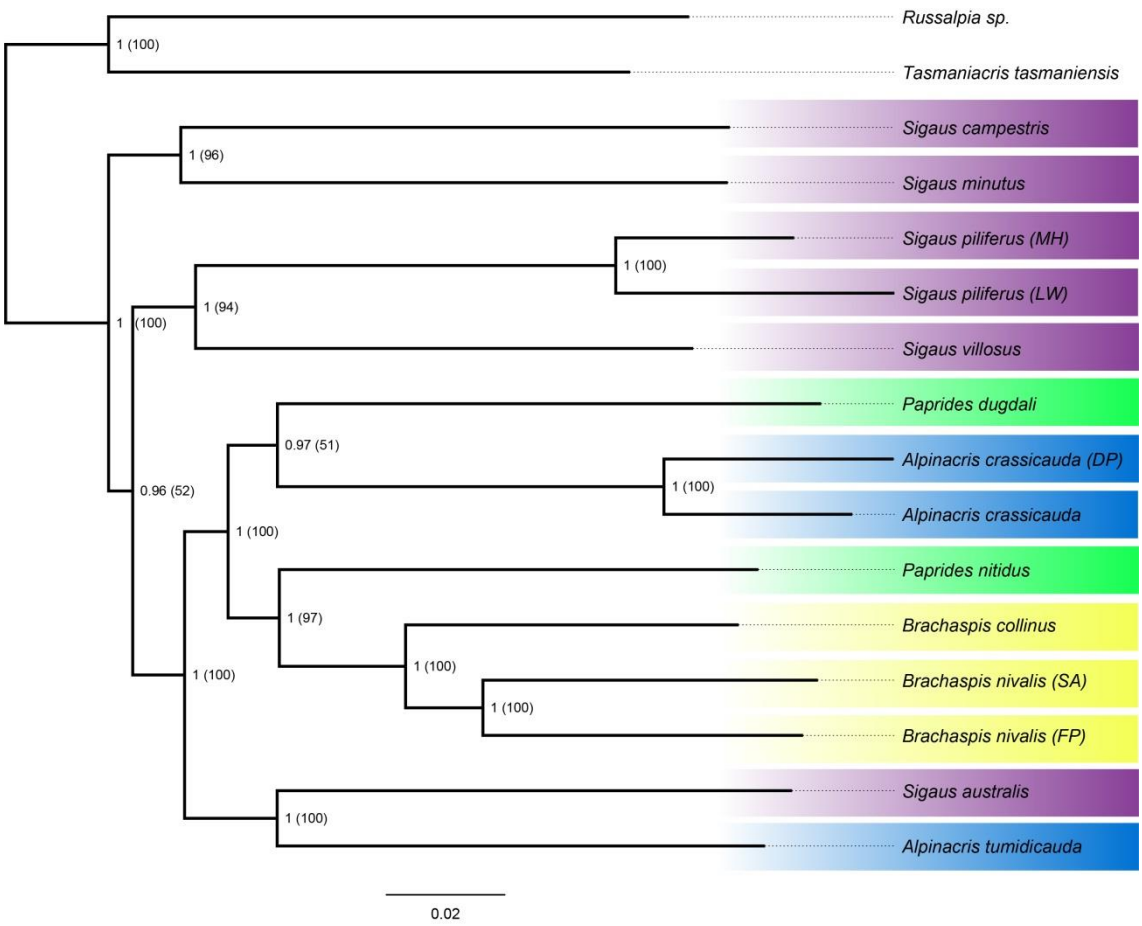

Figure 2.15 Phylogenetic hypotheses for NZ alpine grasshoppers inferred from mitochondrial and nuclear DNA. A combined Bayesian Inference and Maximum Likelihood phylogenetic trees of the 22,164bp total evidence alignment. *Tasmaniacris* tasmaniensis and *Russalpia sp.* were enforced as the outgroup. Posterior probabilities for both analyses are shown, ML values are in brackets (1000 bootstraps). *Alpinacris* individuals are shaded in blue; *Brachaspis* yellow; *Paprides* green; and *Sigaus* purple.

Discussion

Gene tree analysis

To estimate phylogenies of closely related species, it is common practice to use data from one or two mitochondrial genes such as COI and/or 12S (e.g. in New Zealand invertebrates: Doddala *et al*. 2015, Goldberg *et al*. 2015, Hills *et al*. 2012, Trewick 2001, Trewick 2008, Trewick & Morris 2008). However, resolution of deeper level relationships is often hampered by functional constraint on genes and gene regions that limit their phylogenetic resolving power. If I had used DNA sequences from just these two genes, the grasshopper phylogeny presented would have been misleading, and not representative of the evolutionary relationships of these species. Of the 44 gene trees examined, 42 alternative phylogenies were produced; none reflected the full set of relationships shown by the mitochondrial genome or total evidence phylogenies. Failure of data from individual genes to adequately resolve relationships between species was likely due to substitutional saturation or excessive functional conservation, resulting in too much noise or insufficient phylogenetic signal (Halanych & Robinson 1999, Lopez *et al*. 1999, Philippe & Fonterre 1999, Rokas *et al*. 2003, Simon *et al.* 1994). However, when combined, these factors balanced out and enough signal was resolved to produce a robust phylogeny (Rokas *et al*. 2003)

This is the first study to comprehensively examine the phylogenetic relationships of all of New Zealand's endemic alpine grasshoppers, and to consider the relevance of their current systematic treatment. High-throughput DNA sequencing provided a suitable way to rapidly generate sufficient data, with the potential to resolve their evolutionary relationships. Maximum Likelihood and Bayesian Inference do not support the partitioning of 13 New Zealand species into separate monophyletic clades that coincide with the four currently recognised genera. Species of *Alpinacris*, *Paprides* and *Sigaus* were not reciprocally monophyletic, with for example *Sigaus australis* being strongly supported as sister to *Alpinacris tumidicauda*. The three *Brachaspis* taxa were found to form a monophyletic clade sister to *Paprides nitidus*. The phylogenetic analysis providing the best supported nodes (Figure 2.5B), indicates five clades: A) *S. campestris* and *S. minutus*, B) *S. piliferus* (LW), *S. piliferus* (MH) and *S. villosus*, C) *P.*

dugdali, *A. crassicauda* and *A. crassicauda* (DP), D) *P. nitidus*, *B. collinus*, *B, nivalis* (SA) and *B. nivalis* (FP), and E) *S. australis* and *A. tumidicauda.*

Taxonomy of New Zealand's alpine grasshoppers

Classifying New Zealand grasshoppers based on morphology has proven to be difficult, with a number of species being moved between genera, new genera being created to accommodate species and species complexes being exposed (Bigelow 1967, Dowle *et al.* 2014, Hutton 1897, Trewick 2001, Trewick 2008, Trewick & Morris 2008). My findings here support this complexity of classifying New Zealand alpine species, as neither groupings suggested by Bigelow (1967) or former groupings by Hutton (1897) were resolved (Table 2.2). For example, *S. villosus* was not found to be closely related to *Brachaspis*, although previously being classified as *B. villosus*; nor was *S. australis* found to be closely related to members of *Paprides*, despite formerly being classified in this genus. More recent taxonomy (Jamieson 1999), such as the division of *S. childi* (represented here by *S. australis*) from *S. minutus*, also demonstrates how morphological convergence between species can obscure phylogenetic relationships. The morphological characters previously used to distinguish genera (e.g. internal male genitalia) may not be adequately informative. One such example is *Sigaus*, which is described primarily on internal male genitalia. Bigelow (1967) himself noted that within *Sigaus*, apart from sharing similar internal genitalia, there were often other morphological similarities shared by *Sigaus* species that were not found universally between all *Sigaus* members, and these characters were often shared with other genera. Classifying grasshoppers using internal genitalia is common practice, however, in the case of New Zealand's alpine grasshoppers, a dependence on using internal genitalia to describe taxa may have concealed other, more diagnostic characters that may have more effectively resolved their taxonomy (Dirsh 1956a, 1956b, Song 2010).

Bigelow (1967) pointedly explained why he believed that internal male genitalia are the most useful trait in the classification of New Zealand grasshoppers, stating that the complexity of the structures makes them more evolutionarily informative than external traits which tend to display minor variation within and between species. There is similarity in internal genitalia of *Sigaus* and the Tasmanian genus *Russalpia*, members of *Sigaus* are sister to the rest of the New Zealand fauna, and members of *Sigaus* are

found in three of the five clades I established here. Perhaps the similarity in internal genitalia of *Sigaus* species is ancestral rather than a derived trait, and it is therefore not an appropriate identifier of a genus in this case. An intensive traditional and geometric morphometric analysis of an array of morphological features, analysed through modelling software to reduce human bias associated with traditional taxonomy, could help determine if there are morphological similarities among lineages within the clades identified here (Dowle *et al*. 2014, Sivyer *et al*. 2018).

In situations where phylogenetic analysis of substantial data indicates poly- or paraphyletic relationships, lumping and splitting alternatives are available for taxonomic reconciliation. Some examples in the New Zealand fauna are provided by recent treatments of native skinks and geckos which until recently were recognised as consisting of two genera each (Chapple *et al*. 2009, Nielson *et al*. 2011). The ~34 skink species are now recognised within a single endemic genus, *Oligosoma*, whereas ~36 gecko species have been assigned to seven endemic genera (Chapple *et al*. 2009, Nielson *et al*. 2011). Either approach could apply to these acridid grasshoppers. Currently, there are four recognised genera containing 13 species of endemic alpine grasshoppers in New Zealand. A single endemic New Zealand genus could be *Paprides* (Hutton 1897) or *Sigaus* (Hutton 1897) by virtue of precedence and availability (i.e. not used for an unrelated species). Alternatively, phylogenetic relationships could inform generic classification (Table 2.8) (Figure 2.5B). Ideally, correlations of morphological and ecological data with clades would help determine the best taxonomic groupings (if any) between these species. Additionally, as *Sigaus* is classified under the subtribe Russalpiina, it should be considered that all other endemic alpine grasshopper species from New Zealand be classified under this subtribe as well.

Biogeography re-examined

In regards to the distribution of these species, their original classification meant the distribution of species within two particular genera was disjointed. The two species of *Alpinacris* and the two species of *Paprides* in both instances had a species in southern South Island and a species in northern South Island, with no overlap between the species in central South Island. The disjunct distributions of these congeneric pairs

Table 2.8 Four suggestions for the possible re-classification of New Zealand's endemic alpine Acrididae based on the phylogenetic relationships established in this study.

Option 1: One genus named *Paprides* or *Sigaus*	Genus 1	*Alpinacris crassicauda* *Alpinacris tumidicauda* *Brachaspis collinus* *Brachaspis nivalis* *Brachaspis robustus* *Paprides dugdali* *Paprides nitidus* *Sigaus australis* *Sigaus campestris* *Sigaus childi* *Sigaus minutus* *Sigaus piliferus* *Sigaus villosus*
Option 2: Three genera, using three of the four names already in use: *Alpinacris*, *Brachaspis*, *Paprides* and *Sigaus*	Genus 1	*Sigaus campestris* *Sigaus minutus*
	Genus 2	*Sigaus piliferus* *Sigaus villosus*
	Genus 3	*Paprides dugdali* *Alpinacris crassicauda* *Paprides nitidus* *Brachaspis collinus* *Brachaspis nivalis* *Brachaspis robustus* *Sigaus australis* *Sigaus childi* *Alpinacris tumidicauda*
Option 3: Four genera, using the four names already in use: *Alpinacris*, *Brachaspis*, *Paprides* and *Sigaus*	Genus 1	*Sigaus campestris* *Sigaus minutus*
	Genus 2	*Sigaus piliferus* *Sigaus villosus*
	Genus 3	*Paprides dugdali* *Alpinacris crassicauda* *Paprides nitidus* *Brachaspis collinus* *Brachaspis nivalis* *Brachaspis robustus*
	Genus 4	*Sigaus australis* *Sigaus childi* *Alpinacris tumidicauda*
Option 4: Five genera, using the four genus names already in use and one new genus name.	Genus 1	*Sigaus campestris* *Sigaus minutus*
	Genus 2	*Sigaus piliferus* *Sigaus villosus*
	Genus 3	*Paprides dugdali* *Alpinacris crassicauda*
	Genus 4	*Paprides nitidus* *Brachaspis collinus* *Brachaspis nivalis* *Brachaspis robustus*
	Genus 5	*Sigaus australis* *Sigaus childi* *Alpinacris tumidicauda*

have been used to explore biogeography hypotheses in New Zealand (e.g. the beech gap) (Heads 1998, Trewick & Wallis 2001, Wallis *et al*. 2016). But distributions of sister species have been turned upside down with this new evidence. For example, *A. crassicauda* and *A. crassicauda* (DP) formed a clade with *P. dugdali*, showing the same southern and northern South Island disjunction, although involving different species pairs. *Paprides nitidus* is sister to the *Brachaspis* species, which have distributions restricted to Otago and Canterbury respectively. And the sister of the Otago *Alpinacris tumidicauda* is not in the north, but is the widespread *Sigaus australis*, found throughout Otago and Canterbury. Although the distribution of related species is used by biogeographers to infer evolutionary processes (Warren *et al.* 2014), this study is a good example of how taxonomy can be misleading about species relationships, leading to erroneous conclusions.

Sigaus piliferus is the sole alpine grasshopper species in the North Island of New Zealand. Large diversity of species in the South Island might be related to the larger alpine area available and lower extinction rates. Unexpectedly, the two *S. piliferus* populations are most closely related to *S. villosus*, a species restricted to habitat above 1800m and with one of the narrowest distributions of all New Zealand grasshopper species.

When the distributions of the five clades are examined, there are no geographic divides between them; all clades have some member species that occur in sympatry with members from other clades (Figure 2.16). Thus there is no readily apparent ecological or geographical partitioning of species-clades. Now that I have established the evolutionary relationship among species of New Zealand alpine grasshopper one can compare the distribution of related species to better understand their speciation history. Contrasting models of speciation (allopatric and sympatric) make different predictions about ranges of sister species (Warren *et al.* 2014), and the timing of species radiations in response to climatic and geological change can be tested. This new and robust molecular phylogeny is the first step to infer evolutionary processes within this radiation.

Conclusion

High-throughput DNA sequencing has proved a useful tool for assembling DNA sequences from both mitochondrial and nuclear markers even though grasshoppers have very large genomes (5-16pg, Rees *et al*. 1978). Extensive read coverage of both mitochondrial and nuclear markers allowed for a thorough examination of the genetic relatedness of the grasshopper species. It gave me the ability to investigate their phylogeny using both conserved and highly substituted genes, which were explored individually and as a complete total evidence unit. I have been able to determine that the current classification of 13 New Zealand endemic grasshopper species into four distinct genera is not supported by their genetic phylogeny, and that further investigation into the morphology and ecology of these species is required so that appropriate reclassifications of these species and genera can be made.

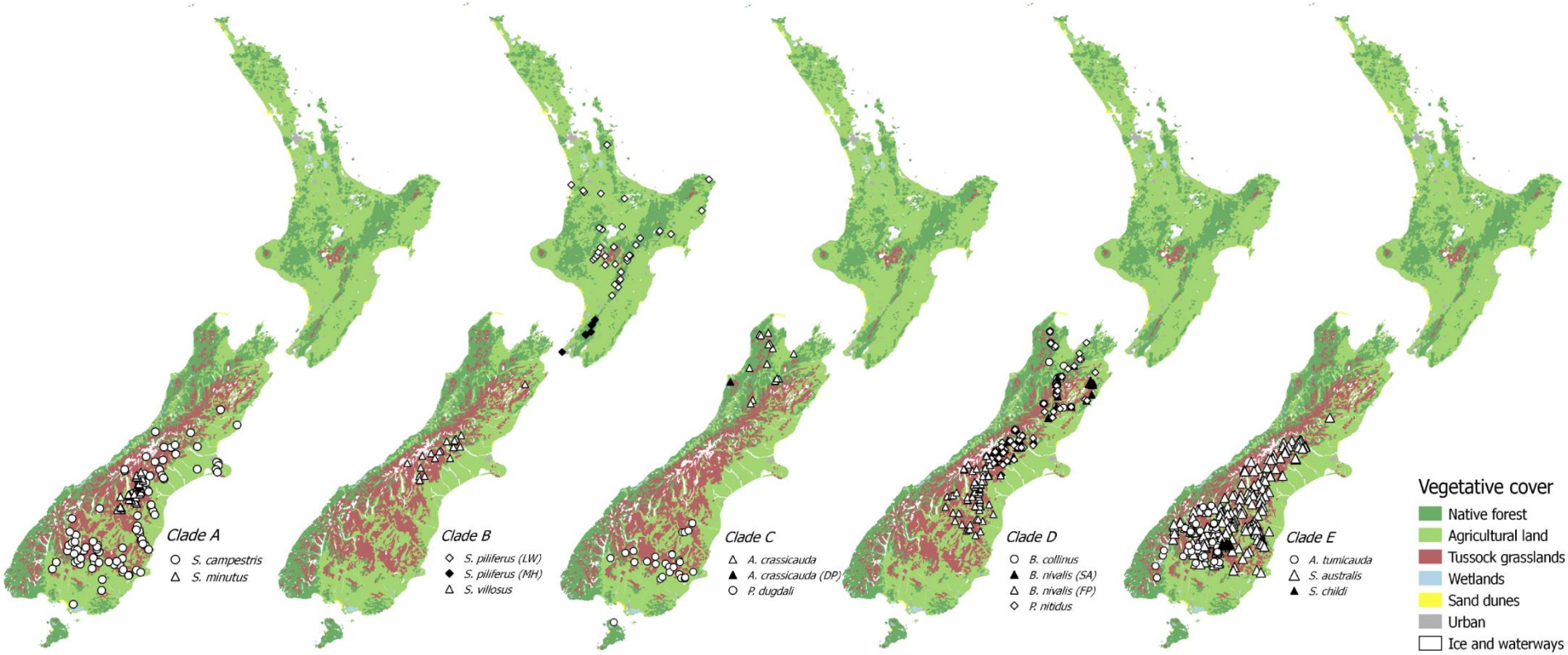

Figure 2.16 The distributions of members within the five phylogenetic clades established in this study. All clades have distributions that overlap, making it difficult to distinguish clades in geographical context.

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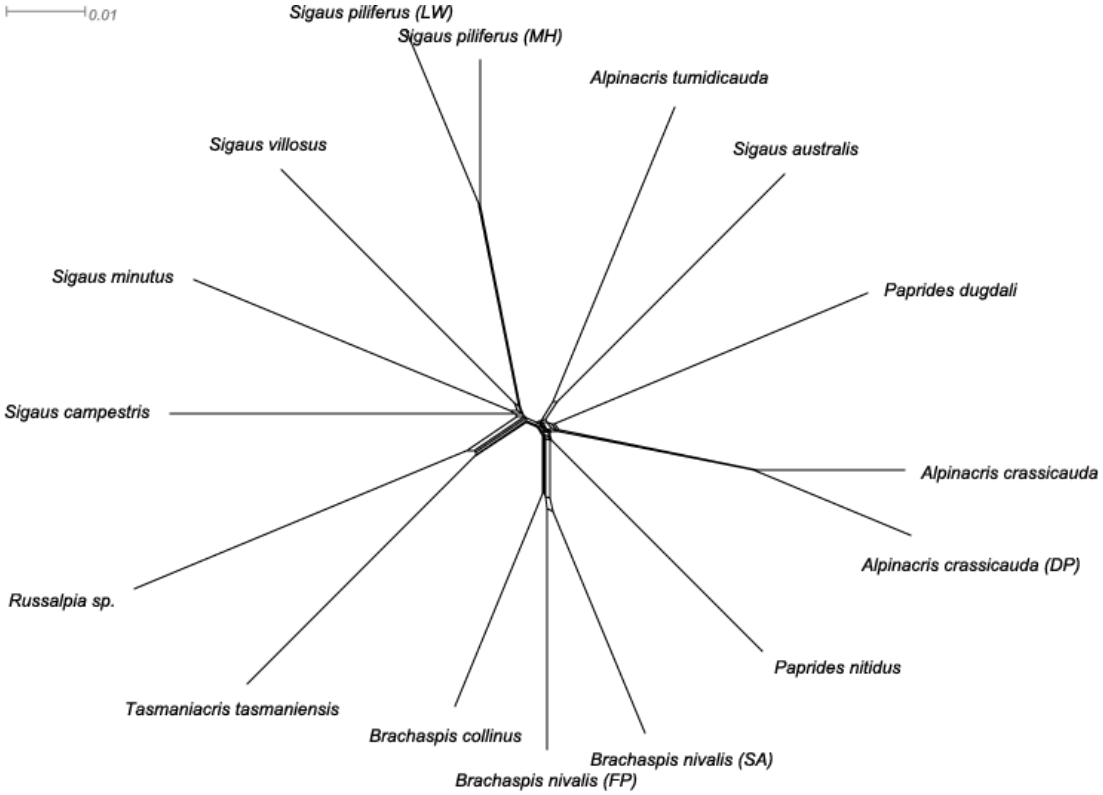
Supplementary Material for Chapter Two

Supplementary Table 2.1 Summary matrix of the 16 grasshopper sequences used in the mitochondrial genome "complete mtDNA+D-loop" alignment. This alignment is 14,516bp in length. The number of nucleotide differences between these sequences is displayed in the top matrix and pairwise percent identity is displayed in the bottom matrix. The two sequences which are the most similar are highlighted in dark grey, the two sequences which are least similar are highlighted in light grey.

	A. crassicauda	*A. crassicauda* (DP)	*A. tumidicauda*	*B. collinus*	*B. nivalis* (SA)	*B. nivalis* (FP)	*P. dugdali*	*P. nitidus*	*Russalpia sp.*	*S. australis*	*S. campestris*	*S. minutus*	*S. piliferus* (LW)	*S. piliferus* (MH)	*S. villosus*	*T. tasmaniensis*
A. crassicauda		688	1838	1669	1589	1790	1739	1578	2077	1736	1847	1927	1965	1928	1916	2018
***A. crassicauda* (DP)**	95.5		1877	1694	1668	1846	1775	1621	2109	1766	1870	1974	1991	1982	1964	2052
A. tumidicauda	88.1	87.8		1528	1645	1685	1675	1599	1939	1511	1723	1791	1867	1815	1805	1888
B. collinus	89.2	89	90.1		1188	1233	1544	1428	1825	1530	1595	1631	1764	1666	1641	1729
***B. nivalis* (SA)**	89.6	89.1	89.3	92.3		1272	1566	1397	1923	1541	1725	1790	1730	1762	1794	1873
***B. nivalis* (FP)**	88.5	88.1	89.1	92	91.8		1647	1516	1774	1641	1680	1657	1878	1637	1637	1704
P. dugdali	88.7	88.5	89.1	90	89.8	89.4		1526	1939	1603	1731	1703	1890	1805	1781	1871
P. nitidus	89.7	89.4	89.6	90.8	90.9	90.2	90.1		1825	1496	1671	1738	1700	1703	1754	1749
Russalpia sp.	86.6	86.4	87.5	88.2	87.6	88.6	87.5	88.2		1922	1858	1811	2085	1810	1860	1592
S. australis	88.7	88.5	90.2	90.1	89.9	89.4	89.6	90.3	87.6		1744	1782	1793	1794	1808	1862
S. campestris	88	87.9	88.8	89.7	88.8	89.2	88.8	89.2	88	88.7		1721	1890	1690	1732	1830
S. minutus	87.6	87.3	88.5	89.5	88.5	89.4	89.1	88.8	88.4	88.5	88.9		1959	1697	1565	1770
***S. piliferus* (LW)**	87.2	87	87.9	88.6	88.7	87.9	87.8	88.9	86.6	88.3	87.8	87.4		1049	1974	2033
***S. piliferus* (MH)**	87.6	87.2	88.3	89.3	88.6	89.5	88.4	89	88.3	88.4	89.1	89.1	93.2		1678	1746
S. villosus	87.7	87.4	88.4	89.5	88.5	89.5	88.6	88.7	88.1	88.4	88.9	90	87.3	89.2		1742
T. tasmaniensis	87	86.8	87.9	88.9	88	89	88	88.8	89.8	88	88.3	88.6	87	88.8	88.8	

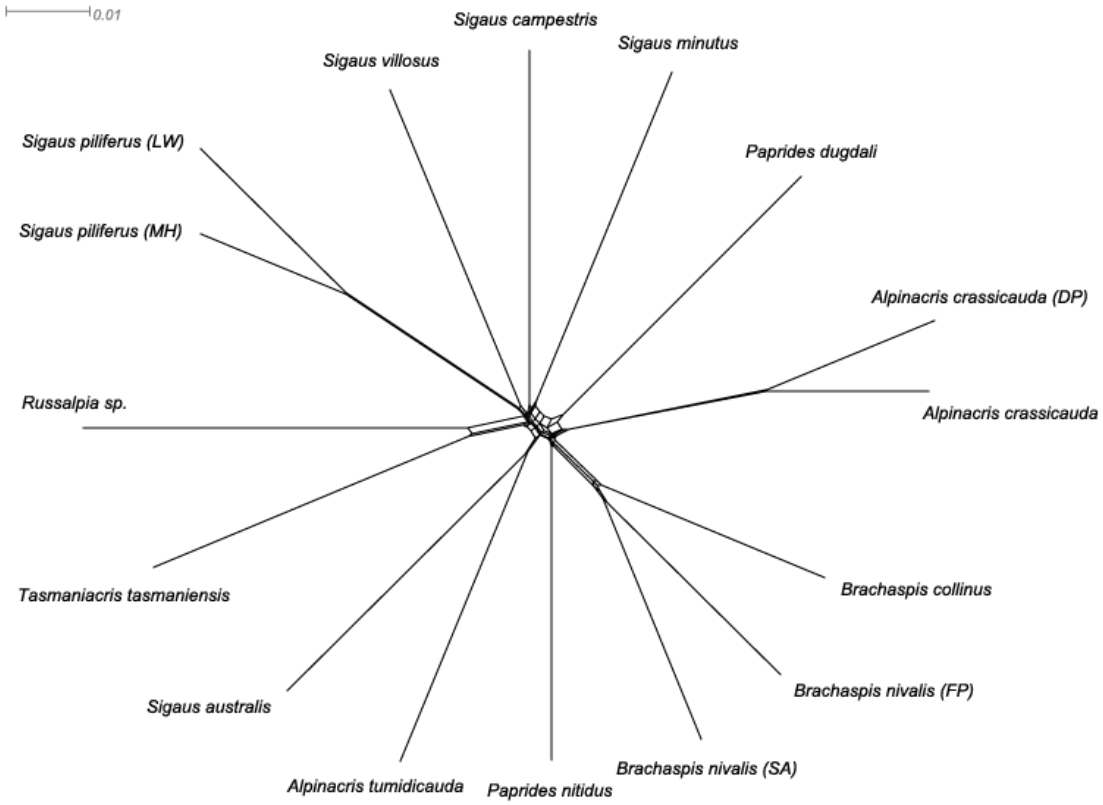
Supplementary Figure 2.1 Splits network of the mitochondrial genome "complete mtDNA+D-loop" alignment. This alignment consists of 16 sequences and is 14,516bp in length. Splits were generated using the Neighbour-Net algorithm in SplitsTree4. This network demonstrates the different phylogenetic tree topologies being signalled from the underlying sequence data.



Supplementary Table 2.2 Summary matrix of the 16 grasshopper sequences used in the mitochondrial genome "complete mtDNA" alignment. This alignment is 14,176bp in length. The number of nucleotide differences between these sequences is displayed in the top matrix and pairwise percent identity is displayed in the bottom matrix. The two sequences which are the most similar are highlighted in dark grey, the two sequences which are least similar are highlighted in light grey.

	A. crassicauda	*A. crassicauda* (DP)	*A. tumidicauda*	*B. collinus*	*B. nivalis* (SA)	*B. nivalis* (FP)	*P. dugdali*	*P. nitidus*	*Russalpia sp.*	*S. australis*	*S. campestris*	*S. minutus*	*S. piliferus* (LW)	*S. piliferus* (MH)	*S. villosus*	*T. tasmaniensis*
A. crassicauda		639	1469	1387	1401	1407	1444	1387	1703	1482	1527	1525	1694	1557	1472	1599
***A. crassicauda* (DP)**	95.6		1517	1409	1470	1469	1479	1414	1743	1501	1579	1565	1711	1608	1530	1636
A. tumidicauda	90	89.6		1245	1301	1275	1363	1285	1543	1225	1398	1365	1528	1415	1340	1457
B. collinus	90.5	90.4	91.5		945	927	1297	1197	1494	1259	1329	1327	1459	1363	1294	1387
***B. nivalis* (SA)**	90.4	89.9	91.1	93.5		905	1319	1210	1535	1322	1396	1395	1493	1378	1350	1447
***B. nivalis* (FP)**	90.4	90	91.3	93.6	93.8		1311	1183	1514	1271	1371	1350	1484	1383	1311	1416
P. dugdali	90.2	89.9	90.7	91.1	91	91.1		1293	1586	1360	1419	1341	1597	1455	1379	1481
P. nitidus	90.5	90.3	91.2	91.8	91.7	91.9	91.2		1487	1285	1386	1372	1481	1367	1352	1370
Russalpia sp.	88.3	88.1	89.4	89.8	89.5	89.6	89.2	89.8		1562	1521	1540	1677	1546	1543	1391
S. australis	89.9	89.7	91.6	91.4	90.9	91.3	90.7	91.2	89.3		1405	1391	1548	1430	1364	1451
S. campestris	89.6	89.2	90.4	90.9	90.4	90.6	90.3	90.5	89.6	90.4		1356	1538	1387	1373	1481
S. minutus	89.6	89.3	90.7	90.9	90.5	90.8	90.8	90.6	89.5	90.5	90.7		1536	1443	1357	1480
***S. piliferus* (LW)**	88.4	88.3	89.6	90	89.8	89.9	89.1	89.9	88.5	89.4	89.5	89.5		728	1495	1589
***S. piliferus* (MH)**	89.4	89	90.3	90.7	90.6	90.5	90.1	90.7	89.4	90.2	90.5	90.1	95		1380	1479
S. villosus	89.9	89.5	90.8	91.1	90.8	91	90.6	90.8	89.4	90.6	90.6	90.7	89.8	90.6		1459
T. tasmaniensis	89.1	88.8	90	90.5	90.1	90.3	89.9	90.6	90.5	90.1	89.9	89.9	89.2	89.9	90	

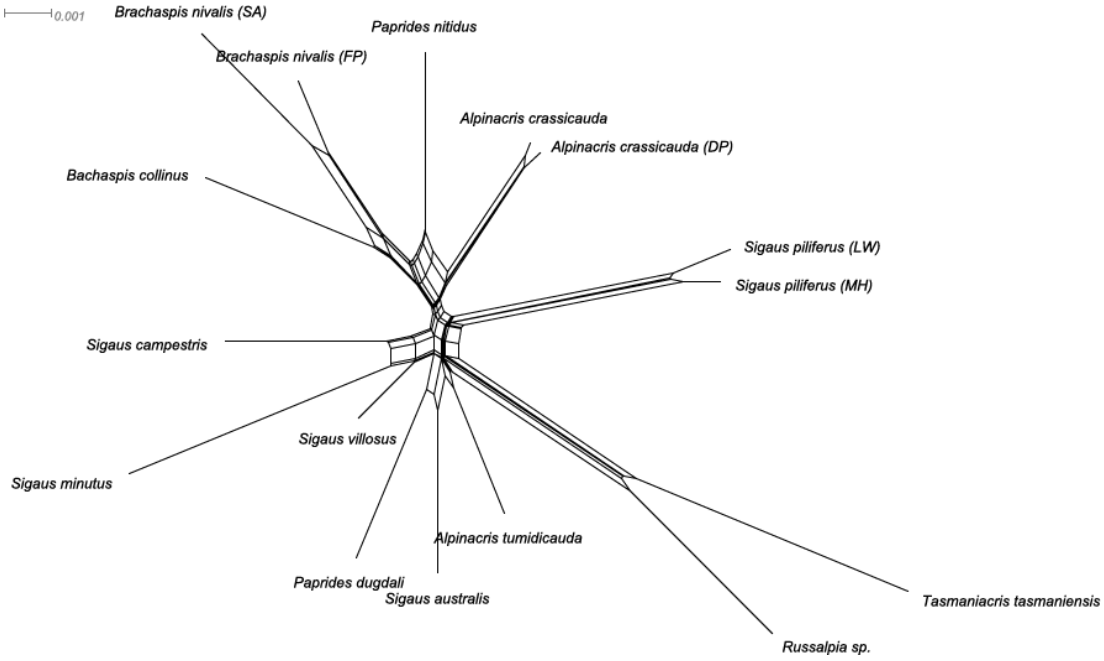
Supplementary Figure 2.2 Splits network of the mitochondrial genome "complete mtDNA" alignment. This alignment consists of 16 sequences and is 14,176bp in length. Splits were generated using the Neighbour-Net algorithm in SplitsTree4. This network demonstrates the different phylogenetic tree topologies being signalled from the underlying sequence data.



Supplementary Table 2.3 Summary matrix of the 16 grasshopper sequences used in the 45S ribosomal cassette alignment . This alignment is 6,713bp in length. The number of nucleotide differences between these sequences is displayed in the top matrix and pairwise percent identity is displayed in the bottom matrix. The two sequences which are the most similar are highlighted in dark grey, the two sequences which are least similar are highlighted in light grey.

	A. crassicauda	*A. crassicauda* (DP)	*A. tumidicauda*	*B. collinus*	*B. nivalis* (SA)	*B. nivalis* (FP)	*P. dugdali*	*P. nitidus*	*Russalpia sp.*	*S. australis*	*S. campestris*	*S. minutus*	*S. piliferus* (LW)	*S. piliferus* (MH)	*S. villosus*	*T. tasmaniensis*
A. crassicauda		21	113	87	98	94	107	94	139	110	119	128	113	106	94	154
***A. crassicauda* (DP)**	99.7		114	89	100	98	108	99	140	111	114	127	116	108	95	157
A. tumidicauda	98.4	98.4		90	114	99	97	100	104	81	90	120	114	117	63	121
B. collinus	98.7	98.7	98.7		70	65	92	84	131	102	107	112	108	103	71	141
***B. nivalis* (SA)**	98.6	98.6	98.4	99		54	111	99	151	121	129	141	115	113	96	159
***B. nivalis* (FP)**	98.6	98.6	98.6	99.1	99.2		95	92	139	105	111	114	102	100	71	143
P. dugdali	98.4	98.4	98.6	98.7	98.4	98.6		98	109	78	89	106	113	111	67	122
P. nitidus	98.6	98.6	98.6	98.8	98.6	98.7	98.6		138	110	108	122	120	118	84	147
Russalpia sp.	98	98	98.5	98.1	97.8	98	98.4	98		107	103	138	130	124	112	79
S. australis	98.4	98.4	98.8	98.5	98.3	98.5	98.9	98.4	98.5		91	113	105	105	80	133
S. campestris	98.3	98.3	98.7	98.5	98.1	98.4	98.7	98.4	98.5	98.7		91	117	118	78	104
S. minutus	98.1	98.2	98.3	98.4	98	98.3	98.5	98.2	98	98.4	98.7		128	125	70	143
***S. piliferus* (LW)**	98.4	98.3	98.4	98.4	98.3	98.5	98.4	98.3	98.1	98.5	98.3	98.1		31	91	146
***S. piliferus* (MH)**	98.5	98.4	98.3	98.5	98.4	98.6	98.4	98.3	98.2	98.5	98.3	98.2	99.6		94	143
S. villosus	98.6	98.6	99.1	99	98.6	99	99	98.8	98.4	98.8	98.9	99	98.7	98.6		117
T. tasmaniensis	97.8	97.7	98.3	98	97.7	97.9	98.2	97.9	98.9	98.1	98.5	97.9	97.9	97.9	98.3	

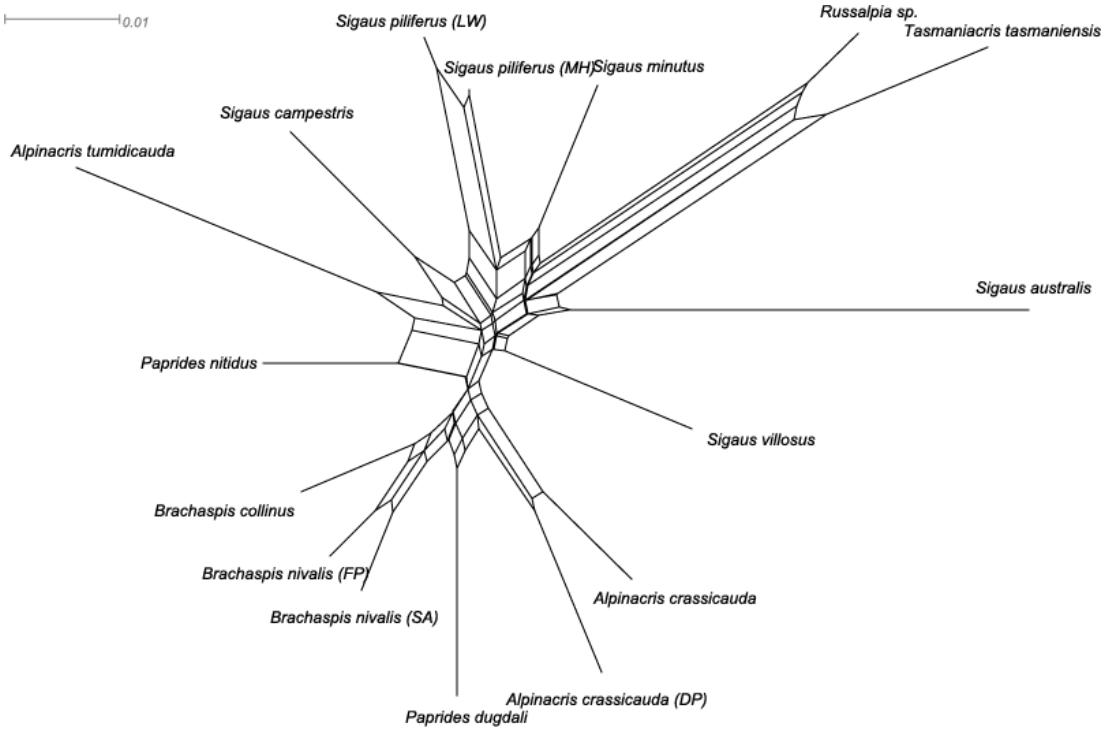
Supplementary Figure 2.3 Splits network of the 45S ribosomal cassette alignment. This alignment consists of 16 sequences and is 6,713bp in length. Splits were generated using the Neighbour-Net algorithm in SplitsTree4. This network demonstrates the different phylogenetic tree topologies being signalled from the underlying sequence data.



Supplementary Table 2.4 Summary matrix of the 16 grasshopper sequences used in the Histone 3 (H3) alignment. This alignment is 744bp in length. The number of nucleotide differences between these sequences is displayed in the top matrix and pairwise percent identity is displayed in the bottom matrix. The two sequences which are the most similar are highlighted in dark grey, the two sequences which are least similar are highlighted in light grey.

	A. crassicauda	*A. crassicauda* (DP)	*A. tumidicauda*	*B. collinus*	*B. nivalis* (SA)	*B. nivalis* (FP)	*P. dugdali*	*P. nitidus*	*Russalpia sp.*	*S. australis*	*S. campestris*	*S. minutus*	*S. piliferus* (LW)	*S. piliferus* (MH)	*S. villosus*	*T. tasmaniensis*
A. crassicauda		10	27	16	21	17	15	19	19	27	37	20	21	20	18	35
***A. crassicauda* (DP)**	98.7		24	14	20	15	13	14	17	25	33	20	20	15	15	33
A. tumidicauda	96.4	96.8		24	26	24	23	26	21	30	36	27	25	30	27	36
B. collinus	97.8	98.1	96.8		20	11	7	16	16	23	39	21	21	21	19	31
***B. nivalis* (SA)**	97.2	97.3	96.5	97.3		21	18	24	20	27	36	19	22	20	19	30
***B. nivalis* (FP)**	97.7	98	96.8	98.5	97.2		4	19	17	24	39	20	23	24	21	32
P. dugdali	98	98.3	96.9	99.1	97.6	99.5		15	15	21	38	18	19	20	18	29
P. nitidus	97.4	98.1	96.5	97.8	96.8	97.4	98		19	25	39	23	22	21	21	32
Russalpia sp.	97.4	97.7	97.2	97.8	97.3	97.7	98	97.4		24	37	23	25	23	21	27
S. australis	96.4	96.6	96	96.9	96.4	96.8	97.2	96.6	96.8		40	25	23	27	23	13
S. campestris	95	95.6	95.2	94.8	95.2	94.8	94.9	94.8	95	94.6		35	30	32	31	46
S. minutus	97.3	97.3	96.4	97.2	97.4	97.3	97.6	96.9	96.9	96.6	95.3		18	16	18	31
***S. piliferus* (LW)**	97.2	97.3	96.6	97.2	97	96.9	97.4	97	96.6	96.9	96	97.6		17	16	32
***S. piliferus* (MH)**	97.3	98	96	97.2	97.3	96.8	97.3	97.2	96.9	96.4	95.7	97.8	97.7		6	32
S. villosus	97.6	98	96.4	97.4	97.4	97.2	97.6	97.2	97.2	96.9	95.8	97.6	97.8	99.2		30
T. tasmaniensis	95.3	95.6	95.2	95.8	96	95.7	96.1	95.7	96.4	98.3	93.8	95.8	95.7	95.7	96	

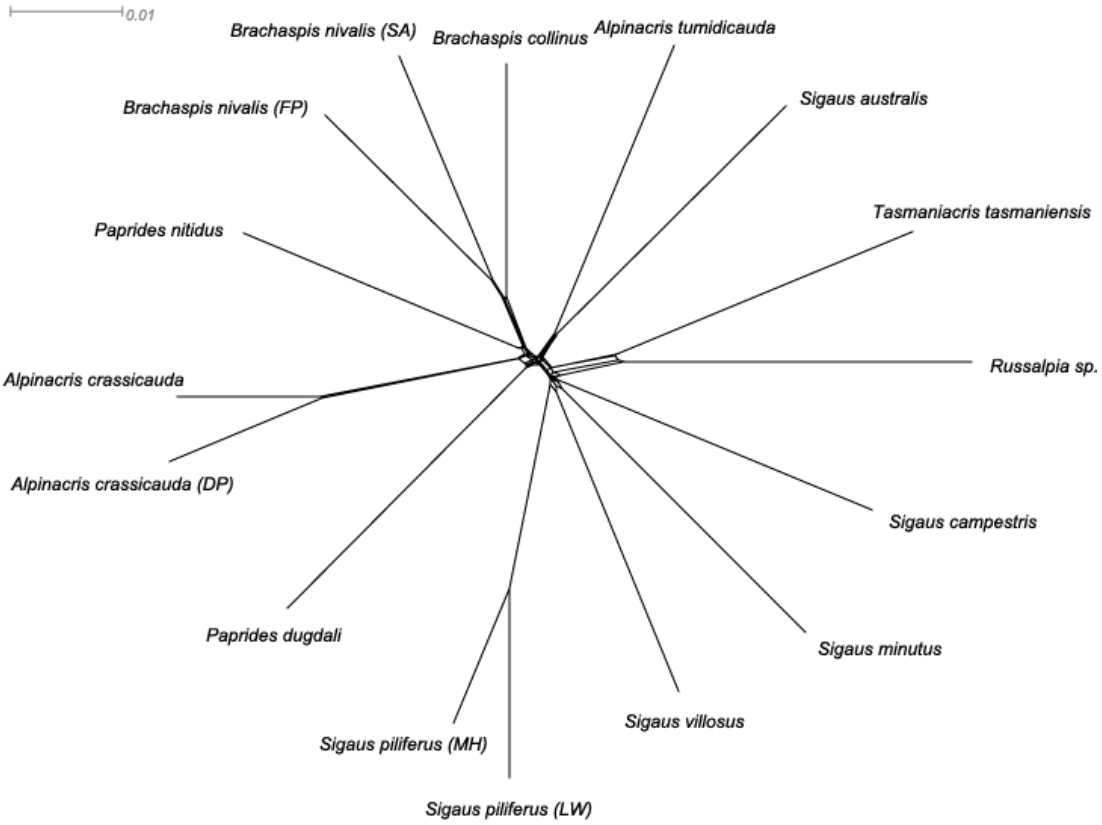
Supplementary Figure 2.4 Splits network of the Histone 3 (H3) alignment. This alignment consists of 16 sequences and is 744bp in length. Splits were generated using the Neighbour-Net algorithm in SplitsTree4. This network demonstrates the different phylogenetic tree topologies being signalled from the underlying sequence data.



Supplementary Table 2.5 Summary matrix of the 16 grasshopper sequences used in the total evidence alignment. This alignment is 22,164bp in length. The number of nucleotide differences between these sequences is displayed in the top matrix and pairwise percent identity is displayed in the bottom matrix. The two sequences which are the most similar are highlighted in dark grey, the two sequences which are least similar are highlighted in light grey.

	A. crassicauda	*A. crassicauda* (DP)	*A. tumidicauda*	*B. collinus*	*B. nivalis* (SA)	*B. nivalis* (FP)	*P. dugdali*	*P. nitidus*	*Russalpia sp.*	*S. australis*	*S. campestris*	*S. minutus*	*S. piliferus* (LW)	*S. piliferus* (MH)	*S. villosus*	*T. tasmaniensis*
A. crassicauda		792	2025	1835	1963	1771	1926	1745	2306	1935	2064	2144	2159	2114	2088	2265
***A. crassicauda* (DP)**	96.5		2041	1841	2000	1835	1958	1776	2324	1964	2051	2163	2156	2146	2115	2296
A. tumidicauda	91.2	91.2		1664	1812	1790	1825	1715	2099	1641	1873	1971	2023	1977	1918	2065
B. collinus	92.1	92	92.8		1307	1266	1638	1514	1963	1655	1731	1763	1871	1777	1739	1903
***B. nivalis* (SA)**	91.5	91.4	92.2	94.4		1315	1746	1618	1921	1760	1814	1791	2004	1737	1727	1883
***B. nivalis* (FP)**	92.3	92	92.2	94.5	94.3		1690	1506	2090	1703	1878	1947	1854	1877	1903	2074
P. dugdali	91.7	91.5	92.1	92.9	92.5	92.7		1656	2077	1722	1837	1811	2038	1932	1864	2045
P. nitidus	92.4	92.3	92.6	93.4	93	93.5	92.8		1990	1639	1800	1882	1833	1830	1858	1934
Russalpia sp.	90.1	90	90.9	91.5	91.7	91	91.1	91.4		2070	1974	1970	2246	1936	1991	1693
S. australis	91.6	91.5	92.9	92.8	92.4	92.6	92.5	92.9	91.1		1879	1936	1947	1936	1937	2055
S. campestris	91.1	91.1	91.9	92.5	92.2	91.9	92.1	92.2	91.5	91.9		1827	2028	1836	1844	1964
S. minutus	90.8	90.7	91.5	92.4	92.3	91.6	92.2	91.9	91.5	91.6	92.1		2114	1829	1651	1944
***S. piliferus* (LW)**	90.6	90.6	91.2	91.9	91.3	91.9	91.2	92	90.3	91.5	91.2	90.9		1092	2090	2229
***S. piliferus* (MH)**	90.9	90.8	91.5	92.3	92.5	91.9	91.7	92.1	91.7	91.6	92.1	92.1	95.3		1773	1926
S. villosus	91	90.9	91.7	92.5	92.6	91.8	92	92	91.4	91.6	92.1	92.9	91	92.4		1888
T. tasmaniensis	90.3	90.1	91.1	91.8	91.9	91.1	91.2	91.7	92.7	91.2	91.6	91.6	90.4	91.7	91.9	

Supplementary Figure 2.5 Splits network of the total evidence alignment. This alignment consists of 16 sequences and is 22,164bp in length. Splits were generated using the Neighbour-Net algorithm in SplitsTree4. This network demonstrates the different phylogenetic tree topologies being signalled from the underlying sequence data.



Lake Rotoiti with St Arnaud Range (Rainbow Ski Area) to the left, South Island

Chapter Three

The time of divergence of a monophyletic radiation: an alpine radiation before alpine habitat in New Zealand's endemic grasshoppers?

Introduction

Climatic and geological change can create new environments that are a source of ecological opportunity; a new place for species to colonise and exploit (Gillespie 2004, Hughes & Eastwood 2006, Ólafsdóttir *et al*. 2007, Simpson 1949). Colonisation of a new environment can be facilitated by the evolution of novel traits, and often results in a range-restricted species escaping from antagonists (Bolnik *et al*. 2010, Robinson & Wilson 1994, Robinson, Wilson & Margosian 2000, Yoder *et al* 2010). Ecological opportunity releases species from the pressures of natural selection, and results in increased density, increased intraspecific competition, and increased trait variation (Simpson 1949, 1953, Schluter 2000, Yoder *et al* 2010). Thus ecological opportunities can be the stimulus for an adaptive radiation where a single ancestral species gives rise to many derived species, each using a different resource and inhabiting its own new niche space (Simpson 1949, Yoder *et al* 2010).

New Zealand has a diverse array of endemic flora and fauna adapted to the alpine environment, although the mountains of New Zealand are estimated to be less than 12 million years old (Table 3.1) (Batt *et al*. 2004, Chamberlain & Poage 2000, Tippett & Kamp 1993). Alpine habitat in New Zealand was likely limited during the warm climate of the Miocene (23 – 5.3Mya) - if present at all (Heenan & McGlone 2013, McGlone *et al*. 2001). However, a cooling period at the end of the Pliocene (2.6Mya) coupled with the rapid uplift of the Southern Alps meant that stable alpine habitat has probably only

been available since approximately 2.6 – 0.9Mya (Heenan & McGlone 2013, McGlone *et* al. 2001, Wallis *et al.* 2016). Many of the taxa found inhabiting the alpine zone of New Zealand are thought to have arrived, colonized and radiated following this period of geological, climatic and habitat diversification. Examples of endemic alpine taxa are summarised in Table 3.1, and includes species of endemic alpine grasshopper (Acrididae).

Alpine radiations in New Zealand

The formation of the Southern Alps, the most extensive mountain range in New Zealand, arose following the collision of the Australian and Pacific plates. As the Australian Plate moves northwards and towards the Pacific Plate, it forces the Pacific Plate downwards and itself upwards, forming the Southern Alps. This has been occurring over approximately 25My, with most uplift occurring in the last 5My (Kamp 1986, Trewick & Bland 2012). With the formation of this mountain range would have come ecological opportunity, as alpine habitat was made available for the first time since the Jurassic/Cretaceous in this region (Cockayne 1928). Some of New Zealand's endemic taxa have adapted and colonised this alpine habitat, evolving from lineages that have been in New Zealand much longer than the age of the mountains (e.g. geckos (Nielson *et al*. 2011), cicada (Buckley & Simon 2007, Marshall *et al*. 2010), carabid beetles (Goldberg *et al*. 2014) and weta (Trewick & Morgan-Richards 2005)). With some lineages resulting in alpine radiations (e.g. cicada (Buckley & Simon 2007, Marshall *et al*. 2010), carabid beetles (Goldberg *et al*. 2014) and geckos (Nielson *et al*. 2011)), but not all (e.g. weta (Trewick & Morgan-Richards 2005)). Other lineages, pre-adapted to alpine environments, may have arrived and established after the alpine habitat formed in New Zealand (e.g. *Phyllache* (Wagstaff &Wege 2002)). For the majority of New Zealand's alpine lineages, however, we do not know when they arrived or whether they should be considered alpine radiations. This is either due to a lack of study, misleading taxonomic classifications, small molecular datasets and/or inadequate divergence date modelling. New Zealand's alpine grasshoppers are an abundant and diverse element of the endemic alpine fauna that may represent a recent arrival and alpine radiation (Bigelow 1967, Trewick 2001, 2008, Trewick & Morris 2008, Trewick & Wallis 2001).

New Zealand's alpine Acrididae

Ten endemic Acrididae species belonging to four endemic genera (*Alpinacris*, *Brachaspis*, *Paprides* and *Sigaus*) are adapted to living in the harsh conditions of New Zealand's alpine zone (Bigelow 1967). These ten species are considered to be "alpine" as their distributions, physiology, morphology and ecology all have an affinity with the alpine zone (Batcheler 1967, Bigelow 1967, Sinclair 2001). Of the ten alpine species, one *Sigaus* species (*Sigaus piliferus*) inhabits the mountains and ranges of North Island New Zealand; the others are restricted to the South Island (Bigelow 1967). These taxa are predominantly found above the treeline, although some have ranges that extend below this. Three additional non-alpine species (*Brachaspis robustus*, *Sigaus childi* and *Sigaus minutus*) are only recorded from lowland grasslands (Bigelow 1967, Jamieson 1999).

The alpine grasshopper species are physiologically adapted to living in the alpine zone, where they are tolerant of freezing solid at any life stage and reanimating when warmer conditions arise; some species are known to have a lower lethal temperature (LLT) of -13.6°C (Batcheler 1967, Hawes 2015, Mason 1971, Sinclair 2001). Commonly, grasshoppers inhabiting alpine environments around the world have a lifecycle that begins with eggs hatching in early spring, individuals completing all instars during summer, adults laying eggs in autumn and then dying when winter returns (Alexander & Hilliard 1964, Liu & Wang 2003). The ability of New Zealand's alpine taxa to overwinter at any life stage allows for multiple generations to be present at a given time, for multiple clutches of eggs to be laid in a season, and for the grasshoppers to live for several years (Batcheler 1967, Mason 1971). It has been hypothesised that their flexible lifecycle is suited to New Zealand's erratic and changeable climate, where snowfall can occur at any time throughout the year and snowpack longevity is very variable (Batcheler 1967).

Globally, grasshoppers are one of the most abundant herbivores in alpine areas – including in New Zealand, and their presence is thought to be closely associated with alpine vegetation (Alexander 1951, Batcheler 1967). New Zealand's grasshoppers are considered to be generalist feeders; however they can be selective grazers where particular plants are present or when particular plants are flowering

Table 3.1 Summary of New Zealand alpine species divergence dating analyses for both fauna and flora. Maximum lineage age is in millions of years (My), and is considered from when the New Zealand lineage split from its nearest common ancestor and/or when the radiation of this lineage began within New Zealand. Dating method codes: Rate = molecular clock method with universal rate, A = prior age estimate calibration, F= fossil calibration, G= geological event calibration. Genes listed under the Sequence data are: 5.8S = 5.8S RNA, 12S = 12S RNA, COI = Cytochrome c oxidase one, COII = Cytochrome c oxidase two, cpDNA = concatenation of more than two chloroplast genes, ETS = Erythroblast transformation-specific transcription factor, H3 = Histone 3, ITS1 = internal transcribed spacer one, ITS2 = internal transcribed spacer two, matK = trnK Maturase K, mtDNA = concatenation of more than two mitochondrial genes, ND1 = NADH dehydrogenase subunit 1, nDNA = concatenation of more than two nuclear genes, rbcL = ribulose-bisphosphate carboxylase gene. Alignment length is noted in brackets in base pairs (bp).

Family	Genus	Maximum Lineage Age (My)	Dating method	Sequence data	Reference
Fauna					
Acrididae	*Alpinacris*	2.6	Rate	12S (370bp)	Trewick & Wallis 2001, Wallis *et al*. 2016
Acrididae	*Brachaspis*	1.97	Rate	COI	Wallis *et al*. 2016
Acrididae	*Paprides*	2.2 – 4.4	Rate	COI (540bp),12S (370bp)	Trewick & Wallis 2001, Wallis *et al*. 2016
Anostostomatidae	*Deinacrida*	17	Rate	COI (510 bp), 12S (440 bp)	Trewick &Morgan-Richards 2005
Anostostomatidae	*Hemideina*	17	Rate	COI (510 bp), 12S (440 bp)	Trewick &Morgan-Richards 2005
Blattodea	*Celatoblatta*	6.6	Rate	COI(405bp)	Chinn & Gemmell 2004
Carabidae	*Mecodema*	13.3	Rate	COI(789bp)	Goldberg *et al*. 2014
Cicadoidea	*Kikihia*	12.4	Rate	mtDNA(2152bp)	Marshall *et al*. 2008
Cicadoidea	*Maoricicada*	11.3	G	mtDNA(2274bp), nDNA(3530bp)	Buckley & Simon 2007
Curculionidae	*Lyperobius*	5.5	Rate	COI(640bp)	Trewick &Wallis 2001
Ephemeroptera	*Leptophlebiidae*	2.6 – 0.01	G	N/A	Hitchings 2009
Grypopterygidae	*Apteryoperla*	5.3 – 2.6	Rate	COI(644bp), H3(322bp)	McCulloch *et al*. 2010
Grypopterygidae	*Holcoperla*	5.3 – 2.6	Rate	COI(644bp), H3(322bp)	McCulloch *et al*. 2010
Grypopterygidae	*Vesicaperla*	5.3 – 2.6	Rate	COI(644bp), H3(322bp)	McCulloch *et al*. 2010
Hepialidae	*Oxycanus*	4	Rate	COI & COII(527bp)	Brown *et al*. 1999

Hepialidae	*Wiseana*	1.5	Rate	COI & COII(527bp)	Brown *et al*. 1999
Lycosidae	*Anoteropsis*	5	G	ND1(420bp)	Vink & Paterson 2003
Notonemouridae	*Cristaperla*	5.3 – 2.6	Rate	COI(644bp), H3(322bp)	McCulloch *et al*. 2010
Notonemouridae	*Halticoperla*	5.3 – 2.6	Rate	COI(644bp), H3(322bp)	McCulloch *et al*. 2010
Notonemouridae	*Spaniocerca*	5.3 – 2.6	Rate	COI(644bp), H3(322bp)	McCulloch *et al*. 2010
Flora					
Asteraceae	*Abrotanella*	3.1	Rate,F	ITS1 & ITS2(~700bp), matK (~900bp)	Wagstaff *et al*. 2006
Asteraceae	*Raoulia*	5.3 – 2.6	Rate	ITS1,5.8S,ITS2(N/A)	Smissen *et al*. 2003a
Boraginaceae	*Myosotis*	3.37	F	ITS(627bp), ETS (287bp)	Meudt *et al.* 2015
Brassicaceae	*Pachycladon*	1.6	A	nDNA(717bp)	Joly *et al*. 2009
Caryophyllaceae	*Scleranthus*	7.7	Rate,F	ITS1 & ITS2(N/A)	Smissen *et al*. 2003b
Gentianaceae	*Gentianella*	2.7	Rate,G,F	ITS1 & ITS2(~230bp)	Von Hagen & Kadereit 2004
Poaceae	*Chionochloa*	7.6	Rate,A	cpDNA(N/A)	Pirie *et al*. 2010
Podocarpaceae	*Phyllocladus*	6.3	Rate,F	rbcL & matK(2845)	Wagstaff 2004
Ranunculaceae	*Ranunculus*	5.0	Rate,A	ITS1 & ITS2(~600bp)	Lockhart *et al*. 2001
Scrophulariaceae	*Hebe*	3.9	Rate,F	rbcL(1402bp), ITS1 & 1TS2(695bp)	Wagstaff *et al*. 2002
Stylidiaceae	*Forstera*	6.0	Rate,F	rbcL(1402bp),ITS1 & ITS2(782bp)	Wagstaff & Wege 2002
Stylidiaceae	*Oreostylidium*	2.0	Rate,F	rbcL(1402bp),ITS1 & ITS2(782bp)	Wagstaff & Wege 2002
Stylidiaceae	*Phyllache*	6.0	Rate,F	rbcL(1402bp),ITS1 & ITS2(782bp)	Wagstaff & Wege 2002

(Watson 1971, White 1975). When available, flower parts of alpine herbs are known to make up a significant proportion of grasshopper diet, while the remainder can consist of both monocotyledonous and dicotyledonous plants and even moss and ferns (Watson 1971). Alpine herbs and shrubs known to be eaten by New Zealand grasshoppers are: *Agrostis dyeri*, *Anistome aromatica*, *Celmisia* species, *Coprosma* species, *Drapetes dieffenbachi*, *Epibolium* species, *Gaultheria depressa*, *Hebe* species, *Luzula* species, *Pratia* species, *Polytichum juniperium Raoulia* species, *Rumex acetosella*, *Senecio bellidiodes* and *Wahlembergia albomarginate* (Watson 1971, White 1975). Although New Zealand grasshoppers have distributions that are closely associated with tussock grasslands, their diet is not influenced by the relative abundance of such plant species (e.g. *Chionochloa*, *Poa*), and tussocks are not commonly found in their diet (Watson 1971). The association between grasshoppers and tussocks is hypothesised to be for shelter (Watson 1971).

Morphologically, the alpine grasshoppers share characteristics typical of other alpine insects found throughout the world (Hodkinson 2005) They are flightless and those found at the highest elevations (e.g. *Brachaspis collinus, Brachaspis nivalis and Sigaus villosus*) are dark and dull in colouration, pubescent and are the largest of all of New Zealand's endemic species (Bigelow 1967, Hodkinson 2005). New Zealand's alpine grasshoppers are considered to be close relatives of taxa inhabiting Tasmania (e.g. *Russalpia* and *Tasmaniacris* species) as they also inhabit alpine environments, have similar male genitalia, are flightless and lack the ability to communicate audibly (Bigelow 1967, Key 1991).

The arrival of grasshoppers in New Zealand and the timing of their diversification is investigated here. All short-horned grasshoppers in New Zealand must have arrived via dispersal over the sea as the split of Zealandia from Gondwana (~85mya) was before the origin of Acrididae from other Acridoidea families ~35-73mya (Scudder 1885, Song *et al*. 2015). The many endemic New Zealand species might result from many independent arrivals or from a single arrival and subsequent diversification. This latter scenario would result in a monophyletic clade of New Zealand species. The New Zealand grasshopper's apparent affinity with alpineness may be an ancestral trait or the result of adaptation *in situ*. A radiation that may have been triggered by geological or climatic

events, such as the Kaikoura Orogeny or the Last Glacial Maximum (LGM), would be expected to have molecular signature that could be dated to the environmental change.

Calibrating trees and estimating time of divergence

Estimating the divergence time of lineages allows us to test hypotheses that seek to explain lineage splits and origin of evolutionary novelties (Rutschmann 2006). However, as with any model, the predictive accuracy of divergence dating analyses is correlated with the quality of the input data. Therefore, to get a meaningful divergence date estimate, it requires a well supported phylogeny, the correct DNA substitution model and incorporation of uncertainty associated with calibration into priors (Hills 2010).

In the past, evolutionary divergence dates were estimated from DNA sequences using a maximum-likelihood strict molecular clock method. This was based on the idealised molecular clock framework as predicted by neutral theory, which proposed that rates of molecular change occur at approximately the same constant rate across time and between species (Kimura 1968, 1980). However, with the accumulation of DNA sequence data from a variety of organisms, it was revealed that this "one size fits all" approach was not appropriate, as some lineages were found to undergo rapid bouts of molecular evolution, while others remain relatively static, even between closely related species (Langley & Fitch, 1974, Welch & Bromham 2005). There are four main factors that can result in variable rates of evolution between lineages and species: generation time, metabolic rate, mutation rate, and effective population size (Rutschmann 2006). With the development of Bayesian statistics in the late 1990's came the relaxed molecular clock approach, in which variation in rates of molecular evolution among branches of a phylogeny can be incorporated into the model (Thorne & Kishino 1998, Drummond *et al*. 2006, Kishino *et al*. 2001).

Accurate estimates of divergence times and rates of evolution obtained from molecular clock analysis are largely dependent on the quality of the calibration data used (Sauquet *et al.* 2012). Point fossil calibrations were used in the past, and uncertainty surrounding these dates was not accounted for in divergence dating models (Rutschmann 2006). However, Bayesian statistics now allows uncertainty around fossil dates to be

incorporated (Drummond *et al*. 2006). Fossils or geological events are commonly used to calibrate divergence dating models. The reliability of the divergence dating results will be reduced if there are no calibration priors; fossil calibrations are too distant relative to the phylogeny being examined; there is uncertainty around the age of fossils used; or when single accurate or single inaccurate calibration points are used instead of multiple calibration points (Ho & Phillips 2009, Near & Sanderson 2004). An advantage of this study is that there is a comprehensive fossil record for Orthopterans, including members of Acrididae (Scudder 1885, Sharov 1968, Riek 1976), additionally, previous divergence dating analyses have trialled a number of fossils for use in calibrating divergence dating models (Song *et al*. 2015). Estimating divergence dates has further improved with high-throughput Next Generation Sequencing (NGS) that permits the assembly of large molecular datasets, greatly improving the robustness of phylogenies (Vaux *et al*. 2017).

Aims of study

With these factors in mind, this study has two aims. First, I investigate the genetic relationship of New Zealand's endemic grasshoppers with a range of species from outside of New Zealand (including members of Acrididae from Australia). This is in order to determine if the New Zealand group is monophyletic and in turn, to establish who their closest relatives are. Second, I will estimate the time of the New Zealand species radiation(s), including an estimate of when their lineages split from their last common ancestor (outside of New Zealand). A radiation that began after the formation of New Zealand's alpine environment would be compatible with an alpine adaptive radiation. These questions will be addressed using DNA sequences of whole mitochondrial genomes and fossil calibrated divergence dating analyses.

Materials and Methods

Taxon sampling

DNA sequence data was acquired for representatives of 75 grasshopper species comprising both published (52 individuals) and unpublished, newly sequenced taxa (20 individuals) (Table 3.2). Complete mitochondrial (mtDNA) genome sequences of species from the superfamily Acridoidea were obtained for phylogenetic analysis and molecular dating using the GenBank database search algorithm BLAST (Altschul *et al.* 1990). Forty-One Acrididae mtDNA genomes from around the world, representing 11 subfamilies and 40 different genera were acquired from GenBank. A further 11 individuals from the superfamilies: Eumasticoidea, Pamphagoidea, Tetrigoidea, Tridactyloidea and three Ensifera were obtained for use as outgroups (Table 3.2).

Next Generation Sequencing (NGS) was used to acquire mtDNA genomes from 20 New Zealand and Australian Acrididae for this study (see Chapter Two for methods). This included 14 individuals representing ten putative New Zealand alpine grasshopper species: *Alpinacris crassicauda*, *Alpinacris crassicauda* (DP), *Alpinacris tumidicauda*, *Brachaspis collinus*, *Brachaspis nivalis* (SA), *Brachaspis nivalis* (FP), *Paprides dugdali, Paprides nitidus, Sigaus australis, Sigaus campestris, Sigaus piliferus* (LW), *S. piliferus* (MH) and *Sigaus villosus*, as well as the lowland species *Sigaus minutus* (Table 3.2). Three species were sampled at two locations to encompass potential diversity: *A. crassicauda*, *B. nivalis* and *S. piliferus*. *Alpinacris crassicauda* (DP) represents a distinct lineage of *A. crassicauda* from Denniston Plateau. *Brachaspis nivalis* (SA) represents the northern clade of *B. nivalis*, while *B. nivalis* (FP) represents the southern clade. The central and northern clade of *S. piliferus* is represented by *S. piliferus* (LW) and the southern clade by *S. piliferus* (MH). Species were identified using morphological traits, with the pronotum margin, subgenital plate and epicrot being particularly informative (Bigelow 1967, Jamieson 1999).

Two other endemic New Zealand grasshopper species from the Australasian genus, *Phaulacridium* were sampled: *Phaulacridium marginale* and *Phaulacridium otagoense.* This genus is represented in Australia and all members are low-elevation species. Because the genus is neither endemic nor alpine, *P. marginale* and *P. otagoense* are not

treated as part of the New Zealand alpine grasshoppers. Nevertheless, their relationship within the Acrididae and with New Zealand's alpine species is examined by their inclusion in this study. Additionally data from four Australian alpine species: *Russalpia* sp. and *Tasmaniacris tasmaniensis* from Tasmania, and *Kosciuscola cognatus* and *Praxibulus* sp. from mainland Australia were also obtained to examine their evolutionary relationships of the New Zealand alpine species. Summaries of mtDNA genomes of the *Phaulacridium* species and mainland Australian species will be reported here as they were not included in Chapter Two (Supplementary Table 3.1, 3.2, 3.3, 3.4). All New Zealand and Tasmanian species included are classified in the subfamily Catantopinae while the mainland Australian species are classified in Oxyinae.

Phylogenetic analysis

Assembling DNA sequences of the complete mtDNA genome of each grasshopper is fully described in Chapter Two. All alignments were created using the alignment plugin MUSCLE (Edgar 2004) in Geneious R9 (v9.1.4) (Kearse *et al*. 2012), and were edited manually before being run through the Gblocks server (Castresana 2000) to remove any remaining ambiguous characters. Two different phylogenetic analyses were performed using different taxon arrangements. The first analysis included the mtDNA genomes of 26 GenBank individuals. These were aligned with nine representatives of the newly sequenced New Zealand/Australia/Tasmania individuals in order to test the appropriate use of Pamphagidae as an outgroup for the following monophyly analysis; members from Ensifera, and the Caeliferan Superfamilies of Tridactyloidea, Tetrigoidea, Eumasticoidea and Acridoidea were included (Table 3.2). Once a suitable outgroup was established, the second analysis was performed – determining if the New Zealand taxa form a monophyletic clade and in turn, establishing whom the closest relatives of New Zealand's endemic grasshoppers are. This alignment included the mtDNA genomes of 43 international Acrididae species and the 20 newly sequenced New Zealand/Australia/Tasmania individuals. Three members of Pamphagidae were used in the outgroup.

Both alignments were analysed using Maximum Likelihood (ML) (RAxML v 8 (Stamatakis 2014)) and Bayesian Inference (BI) (MrBayes v 3.2.2 (Ronquist & Huelsenbeck 2014)) through the CIPRES Science Gateway Server (Miller, Pfeiffer &

Table 3.2 List of grasshopper sequences used in the phylogenetic and divergence dating analyses in this study, along with their taxonomic classifications, global range, and GenBank accession numbers. Grasshoppers that were newly sequenced for this study are marked with a *. New Zealand's endemic alpine grasshoppers are highlighted in green, New Zealand's endemic lowland grasshoppers are highlighted in pink, alpine grasshoppers from Tasmania, are highlighted in orange, and alpine grasshoppers from mainland Australia are highlighted in blue.

Species	Location	Superfamily	Family	Subfamily	GenBank Accession
Acrida cinerea	East and Southeast Asia	Acridoidea	Acrididae	Acridinae	NC_014887
Acrida willemsei	East and Southeast Asia				NC_011303
Phlaeoba albonema	East Asia				NC_011827
Phlaeoba tenebrosa	South and East Asia				NC_029150
Calliptamus abbreviatus	Central Asia			Calliptaminae	NC_030626
Calliptamus italicus	Europe, Western and Central Asia				NC_011305
Peripolus nepalensis	Central and South Asia				NC_029135
Caryanda sp.	Unknown			Caryandinae	NC_030165
Alpinacris crassicauda	New Zealand			Catantopinae	*
***Alpinacris crassicauda* (DP)**	New Zealand				*
Alpinacris tumidicauda	New Zealand				*
Brachaspis collinus	New Zealand				*
***Brachaspis nivalis* (SA)**	New Zealand				*
***Brachaspis nivalis* (FP)**	New Zealand				*
Paprides dugdali	New Zealand				*
Paprides nitidus	New Zealand				*
Phaulacridium marginale	New Zealand				*
Phaulacridium otagoense	New Zealand				*
Sigaus australis	New Zealand				*
Sigaus campestris	New Zealand				*
Sigaus minutus	New Zealand				*
***Sigaus piliferus* (LW)**	New Zealand				*
***Sigaus piliferus* (MH)**	New Zealand				*

Sigaus villosus	New Zealand		*
Russalpia sp.	Tasmania		*
Tasmaniacris tasmaniensis	Tasmania		*
Traulia szetschuanensis	East Asia		NC_013826
Xenocatantops brachycerus	East and Southeast Asia		NC_021609
Chondracris rosea	East, Southeast and South Asia	Cyrtacanthacridinae	NC_019993
Schistocerca gregaria	Middle East, Northern and Southern Africa		NC_013240
Shirakiacris shirakii	East and Southeast Asia	Eyprepocnemidinae	NC_021610
Arcyptera coreana	East Asia	Gomphocerinae	NC_013805
Ceracris kiangsu	East Asia		NC_019994
Ceracris versicolor	Southeast Asia		NC_025285
Chorthippus chinensis	East Asia		NC_011095
Euchorthippus fusigeniculatus	East Asia		NC_014449
Gomphocerippus rufus	Europe, Central and East Asia		NC_014349
Gomphocerus licenti	East Asia		NC_013847
Gomphocerus sibiricus	Central and West Asia, Europe		NC_021103
Gomphocerus sibiricus	Central Asia		NC_015478
Gonista bicolor	East and Southeast Asia		NC_029205
Orinhippus tibetanus	South Asia		NC_023467
Pacris xizangensis	South Asia		NC_023919
Fruhstorferiola kulinga	East Asia	Melanoplinae	NC_026716
Fruhstorferiola sp.	East asia		KU355786
Kingdonella bicollina	South Asia		NC_023920
Ognevia longipennis	Central and East Asia		NC_013701
Prumna arctica	East Asia		NC_013835
Qinlingacris elaeodes	East Asia		KM363599
Qinlingacris taibaiensis	East Asia		NC_027187

Angaracris barabensis	West Siberia, Central and East Asia			Oedipodinae	NC_025558
Angaracris rhodopa	West Siberia, Central and East Asia				NC_025946
Bryodema luctuosum	South and East Asia				HQ833839
Gastrimargus marmoratus	South Africa, Central and Southeast Asia				NC_011114
Locusta migratoria	Global				JN858150
Oedaleus decorus	Central and East Asia				NC_011115
Kosciuscola cognatus	South Eastern Australia			Oxyinae	*
Oxya chinensis	East and Southeast Asia				NC_010219
Oxya hyla inricata	East and Southeast Asia				KP313875
Praxibulus sp.	Eastern Australia				*
Spathosternum prasiniferum	Southeast Asia			Spathosterninae	KM588074
Asiotmethis jubatus	West Siberia and Central Asia	Pamphagoidea	Pamphagidae	Thrinchinae	NC_025904
Filchnerella helanshanensis	East Asia				NC_020329
Prionotropis hystrix	South Europe				NJX913764
Erianthus versicolor	Southeast Asia	Eumasticoidea	Chorotypidae	Erianthinae	JQ975394
Pielomastax zhengi	East Asia		Episactidae	Episactinae	NC_016182
Paramastax nigra	Western South America		Eumastacidae	Paramastacinae	JX913772
Alulatettix yunnanensis	East Asia	Tetrigoidea	Tetrigidae	Tetriginae	NC_018542
Tetrix japonica	East Asia				NC_018543
Cylindraustralia sp.	Australia	Tridactyloidea	Cylindrachetidae	-	KM657334
Mirhipipteryx andensis	Central and South America		Ripipterygidae	Ripipteryginae	NC_028065
Ellipes minuta	North, Central and South America		Tridactylidae	Tridactylinae	NC_014488
Ensifera					
Comicus campestris	Southern Africa	Schizodactyloidea	Schizodactylidae	Comicinae	NC_028062
Gryllotalpa orientalis	Cosmopolitan	Gryllidea	Gryllotalpoidea	Gryllotalpinae	AY660929
Teleogryllus emma	Cosmopolitan	Grylloidea	Gryllidae	Gryllinae	EU557269

Schwartz 2010). ML analyses were conducted using 1000 random starting trees under a GAMMA model and GTR substitution matrix. Node support was assessed using 1000 non-parametric bootstrap iterations, applying fast bootstrapping, alongside a subsequent ML search. Phylogenies were visualised in FigTree v 1.4.3 (Rambaut 2016). For the BI analyses, each analysis was run using a GTR substitution model combined with the proportion of invariable sites model Invgamma; models were run for 30 million generations with four MCMC chains in each, and a burnin of one million generations. Outputs were analysed in Tracer v 1.6 (Rambaut *et al*. 2014), before trees were produced and visualised in FigTree.

Dating analysis

Time calibration of these grasshoppers used BEAST2 (v 2.4.4) (Bouckaert *et al*. 2014). An .xml file was produced in BEAUti2 (Bouckaert *et al*. 2014) where the directions for BEAST2 to run a GTR site model, a Relaxed Clock Log Normal clock model and a Birth Death model as the tree prior were specified. A thorough divergence dating analysis was performed; a number of BEAST2 runs were implemented using different molecular datasets, numbers of individuals and fossil calibration points in order to get the most well supported divergence dates for the New Zealand and Australian taxa. Five different fossil calibrations were chosen for the divergence dating analysis (Table 3.3). They were selected because they are the oldest definitive fossils for their respective Superfamilies, and for *Eolocustopsis primitiva* it is the oldest definitive fossil for Caelifera and Acridomorpha (Riek 1976, Scudder 1885, Sharov 1968). Additionally these fossils have already been trialled in a divergence dating analysis (Song *et al*. 2015). All calibration points were constrained by monophyly and age, and a normal distribution was used as their distribution prior. The .xml file produced in BEAUti2 was run through BEAST2 for 10 or 100 million generations, sampling every 1000 generations. Tracer was used to investigate the Bayesian output from BEAST2. Maximum clade credibility trees with median heights were generated in TreeAnnotator v 2.4.4 (Bouckaert *et al*. 2014) and edited in FigTree, where approximate dates of divergence and 95% HPD intervals could be visualized. The processing power of CIPRES Science Gateway v 3.3 (Miller *et al*. 2010) was used for all phylogenetic tree construction and divergence dating analyses.

Table 3.3 Fossils used to calibrate divergence dating analyses. Mya = Million years ago. min – max = the minimum and maximum age of each fossil based on the stratigraphic layer they were found in. SD = standard deviation. Table is modified from Song *et al.* 2015.

Fossil	Superfamily	Species	Notes	Fossil description	Median age (Mya)	Fossil age (min – max)	Reference
1	Locustopsoidea	*Eolocustopsis primitiva*	Oldest definitive Caelifera & Acridomorpha	Forewing (incomplete), forewing length = 17mm, found in Changhsingian delta plain shale South Africa	253.15	252.3–254.0 (SD = 1.2)	Riek (1976)
2	Tridactyloidea	*Monodactylus curtipennis*	Oldest definitive Tridactyloidea	Exoskeleton (whole insect), body length = 11.5mm, found in Aptian lacustrine siltstone/marl, Zaza formation, Russia	119.0	112.6–125.4 (SD = 9.0)	Sharov (1968)
3	Tetrigoidea	*Prototetrix reductus*	Oldest definitive Tetrigoidea	Forewing (complete), forewing length = 6.2mm, found in Aptian lacustrine siltstone/marl, Zaza formation, Russia	119.0	112.6–125.4 (SD = 9.0)	Sharov (1968)
4	Eumasticoidea	*Archaeomastax jurassicus*	Oldest definitive Eumasticoidea and Eumastacidae	Fore and hindwings, forewing length = 10mm, found in Callovian/Oxfordian lacustrine siltstone, Karabastau formation, Kazakhstan	160.2	155.7–164.7 (SD = 6.4)	Sharov (1968)
5	Acridoidea	*Tyrbula russelli*	Oldest definitive Acridoidea and Acrididae	Exoskeleton (complete female), body length = 23mm, found in Chadronian lacustrine shale, Florissant formation, Colorado	35.55	33.9–37.2 (SD =2.3)	Scudder (1885)

Results

Phylogenetic analysis

After the removal of gaps and ambiguities the mitochondrial genome alignment lengths for the outgroup test and monophyly analysis were 10,009bp and 13,878bp respectively. ML and BI trees were identical in topology for the outgroup dataset (Figure 3.1). No paraphyly or polyphyly between subfamilies was found. Pamphagidae was inferred to be a sister clade to the Acrididae clade, with the split between these two being well supported by a Maximum Likelihood Bootstrap (MLB) value of 100 and a Bayesian Inference Posterior Probability (BIPP) of 1. Sister to these two clades was the Eumasticoidea group (MLB 98, BIPP 1), then Tetrigoidea (MLB 100, BIPP 1), and the final Caeliferan clade inferred to be the sister to all former clades was Tridactyloidea (MLB 100, BIPP 1). This is supported by the reconstruction of other Orthopteran phylogenies (Song *et al*. 2015).

After establishing Pamphagidae as a suitable outgroup to the Acrididae clade, the monophyly analyses were carried out. Both the ML and BI trees were similar in topology, the only difference being a polytomy of branches early in the BI tree (Figure 3.2, 3.3). Forty-one out of 62 nodes had MLB and BIPP values of 100 and 1 respectively. The four New Zealand alpine genera of interest (*Alpinacris*, *Brachaspis*, *Paprides* and *Sigaus*) formed a monophyletic clade (ML 100, BIPP 1), although the monophyly of individuals within each of these genera was not supported (as found in Chapter Two). The Tasmanian species (*T. tasmaniensis* and *Russalpia sp*.) were found to form a clade sister to the New Zealand alpine genera (ML 90, BIPP 1). The two members of the lowland New Zealand genus (*Phaulacridium*) formed a monophyletic clade, sister to the clade of New Zealand and Tasmanian individuals (ML 100, BIPP 1). Two other mainland Australian alpine individuals that were sequenced for this study (*K. cognatus* and *Praxibulus sp.*) were not found to be closely related to either the New Zealand taxa (lowland or alpine) or the Tasmanian taxa, being placed in a clade with other Oxyinae members (*Oxya chinensis* and *Oxya hyla intricata*) (MLB 72, BIPP 0.99).

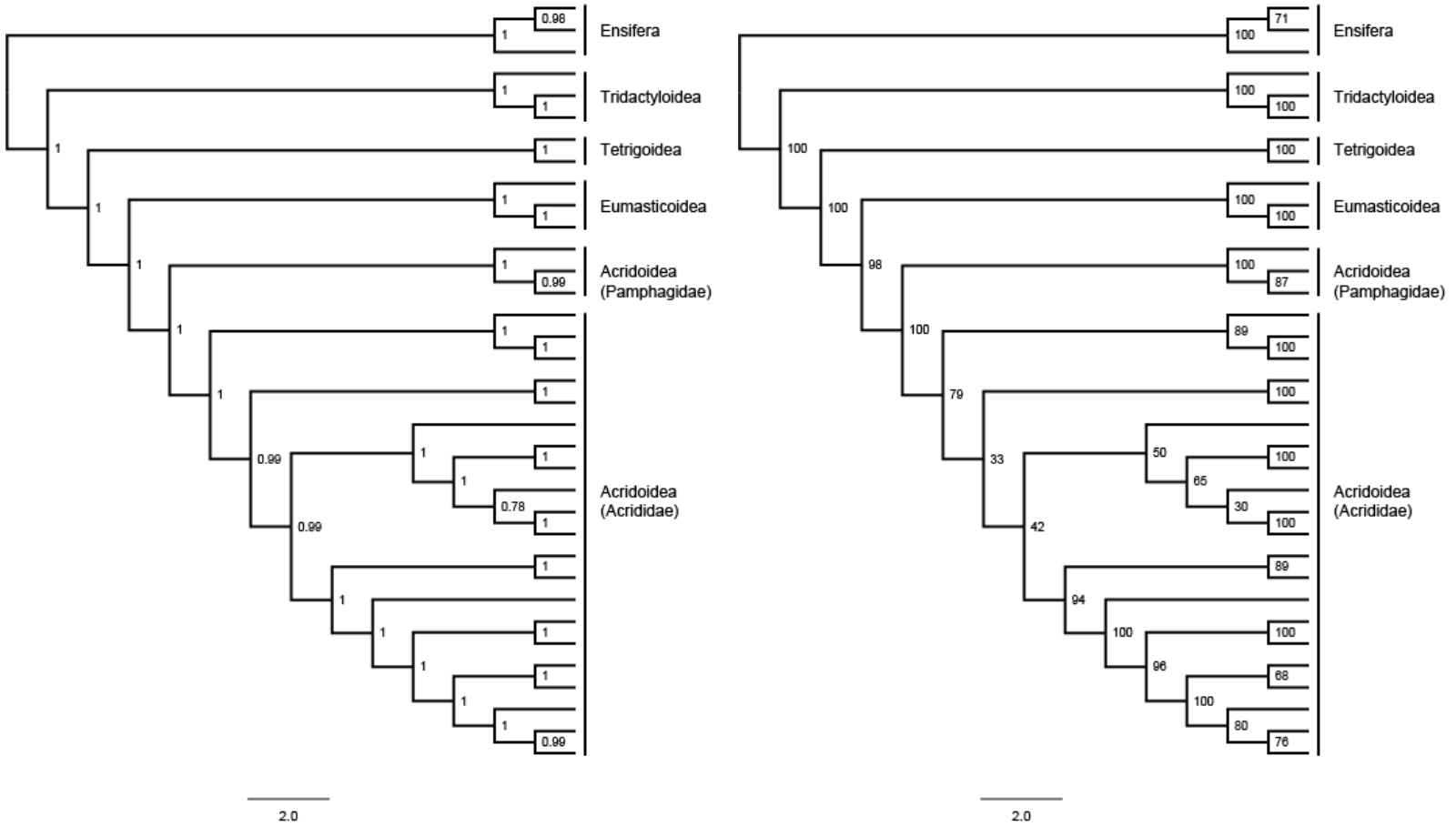


Figure 3.1 Phylogenetic hypotheses of suitable outgroups for Acrididae. Bayesian Inference (BI) (left) and Maximum Likelihood (ML) (right) phylogenetic trees of mitochondrial genome alignments. This alignment consisted of 35 Ensiferan sequences and was 10,009bp in length. Three Ensiferans were enforced as the outgroup. BI posterior probabilities and ML bootstraps (1000 bootstraps) are shown at nodes.

Figure 3.2 Phylogenetic hypothesis of Acridoidea relationships inferred from mitochondrial DNA using a Maximum Likelihood (ML) analysis. This alignment consisted of 60 Acrididae mitochondrial genome sequences, each 13,878bp in length. Three Pamphagidae sequences were enforced as the outgroup. Boostrap support values are shown at nodes. New Zealand's endemic alpine grasshoppers are highlighted in green, New Zealand's endemic lowland grasshoppers in pink, alpine grasshoppers from Tasmania in orange, alpine grasshoppers from mainland Australia in blue.

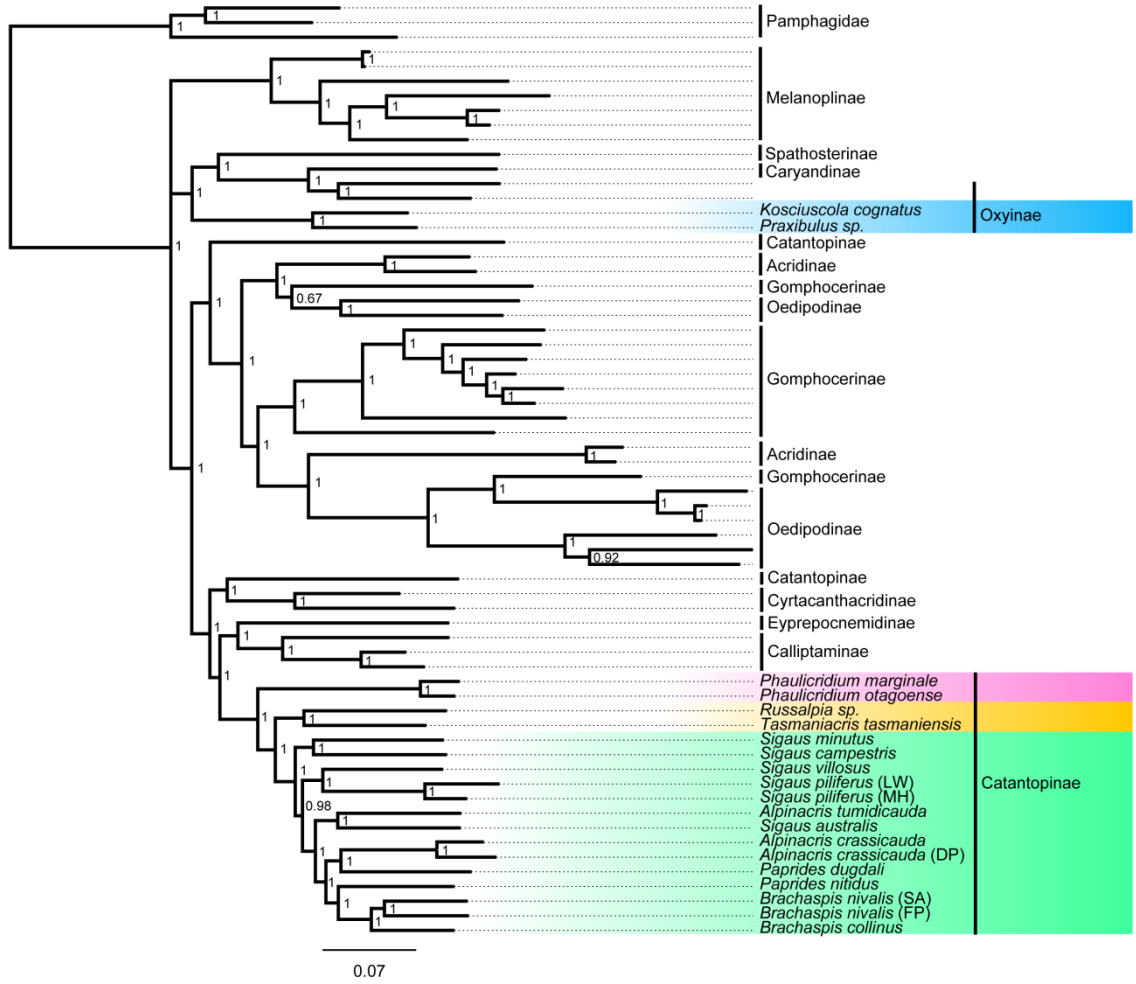


Figure 3.3 Phylogenetic hypothesis of Acridoidea relationships inferred from mitochondrial DNA using a Bayesian Inference (BI). This alignment consisted of 60 Acrididae mitochondrial genome sequences and was 13,878bp in length. Three Pamphagidae sequences were enforced as the outgroup. BI posterior probabilities are shown at nodes. New Zealand's endemic alpine grasshoppers are highlighted in green, New Zealand's endemic lowland grasshoppers in pink, alpine grasshoppers from Tasmania in orange, alpine grasshoppers from mainland Australia in blue.

Divergence dating analysis

After performing 89 BEAST2 analyses, two final trees were chosen based on an evaluation of Bayesian Inference statistics in Tracer (Table 3.4). The first tree was produced from an alignment of 13 concatenated mtDNA protein coding genes that was 10,009bp in length (Table 3.5) (Figure 3.4). It consisted of 27 individuals comprising: five New Zealand alpine species, two Tasmanian species, two mainland Australian species, one *Phaulacridium*, and nine other Acrididae representatives, alongside three Eumasticoidea, two Tetrigoidea and three Tridactyloidea. Two fossil calibrations were used (Table 3.3: fossils 2 & 5). Nodes of most interest include the split of *Phaulacridium* from the Tasmanian and New Zealand alpine group at 22 Mya (Node F: 95% HPD = 17.11 – 27Mya), the split between Tasmanian and New Zealand alpine individuals 19.5 Mya (Node G: 95% HPD = 14.83 – 24.33Mya) and the initial split between members of the New Zealand alpine clade 17 Mya (Node H: 95% HPD = 12.37 – 21.44Mya). The divergence dates established in this analysis were well supported, and were consequently used as secondary calibrations for the dating of a more thorough Acrididae phylogeny, that focuses on dating the radiation of all the New Zealand alpine taxa (Hedges *et al*. 2004).

The second tree was also produced from an alignment of mtDNA protein coding genes that was 10,015bp in length (Table 3.6) (Figure 3.5). It consisted of 32 individual's comprising: 14 New Zealand alpine species, two Tasmanian species, two *Phaulacridium* species, two mainland Australian species, 10 other Acrididae representatives and three Eumasticoidea individuals. One prior age calibration was used at the root of the Acrididae clade, obtained from the first BEAST2 tree (Node D: 35.5Mya, SD = 3.8). There are minor differences in the divergence dates of the three previously mentioned splits: the split of *Phaulacridium* from the Tasmanian and New Zealand alpine group at 20.82Mya (Node C: 95% HPD = 15.49 – 26.24Mya), the split between Tasmania and New Zealand alpine individuals 18.6Mya (Node E: 95% HPD = 13.77 – 23.49Mya) and the initial split between members of the New Zealand alpine clade 16.61Mya (Node G: 95% HPD = 12.39 – 21.11Mya) (Table 3.6).

Within the main group of interest, the New Zealand alpine species, the root split at node G of *S. campestris* and *S. minutus* from the remaining alpine species is very close

Table 3.4 Summary of the 89 BEAST2 analyses investigated for this study. NZ Species notes if run contained all New Zealand (NZ) species or just representatives (Reps). Fossil calibration numbers refer to fossils in Table 3.3, whilst fossil letters refer to nodes in Table 3.5. The Alignment type column notes whether the sequences consisted of protein coding genes (CDS), ribosomal RNA genes (rRNA) and/or transfer RNA (tRNA) genes. Effective Sample Size (ESS) summary statistics for Posterior, Prior and Tree Likelihood are provided; values greater than 200 are considered enough to make analyses useful. Divergence dates of the Tasmania/ New Zealand split and the NZ split are displayed, along with their respective Highest Posterior Density interval (HPD) values. The two BEAST2 runs highlighted in purple are the two final divergence date models displayed in this study (Figure 3.4 and 3.5).

NZ Species	Number of Sequences	Fossil Calibrations	Alignment Type	Number of Iterations (in millions)	Posterior	Prior	Tree Likelihood	Tasmania/ NZ split	Tasmania/NZ split 95% HPD	NZ Split	NZ split 95% HPD
All	53	5	CDS, rRNA, tRNA	10	12	233	12	19.96	16.35 – 23.82	17.71	14.44 – 21.27
Reps	44	5	CDS, rRNA, tRNA	10	138	35	53	19.33	14.59 – 24.15	16.92	12.43 – 21.70
All	56	2,5	CDS, rRNA, tRNA	10	15	47	15	20.81	17.17 – 24.88	18.61	15.52 – 22.25
Reps	47	2,5	CDS, rRNA, tRNA	10	13	67	11	20.92	14.95 – 24.91	18.57	13.23 – 22.67
All	55	3,5	CDS, rRNA, tRNA	10	4	15	5	25.87	21.23 – 30.91	22.72	14.75 – 30.58
Reps	46	3,5	CDS, rRNA, tRNA	10	3	6	3	21.72	13.40 – 26.47	16.49	8.37 – 20.83
All	58	2,3,5	CDS, rRNA, tRNA	10	115	26	844	24.33	18.36 – 29.42	21.92	15.97 – 27.01
Reps	59	2,3,5	CDS, rRNA, tRNA	10	16	23	13	14.95	11.73 – 20.73	13.41	9.63 – 19.76
All	61	2,3,4,5	CDS, rRNA, tRNA	10	4	12	4	25.30	17.92 – 31.03	21.83	16.45 – 29.43
Reps	52	2,3,4,5	CDS, rRNA, tRNA	10	4	9	4	12.85	5.14 – 27.34	9.58	3.555 – 20.70
All	64	1,2,3,4,5	CDS, rRNA, tRNA	10	9	6	9	21.85	16.09 – 31.44	19.84	12.33 – 28.32
Reps	55	1,2,3,4,5	CDS, rRNA, tRNA	10	4	4	4	19.00	10.70 – 25.26	14.35	8.72 – 19.47
All	53	5	CDS, rRNA	10	7	46	6	20.22	16.53 – 24.05	17.83	14.53 – 21.10
Reps	44	5	CDS, rRNA	10	218	507	189	19.31	15.53 – 22.97	17.18	13.48 – 20.76
All	56	2,5	CDS, rRNA	10	5	11	5	22.34	15.43 – 29.83	19.91	10.51 – 25.34
Reps	47	2,5	CDS, rRNA	10	38	22	71	18.86	13.28 – 26.19	15.37	9.62 – 20.98
All	55	3,5	CDS, rRNA	10	23	103	22	20.72	17.38 – 24.58	18.45	15.14 – 22.00
Reps	46	3,5	CDS, rRNA	10	31	128	22	20.34	16.26 – 24.99	18.16	13.89 – 22.56
All	58	2,3,5	CDS, rRNA	10	17	41	17	26.07	22.40 – 31.23	24.14	20.56 – 29.73
Reps	59	2,3,5	CDS, rRNA	10	10	32	10	21.21	14.73 – 29.21	17.37	11.40 – 23.96
All	61	2,3,4,5	CDS, rRNA	10	3	27	3	23.83	18.98 – 28.75	20.66	16.21 – 26.45
Reps	52	2,3,4,5	CDS, rRNA	10	3	10	3	23.07	14.55 – 30.41	18.87	11.44 – 26.99
All	64	1,2,3,4,5	CDS, rRNA	10	4	9	4	23.40	19.17 – 30.63	20.79	17.1 – 27.07

Reps	55	1,2,3,4,5	CDS, rRNA	10	5	14	5	16.70	11.97 – 23.35	13.23	7.92 – 20.59
All	53	5	CDS	10	86	500	85	20.66	17.25 – 24.05	18.56	15.40 – 21.74
Reps	44	5	CDS	10	1037	502	988	19.07	15.28 – 23.26	16.85	13.14 – 20.68
All	56	2,5	CDS	10	3	24	3	22.90	14.54 – 27.70	20.82	14.12 – 26.15
Reps	47	2,5	CDS	10	18	26	21	19.63	12.99 – 29.05	16.03	10.53 – 25.80
All	55	3,5	CDS	10	15	63	14	20.79	17.64 – 23.95	18.81	15.62 – 21.79
Reps	46	3,5	CDS	10	188	138	266	20.38	16.28 – 24.01	18.10	14.45 – 21.49
All	58	2,3,5	CDS	10	15	41	11	25.84	22.13 – 29.98	23.50	19.98 – 27.99
Reps	59	2,3,5	CDS	10	18	10	53	18.15	12.06 – 23.46	14.88	10.17 – 19.63
All	61	2,3,4,5	CDS	10	7	8	7	25.43	18.88 – 31.35	22.61	16.13 – 28.56
Reps	52	2,3,4,5	CDS	10	11	8	11	22.96	16.35 – 29.98	19.89	14.67 – 28.11
All	64	1,2,3,4,5	CDS	10	6	25	6	25.17	20.30 – 30.08	22.28	18.05 – 27.65
Reps	55	1,2,3,4,5	CDS	10	14	10	16	17.04	10.56 – 21.28	13.74	7.24 – 18.43
Reps	53	5	CDS	100	240	1791	265	19.04	14.94 – 23.31	16.84	12.79 – 20.99
Reps	53	5	CDS	10	383	194	633	16.21	12.81 – 20.03	14.41	11.17 – 18.01
Reps	53	5	CDS	100	379	994	336	16.21	12.45 – 20.28	14.40	10.89 – 18.24
Reps	56	2,5	CDS	100	129	1426	123	20.18	16.25 – 24.32	17.93	14.13 – 21.98
Reps	56	2,5	CDS	100	2676	634	4487	18.24	14.64 – 22.0	16.29	12.73 – 19.88
All	53	5	CDS	10	543	161	1141	16.92	13.66 – 19.94	15.28	12.39 – 18.09
Reps	44	5	CDS	10	897	140	1887	16.59	13.19 – 19.95	14.89	11.70 – 18.10
All	43	5	CDS	10	867	283	1274	21.26	17.2 – 25.21	19.08	15.49 – 22.70
Reps	34	5	CDS	10	1139	230	1789	20.82	16.64 – 25.01	18.67	14.50 – 22.45
Reps	37	4,5	CDS	10	392	64	1154	20.93	16.87 – 24.92	18.61	14.71 – 22.92
Reps	36	3,5	CDS	10	178	66	81	19.52	12.85 – 26.78	15.68	7.44 – 24.18
Reps	45	2,4,5	CDS	10	10	5	8	19.60	13.61 – 29.82	15.48	9.75 – 20.93
Reps	45	2,4,5	CDS	10	16	8	900	19.97	8.64 – 23.71	17.14	7.39 – 20.95
Reps	45	4,5	CDS	10	9	17	9	22.46	18.06 – 26.40	19.66	15.18 – 23.94
Reps	45	4,5	CDS	10	415	90	882	21.46	16.72 – 25.97	18.62	14.43 – 23.07
Reps	45	1,4,5	CDS	10	119	32	661	22.71	17.43 – 27.59	19.78	14.83 – 24.90
Reps	45	1,4,5	CDS	10	278	73	1114	24.32	19.57 – 28.61	21.34	16.43 – 25.77
Reps	45	1,4,5	CDS	10	185	55	1364	21.61	15.97 – 27.08	18.84	12.97 – 24.03
Reps	42	1,4,5	CDS	10	255	57	833	21.05	16.75 – 25.41	18.23	14.47 – 22.30
Reps	42	1,4,5	CDS	10	39	47	36	20.61	12.98 – 24.76	18.10	12.31 – 22.77
Reps	42	1,5	CDS	10	209	72	1302	18.62	14.10 – 22.94	16.27	12.53 – 20.55
Reps	42	1,2,5	CDS	10	419	83	1191	18.40	14.49 – 22.07	16.13	12.69 – 19.80
Reps	30	1,5	CDS	10	433	89	947	22.40	16.86 – 26.92	19.20	14.70 – 23.41
Reps	30	1,4,5	CDS	10	229	75	1184	22.59	17.10 – 28.13	19.12	13.44 – 25.09
Reps	30	1,2,5	CDS	10	5	133	5	21.31	17.15 – 25.34	18.71	14.41 – 22.60
Reps	30	1,2,5	CDS	10	53	53	81	20.58	13.10 – 29.44	17.13	8.47 – 25.68
Reps	27	1,5	CDS	10	373	101	905	20.91	16.08 – 26.17	18.18	13.88 – 23.13
Reps	27	1,4,5	CDS	10	692	264	750	21.27	16.59 – 26.77	18.43	13.50 – 23.18

Reps	27	1,2,5	CDS	10	73	187	60	22.02	17.72 – 27.9	19.26	14.18 – 24.31
Reps	27	1,2,5	CDS	10	750	161	1049	20.86	15.72 – 26.24	18.02	12.92 – 23.54
Reps	27	1,2,5	CDS	100	4639	1014	8812	20.96	15.55 – 26.18	18.01	12.89 – 23.21
Reps	27	1,2,5	CDS	10	771	145	1321	20.45	16.13 – 24.95	17.91	13.88 – 22.21
Reps	27	1,2,4,5	CDS	10	7	77	7	21.48	16.44 – 26.28	18.75	13.93 – 23.32
Reps	27	1,2,5	CDS	100	5279	1074	7896	20.22	15.91 – 24.81	17.56	13.41 – 22.27
Reps	27	2,5	CDS	10	1028	211	1090	19.11	15.3 – 23.25	16.64	12.92 – 20.69
Reps	27	2,5	CDS	100	3778	707	8829	19.46	14.82 – 24.23	16.90	12.37 – 21.43
Reps	30	1,2,5	CDS	10	870	195	1150	21.28	17.05 – 25.21	18.60	14.58 – 22.64
Reps	30	1,2,5	CDS	100	8158	1430	11766	21.12	16.96 – 25.40	18.39	14.21 – 22.61
Reps	30	2,5	CDS	10	748	170	1131	20.89	16.80 – 24.83	18.17	13.98 – 22.09
Reps	30	2,5	CDS	100	5525	923	10868	20.34	16.10 – 24.52	17.74	13.64 – 21.84
Reps	30	1,2,5	CDS	10	9	252	9	20.49	16.71 – 25.41	17.70	13.90 – 22.77
Reps	30	1,2,5	CDS	100	4504	1790	9030	21.17	16.55 – 26.05	18.37	13.82 – 23.19
Reps	30	2,5	CDS	10	424	120	1074	20.84	16.87 – 28.00	18.11	14.05 – 22.73
Reps	30	2,5	CDS	100	6459	870	9806	21.17	16.97 – 25.26	18.52	14.48 – 22.63
Reps	30	2,5	CDS	10	471	69	1411	19.63	14.53 – 24.95	16.88	11.80 – 21.65
Reps	30	2,5	CDS	100	6758	1068	11362	19.81	15.23 – 24.84	17.19	12.6 – 21.62
Reps	34	5	CDS	10	5	3	5	8.72	1.03 – 33.40	7.27	10.64 – 30.09
Reps	34	5	CDS	10	918	227	1263	25.37	19.36 – 31.00	22.79	17.31 – 28.03
All	32	C,D	CDS	10	7	7	208	-	-	-	-
All	32	C,D,G	CDS	10	4	4	1480	-	-	-	-
All	32	C	CDS	10	105	101	1285	-	-	-	-
All	32	D	CDS	10	242	144	578	18.52	13.7 – 23.7	16.46	12.27 – 24.29
All	32	D	CDS	100	4999	1484	15734	18.6	13.77 – 23.48	16.61	12.39 – 21.11

Table 3.5 Divergence dating analysis node ages, in reference to nodes in Figure 3.4. Median node age is in millions of years (My). Posterior probabilities are inferred from Bayesian Inference (BI). HPD = Highest Posterior Density interval estimates.

Node	Median Node Age (My)	95% HPD (Node Age)	Posterior Probability
A	131.16	127.72 – 134.55	1
B	106.9	82.34 – 130.31	0.81
C	86.16	61.76 – 109.0	1
D	35.5	31.76 – 39.36	1
E	28.65	22.16 – 35.05	0.87
F	22.09	17.11 – 27.0	1
G	19.46	14.83 – 24.23	1
H	16.9	12.37 – 21.43	1

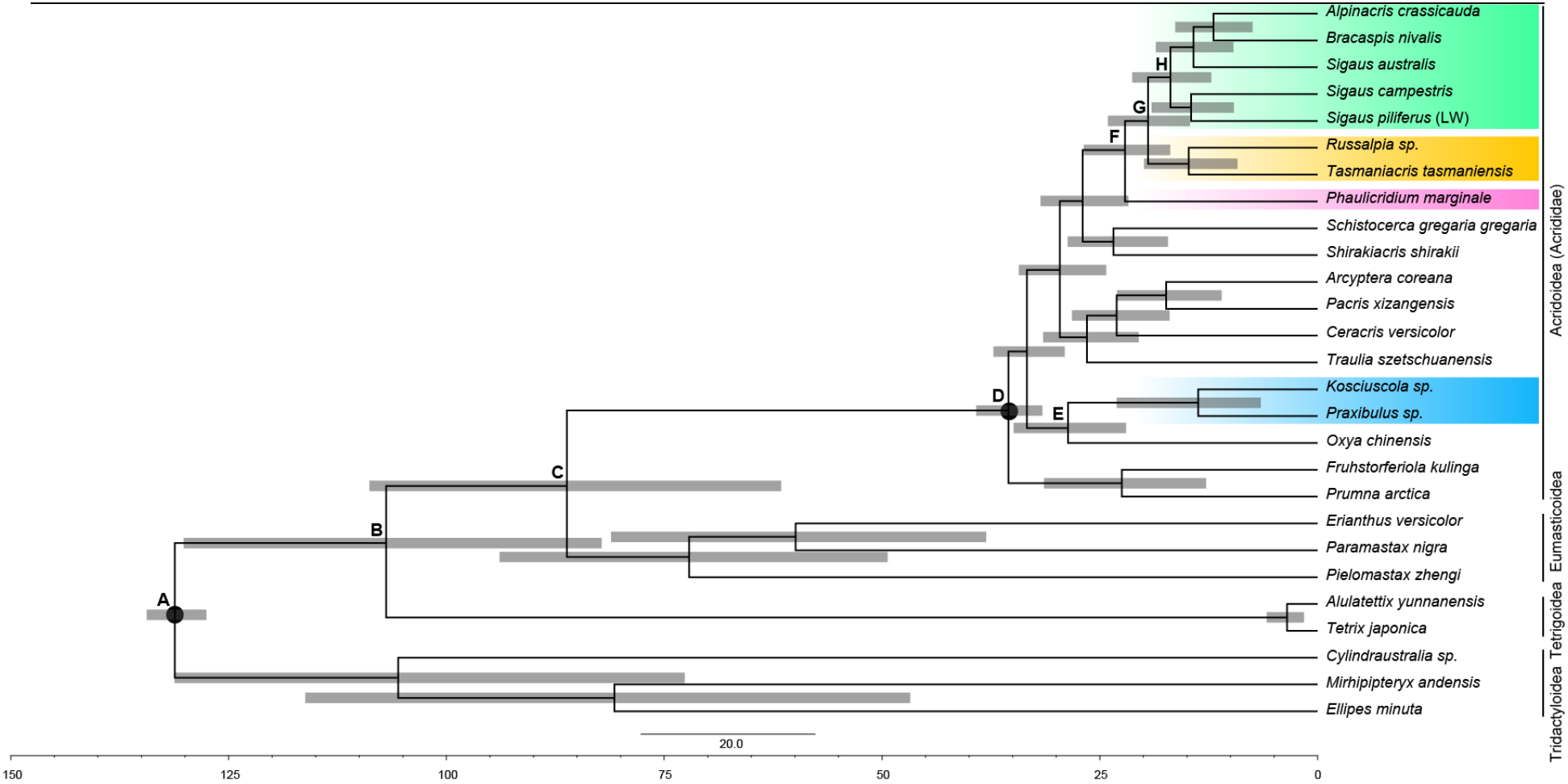


Figure 3.4 Phylogenetic hypothesis of Acridoidea relationships and divergence dates inferred from mitochondrial DNA using Bayesian Inference (BI). This alignment consisted of 27 Acrididae mitochondrial genome sequences and was 10,009bp in length. No sequences were enforced as the outgroup. BI posterior probabilities, divergence date estimates and 95% HPDs of divergence date estimates are provided in Table 3.5. New Zealand's endemic alpine grasshoppers are highlighted in green, New Zealand's endemic lowland grasshoppers in pink, alpine grasshoppers from Tasmania in orange, alpine grasshoppers from mainland Australia in blue. Black circles at nodes A and D indicate where fossil calibrations were applied.

Table 3.6 Divergence dating analysis node ages, in reference to nodes in Figure 3.5. Median node age is in millions of years (My). Posterior probabilities are inferred from Bayesian Inference (BI). HPD = Highest Posterior Density estimates.

Node	Median Node Age (My)	95% HPD (Node Age)	Posterior Probability
A	34.2	26.6 – 41.8	1
B	10.32	6.73 – 14.27	1
C	20.82	15.49 – 26.24	1
D	5.25	3.27 – 7.65	1
E	18.6	13.77 – 23.49	1
F	15.07	10.75 – 19.55	1
G	16.61	12.39 – 21.11	1
H	14.29	10.29 – 18.40	1
I	16.05	12.01 – 20.49	0.49
J	14.37	10.47 – 18.48	1
K	5.73	3.66 – 8.09	1
L	14.89	10.94 – 18.90	1
M	12.62	9.01 – 16.31	1
N	13.98	10.25 – 17.79	1
O	12.61	9.12 – 16.12	1
P	9.02	6.29 – 11.91	1
Q	7.8	5.36 – 10.54	0.95
R	12.42	8.92 – 15.92	1
S	4.88	3.1 – 6.87	1

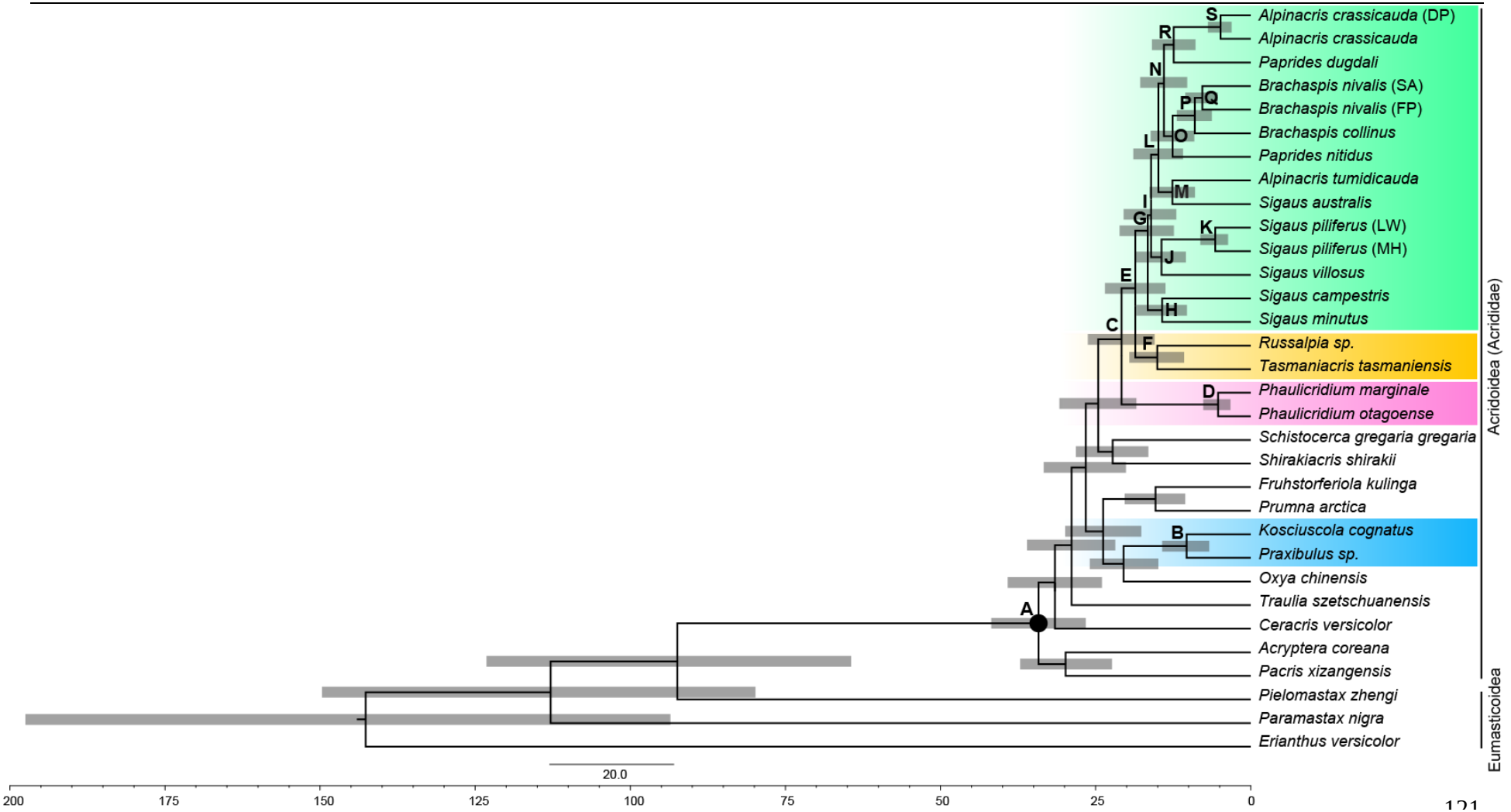


Figure 3.5 Phylogenetic hypothesis of Acridoidea relationships and divergence dates inferred from mitochondrial DNA using Bayesian Inference (BI). This alignment consisted of 32 Acrididae mitochondrial genome sequences and was 10,015bp in length. No sequences were enforced as the outgroup. BI posterior probabilities, divergence date estimates and 95% HPDs of divergence date estimates are provided in Table 3.6. New Zealand's endemic alpine grasshoppers are highlighted in green, New Zealand's endemic lowland grasshoppers in pink, alpine grasshoppers from Tasmania in orange, alpine grasshoppers from mainland Australia in blue. Black circles at nodes A and D indicate where fossil calibrations were applied.

followed by another divergence at node I (~16Mya), although this node has a low BIPP value (0.49). Between ~12 – 14Mya, there appears to be a radiation event, with seven divergences (Nodes: H, J, L, M, N, O, R). At ~9Mya, the *Brachaspis* clade is formed. The most recent divergences occurred ~4 – 6Mya, between *A. crassicauda* and *A. crassicauda* (DP) (Node: S) and *S. piliferus* (LW) and *S. piliferus* (MH) (Node: K). The divergence date estimate for the two Tasmanian species (Node: F) is just prior to the radiation event in New Zealand, while the two *Phaulacridium* species (Node: D) are younger, having a divergence date at a similar time to the *A. crassicauda* and *S. piliferus* individuals. The mainland Australian alpine species (Node: B) diverged ~10Mya.

Discussion

To test predictions about how groups of species have evolved, it is imperative to establish a robust phylogeny, and estimate the timing of divergence events using reliable calibrations (Hills 2010, Ho & Phillips 2009, Near & Sanderson 2004, Sauquet *et al.* 2012, Vaux *et al.* 2017). This study had two aims. The first was to determine the ancestry of New Zealand's endemic alpine grasshoppers and to establish if they form a monophyletic clade. The second was to estimate the time of their divergence from their closest relatives, as well as each other, in order to determine if the radiation of this group coincided with geological and/or climatic events. I investigated these aims using published mitochondrial genomes of global Acrididae species and 20 newly sequenced mitochondrial genomes of New Zealand and Australian alpine grasshoppers. Molecular phylogenetic analysis inferred a monophyletic clade of New Zealand endemic alpine taxa. Of all the grasshoppers sampled, the New Zealand taxa are most closely related to Tasmanian alpine species. However, the New Zealand/Tasmanian alpine clade is not sister to other Australian alpine species; this suggests that adaptation to alpine habitat has evolved more than once within the Australian grasshoppers. Furthermore, it was estimated that the ancestor of New Zealand's alpine grasshoppers split from their Tasmanian relatives approximately 19Mya.

Australian relatives

New Zealand's endemic alpine grasshoppers are currently divided into four genera (Bigelow 1967, Hutton 1898). Based on genitalia the genus *Sigaus* was considered sister to the Tasmania alpine genus *Russalpia* (and classified in the subtribe Russalpiina) (Bigelow 1967). In contrast species from the genera *Brachaspis* and *Paprides* were thought to resemble the Tasmanian alpine genus *Tasmaniacris* (Key 1991). These morphological similarities could have indicated multiple arrivals of grasshoppers to New Zealand from Tasmania. For example, an arrival of ancestors of *Russalpia* and another by ancestors of *Tasmaniacris*, that then diverged into the assorted lineages we see in New Zealand today. However, the findings here suggest just one successful arrival of an ancestor to the alpine grasshoppers of New Zealand, and that these grasshoppers share a common ancestor with the Tasmanian alpine genera *Russalpia* and *Tasmaniacris*. Thus alpine grasshopper diversity in New Zealand has

apparently resulted from a single colonisation event and subsequent speciation within New Zealand.

There are two other species of endemic grasshopper in New Zealand, *Phaulacridium marginale* and *Phaulacridium otagoense*, both of which have lowland distributions and are classified under the Tribe Cantantopini (Key 1992, Westerman & Ritchie 1984). Representatives of the genus *Phaulacridium* are found in Australia, and the five species of this genus form a monophyletic clade with respect to *Minyacris* species from Australia (Goldberg *et al.* 2015). In the current study *Phaulacridium* is sister to the New Zealand/Tasmanian clade suggesting that there were at least two successful arrivals of grasshoppers to New Zealand. Further sampling of global Acrididae species could reveal species that nest within the New Zealand and/or New Zealand/Tasmanian clade, although this would not change the date of their split from their Tasmanian relatives.

Divergence dating and alpine ancestry

Using fossil calibrated molecular clock analyses the mtDNA phylogeny revealed that the New Zealand grasshoppers split from their Tasmanian relatives approximately ~19Mya. It has been determined that the New Zealand alpine species likely arrived in New Zealand during a geological and climatic period when no alpine habitat would have been available (McGlone *et al*. 2001). New Zealand, 17–20Mya, is thought to have been moist and warm-temperate in climate, and covered in subtropical forest (McGlone *et al*. 2001); Acrididae are ectotherms, and require UV radiation for thermoregulation - open spaces for basking are a necessity (Harris *et al*. 2015, Pepper & Hastings 1952). For the ancestors of New Zealand's alpine grasshoppers to have been able to establish themselves, there must have been open spaces available (e.g. the edges of forests, around rivers and lakes, and/or along the coast); however, these would not have been in the form of alpine tussock grasslands. The predominant tussock genus *Chionochloa* is estimated to have arrived and radiated in New Zealand ~3–11.4Mya (Pirie *et al*. 2010) This would suggest that the ancestors of today's New Zealand grasshoppers were not dependent on living in the alpine zone, and were suitably adapted to living in subtropical conditions on arrival. This could have implictions for how they respond to future climate change.

There appears to be a radiation of New Zealand taxa ~13–15Mya, which could suggest a change in landscapes at this time in New Zealand (e.g. open grasslands), allowing for species to expand their ranges and diversify. However, there is no indication that the grasshoppers radiated during the main mountain building events of the Kaikoura Orogeny 2–5Mya, with most grasshopper species already being present by the time that mountains and stable alpine habitats became available in New Zealand (Kamp 1986, Trewick & Bland 2012, Wallis *et al.* 2016). This begs the question, why are the majority of New Zealand's alpine grasshoppers found in the alpine zone today, when it appears that on arrival, they successfully colonised lowland habitats?

New Zealand's alpine grasshoppers have a clear association with the alpine zone, and this phylogeny indicates that it could be an ancestral trait given that the sister clade in Tasmania is also alpine adapted. Tasmania's mountains are thought to have been present throughout the Cenozoic (66 – 0Mya), however a suitable cool climate for an alpine environment is unlikely to have been available in Tasmania until the late Oligocene/early Miocene (~23.7Mya) (Hill 1988, Macphail *et al* 1991). It is probable that Tasmania's alpine grasshoppers evolved during the Miocene, potentially through the adaptation of lowland Tasmanian populations to the newly formed alpine zone. Alternatively, alpine ancestors from mainland Australian mountain populations could have colonised the newly formed Tasmanian alpine habitat; however the findings of this study indicate this would not have been by members of the alpine genera *Kosciuscola* or *Praxibulus*. Stable alpine habitat was not available in New Zealand until ~2.6–0.9Mya (Heenan & McGlone 2013, Wallis *et al*. 2016). I have estimated the ancestor of New Zealand's alpine grasshoppers to have split from their Tasmanian relatives ~19Mya. At this time of the Miocene, it is plausible that the ancestors of New Zealand's alpine grasshoppers were adapted to the alpine environment and had an alpine distribution in Tasmania. Regardless, their ancestor arrived in New Zealand at a time when no mountains were present, let alone a stable alpine habitat for them to colonise.

New Zealand's alpine lineages

Estimating the time of divergence has been attempted for a number of New Zealand alpine lineages, with varying levels of reliability (Table 3.1). Many of these studies use genetic datasets that are less than 1000bp in length; the majority use fossils outside of

their focal study group for rate calibration, and some use taxonomic relationships that have since been revised; thus the quality of their date estimates is questionable. Most of the studies estimated New Zealand alpine lineages to be about the same age as stable alpine habitat (70% suggested less than 6 mya; Table 3.1). And all nine studies that used fossil calibrations rather than applying a universal rate of DNA mutation estimated a maximum age of their lineage as < 8 mya (Table 3.1). Greater DNA sequence lengths, such as can be obtained through NGS, will greatly improve the robustness of the phylogenies being examined (previously the study with the longest DNA sequences was < 6,000bp) (Buckley & Simon 2017, Hills 2010, Vaux *et al*. 2007). In addition, using fossils of recent common ancestors provides a more reliable constraint on the model of evolution of the group being studied than applying a single rate of DNA evolution based on unrelated taxa (Sauquet *et al*. 2012). These two factors, along with the implementation of a powerful Bayesian analysis, are why the current study provides a more reliable estimate for the time of divergence of a New Zealand alpine lineage than previous work. This includes prior divergence dating analyses of New Zealand's alpine grasshoppers. Previous studies have dated New Zealand's alpine grasshopper lineages at 2.2 - 4.4My (Trewick & Wallis 2001, Wallis *et al*. 2016), however these divergence date estimates relied on a fixed universal rate of DNA mutation and on taxonomic division of the group into four distinct genera, for which there is little evidence (Chapter Two).

Conclusion

This study explored a variety of fossil calibrations, combinations of taxa and sequence datasets. Throughout the analysis, divergence date estimates varied little between analyses (Table 3.4). The common ancestor for the New Zealand alpine grasshopper species existed long before there was alpine habitat available in New Zealand – and this requires an extraordinary explanation. Either there were multiple independent evolutionary events of freeze tolerance (for each New Zealand grasshopper lineage) and adaptation to inhabiting the alpine zone, or retention of freezing ability without selection for millions of years (retained from their common ancestor from Tasmania). Neither scenario seems likely. Instead, should we perhaps be looking at Antarctica as a possible source of New Zealand alpine grasshoppers? Could Tasmanian grasshoppers have colonised Antarctica ~ 20 mya, prior to the peak of the Antarctic greening,

remained adapted to cooler climates ~14Mya (Feakins *et al*. 2012, Lewis *et al*. 2008), and then moved to New Zealand as open habitats became available?

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Supplementary Material for Chapter Three

Supplementary Table 3.1 The New Zealand and Australian grasshopper individuals for which mitochondrial genomes, 45S ribosomal cassettes and H3 sequences were acquired, summarised and used in the phylogenetic analyses of this study. Specimen codes are linked with specimens kept in the Phoenix Collection at Massey University, Palmerston North.

Origin	Genus	Species	Species I.D.	Location
New Zealand	*Phaulacridium*	*Ph. marginale*	GH762	Ahimanawa Range, Hawke's Bay, North Island
		Ph. otagoense	GH1576	Lake Dunstan, Cromwell, South Island
Australia	*Kosciuscola*	*K. cognatus*	GH1721	Mt Kosciuszko, Guthega, NSW
	Praxibulus	*P. sp.*	GH1772	Mt Kosciuszko, Guthega, NSW

Supplementary Table 3.2 Summary of the Next Generation Sequencing (NGS) 100 base pair (bp) paired read data from which the mitochondrial genomes of two New Zealand grasshoppers and two Australian grasshoppers were assembled.

Species	# Paired Reads	Max read depth	Mean read depth	Read depth Std. Dev.
Ph. marginale	48,365	638	378.8	49.5
Ph. otagoense	16,858	478	136.2	37.9
K. cognatus	31,829	334	253.4	50.8
P. sp.	31,663	398	258.8	44.2

Supplementary Table 3.3 Summary of the Next Generation Sequencing (NGS) paired read data from which mitochondrial genomes of two New Zealand grasshoppers and two Australian grasshoppers were assembled, alongside summaries of their compositions.

Species Name	mtDNA Sequence Lengths	Protein Coding Regions	rRNAs	tRNAs	D-Loop	Intergenic Regions	GC%
Ph. marginale	15,023	11,151	2,126	1,480	163	103	27.1
Ph. otagoense	15,025	11,151	2,134	1,458	174	108	27.0
K. cognatus	15,684	11,148	2,165	1,472	840	59	25.3
P. sp.	15,299	11,148	2,165	1,472	429	85	25.3

Supplementary Table 3.4 Summary of the Next Generation Sequencing (NGS) paired read data from which 45S ribosomal (rDNA) cassettes and H3 sequences of two New Zealand grasshoppers and two Australian grasshoppers were assembled, alongside summaries of their compositions.

Species Name	45S # Paired Reads	45S Max read Depth	45S Mean read depth	45S Read depth Std. Dev.	45S Sequence Lengths	45S rRNAs	45S ITS	45S GC%	H3 Sequence Lengths	H3 GC%
Ph. marginale	103,042	16,833	1,694.1	3,001.4	6,892	6,243	632	57.4	818	66.4
Ph. otagoense	52,346	4,420	762.6	449	6,887	6,243	644	57.3	802	67
K. cognatus	13,014	385	288.8	59.3	6,865	6,298	601	57.8	809	67.9
P. sp.	49,705	1,434	1,051.7	230.7	6,911	6,259	606	57.8	810	68.8

Tarn near Mount McRae, St Arnaud Range (Rainbow Ski Area), South Island

Chapter Four

Ecological niche modelling of endemic grasshoppers to infer climate change effects on New Zealand's alpine fauna

Introduction

Atmospheric levels of carbon dioxide now exceed 400 parts per million (ppm), increasing global oceanic and land surface temperatures by 0.85°C on average in the last 120 years (Intergovernmental Panel on Climate Change (IPCC) 2014, National Oceanic Atmospheric Administration (NOAA) 2017). Concentrations of greenhouse gases are projected to raise global temperatures further, from 1°C to 3.7°C, depending on Representative Concentration Pathway (RCP) trajectories (IPCC 2014) (Table 4.1). Such temperature increases are having, and will have, profound impacts on biodiversity, especially on species that have narrow temperature requirements (Bulgarella *et al.* 2014, Chen *et al.* 2011, Hickling *et al.* 2006, Levinsky *et al.* 2007, Moritz *et al.* 2008, Parmesan 2006, Rodríguez-Trelles & Rodríguez 1998, Root *et al.* 2003, Thomas *et al.* 2004). Changes in species distributions as a result of anthropogenic climate change are of ecological, conservation and economic interest (Bellard *et al*. 2016, Buerki *et al*. 2015, Buczkowski & Bertelsmeier 2017, Fletcher *et al*. 2016, Gama *et al*. 2015, Ihlow *et al*. 2016, Lazo *et al*. 2016, Lei *et al*. 2017, Martins *et al*. 2016, Molloy *et al*. 2017, Schinhen *et al*. 2014, Yan *et al*. 2017).

Defining an organisms niche

An organism's ecological niche is considered to be all the "conditions" that allow it to exist in a set space and time; all the abiotic and biotic factors that allow a species to maintain its population growth at or above zero, and therefore exist (Elton 1927,

Table 4.1 The four Representative Concentration Pathway (RCP) trajectories proposed by the Intergovernmental Panel on Climate Change Fifth Assessment Report. Trajectories are based on four greenhouse gas concentration scenarios, which are estimated on whether emissions are reduced or not, and when they will be reduced, before the year 2090 (2081 – 2100).

Climate Change Scenario	Mean Temperature Increase (°C)	Temperature Increase Range (°C)
RCP2.6	1.0	0.3 – 1.7
RCP4.5	1.8	1.1 – 2.6
RCP6.0	2.2	1.4 – 3.1
RCP8.5	3.7	2.6 – 4.8

Grinnell 1924). Also known as its Realised Ecological Niche, an organism's niche is the outcome of its Realised Environmental Space, Fundamental Niche Space and Potential Niche Space interacting (Jackson & Overpeck 2000). A Realised Environmental Space consists of all the environmental conditions that are occurring in an organism's surroundings at a set time, and Fundamental Niche Space encompasses the specific environmental zone within which an organism can maintain a stable population size (e.g. appropriate climatic conditions); where these two variables overlap they create an organisms Potential Niche Space (Figure 4.1) (Grinnell 1917, Jackson & Overpeck 2000). This Potential Niche Space becomes further limited to an organism's Realised Ecological Niche when biotic interactions with other organisms are considered (e.g. predation, competition, disease, parasitism, mutualism) (Figure 4.1) (Elton 1927, Grinnell 1924, Jackson & Overpeck 2000).

General climatic changes will alter available Realised Environmental Space, and evolutionary processes will alter an organism's Fundamental, and therefore, Potential Niche Space; changes in biotic variables (e.g. arrival of invasive species) will result in further changes to its Realised Ecological Niche (Jackson & Overpeck, 2000). Organisms will continually be under pressure from inter- and intra-specific competition, and will need to adjust their niches to stay viable (Gause 1932, Jackson & Overpeck 2000, Van Valen 1973). With anthropogenic climate change, selection pressure is likely to change more rapidly, threatening populations, species and ecosystems.

Ecological Niche Models

Ecological Niche Models (ENMs), also known as species distribution models, are a useful tool for predicting the potential niche space that species can occupy (Pearson & Dawson 2003, Phillips & Dudik 2008, Thuiller 2003). There has been an increase in the modelling of species distributions with the availability of modelling software (GARP, MaxEnt, ModEco) and R packages (biomod2, dismo, sdm); while the more recent introduction of ensemble models has improved their predictive accuracy (Anderson *et al*. 2003, Hijmans & Elith 2013, Naimi & Araújo 2016, Phillips *et al.* 2005, Sivyer *et al.*2018, Thuiller 2003, Thuiller *et al.* 2009, 2016). Ecological Niche Models use the known distribution of a species to determine what environmental

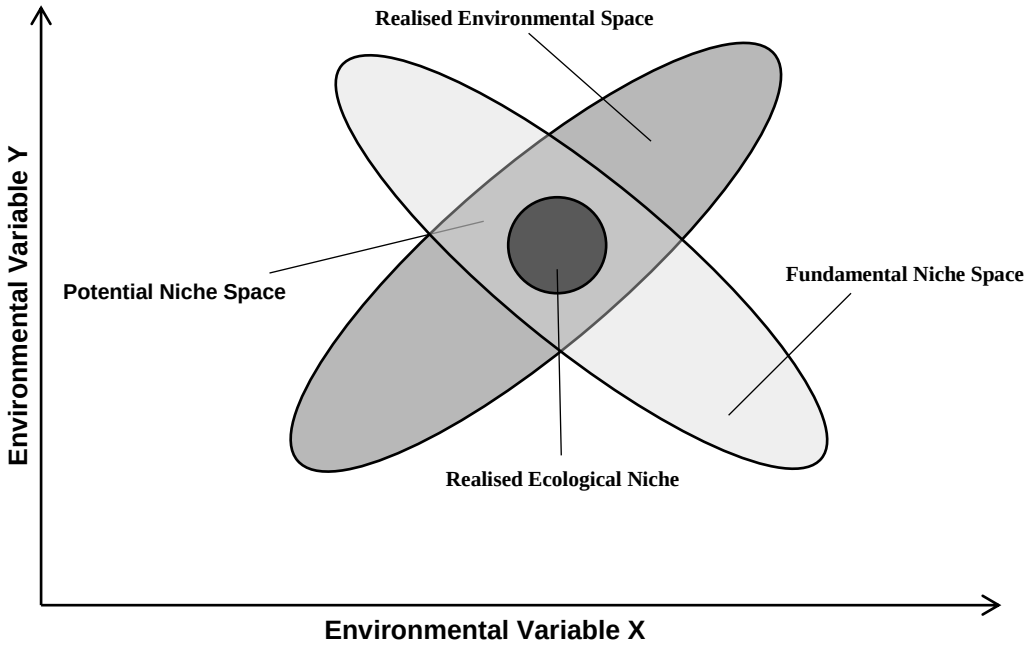

Figure 4.1 A 2-dimensional diagram demonstrating how a species realised environmental space and fundamental niche space interact to form a species potential niche space; this is further restricted to a species realised ecological niche space once biotic interactions are considered (modified from Jackson & Overpeck 2000).

variables are key to limiting the distribution of that species. The Potential Niche Space of a species models the current suitable environmental conditions for a species, based on its occurrence data. This information is used to hindcast or forecast the potential distribution of species, demonstrating where these same suitable conditions may have occurred in the past, or will occur in the future (Anderson *et al*. 2003). Available environmental data usually comprises of climate variables and landuse information. To hindcast or forecast potential species distributions, predictions of these variables from the past or for the future are required, and can be acquired from online databases that have inferred climate based on models (e.g. Worldclim – Global Climate Data) (Hijmans *et al*. 2005).

A species' ecological niche is complex, and ENMs by no means encompass all of the dimensions that go in to the formation of a species niche (Davis *et al.* 1998, Pearson *et al*. 2006). However, ENMs are informative and are a step towards understanding how a species may be interacting with its abiotic and biotic environment (Anderson *et al*. 2003, Pearson *et al*. 2006). Ecological Niche Models are primarily used to aid in the management of endangered and invasive species, as ENMs can highlight where suitable habitat is and where it is most likely to decrease or increase in light of future climate or landuse change scenarios (Bellard *et al*. 2016, Buerki *et al*. 2015, Buczkowski & Bertelsmeier 2017, Duque-Lazo *et al*. 2016, Fletcher *et al*. 2016, Gama *et al*. 2015, Ihlow *et al*. 2016, Lei *et al*. 2017, Martins *et al*. 2016, Molloy *et al*. 2017, van Schingen *et al*. 2014, Yan *et al*. 2017). Less commonly, is the use of ENMs to aid in species delimitation, alongside genetic and morphological evidence (Leaché *et al*. 2009, Raxworthy *et al*. 2007, Rissler & Apodaca 2007). Once niches are established and changes predicted, appropriate management solutions such as implementing or increasing protection of a species and particular habitats, or increasing pest management, can be applied (Bellard *et al*. 2016, Buerki *et al*. 2015, Buczkowski & Bertelsmeier 2017, Duque-Lazo *et al*. 2016, Fletcher *et al*. 2016, Gama *et al*. 2015, Ihlow *et al*. 2016, Lei *et al*. 2017, Martins *et al*. 2016, Molloy *et al*. 2017, van Schingen *et al*. 2014, Yan *et al*. 2017).

Climate change in New Zealand

It is predicted that average temperatures in New Zealand will increase by 0.7°C (RCP2.6) to 3.0°C (RCP8.5) by 2090 (relative to 1986-2005 temperatures) (Ministry for the Environment, 2016). Precipitation is predicted to reduce in the north and east of the North Island and increase in most other areas, particularly the West Coast of the South Island, where it could increase by up to 40% (Ministry for the Environment, 2016). Droughts are predicted to increase in severity and intensity, particularly in the north and east of the North Island, and around the main divide of the South Island (Ministry for the Environment, 2016). Additionally, mean sea level pressure and daily extreme wind speeds are also predicted to increase in most parts of New Zealand, whilst humidity is predicted to decrease – except along the West Coast of the South Island. In high elevation areas of the South Island, snow days are predicted to decrease by 30 days by 2090 (RCP8.5) (Ministry for the Environment, 2016). These findings were simulated based on RCP trajectories established by the Intergovernmental Panel on Climate Change (IPCC) fifth Assessment Report (IPCC 2014). Representative Concentration Pathways reflect the projected outcomes of four different greenhouse gas concentration scenarios: RCP2.6 assumes a decline in greenhouse gas emissions following a peak in 2020; RCP4.5 assumes a decline in greenhouse gas emissions following a peak in 2040; RCP6.0 assumes a decline in greenhouse gas emissions following a peak in 2080; and RCP8.5 assumes an uninhibited increase in greenhouse gas emissions throughout the 21st century (Table 4.1) (IPCC 2014). Representative Concentration Pathway 2.6 and RCP8.5 are used in this study to investigate the two most extreme scenarios for which future climate change may impact alpine grasshoppers in New Zealand.

Alpine grasshoppers as representatives of alpine fauna

New Zealand is inhabited by a diverse fauna of endemic Acrididae (Bigelow 1967). Of 13 species, 10 are predominantly found in alpine environments, where they inhabit tussock, herb and scree habitats (Bigelow 1967). Some of these grasshopper species are found only inhabiting the alpine zone (e.g. *Alpinacris crassicauda* and *Sigaus villosus*), others have ranges that extend from the alpine zone to below the treeline (e.g. *Paprides nitidus* and *Sigaus australis*), and there are also members that are only found inhabiting lowland grasslands (e.g. *Sigaus childi* and *Sigaus minutus*). With future climate change, New Zealand's alpine zone is predicted to be reduced by ~50%, and is estimated to lose

33-50% of its indigenous alpine flora species with 3°C of warming (Halloy & Mark 2003); it is not known how New Zealand's alpine fauna will be affected. By modelling New Zealand's endemic alpine grasshopper diversity and the niches they inhabit, we may be able to better understand how New Zealand's alpine fauna will respond to future climate change.

Aims

What limits the current distribution of New Zealand's endemic alpine grasshoppers? If the factors controlling their distribution are mostly captured by abiotic factors then species distributions are expected to closely match their environmental envelope (e.g. their Realised Niche Space will match their Potential Niche Space) and the effects of climate change on species ranges should be predictable (if no adaptation). However, if biotic interactions are important factors controlling distribution, then species distributions may poorly correlate with their predicted environmental envelope and it will be more difficult to predict the effect of climate change on their future distributions. Here I use current species distributions and Ecological Niche Modelling tools to infer past (i.e. LGM), current and future (i.e. RCP2.6 and RCP8.5) distributions of New Zealand's endemic alpine grasshoppers. In order to predict how climate has, and will, impact their distributions, this comprehensive study will explore niche models at two taxonomic levels, including examination of the diverging mitochondrial lineages of *Brachaspis nivalis* and *Sigaus piliferus*. Knowing how these alpine grasshoppers may respond to future climate scenarios will be valuable knowledge in the conservation management of alpine habitats in New Zealand. Interpretation of future effects will draw upon inference from past change. Specifically, it is expected that climate warming since the Last Glacial Maximum (LGM) is likely to have reduced the habitat available to these alpine grasshoppers. This is predicated upon the assumption that occupiable area reduces with elevation.

Materials and Methods

Presence absence data

Location records were acquired for 12 species of New Zealand's endemic alpine grasshoppers and four mitochondrial lineages: *Alpinacris crassicauda, Alpinacris tumidicauda, Brachaspis collinus, Brachaspis nivalis* (SA), *Brachaspis nivalis* (FP), *Paprides dugdali, Paprides nitidus, Sigaus australis, Sigaus campestris, Sigaus childi, Sigaus minutus, Sigaus piliferus* (LW), *Sigaus piliferus* (MH) and *Sigaus villosus*. *Brachaspis nivalis* (SA) represents the northern clade of *B. nivalis*, while *B. nivalis* (FP) represents the southern clade. The central and northern clade of *S. piliferus* is represented by *S. piliferus* (LW) and the southern clade by *S. piliferus* (MH). Journal articles, books and Crown Pastoral Lease Tenure Reviews (CPLTR) were reviewed for location information. Crown Pastoral Lease Tenure Reviews (CPLTR) are produced by Land Information New Zealand (LENZ) and contain conservation reports and ecological surveys carried out by the Department of Conservation on stations throughout the South Island of New Zealand. Further records were retrieved from the Phoenix Lab collection at Massey University. Longitudinal and latitudinal coordinates for each location were obtained from NZ Topo Map (www.topomap.co.nz).

Location records for two other endemic lowland grasshopper species: *Phaulicridium marginale* and *Phaulicridium otagoense* were also included in the data set (Sivyer *et al*. 2018). Both of these species have a lowland distribution and the addition of their location data points increases the accuracy of the niche models by providing additional absence information. All records were pooled into a binary table where for each location the presence or absence of each species was recorded. The presence points of each species were mapped in turn using QGIS v.2.16.1 (QGIS Development Team 2017). This was in order to demonstrate what information was provided to the models (in the form of presence points for each species) and also so that the distribution of each species could be visualised. The map of New Zealand used to project these distributions onto was created using the "Vegetative Cover Map of New Zealand" file obtained from the LRIS Portal (https://lris.scinfo.org.nz/) (Leathwick *et al*. 2012, Newsome 1987). The files were edited in QGIS, where broad vegetation groupings were regrouped and coloured by: native forest, agricultural land, tussock grasslands, urban, wetlands, sand-dunes and ice and waterways categories.

Predictor variable layers

In order to define and project the potential niche of each grasshopper, 19 Bioclimatic variables were obtained from the Worldclim website (http://www.worldclim.org/) for three different time periods – the Last Glacial Maximum (LGM) (~15,000), 'current' (e.g. data averaged from 1960-1990) and 'future' (e.g. data predicted and averaged from 2061-2080) (Table 4.2). These were at a resolution of 2.5 arc minutes (approx. $5km^2$) for the LGM and 30 arc-seconds (approx. $1km^2$) for the current and future layers. Two future predicted climate layers were acquired for the 'future' time period, RCP2.6 and RCP8.5, representing potential low and high greenhouse gas concentration scenarios, respectively. All climate layers acquired were produced using the MIROC-ESM global climate model (Watanabe *et al*. 2011). The Worldclim files were cropped to the extent of New Zealand (Latitudes: -49,-32; Longitudes: 165,180) using QGIS. A Variance Inflation Factor (VIF) analysis refined the list of 19 climate variables down to seven using the R package 'VIF' (Lin *et al*. 2011). Variance Inflation Factor analyses use stepwise selection to identify and remove collinear variables. This is done in order to increase parsimony and minimize over-fitting during the modelling process (Fletcher *et al*. 2016).

Soil and vegetation (i.e. vege) layers were acquired. These two variables are known to influence the distribution of some grasshopper species (Nattier *et al*. 2013; Weiss *et al.* 2013). The soil and vege data layers were rasterized, clipped and re-scaled from their original files "Fundamental Soil Layers New Zealand Soil Classification" and "Vegetative Cover Map of New Zealand", obtained through the Land Resource Information System (LRIS) portal (Landcare Research New Zealand Limited 2010, Leathwick *et al*. 2012). No past (e.g. LGM) or future (e.g. 2070) GIS files exist for soil and vegetation cover in New Zealand, these layers represent the current approximate state of vegetation and soil in New Zealand, and were therefore used as static layers throughout the modelling process. Ecological Niche Models that include such static layers are known to perform as well as, or better than, models where only dynamic variables are included (Stanton *et al*. 2012).

Table 4.2 The 21 predictor variables initially investigated for use in the Ecological Niche Models. This list was refined to nine variables following a Variance Inflation Factor (VIF) analysis, with variables retained for modelling shaded in grey.

Variable Code	Variable Description
Bio1	Annual mean temperature
Bio2	Mean diurnal range (Mean of monthly (max temp - min temp))
Bio3	Isothermality (BIO2/BIO7) (* 100)
Bio4	Temperature seasonality (standard deviation *100)
Bio5	Max temperature of warmest month
Bio6	Min temperature of coldest month
Bio7	Temperature annual range (BIO5-BIO6)
Bio8	Mean temperature of wettest quarter
Bio9	Mean temperature of driest quarter
Bio10	Mean temperature of warmest quarter
Bio11	Mean temperature of coldest quarter
Bio12	Annual precipitation
Bio13	Precipitation of wettest month
Bio14	Precipitation of driest month
Bio15	Precipitation seasonality (Coefficient of Variation)
Bio16	Precipitation of wettest quarter
Bio17	Precipitation of driest quarter
Bio18	Precipitation of warmest quarter
Bio19	Precipitation of coldest quarter
Soil	Fundamental soil layers of New Zealand
Vege	Vegetative cover of New Zealand

Ecological niche models

The R package 'biomod2' v. 3.3-7 (Thuiller *et al*. 2016) was used to model the environmental niche of each species. Ecological Niche Models were produced at two different resolutions of taxonomy. The first degree of taxonomic modelling was at a species level, modelling the ecological niches of each of the 12 alpine grasshopper species. In this instance, *Brachaspis nivalis* (SA) and *Brachaspis nivalis* (FP) were combined into *Brachaspis nivalis* (All), and *Sigaus piliferus* (LW) and *Sigaus piliferus* (MH) were combined into *Sigaus piliferus* (All). The second degree of taxonomic modelling was at the mitochondrial lineage level, where the ecological niches of *Brachaspis nivalis* (SA) and *Brachaspis nivalis* (FP), and *Sigaus piliferus* (LW) and *Sigaus piliferus* (MH) were compared with that of *Brachaspis nivalis* (All) and *Sigaus piliferus* (All), respectively. This analysis was undertaken in order to reveal if these distinct mitochondrial lineages are diverging ecologically.

Modelling methods

Ten different modelling methods were selected to analyse the presence and absence data of the grasshoppers against 'current' predictor variables: Generalized Linear Model (GLM), Generalised Boosting Model/Boosted Regression Tree (GBM), Generalised Additive Model (GAM), Classification Tree Analysis (CTA), Artificial Neural Network (ANN), Surface Range Envelope (SRE), Flexible Discriminent Analysis (FDA), Multiple Adaptive Regression Splines (MARS), Random Forest (RF) and Maximum Entropy (MAXENT). Descriptions of each model and their use within 'biomod2' are explained indepth in Diniz-Filho *et al*. (2009). All modelling parameters were kept at default values and 80% of the data was used to calibrate the models, with the remainding 20% being used to test them; each model was repeated three times resulting in a total of 30 models per species. Output values of variable importance were calculated as 1 - the mean correlation score of each variable, with scores closest to 1 indicating a variable is of high importance.

Investigation of model accuracy was carried out using two different evaluation methods: Reciever Operating Characteristic (ROC) (i.e. Area Under the Curve (AUC)) and True Skill Statistic (TSS). Models with ROC values of 0.9–1 and TSS values of 0.8–1 are considered to have 'excellent' predictive power (accuracy) (Thuiller *et al.* 2009). An

ensemble model was then created from a subset of these models based on their ROC values (>0.9). Using the ensemble model, ensemble forecasts were projected for the LGM and the 2061-2080 datasets. Plots were produced for each time period using the Ensemble Model mean weights model (EMmw). EMmw variable importance was calculated by applying the weights produced in the ensemble model to the associated models in the 30 model data set. As models were run three times for each predictor variable, these were then averaged, and EMmw variable importance was calculated by summing the total of these averages for each predictor variable, and dividing by the number of modelling methods used (i.e. 10) (Fletcher *et al*. 2016). Final scores of variable importance were converted into percentages of total variable importance for each modelling method.

Binary vectors of each ENM were generated for range change and fragmentation statistic analyses. These binary vectors were generated from the 30 niche model dataset, where for each pixel ($1m^2$), if in >50% of models it scored greater than the predetermined cutoff value, it was ranked as a 1, and all other pixels as 0's. When comparing binary vetors between current and future models, Biomod2 ranked pixels as: 'Never occupied' (i.e. pixels were unoccupied and remain unoccupied between models), 'Always occupied' (i.e. pixels were occupied and remain occupied between models), 'Lost' (i.e. pixels were occupied but become unoccupied in the future RCP scenario) or 'Gained' (i.e. pixels that were unoccupied in the 'current' model become occupied in the RCP model), from which range change statististics were then calculated. Fragstats, implemented in R package 'SDM Tools' v 1.1-221 (VanDerWal *et al*. 2014), also used these binary files to estimate various fragmentation statistics (e.g. patch size, number of patches, core area of patches), which were compared between different RCP scenarios from their 'current' niche models. Pixels that were connected within each of the binary files were given unique patch identities, and the area and number of these patches was calculated for each scenario. In this study, all patch fragments that were less than $100m^2$ were excluded from the fragmentation statistics analysis. A flowchart of the methods implemented in this study can be seen in Figure 4.2.

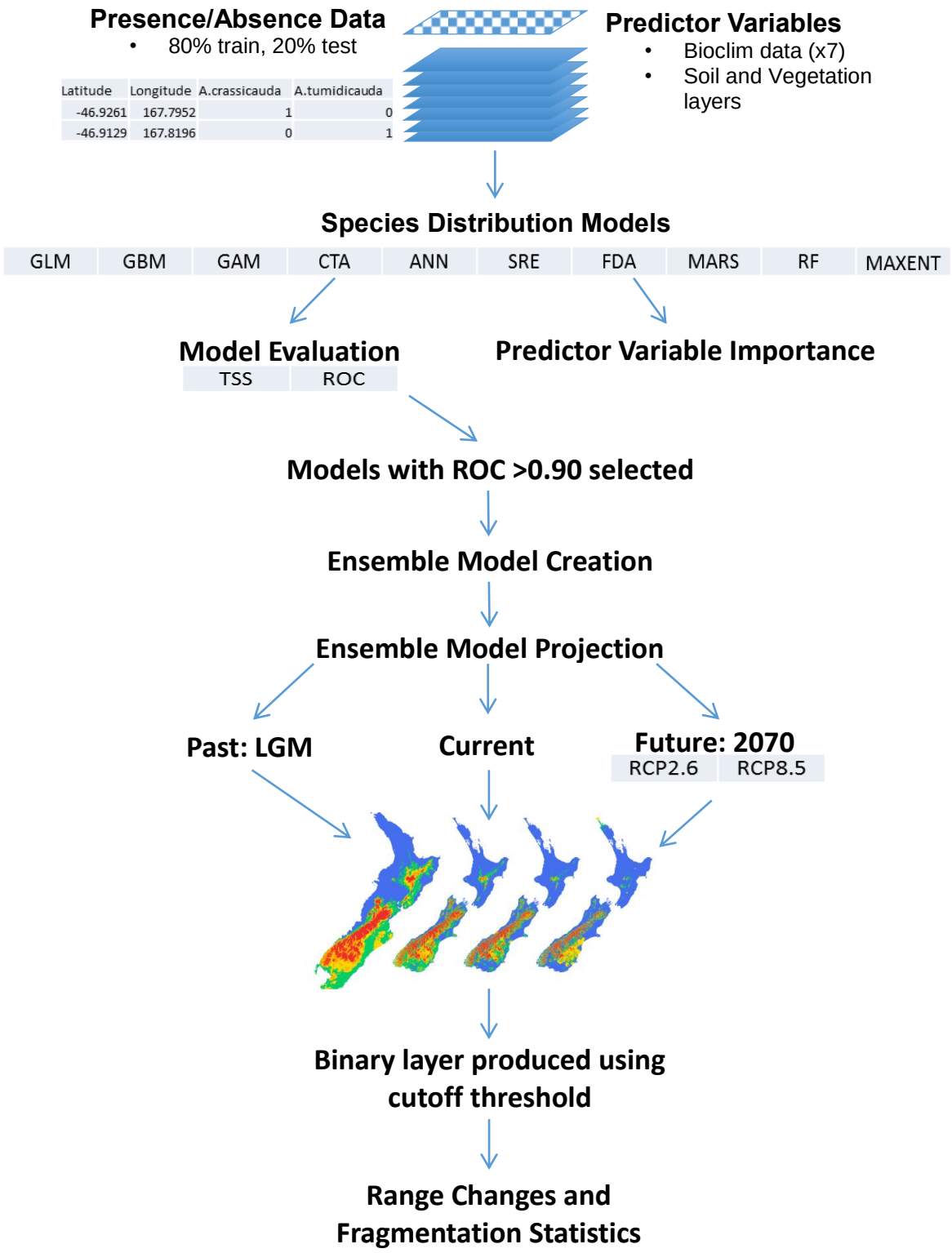

Figure 4.2 Flowchart of the Ecological Niche Modelling method applied in this study.

Results

Species distributions

To supplement the l78 location records published by Bigelow (1967), I interrogated 61 journal articles and 340 Crown Pastoral Lease Tenure Reviews (CPLTR) for location information. A total of 250 and 287 locations were recorded from the journal publications and CPLTRs respectively; 178 unpublished locations were retrieved from the Phoenix Collection at Massey University. Additionally, 245 *Phaulacridium* records were acquired (Sivyer *et al*. 2018). In total the GPS coordinates of 949 locations were acquired once duplicates were removed (Appendix One). Distribution maps for each of the grasshopper species were produced using these location records (Figure 4.3, 4.4, 4.5, 4.6). As Bigelow (1967) was the last person to map the distributions of many of New Zealand's grasshopper species, these maps provide the most comprehensive distributions of New Zealand's endemic alpine grasshoppers to date. Although there are no major changes in the distribution of each grasshopper species (c.f. Bigelow 1967) apparent gaps have been filled so that there is more connectivity within species ranges.

Ecological niche models by species:

Model evaluations

Individual ENMs were run for 12 of New Zealand's endemic alpine grasshopper species. Different numbers of presence data points were available for each species, with 10/12 species having >30, whilst two had <30 presence data points (Table 4.3). A minimum of 30 occurrences are recommended for ENMs, below which model accuracy decreases and variability of model accuracy increases (Breiner *et al.* 2015, Hernandez *et al*. 2006, Stockwell & Peterson 2002, Wisz *et al*. 2008).Thus the more presence points available for the model to use, the more predictively accurate the model will be, however, some species have restricted ranges and therefore few points. The majority of species had models with ROC values >0.9, the exceptions being *B. nivalis* (All), *S. australis* and *S. campestris*, for whom no individual model had an ROC >0.9 (Table 4.4) (Figure 4.7, 4.8). The models that most commonly had the highest model evaluation scores between species were GBM, followed by MAXENT and RF. CTA, ANN and SRE models consistently had low evaluation model scores between species

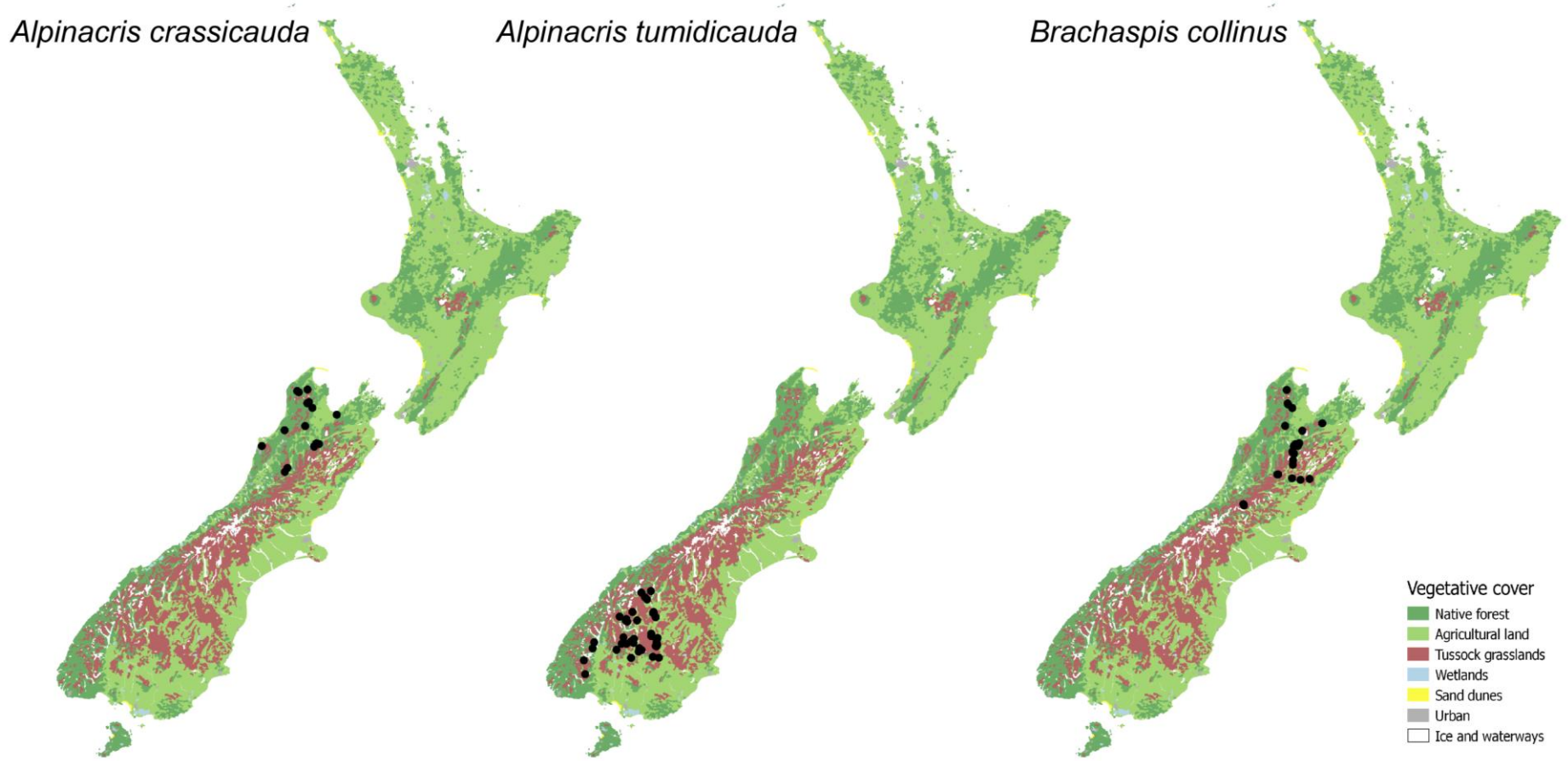

Figure 4.3 The current distributions of *Alpinacris crassicauda, Alpinacris tumidicauda* and *Brachaspis collinus*. These location records were used as presence data points for the Ecological Niche Modelling of these species.

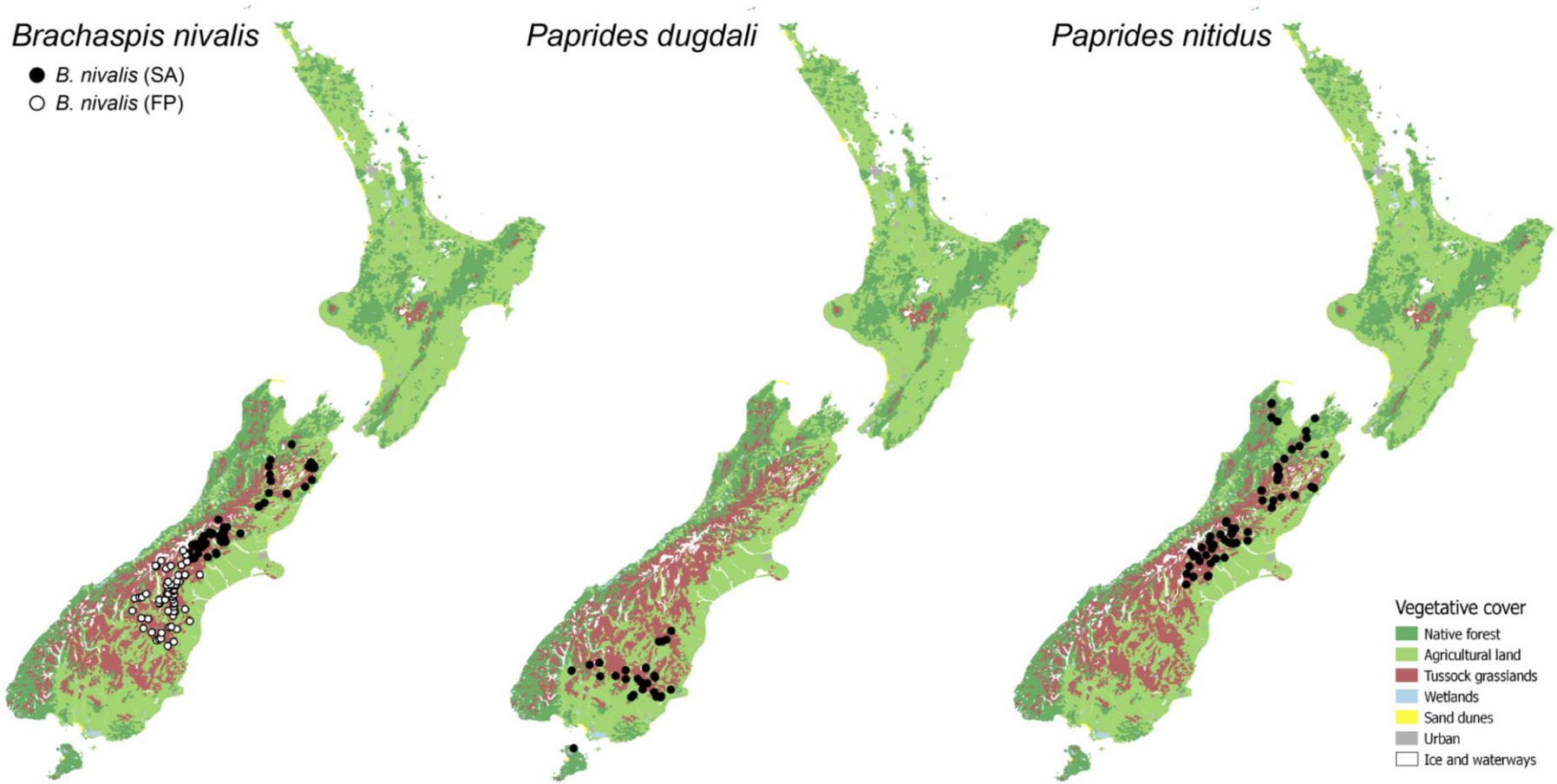

Figure 4.4 The current distributions of *Brachaspis nivalis, Paprides dugdali* and *Paprides nitidus*. These location records were used as presence data points for the Ecological Niche Modelling of these species (and mitochondrial lineages of *B. nivalis*).

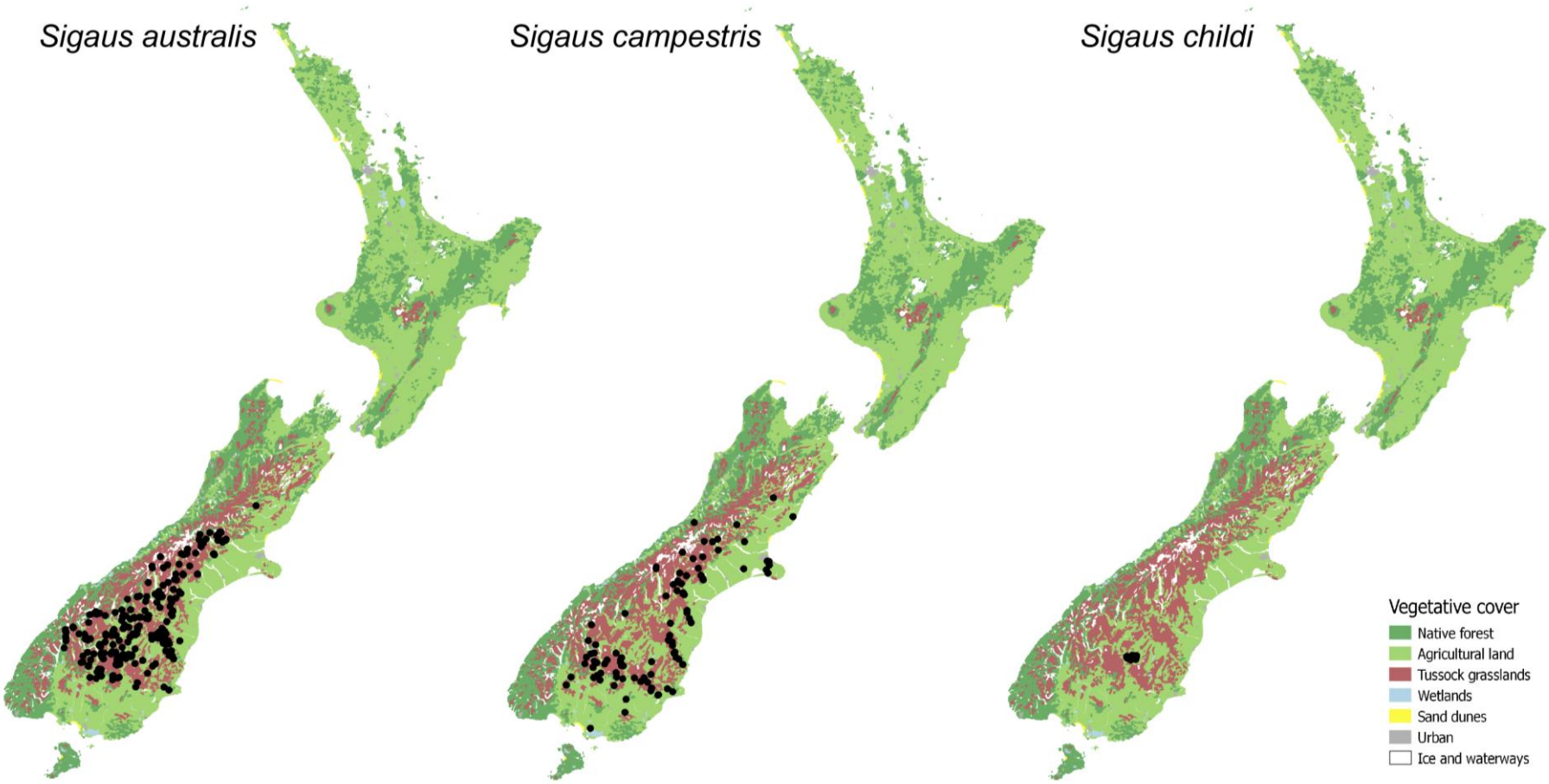

Figure 4.5 The current distributions of *Sigaus australis, Sigaus campestris* and *Sigaus childi*. These location records were used as presence data points for the Ecological Niche Modelling of these species.

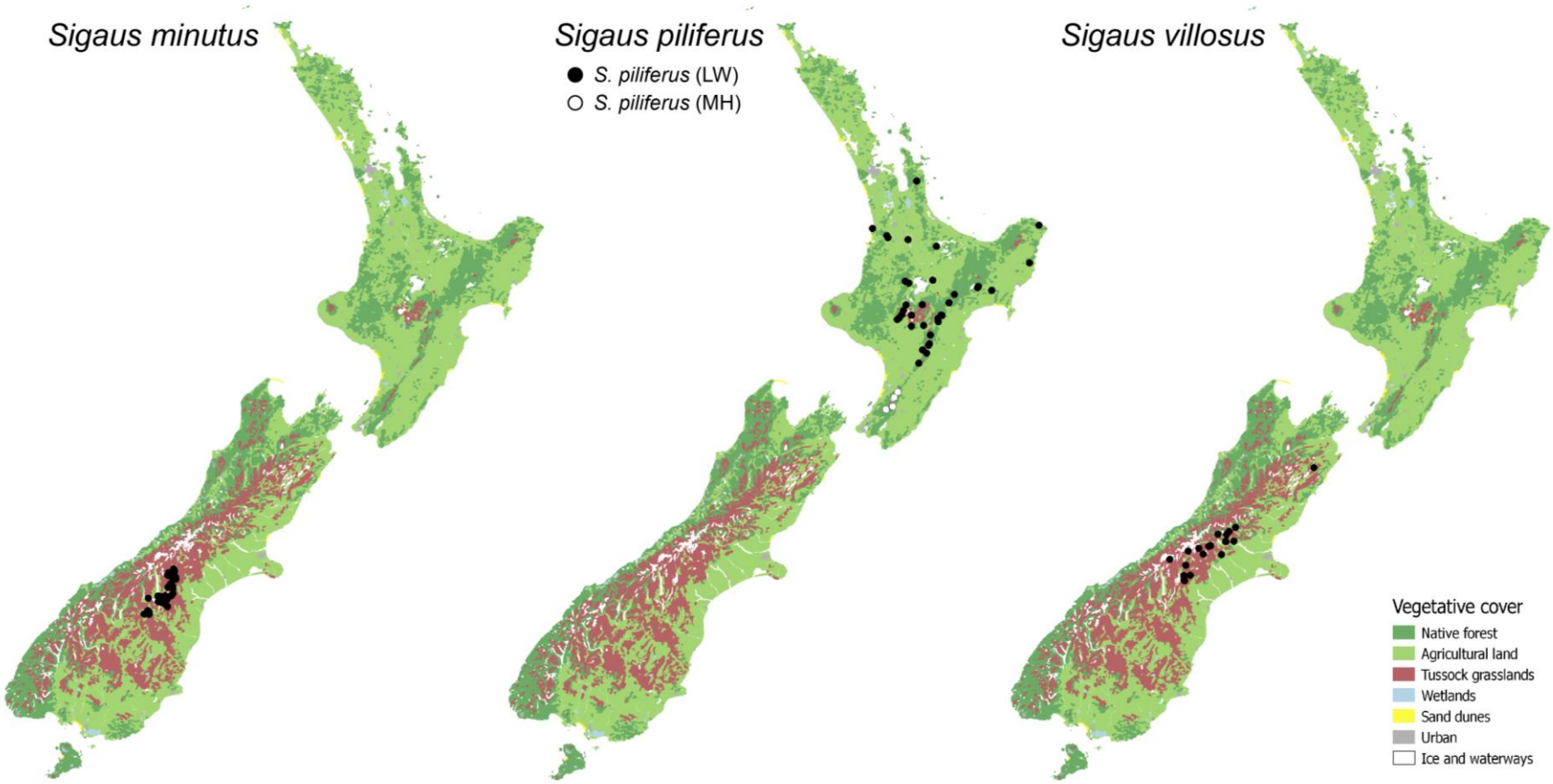

Figure 4.6 The current distributions of *Sigaus minutus, Sigaus piliferus* and *Sigaus villosus*. These location records were used as presence data points for the Ecological Niche Modelling of these species (and mitochondrial lineages of *S. piliferus*).

Table 4.3 A list of species and mitochondrial lineages examined in this study, including the number of presence and absence points available for each species to be used in the Ecological Niche Modelling analysis. Species with less than 30 presence points are marked with a *.

Species	Presence	Absence
Alpinacris crassicauda	16*	933
Alpinacris tumidicauda	49	900
Brachaspis collinus	30	919
Brachaspis nivalis (All)	129	817
Brachaspis nivalis (SA)	59	890
Brachaspis nivalis (FP)	70	879
Paprides dugdali	29*	920
Phaulacridium marginale	205	744
Paprides nitidus	91	858
Phaulacridium otagoense	26*	923
Sigaus australis	278	671
Sigaus campestris	100	849
Sigaus childi	41	908
Sigaus minutus	40	909
Sigaus piliferus (All)	45	901
Sigaus piliferus (LW)	36	913
Sigaus piliferus (MH)	8*	941
Sigaus villosus	23*	926

Table 4.4 The two model evaluation metrics (Reciever Operating Characteristic (ROC) and True Skill Statistic (TSS)) of the ten modelling methods implemented during the Ecological Niche Modelling (ENMs) analyses for each species. Model evaluation scores for the final Ensemble Model Mean Weights model (EMmw) are also included. ROC values of 0.9–1 and TSS values of 0.8–1 are considered to have 'excellent' predictive accuracy. Cutoff values are thresholds that maximise the percentage of presences and absences correctly predicted by the model. These are used to transform vectors of probabilities (i.e. the initial ENMs) into binary vectors for range change and fragmentation statistical analyses. Sensitivity (Sens.) is the percentage of presences correctly predicted by the EMmw model and Specificity (Spec.) is the number of absences correctly predicted by the EMmw model. Cells shaded a darker purple indicate highest model evaluation scores, with white indicating evaluation scores of 85 or less.

Species	Evaluation Method	Modelling Method										EMmw Model	Cutoff (Sens.;Spec.)
		GLM	GBM	GAM	CTA	ANN	SRE	FDA	MARS	RF	MAXENT		
A. crassicauda	ROC	**0.96**	**0.99**	**0.61**	**0.96**	**0.97**	**0.60**	**0.96**	**0.76**	**0.91**	**0.98**	1.00	573.5
	TSS	0.95	0.97	0.22	0.91	0.90	0.20	0.92	0.54	0.84	0.97	0.98	(100; 98)
A. tumidicauda	ROC	0.92	0.93	0.94	0.89	0.90	0.84	0.93	0.92	0.92	0.94	0.97	390.5
	TSS	0.78	0.75	0.83	0.80	0.77	0.68	0.78	0.79	0.78	0.81	0.88	(100; 88)
B. collinus	ROC	0.97	0.97	0.96	0.90	0.96	0.76	0.98	0.95	0.97	0.97	0.99	531.5
	TSS	0.95	0.95	0.93	0.80	0.87	0.52	0.92	0.90	0.92	0.91	0.97	(100; 97)
B. nivalis (All)	ROC	0.77	0.83	0.79	0.73	0.74	0.71	0.76	0.79	0.81	0.81	0.94	394
	TSS	0.53	0.59	0.53	0.48	0.47	0.42	0.45	0.53	0.55	0.51	0.77	(98; 80)
B. nivalis (SA)	ROC	0.92	0.93	0.88	0.86	0.89	0.75	0.89	0.93	0.93	0.93	0.98	552
	TSS	0.79	0.83	0.70	0.68	0.73	0.51	0.65	0.80	0.77	0.77	0.90	(98; 92)
B. nivalis (FP)	ROC	0.88	0.87	0.85	0.75	0.87	0.69	0.85	0.83	0.88	0.88	0.96	460
	TSS	0.70	0.72	0.63	0.55	0.68	0.38	0.66	0.69	0.73	0.70	0.83	(94; 89)
P. dugdali	ROC	0.84	0.93	0.91	0.83	0.80	0.65	0.90	0.89	0.92	0.95	0.99	495
	TSS	0.64	0.81	0.75	0.65	0.57	0.30	0.76	0.77	0.85	0.87	0.94	(100; 94)
P. nitidus	ROC	0.89	0.90	0.85	0.88	0.82	0.82	0.89	0.90	0.89	0.90	0.95	287
	TSS	0.79	0.78	0.65	0.76	0.60	0.64	0.74	0.79	0.75	0.81	0.83	(99; 84)
S. australis	ROC	0.83	0.84	0.80	0.77	0.79	0.71	0.81	0.82	0.82	0.80	0.92	536.5
	TSS	0.55	0.56	0.53	0.53	0.49	0.41	0.53	0.53	0.54	0.50	0.65	(80; 85)
S. campestris	ROC	0.78	0.74	0.74	0.66	0.69	0.58	0.74	0.72	0.75	0.74	0.93	471.5
	TSS	0.49	0.47	0.43	0.37	0.32	0.15	0.43	0.43	0.48	0.44	0.71	(86; 85)
S. childi	ROC	0.99	0.99	0.99	0.97	0.99	0.95	0.99	0.99	1.00	0.91	1.00	511.5
	TSS	0.99	0.97	0.99	0.93	0.98	0.91	0.95	0.98	0.99	0.80	0.99	(100; 99)
S. minutus	ROC	0.95	0.97	0.93	0.90	0.95	0.83	0.95	0.94	0.97	0.95	0.99	408.5
	TSS	0.86	0.92	0.82	0.79	0.85	0.66	0.85	0.88	0.92	0.86	0.93	(100; 92)
S. piliferus (All)	ROC	0.95	0.96	NA	0.88	0.64	0.88	0.96	0.94	0.97	0.84	0.99	523.5
	TSS	0.86	0.85	NA	0.76	0.60	0.75	0.84	0.88	0.90	0.67	0.97	(100; 97)
S. piliferus (LW)	ROC	0.92	0.98	NA	0.97	0.93	0.73	0.95	0.85	0.96	0.75	0.99	345
	TSS	0.77	0.94	NA	0.93	0.77	0.46	0.82	0.72	0.82	0.50	0.95	(100; 95)
S. piliferus (MH)	ROC	0.91	0.99	NA	0.89	0.97	0.83	1.00	0.91	0.98	1.00	1.00	534.5
	TSS	0.83	0.99	NA	0.77	0.91	0.67	0.99	0.83	0.96	0.99	1.00	(100; 100)
S. villosus	ROC	0.94	0.95	0.93	0.91	0.94	0.82	0.93	0.90	0.93	0.97	0.99	420.5
	TSS	0.88	0.90	0.83	0.82	0.81	0.64	0.83	0.82	0.81	0.89	0.96	(100; 96)

(Table 4.4) (Figure 4.7, 4.8). For the majority of species, the EMmw ensemble model produced had an improved ROC value when compared to their individual model runs (Table 4.4). The exception being *S. childi*, whose RF model and EMmw model both had ROC values of 1. The EMmw models for *B. nivalis* (All), *S. australis* and *S. campestris* had ROC scores >0.9, greatly improving on the predictive accuracy of their individual models. Ten species (*A. crassicauda*, *A. tumidicauda*, *B. collinus, B. nivalis* (All), *P. dugdali*, *P. nitidus, S. childi*, *S. minutus*, *S. piliferus* (All) and *S. villosus*) had Sensitivity scores >90/100, and seven of these species also had Specificity scores >90/100 (*A. crassicauda*, *B. collinus, P. dugdali*, *S. childi*, *S. minutus*, *S. piliferus* (All) and *S. villosus*). Two species that scored <90/100 for both Sensitivity and Specificity were *S. australis* and *S. campestris*. Sensitivity is the percentage of presences correctly predicted by the EMmw model and Specificity is the number of absences that are correctly predicted (Allouche *et al*. 2006)

Predictor variable importance

Predictor variable importance for each of the ten modelling methods can be found in Supplementary Table 4.1, here I will report the predictor variable importance for EMmw models only. Different predictor variables were important in explaining the distributions of the 12 grasshopper species, but vegetation did not rank as an important predictor variable for any species. Soil best explained the distribution of only one species, *S. campestris* (Table 4.5). The predictor variable that best explained the distribution of three species (*A. crassicauda*. *B. collinus* and *S. minutus*) was Mean diurnal range. For two species (*S. australis* and *S. villosus*), Annual mean temperature was the most important predictor variable. For another two species (*A. tumidicauda* and *S. childi*) it was Precipitation of driest month. Isothermality and Temperature of wettest quarter were the most important predictor variables for *B. nivalis* (All) and *S. piliferus* (All), respectively. Mean temperature of driest quarter best explained the distribution of *P. nitidus* whilst Precipitation seasonality best explained the distributions of *P. dugdali*.

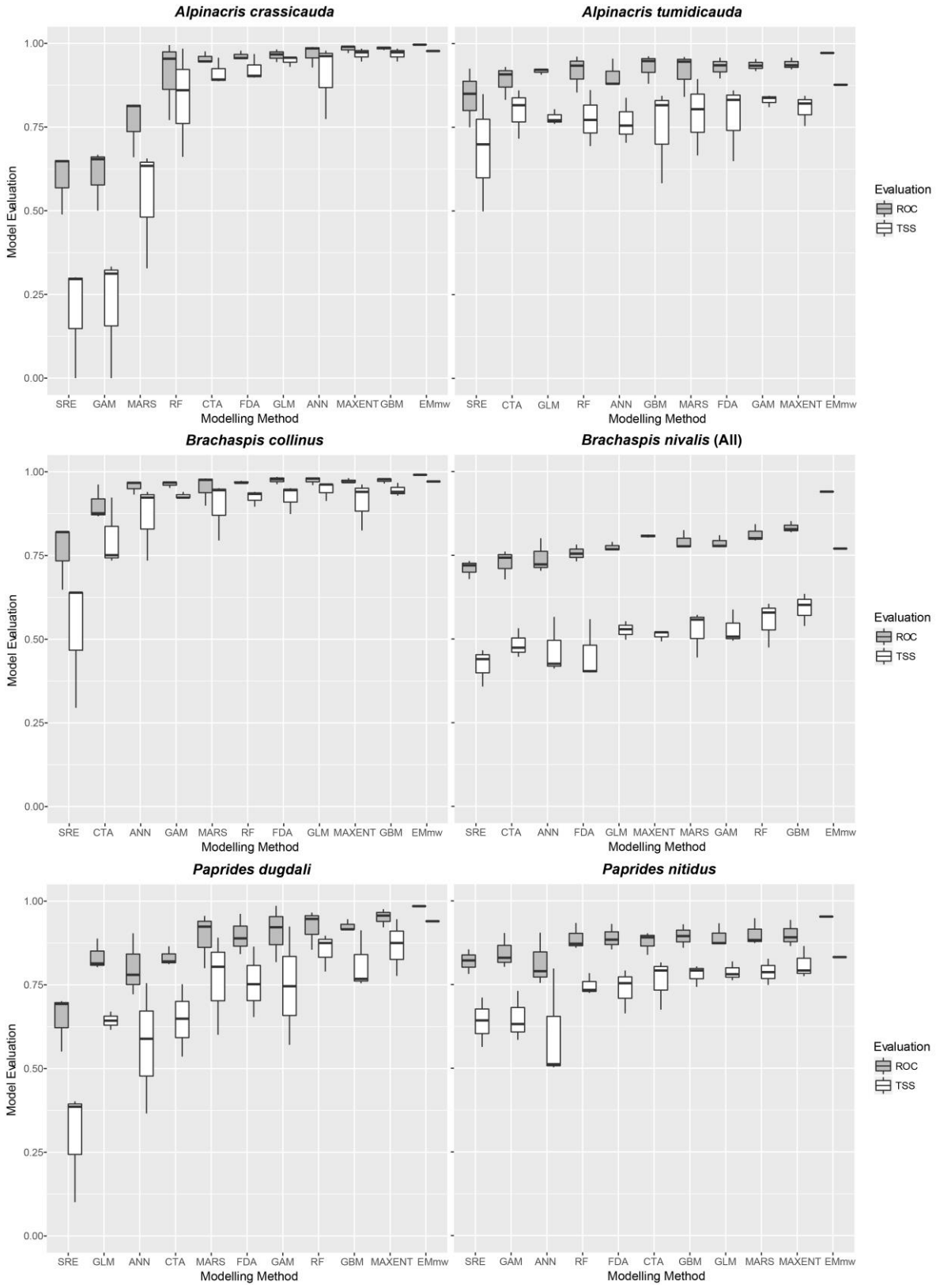

Figure 4.7 Boxplots of Reciever Operating Characteristic (ROC) and True Skill Statistic (TSS) model evaluation scores for each modelling method including Ensemble Model Mean Weights models (EMmw). ROC values of 0.9–1 and TSS values of 0.8–1 are considered to have 'excellent' predictive accuracy. GLM = Generalized Linear Model, GBM = Generalised Boosting Model/Boosted Regression Tree, GAM = Generalised Additive Model, CTA = Classification Tree Analysis, ANN = Artificial Neural Network, SRE = Surface Range Envelope, FDA = Flexible Discriminent Analysis, MARS = Multiple Adaptive Regression Splines, RF = Random Forest and MAXENT = Maximum Entropy.

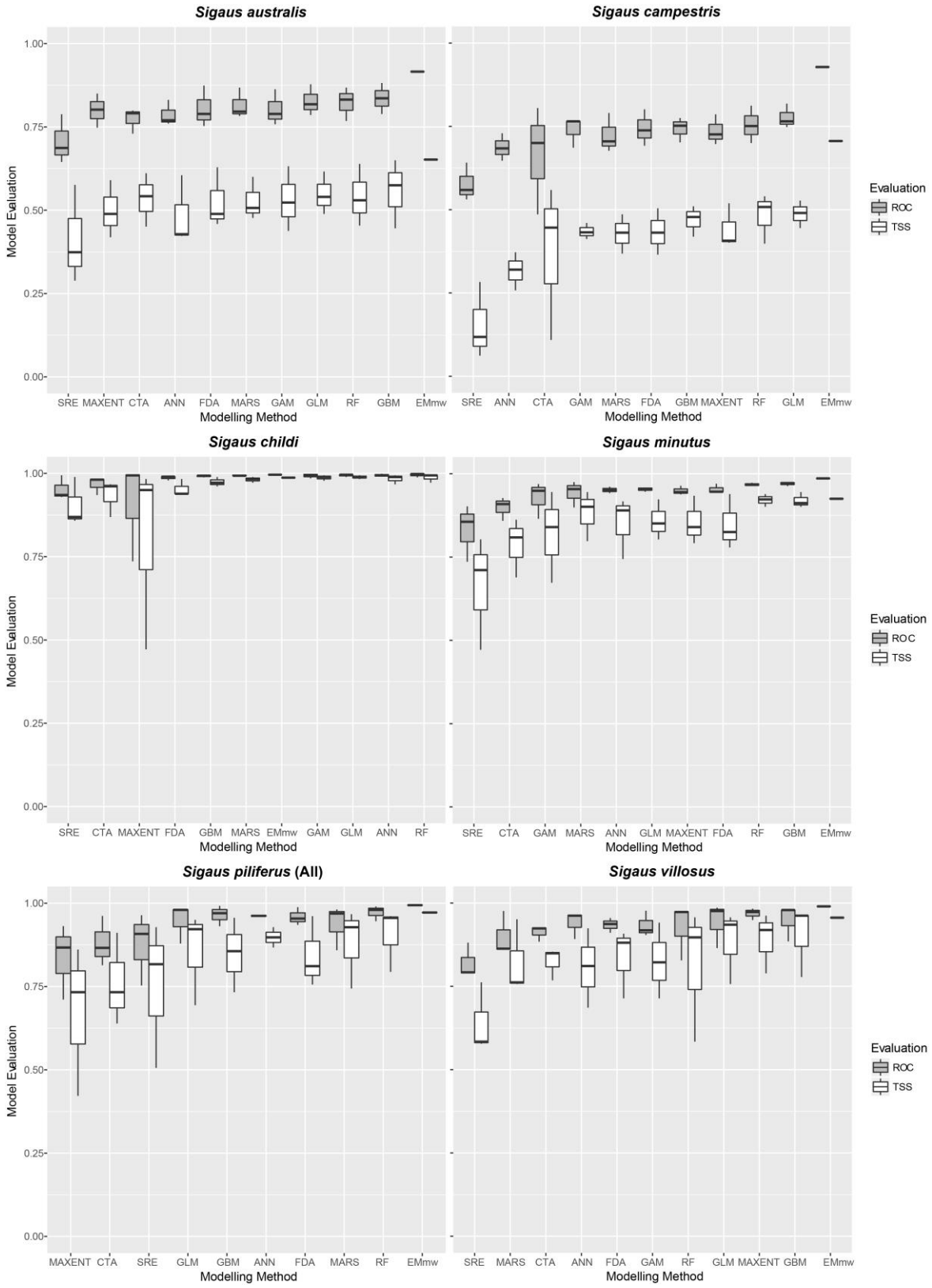

Figure 4.8 Boxplots of Reciever Operating Characteristic (ROC) and True Skill Statistic (TSS) model evaluation scores for each modelling method including Ensemble Model Mean Weights models (EMmw). ROC values of 0.9–1 and TSS values of 0.8–1 are considered to have 'excellent' predictive accuracy. GLM = Generalized Linear Model, GBM = Generalised Boosting Model/Boosted Regression Tree, GAM = Generalised Additive Model, CTA = Classification Tree Analysis, ANN = Artificial Neural Network, SRE = Surface Range Envelope, FDA = Flexible Discriminent Analysis, MARS = Multiple Adaptive Regression Splines, RF = Random Forest and MAXENT = Maximum Entropy.

Current ecological niche models

Ecological niche models were produced for the 12 grasshopper species (Figure 4.9, 4.10, enlarged versions in Supplementary Figures 4.1, 4.2, 4.3, 4.4). Models were produced for three time periods, including two different future greenhouse gas concentration scenarios (e.g. RCP2.6 and RCP8.5), resulting in four ENMs in total for each species.

Initial modelling of the current niche of each species revealed their Potential Niche Spaces. This was compared with the known distribution of each species (Realised Niche Space) in order to determine if they matched. For eight species (*A. tumidicauda, B. collinus, B. nivalis* (All), *P. dugdali, P. nitidus, S. australis, S. childi, S. minutus* and *S. villosus*) their Potential Niche Space closely matched their Realised Niche Space, indicating that their distribution is likely constrained by the climate, vegetation and/or soil type variables input into the models. For the remaining three species (*A. crassicauda*, *S. campestris* and *S. piliferus* (All)) their Potential Niche Spaces were found to be larger than their Realised Niche Spaces. Of these three, all species had Potential Niche Spaces that expanded onto the opposing island (North or South) of which they are currently found.

The predicted niches of *S. campestris* and *S. piliferus* (All) were found to be larger than their current distribution within their respective islands. The model predicts suitable habitat to be present in the south west coast of the South Island and in the north of the South Island for *S. campestris*, further west and north than it has currently been sampled. Suitable habitat is predicted to be available for *S. piliferus* (All) in the west (i.e. Mt Taranaki), and also along the southern east coast of the North Island. Further sampling in these areas would reveal if in fact these species are found where the model predicts they "should" be.

Past ecological niche models

Information from these initial current ENMs were used to predict where suitable niche space would have been available for each species in the past (LGM) and where it will be available in the future. For two species (*A. crassicauda* and *B. collinus*), the suitable

Table 4.5 Predictor variable importance scores reported as percentages of total probability scores for the Ensemble Model Mean Weights model (EMmw) of each species. Cells shaded dark purple indicate highest predictor variable importance scores, with white indicating least important predictor variable scores. "Total" row is the number of times a predictor variable was the highest scoring predictor variable across species.

Species	EMmw Predictor Variable Importance (%)								
	Annual mean temp.	Mean diurnal range	Isothermality	Mean temp. of wettest quarter	Mean temp. of driest quarter	Precipitation of driest month	Precipitation seasonality	Soil	Vege
A. crassicauda	8.7	**43.4**	5.9	8.5	7.2	14.7	4.1	3.5	4.0
A. tumidicauda	21.8	14.2	3.0	6.7	10.6	**23.3**	15.3	2.8	2.3
B. collinus	15.9	**44.7**	2.1	9.3	6.5	5.8	6.9	7.2	1.5
B. nivalis (All)	10.6	5.4	**28.1**	8.9	25.2	6.4	5.5	7.3	2.6
B. nivalis (SA)	23.5	**31.2**	1.2	6.1	16.8	6.4	8.6	2.4	3.9
B. nivalis (FP)	8.8	**33.7**	19.2	5.8	16.2	4.2	4.4	4.3	3.3
P. dugdali	12.9	9.4	1.2	12.8	19.8	18.4	**20.9**	2.5	1.9
P. nitidus	24.3	13.6	8.1	4.7	**31.0**	5.1	10.4	1.3	1.6
S. australis	**34.9**	18.6	4.2	4.3	18.6	5.0	7.1	4.9	2.5
S. campestris	13.1	17.1	6.0	8.3	6.9	18.0	5.4	**22.8**	2.4
S. childi	4.0	24.6	1.6	13.2	7.5	**40.5**	3.1	2.8	2.8
S. minutus	5.5	**27.7**	2.5	4.4	10.0	26.5	14.4	5.4	3.6
S. piliferus (All)	19.2	2.9	7.7	**19.7**	13.4	15.7	18.0	1.9	1.6
S. piliferus (LW)	16.1	8.2	5.2	15.6	19.5	**20.4**	6.5	6.2	2.4
S. piliferus (MH)	11.2	15.7	1.6	9.3	5.9	8.8	**40.8**	5.7	1.1
S. villosus	**47.4**	5.9	2.7	7.2	11.9	5.8	14.2	1.2	3.6
Total	3	5	1	1	1	3	2	1	0

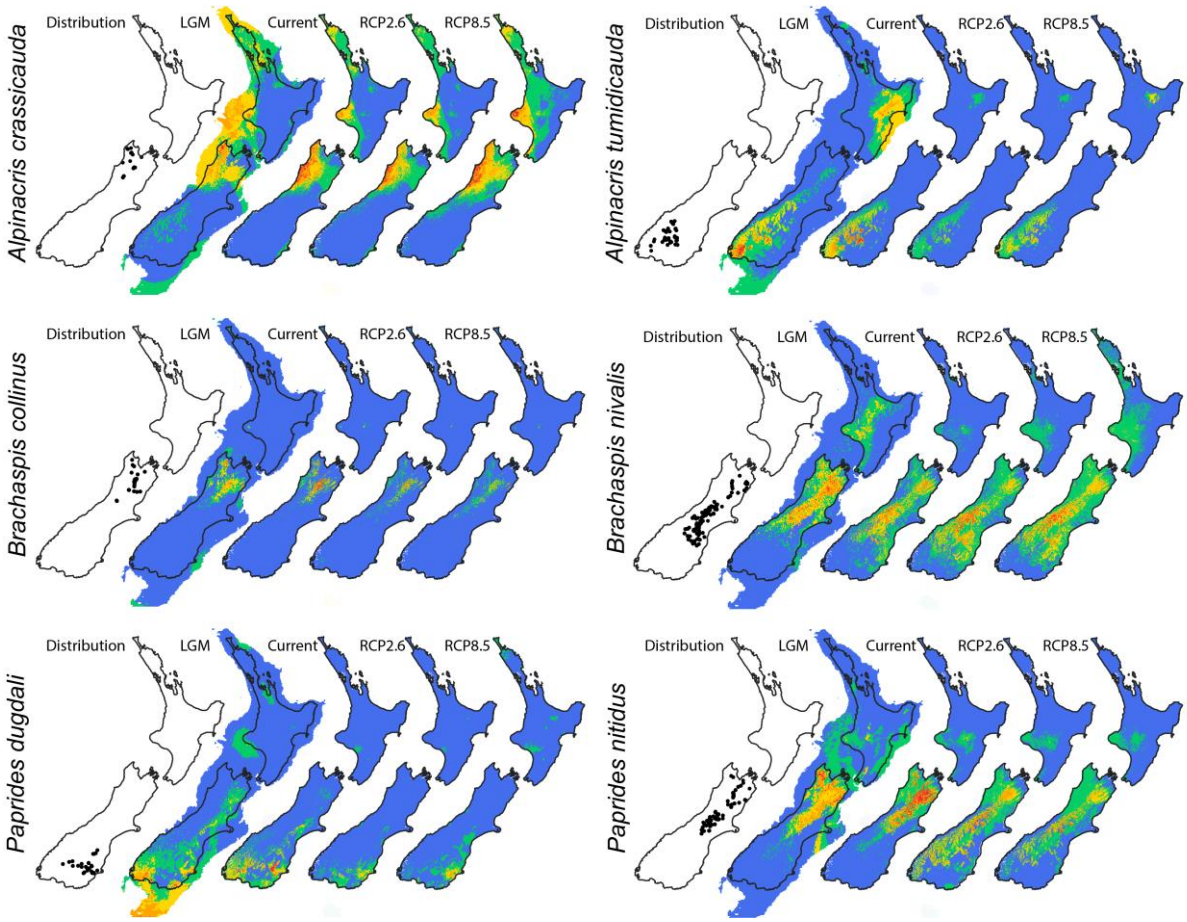

Figure 4.9 The realised niche space of *Alpinacris*, *Brachaspis* and *Paprides* grasshopper species alongside their predicted potential niche spaces, generated through Ecological Niche Modelling. Ensemble Model Mean Weights models were used to generate these predictions. Maps from left to right: the first map indicates where each species is currently distributed and the presence data input into the model; predicted suitable niche space during the Last Glacial Maximum (LGM); predicted suitable niche space for current climate predictions; and the predicted suitable niche space for two future climate change scenarios based on RCP2.6 and RCP8.5 trajectories, respectively. Hot colours indicate a high probability of suitable habitat availability, cool colours indicate a low probability of suitable habitat availability.

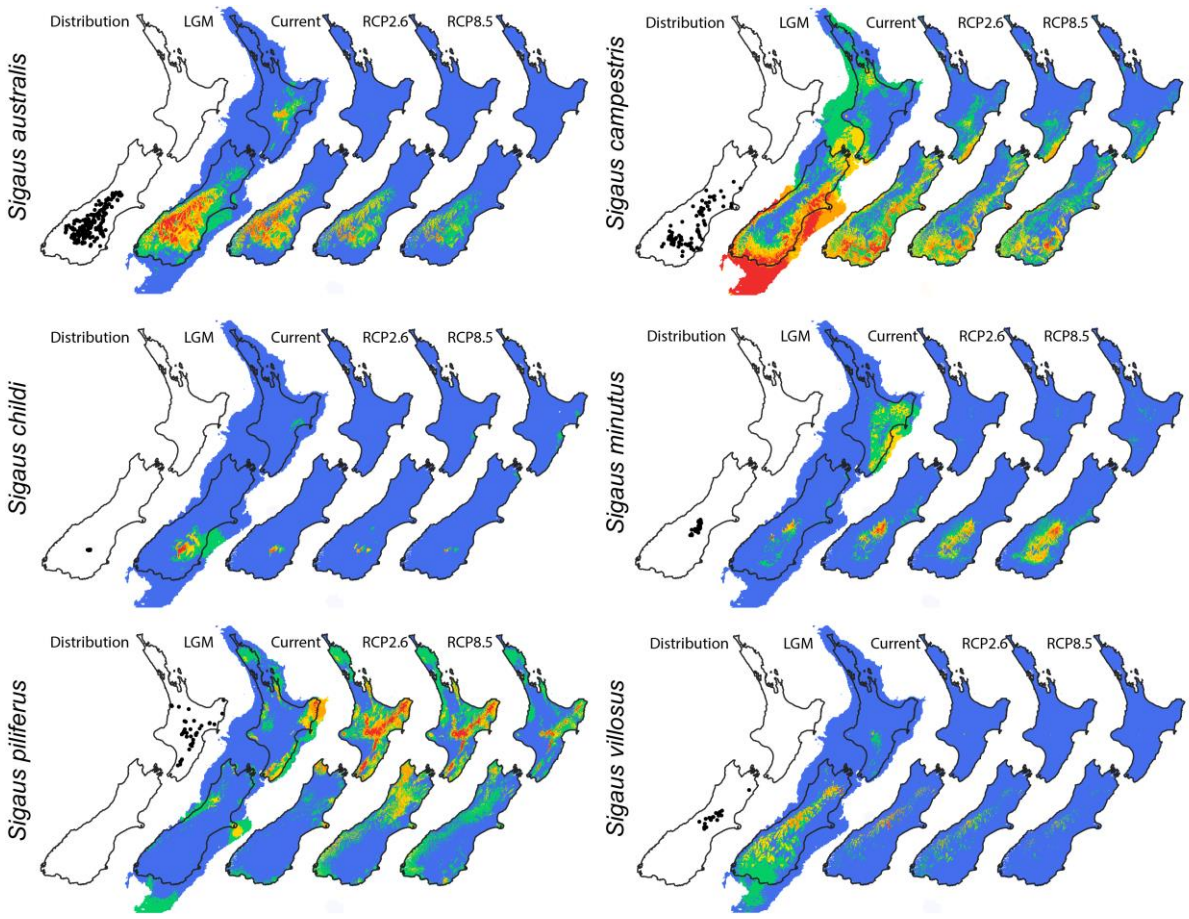

Figure 4.10 The realised niche space of *Sigaus* grasshopper species alongside their predicted potential niche spaces, generated through Ecological Niche Modelling. Ensemble Model Mean Weights models were used to generate these predictions. Maps from left to right: the first map indicates where each species is currently distributed and the presence data input into the model; predicted suitable niche space during the Last Glacial Maximum (LGM); predicted suitable niche space for current climate predictions; and the predicted suitable niche space for two future climate change scenarios based on RCP2.6 and RCP8.5 trajectories, respectively. Hot colours indicate a high probability of suitable habitat availability, cool colours indicate a low probability of suitable habitat availability.

niche space predicted to have been available to them in the LGM was very similar to what is available to them at present. For *A. crassicauda*, this would indicate that it has had ~15,000 years to inhabit suitable niche space in the North Island (Clark *et al.* 2009), but for various reasons has been constrained to its current Realised Niche Space in the South Island. For nine species (*A. tumidicauda*, *B. nivalis* (All), *P. dugdali*, *P. nitidus*, *S. australis*, *S. campestris*, *S. childi*, *S. minutus*, *and S. villosus*) it is predicted that there would have been more suitable niche space available to them during the LGM when compared to their current ENMs. For *A. tumidicauda*, *B. nivalis* (All), *S. australis*, *S. minutus* and *S. villosus*, this includes suitable niche space in the North Island, where they are currently not found. *Sigaus piliferus* (All) is predicted to have had much less suitable niche space available to it during the LGM when compared to its current ENM.

Future ecological niche models

Change in the size of suitable niche space from what is currently predicted to be available, to what will be available in the future, was calculated for both RCP scenarios and for each species (Figure 4.11, 4.12) (Supplementary Table 4.2). When calculated to allow for species to disperse there were mixed results across species, with some species losing, and others gaining suitable niche space (Table 4.6) (Figure 4.13). With a mean temperature increase of 1°C by ~2070 (RCP2.6), nine species (*A. crassicauda*, *A. tumidicauda*, *B. collinus*, *B. nivalis* (All), *P. dugdali*, *S. australis*, *S. campestris*, *S. childi* and *S. piliferus* (All)) are predicted to lose suitable niche space, whilst three species are predicted to gain suitable niche space *(P. dugdali*, *S. minutus* and *S. villosus*). Of those that will lose suitable niche space, five species (*A. tumidicauda*, *B. collinus*, *B. nivalis* (All), *P. nitidus* and *S. australis*) will lose over 50%, with the greatest loss being 95% of suitable niche space (*B. collinus* and *B. nivalis* (All)). *Sigaus villosus*, however, is predicted to gain 168% of suitable niche space, whilst *P. dugdali* and *S. minutus* will gain 12% and 4%, respectively. With a mean temperature increase of 3.7°C by ~2070 (RCP8.5), eight species (*A. tumidicauda*, *B. collinus*, *B. nivalis* (All), *P. nitidus*, *S. australis*, *S. campestris*, *S. piliferus* (All) and *S. villosus*) are predicted to lose suitable niche space, with seven species (*B. collinus*, *B. nivalis*, *P. nitidus*, *S. australis*, *S. campestris*, *S. piliferus* (All) and *S. villosus*) predicted to lose over 50%, and one species (*A. tumidicauda*) predicted to lose 35% of its predicted suitable niche space. Alternatively, four species (*A. crassicauda*, *P. dugdali*, *S. childi*, and *S. minutus*)

will gain suitable niche space, with three species (*A. tumidicauda*, *S. childi* and *S. minutus*) gaining over 150%.

However, all grasshopper species in this study are flightless and dispersal between habitat patches will therefore be limited. When niche space was calculated to exclude newly available niche space that would require migration, all species were predicted to lose at least 20% of suitable niche space in the future. As the grasshoppers are flightless, and New Zealand is divided in to two main islands, it is important to consider that even though new suitable niche space may become available in the future, the grasshopper species may not necessarily be able to disperse into these new areas. With a mean temperature increase of 1°C by ~2070 (RCP2.6), nine species (*A. crassicauda*, *A. tumidicauda*, *B. collinus*, *B, nivalis* (All), *P. dugdali*, *P. nitidus*, *S. australis*, *S. childi* and *S. minutus*) will lose >50% of suitable niche space if they are unable to migrate. Three other species (*S. campestris*, *S. piliferus* (All) and *S. villosus*) will lose over 20% of their predicted suitable niche space. The greatest loss will be by *S. childi* (-100%). With a mean temperature increase of 3.7°C by ~2070 (RCP8.5), eight species (*B. collinus*, *B. nivalis* (All), *P. nitidus*, *S. australis*, *S. campestris*, *S. childi*, *S. piliferus* (All) and *S. villosus*) will lose over 50% of their predicted suitable niche space, and three species (*A. tumidicauda*, *P. dugdali* and *S. minutus*) will lose over 35%. *Paprides nitidus* is predicted to have the greatest loss in this scenario (-97%), followed closely by *S. villosus* (-94%) and *S. australis* (-93%).

Fragmentation statistics

Fragmentation statistics were generated for 'current' and the two future RCP scenarios based on vector binary maps (Figure 4.11, 4.12) (Table 4.7). Patches of connected 'suitable habitat' pixels were identified in each map and given unique IDs from which FragStats were calculated. The most common trend between 'current' FragStats and the FragStats of each future scenario was that with a decrease in suitable habitat area (i.e. decrease in total area of patches), there was a decrease in the mean area of patches, and the number of patches. This occurred in 11/24 of the scenarios across species: *A. crassicauda* (RCP2.6), *A. tumidicauda* (RCP2.6), *B. collinus* (RCP2.6 & RCP8.5), *P. dugdali* (RCP2.6 & RCP8.5), *S. australis* (RCP8.5), *S. campestris* (RCP2.6 & RCP8.5), *S. piliferus* (RCP8.5) and *S. villosus* (RCP8.5). In three scenarios (*A.tumidicauda*

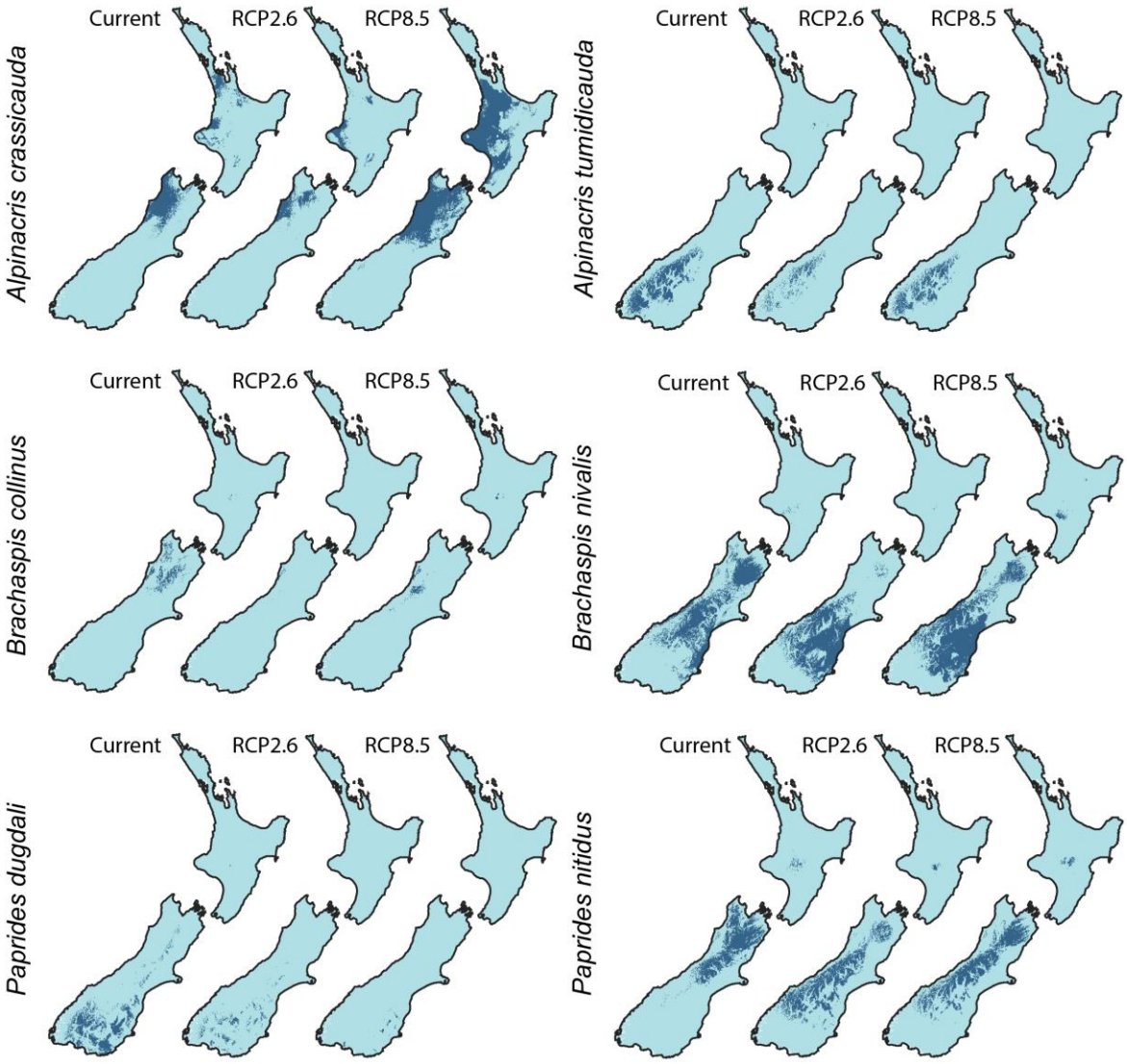

Figure 4.11 Binary vector layers generated for *Alpinacris, Brachaspis* and *Paprides* species, for current predictions and for the two future RCP scenarios. Binary vectors are generated by transforming vectors of probabilities (i.e. initial Ecological Niche Models) using a cutoff threshold (Table 4.5). Each pixel of the map is scored as a 1 (suitable habitat) or a 0 (unsuitable habitat) for each scenario. Dark = suitable, light blue = unsuitable.

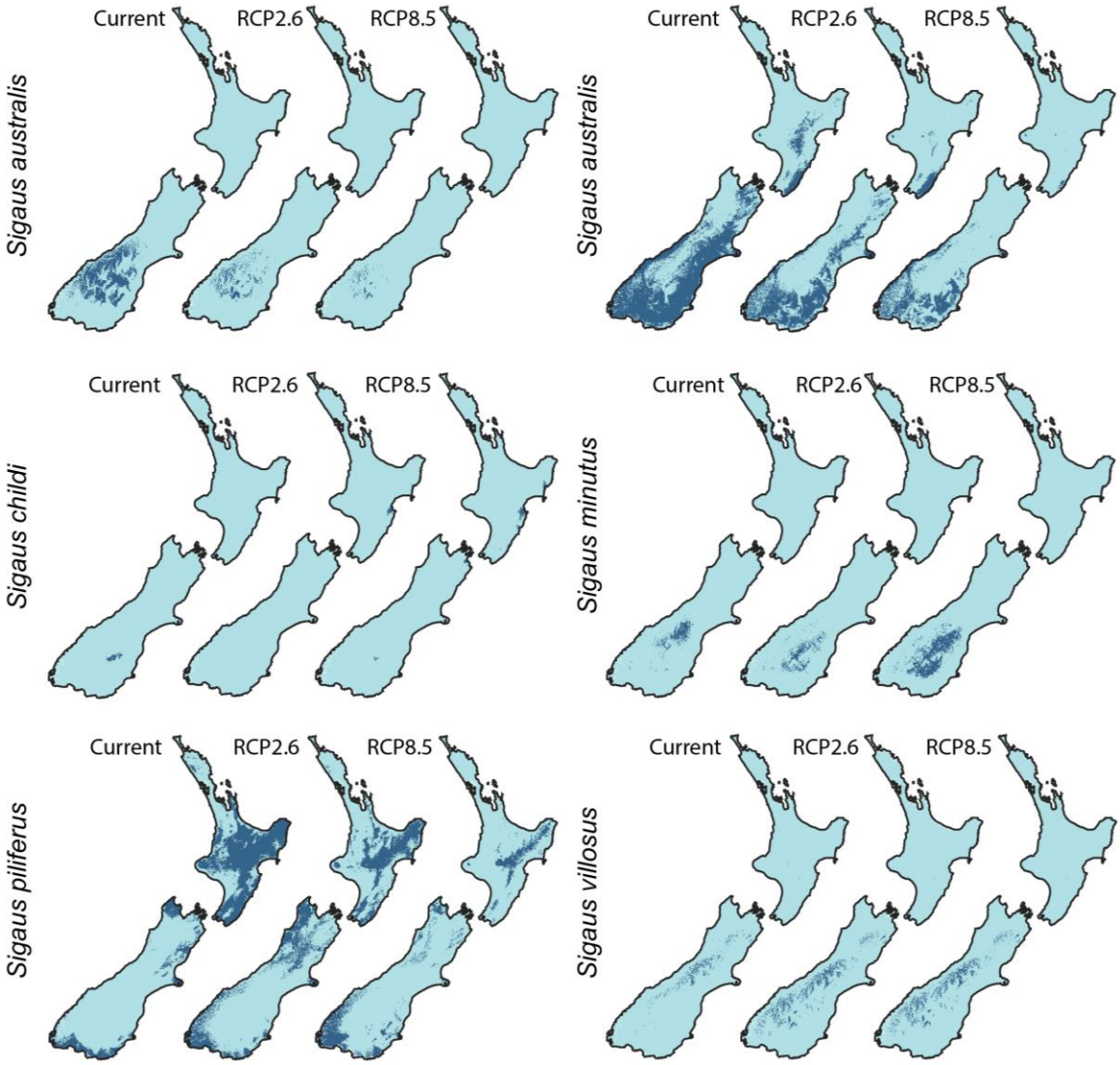

Figure 4.12 Binary vector layers generated for *Sigaus* species, for current predictions and for the two future RCP scenarios. Binary vectors are generated by transforming vectors of probabilities (i.e. initial Ecological Niche Models) using a cutoff threshold (Table 4.5). Each pixel of the map is scored as a 1 (suitable habitat) or a 0 (unsuitable habitat) for each scenario. Dark = suitable, light blue = unsuitable.

Table 4.6 Predicted changes to suitable habitat for the two future scenarios when compared to area of suitable habitat available in current models. Percentages are based on the loss/gain/ stability of pixels between vector binary maps (Figure 4.11 and 4.12). Range changes with dispersal allowed includes "gained" pixels, whilst range change with no dispersal allowed does not. Dark purple indicates the highest loss of suitable habitat (-90 - 100%), white indicates the least amount of loss, or gain of suitable habitat.

Species	% Range change from current models (with dispersal)		% Range change from current models (no dispersal)	
	RCP2.6	RCP8.5	RCP2.6	RCP8.5
A. crassicauda	-43	208	-61	-11
A. tumidicauda	-74	-35	-74	-37
B. collinus	-95	-66	-96	-89
B. nivalis (All)	-95	-66	-96	-89
B. nivalis (SA)	-65	-84	-89	-87
B. nivalis (FP)	60	132	-16	-16
P. dugdali	12	17	-61	-35
P. nitidus	-75	-97	-82	-97
S. australis	-74	-93	-75	-93
S. campestris	-45	-67	-45	-67
S. childi	-47	154	-100	-89
S. minutus	4	191	-80	-44
S. piliferus (All)	-15	-58	-45	-77
S. piliferus (LW)	37	-20	-24	-73
S. piliferus (MH)	1482	931	-75	-14
S. villosus	168	-77	-20	-94

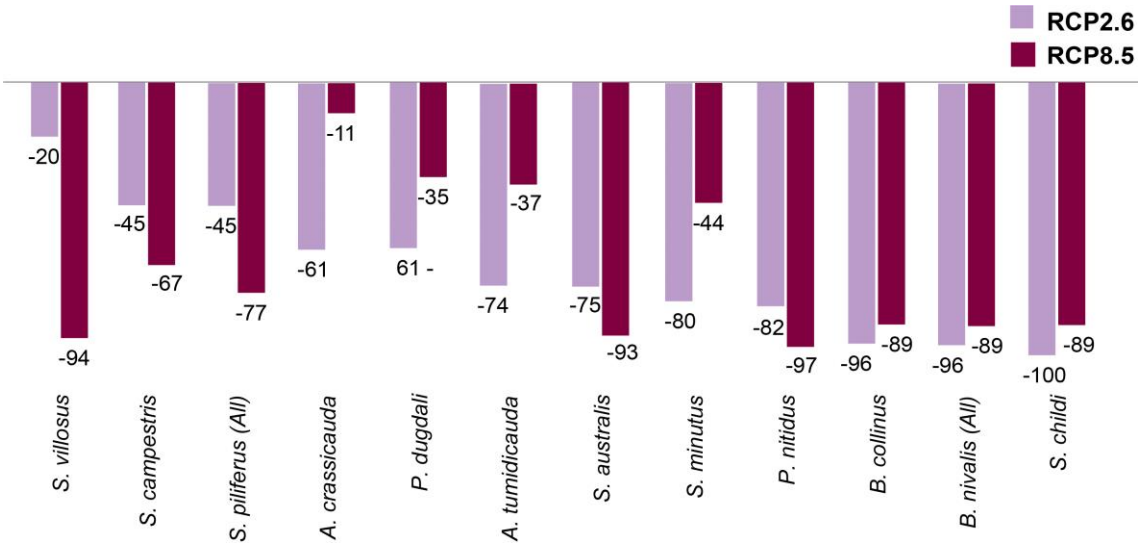

Figure 4.13 Bar graph of predicted loss of suitable habitat the two future scenarios when compared to area of suitable habitat available in current models. Percentages are based on the loss/stability of pixels between vector binary maps (Figure 4.11 and 4.12). The model shown does not allow species to disperse to "gained" pixels, including pixels that are adjacent to stable pixels.

Table 4.7 Fragmentation statistics generated from binary vector files (Figure 4.11 and 4.12). Only patches of pixels exceeding 100 pixels were included.

Species	Scenario	Number of patches	Total area of patches	Min. patch area	Mean patch area	Max. patch area	Total core area
A. crassicauda	Current	12	35156	108	2930	24229	22333
	RCP2.6	8	20771	108	2596	8707	12724
	RCP8.5	19	118524	123	6238	60401	94409
A. tumidicauda	Current	13	29533	142	2272	11737	17068
	RCP2.6	4	4572	303	1143	3186	669
	RCP8.5	14	18731	104	1338	5143	6246
B. collinus	Current	15	7231	106	482	2305	1883
	RCP2.6	2	353	138	177	215	45
	RCP8.5	5	2321	170	464	1040	773
B. nivalis (All)	Current	21	67073	108	3194	39871	36818
	RCP2.6	11	65936	103	5994	61633	42556
	RCP8.5	15	88304	112	5887	72469	59255
B. nivalis (SA)	Current	11	50204	109	4564	40311	31254
	RCP2.6	29	13223	103	456	2706	4922
	RCP8.5	13	6500	104	500	3764	2383
B. nivalis (FP)	Current	9	45781	118	5087	42368	24234
	RCP2.6	4	80549	160	20137	79883	61266
	RCP8.5	8	89226	108	11153	86590	71498
P. dugdali	Current	28	26236	105	937	6005	13230
	RCP2.6	17	4909	106	289	937	1138
	RCP8.5	4	698	123	175	202	203
P. nitidus	Current	4	40058	107	10015	39039	24630
	RCP2.6	19	44780	127	2357	22834	22143
	RCP8.5	24	47599	112	1983	35362	27779
S. australis	Current	16	37840	108	2365	17849	22425
	RCP2.6	18	7328	108	407	1475	2172
	RCP8.5	4	1101	119	275	435	280
S. campestris	Current	30	146228	103	4874	119896	104696
	RCP2.6	21	78914	104	3758	56376	46952
	RCP8.5	26	45806	101	1762	34764	22446
S. childi	Current	2	1534	356	767	1178	884
	RCP2.6	1	811	811	811	811	477
	RCP8.5	7	3653	108	522	1831	1903
S. minutus	Current	6	6694	119	1116	5709	3113
	RCP2.6	6	6312	121	1052	4734	1871
	RCP8.5	3	21754	120	7251	21359	12240
S. piliferus (All)	Current	28	119798	102	4279	88505	91417
	RCP2.6	38	97964	101	2578	36023	58220
	RCP8.5	26	45687	103	1757	19435	26681
S. piliferus (LW)	Current	26	160451	112	6171	107401	127393
	RCP2.6	33	220633	111	6686	107656	156398
	RCP8.5	31	124666	102	4021	76402	81634
S. piliferus (MH)	Current	5	1889	113	378	918	631
	RCP2.6	27	44515	119	1649	17118	26589
	RCP8.5	22	27927	138	1269	8486	15696
S. villosus	Current	10	2425	102	243	756	355
	RCP2.6	25	12192	103	488	5057	2715
	RCP8.5	1	182	182	182	182	15

(RCP8.5), *S. australis* (RCP2.6) and *S. piliferus* (All) (RCP2.6)) where there was a decrease in the total area of their patches, there was a decrease in mean patch size and increase in the number of patches. And for another three scenarios (*P. nitidus* (RCP2.6 & RCP8.5) and *S. childi* (RCP8.5)) where there was an increase in their total patch area, there was an increase in the number of patches, but decrease in the mean area of these patches.

Ecological niche models of mitochondrial lineages:

Model evaluations

Distribution data for *B. nivalis* (All) was divided into *B. nivalis* (SA) and *B. nivalis* (FP) as recommended by Trewick (2001), resulting in 59 and 70 presence points for these datasets, respectively (Table 4.3). For *B. nivalis* (SA) GBM, MARS and GLM were the highest scoring individual models, for *B. nivalis* (FP) it was GBM, RF and MAXENT (Figure 4.14). Model evaluation scores were higher overall than for the combined *B. nivalis* (All) dataset, including for the EMmw ensemble models, with the EMmw models for *B. nivalis* (SA) and *B. nivalis* (FP) scoring ROC values of 0.98 and 0.96, respectively, compared with the *B. nivalis* (All) EMmw ROC value of 0.94 (Table 4.4). Specificity and Sensitivity values were highest for *B. nivalis* (SA) (98/100; 92/100) when compared with *B. nivalis* (All) (98/100; 80/100) and *B. nivalis* (FP) (94/100; 89/100).

Distribution data for *S. piliferus* (All) was divided into *S. piliferus* (LW) and *S. piliferus* (MH) as recommended by Trewick & Morris (2008), resulting in 36 and 9 presence points for each new dataset, respectively (Table 4.3). GBM, CTA and GLM were the highest scoring models for *S. piliferus* (LW), whilst RF, MAXENT and GLM were the highest scoring models for *S. piliferus* (MH) (Figure 4.15). Overall model evaluation scores were high for both mitochondrial lineages, EMmw ensemble models were only slightly higher scoring or equal to individual model scores, with ROC values of 0.99 and 1.00 for *S. piliferus* (LW) and *S. piliferus* (MH), respectively. Sensitivity and Specificity values were high in all instances (*S. piliferus* (All) = 100/100; 97/100, *S. piliferus* (LW) = 100/100; 95/100, *S. piliferus* (MH) = 100/100; 100/100) (Table 4.4).

Predictor variable importance

Isothermality was predicted to be the most important variable for the complete dataset *B. nivalis* (All), however, Mean diurnal range was considered to be the most important predictor variable for both *B. nivalis* (SA) and *B. nivalis* (FP), scoring 31.19 and 33.70, respectively (Table 4.5). This was followed by Annual mean temperature (23.47) and Mean temperature of driest quarter (16.78) for *B. nivalis* (SA), and Isothermality (19.18) and Mean temperature of driest quarter (16.22) for *B. nivalis* (FP). Precipitation of driest month and Precipitation seasonality were considered to be the most important predictor variables for *S. piliferus* (LW) and *S. piliferus* (MH), respectively, also different to what was most important for *S. piliferus* (All) – Mean temperature of driest month (Table 4.5). The second and third most important predictor variables for *S. piliferus* (LW) and *S. piliferus* (MH) were Mean temperature of driest quarter and Annual mean temp, and Mean diurnal range and Annual mean temperature, respectively.

Ecological niche models

The niche models produced for *B. nivalis* (SA) and *B. nivalis* (FP) are visibly different to *B. nivalis* (All) (Figure 4.14). For the current ENM, the most suitable niche space for *B nivalis* (SA) is in the top half of the South Island, and closely matches the distribution of this mitochondrial lineage. The most suitable predicted niche space for *B. nivalis* (FP) is in the lower half of the South Island, and also closely matches the distribution of this mitochondrial lineage. During the LGM, there would have been more suitable niche space available to *B. nivalis* (SA) than currently and also when compared to *B. nivalis* (FP). *Brachaspis nivalis* (FP) was predicted to have had a more restricted area available to it during the LGM. In the future, however, *B. nivalis* (FP) is predicted to experience an increase in suitable niche space available, under both RCP2.6 and RCP8.5 scenarios. If dispersal is allowed, it is estimated that *B. nivalis* (SA) will lose 65% of its suitable niche space under the RCP2.6 scenario, and 84% under the RCP8.5 scenario (Table 4.6). *Brachaspis nivalis* (FP), however, was estimated to gain 60% and 132% under these same two scenarios. If these taxa are not able to disperse, neither maintains their current niche space, and *B. nivalis* (SA) could lose up to 89% of suitable niche space. *Brachaspis nivalis* (FP) will lose no more than 16% under either RCP scenario.

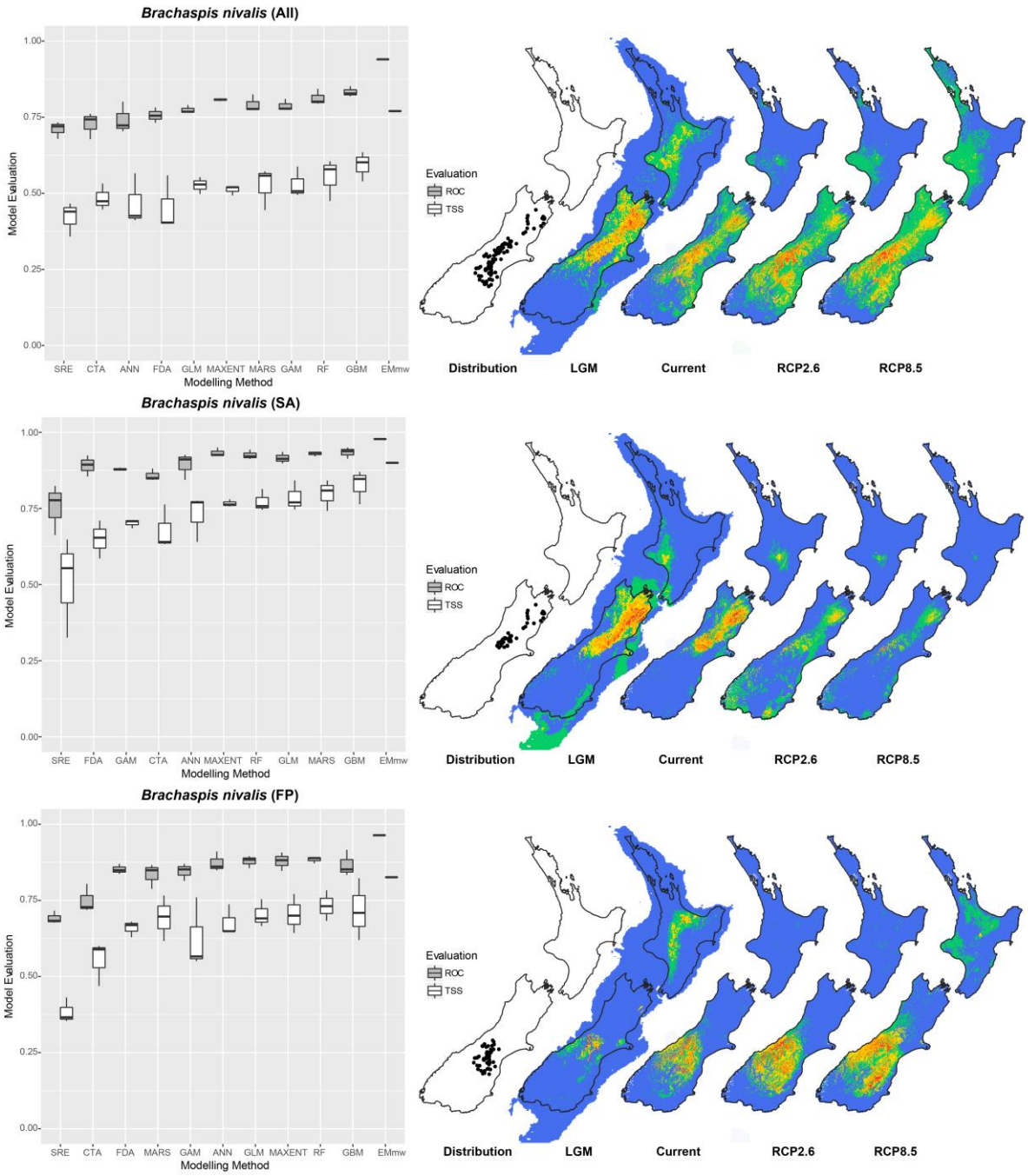

Figure 4.14 Model evaluations and Ecological Niche Models (ENMs) for *Brachaspis nivalis* (All) and two of its mitochondrial lineages *B. nivalis* (SA) and *B. nivalis* (FP). Maps from left to right: the first map indicates where each species is currently distributed and the presence data input into the model; predicted suitable niche space during the Last Glacial Maximum (LGM); predicted suitable niche space for current climate predictions; and the predicted suitable niche space for two future climate change scenarios based on RCP2.6 and RCP8.5 trajectories, respectively. Ensemble Model Mean Weights models were used to generate these predictions. Hot colours indicate a high probability of suitable habitat availability, cool colours indicate a low probability of suitable habitat availability.

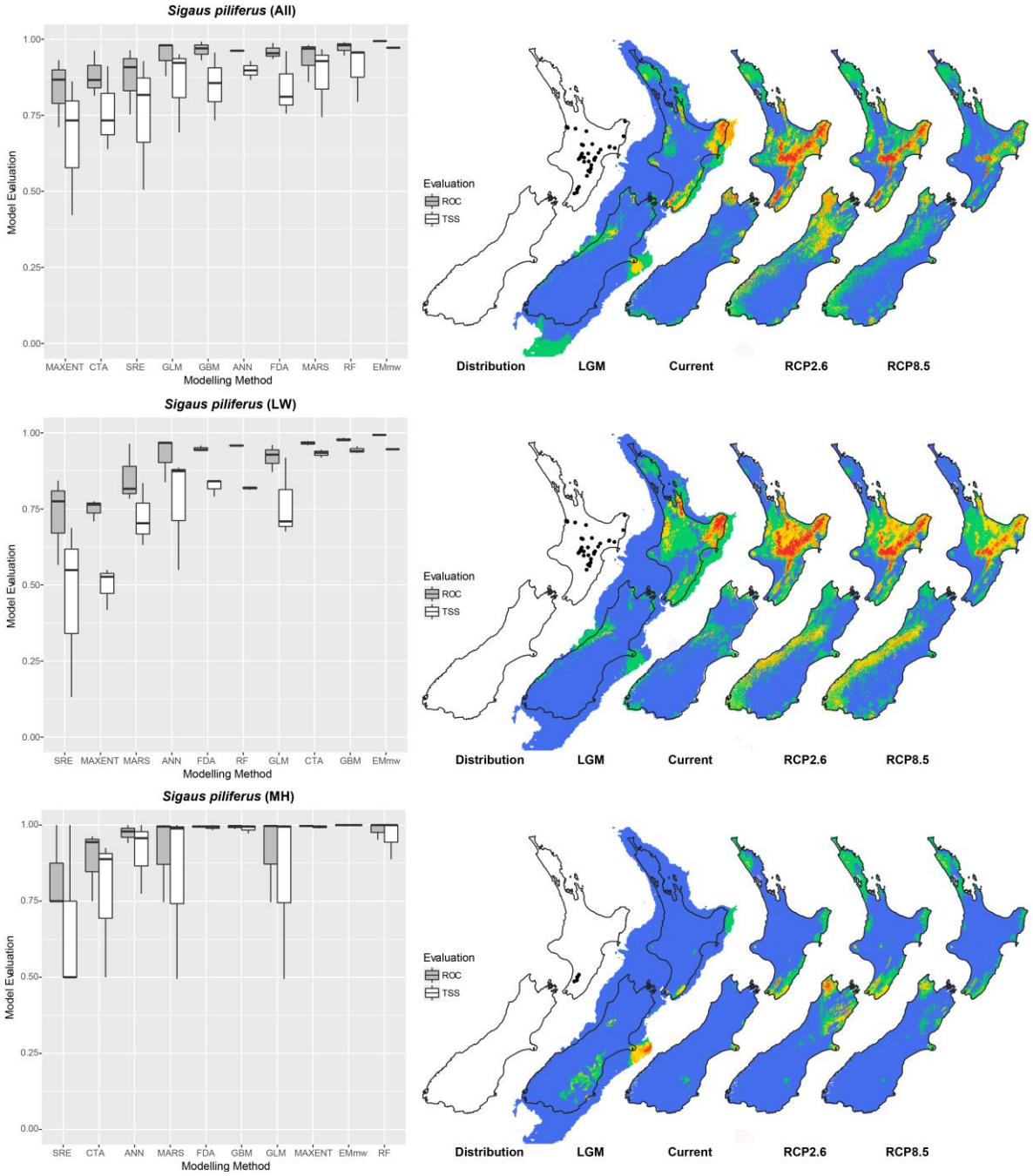

Figure 4.15 Model evaluations and Ecological Niche Models (ENMs) for *Sigaus piliferus* (All) and two of its mitochondrial lineages *S. piliferus* (LW) and *S. piliferus* (MH). Maps from left to right: the first map indicates where each species is currently distributed and the presence data input into the model; predicted suitable niche space during the Last Glacial Maximum (LGM); predicted suitable niche space for current climate predictions; and the predicted suitable niche space for two future climate change scenarios based on RCP2.6 and RCP8.5 trajectories, respectively. Ensemble Model Mean Weights models were used to generate these predictions. Hot colours indicate a high probability of suitable habitat availability, cool colours indicate a low probability of suitable habitat availability.

The current ENM produced for *S. piliferus* (LW) is very similar to the ENM produced for *S. piliferus* (All) (Figure 4.15). The ENM for *S. piliferus* (MH), however, is visibly different and smaller than either *S. piliferus* (All) or *S. piliferus* (LW). During the LGM, the ENM predicts that less suitable niche space would have been available for *S. piliferus* (LW). For *S. piliferus* (MH), it is predicted that more suitable niche space would have been available than currently, however, the majority of this would have been in the South Island. For future predicted RCP scenarios, both *S. piliferus* (LW) and *S. piliferus* (MH) are predicted to gain suitable niche space under an RCP2.6 scenario with dispersal allowed, with *S. piliferus* (MH) predicted to gain as much as 1482% (Table 4.6). Under RCP8.5, however, *S. piliferus* (LW) will lose 20%. When dispersal is not allowed, neither lineage maintains its current niche space, with both predicted to lose at least 14% under future RCP scenarios.

Fragmentation statistics

Fragmentation statistics were generated for the two pairs of mitochondrial lineages (Table 4.7). For *B. nivalis* (SA), in both RCP scenarios it is predicted that there will be a decrease in the total area of suitable habitat patches, as well as a decrease in the mean area and number of patches. For *B. nivalis* (FP) the trend is the opposite, with it being predicted that there will be an increase in the total area, mean area of patches and the number of patches of suitable habitat for both RCP scenarios. For *S. piliferus* (LW), FragStat predictions for each of the RCP scenarios are different, with it being predicted that under the RCP2.6 scenario, there will be an increase in the total area of patches, the mean area of the patches and the total number of patches. Alternatively, for the RCP8.5 scenario, it is predicted that there will be a decrease in the total area of patches and the mean area of patches, whilst the number of patches will increase. For *S. piliferus* (MH), for both RCP scenarios, it is predicted that there will be an increase in total patch area, mean patch area and the number of patches of suitable habitat. For both pairs of mitochondrial lineages, these results are different when compared to the FragStats of *B. nivalis* (All) and *S. piliferus* (All).

Discussion

Changes in Earth's climate are intrinsically linked to the speciation, extinction and distribution of species (Ali & Aitchison 2014, Ayoub & Riechert 2004, Darwin 1859). Climatic change drives species to adapt to change; they may become isolated, lose appropriate habitat and encounter new competition as ranges shift (Iwase *et al.* 2012, Koch & Barnosky 2006, Milá *et al.* 2007, Shapiro *et al.* 2004, Wroe *et al.* 2013). Whether a species survives such changes or goes extinct depends on the severity and speed of change in climate, and the adaptive capacity of the species involved. Future anthropogenic climate change will exert adaptive pressure on a number of taxa, but those on steep climatic gradients will be most rapidly influenced. This applies to New Zealand's endemic alpine grasshoppers. It is evident that climate is a limiting factor in the distribution of the majority of alpine grasshopper species investigated, as revealed by comparison of their current distributions with modelled potential niche spaces. Additionally, it was revealed that for the majority of the grasshopper species, there has been a reduction in suitable niche space since the Last Glacial Maximum. In regards to future climate change, although most grasshoppers will be negatively affected, the predicted response varies amongst species. Furthermore it was determined that for most species, where there is a decrease in the total area of suitable habitat patches, there is also a decrease in the mean size of these patches and the total number of patches.

Ecological niche modelling

The predictive accuracy of ecological niche models is dependent on the input data provided to the model; this includes the number of presence points available for a species (Breiner *et al.* 2015, Hernandez *et al*. 2006, Stockwell & Peterson 2002, Wisz *et al*. 2008). The species in this study were not represented by the same number of presence data points, and this reflects species range sizes and sampling success; additional data points for some species (*P. dugdali*, *S. piliferus* (MH) and *S. villosus*) could improve the reliability of their models. With the available data, the ecological niche models generated were well supported by both ROC and TSS model evaluation metrics. The final ensemble models generally out-performed the individual models; this is consistent with other studies (Araujo & New 2007, Breiner *et al.* 2015, Marmion *et al*. 2009). Ensemble Model Mean Weights models were therefore used for hindcasting

and forecasting past and future climate change models; the use of ensemble models is particularly recommended for low prevalence species (Breiner *et al.* 2015).

The ensemble models had relatively high sensitivity and specificity values, and were thus able to discriminate suitable habitat for each species with high predictive accuracy; this included for species with low numbers of presence data points. Interestingly, the species that had the highest number of presence data points (e.g. *B. nivalis*, *P. nitidus*, *S. australis* and *S. campestris*) had the lowest ROC and TSS model evaluation scores for both individual and ensemble models, and this correlated with low sensitivity and specificity scores. The ROC evaluation method is independent of prevalence, thus it would indicate that higher sampling rates result in reduced modelling performance, and vice versa. This has been described previously, and may relate to the tendency for the distributions of rare or spatially restricted species to be more easily predicted than more common or widely distributed species (Allouche *et al*. 2006, Araujo & Williams 2000, Bouska *et al*. 2015, Hernandez *et al*. 2006, McPherson & Jetz 2007, Segurado & Araujo 2004, Syphard & Franklin 2010). The distribution of widespread species is likely more heterogeneous than species with narrow ranges, and thus more environmental variation may be detected by ENM models. Alternatively, species with large ranges may encompass several genetic lineages that are independently responding to change; in such instances, it may be beneficial to model each lineage separately to improve the predictive accuracy of models (Leaché *et al*. 2009, Raxworthy *et al*. 2007, Rissler & Apodaca 2007).

Of the climate, vegetation and soil variables input into the models, climate was predominantly the most important at explaining the distribution of the grasshopper species. For most species, one predictor variable tended to be the most important predictor variable for that species. For some species (*A. tumidicauda*, *B. nivalis* (All), *P. dugdali*, *S. piliferus* (All)), however, two, three or four variables had near equal predictive importance. This suggests that these species may inhabit more complex niches and/or that numerous variables influence their distributions. In the majority of instances, the current distribution of grasshopper species matched the ecological niche space predicted by the ENMs. This further indicates the reliability of the niche models, and also informs us that the grasshoppers' current distributions are being restricted by the variables I have used in this study. Climate is evidently a key driver in the

distribution of New Zealand's endemic alpine grasshoppers. We can therefore be reassured that inferring the past and future distributions of these grasshoppers via hindcasting and forecasting climate predictions will not be misleading.

Where current distribution of a species is incongruent with its predicted suitable niche space under current conditions, it indicates that other variables are limiting the distribution of these species (Davis *et al*. 1998, Pearson *et al*. 2006). This could involve abiotic variables not included in the analysis, or biological interactions such as competition, predation, disease and/or parasitism. Biotic variables are notoriously difficult to account for in ecological niche modelling (Pearson *et al*. 2006).

The Last Glacial Maximum

The majority of the grasshopper species in this analysis appeared to have smaller niche space now than in the LGM (Figure 4.9, 4.10). This is consistent with expectations of species adapted to alpine living, as alpine environments have been contracting since the LGM (Galbreath 2009). For five of the South Island species (*A. tumidicauda*, *B. nivalis* (All), *S. australis*, *S. minutus* and *S. villosus*) it was found that suitable habitat could have been available in the North Island during the LGM. These species are not found in the North Island today, and this is consistent with their current model. It is possible, therefore, that these species were found in the North Island in the past, but were extirpated by climate warming in the Holocene. Interestingly, newly established phylogenetic relationships of this group (Chapter Two), indicates that *S. villosus* is the sister species of the sole, North Island occupying, *S. piliferus*. Prior to the LGM, it is possible that these two species had connected distributions in the North Island, from which *S. villosus* has since retreated to the South Island with warming temperatures.

Alpinacris crassicauda was predicted to have had suitable niche space in the North Island (where it is currently not found) during the LGM, and at present. The North and South Island of New Zealand were connected during the LGM as a result of lower sea levels (Trewick & Bland 2012), but there is no evidence that *A. crassicauda* has successfully migrated to the North Island. This is most likely due to environmental barriers, or a lack of suitable habitat connectivity for this species to migrate. The land between the two islands would have been low lying and so might have supported scrub

and/or forest vegetation (Shepherd & Perrie 2011). There has been little predicted change in available suitable niche space for *A. crassicauda* and *B. collinus* since the LGM to present. This suggests that these species have been able to make suitable, *in situ*, adaptations to the climatic changes that their habitats have undergone in the last ~15,000 years (Clark *et al*. 2009). This suggests that these species may be particularly resilient to future climatic change; however, both of these species are predicted to lose significant amounts of suitable niche space under future climate change scenarios (Figure 4.13, Table 4.6).

Future climate

Predictions of how species may fare with future climate change vary, depending on the RCP scenario, and whether or not migration is accounted for. Most species of New Zealand alpine grasshopper are predicted to lose at least 30% of suitable niche space under both RCP scenarios (Figure 4.13, Table 4.6). Some species may gain suitable niche space under RCP2.6, whilst also being predicted to lose suitable niche space under RCP8.5, and vice versa. Furthermore, closely related species (as established in Chapter Two) do not demonstrate similar responses to change. The general consensus, however, is that the majority of the grasshopper species will be negatively impacted by future climate change. This is comparable with models of alpine systems around the world (Descombes *et al*. 2016, Biella *et al*. 2017, Guerrina *et al*. 2016, Pradervand *et al*. 2014, Schimt *et al*. 2013, Thuiller *et al.* 2005, Xue *et al*. 2014).

The degree of suitable niche space loss will be relative to the species involved. For species that have wide current distributions (e.g. *S. australis*, *P. nitidus*), a reduction of 10% of suitable niche space would likely be negligible, when compared to a narrowly distributed species (e.g. *S. childi*) losing this same amount. Despite being predicted to gain suitable niche space under various scenarios, species with lowland distributions (e.g. *S. childi* and *S. minutus*), will be at greatest risk of landuse change (e.g. conversion of tussock grasslands to agricultural land, invasion of wilding pines), with the end result likely to be as detrimental as for high elevation species (Sivyer *et al*. 2018). The effects of landuse change that has occurred in the last 60 years may be hidden, as some locations were sampled as far back as the 1960's and may not have been revisited and checked for species since. It is possible that in the last ~60 years some species have

already disappeared from these locations, particularly those that inhabit lowland areas which have recently been intensively modified (e.g. *S. minutus*). Such landuse change will also affect any species hoping to adapt and expand their range from high to lower elevations with future climate change.

Mitochondrial lineages

Another objective of this study was to investigate the ecological niche models of two pairs of mitochondrial lineages – *B. nivalis* (SA) and *B. nivalis* (FP), and *S. piliferus* (LW) and *S. piliferus* (MH). As mitochondrial lineages, they are genetically distinct; this makes it interesting to examine if they are ecologically distinct also. Both pairs of mitochondrial lineages are distributed in allopatry, occurring in either the northern/central region or southern region of their respective islands. From this study, it is apparent that *B. nivalis* (SA) and *B. nivalis* (FP) occupy distinct niche spaces, despite being predominantly influenced by the same climatic predictor variable. This difference in geographic ranges is associated with different predicted responses to climate change. The potential range of *Brachaspis nivalis* (SA) has contracted since the LGM, and was predicted to continue to do so under future climate change scenarios. In contrast, *B. nivalis* (FP) is undergoing range trends in the other direction, with the expansion of suitable niche space since the LGM and expansion underfuture climate change scenarios; this suggests that it will be the more resilient lineage in the future. Such ecological distinctness could be considered alongside genetic evidence in the species delimitation of these lineages (Leaché *et al*. 2009, Raxworthy *et al*. 2007, Rissler & Apodaca 2007).

A similar pattern was found between the two *S. piliferus* lineages, although the divide between suitable niches is not as abrupt. The suitable niche space of *S. piliferus* (LW) was predicted to have decreased since the LGM, whilst for *S. piliferus* (MH) it increased considerably, and will continue to do so. In the present, their niches are predicted to be overlapping (despite their allopatric distribution), and will continue to be under both RCP scenarios. This suggests the possibility of overlap in the distributions of these two lineages in the future, and it will be interesting to see if introgression occurs between these two lineages as a result of potential future niche dynamics.

In contrast to these two pairs of mitochondrial lineages, *S. childi* occurs in sympatry with *Sigaus australis* which has a wide distribution. *Sigaus childi* has a narrow distribution in Central Otago of the South Island; the ecological niche models generated in this study for these two species reinforced this. *Sigaus childi* is found in the centre of *S. australis'* wide ranging distribution. *Sigaus childi* is adapted to arid environments in Central Otago, whilst *S. australis* is primarily adapted to alpine living, but is also found at low elevations. Under the most extreme RCP scenario, *S. childi* was predicted to gain substantial niche space, as arid conditions expand in the Otago region. Alternatively, *S. australis* was predicted to lose suitable niche space under all scenarios. *Sigaus childi* has the potential to expand its range and out-compete *S. australis* in the future (Dowle *et al.* 2014).

Conclusion

Here, through the investigation of the complete diversity of New Zealand's endemic alpine grasshoppers, we can see just how complex it is to predict the outcomes of future climate change for species, regardless of if they share similar environments or are closely related. I have determined that the overall trend for this group will be a decrease in suitable niche space. However, there are some exceptions to this, and some species will be impacted differently depending on the RCP scenarios. This is a good example of how there is no "one size fits all" response of species to future climate change, and thus, unlikely to be a "one size fits all" solution, either. It is difficult to think of a resolution for a group whom for many inhabit an environment that is restricted by elevation and latitude. The results of this study could be used for the future conservation management of these species (e.g. translocating species to where suitable habitat may be/become available). However, in stating the obvious, halting or greatly reducing the impacts of climate change will be the best hope for the conservation of many of these species, and therefore also the best hope for the conservation of alpine biota in New Zealand.

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Supplementary Material Chapter Four

Supplementary Table 4.1 Predictor variable importance scores reported as percentages of total probability scores for each species for each modelling method. Cells shaded dark purple indicate highest predictor variable importance scores, with white indicating less important predictor variable scores.

Species	Predictor Variable	Modelling method										
		GLM	GBM	GAM	CTA	ANN	SRE	FDA	MARS	RF	MAXENT	EMmw
A. crassicauda	Annual mean temp.	17.3	0.9	25.3	5.1	14.2	5.2	1.1	15.4	2.2	0.0	8.7
	Mean diurnal range	18.5	51.3	37.8	73.2	17.1	28.9	57.3	28.7	41.8	79.5	43.4
	Isothermality	7.0	1.7	0.0	0.0	2.2	3.7	3.6	23.2	14.8	3.0	5.9
	Mean temp. wettest 1/4	8.3	3.2	22.8	0.0	13.0	5.6	8.4	13.7	9.7	0.0	8.5
	Mean temp. driest 1/4	20.6	6.4	0.0	7.7	19.3	13.3	0.0	0.0	5.2	0.0	7.2
	Precipitation driest month	11.3	27.0	7.2	13.9	12.2	18.1	13.9	12.9	18.8	12.0	14.7
	Precipitation seasonality	9.7	5.3	0.0	0.0	5.7	15.0	0.0	0.0	1.8	3.7	4.1
	Vege	3.8	0.6	0.0	0.0	8.8	1.1	11.2	4.1	4.6	0.5	3.5
	Soil	3.4	3.6	7.0	0.0	7.5	9.2	4.5	2.1	1.1	1.3	4.0
A. tumidicauda	Annual mean temp.	14.2	8.3	53.5	0.0	14.9	13.3	41.3	39.6	24.8	8.2	21.8
	Mean diurnal range	36.0	7.7	13.9	0.0	6.4	18.7	7.4	20.0	8.5	23.4	14.2
	Isothermality	5.1	0.1	7.6	0.0	0.4	2.2	2.5	5.0	2.9	4.3	3.0
	Mean temp. wettest 1/4	0.9	7.4	0.0	0.0	14.9	11.2	18.2	1.6	11.0	2.2	6.7
	Mean temp. driest 1/4	0.9	18.6	0.0	0.6	23.3	19.1	18.3	11.1	12.0	1.6	10.6
	Precipitation driest month	35.4	37.9	8.1	44.1	14.0	16.7	11.9	17.5	12.2	35.0	23.3
	Precipitation seasonality	4.6	18.4	16.9	55.4	8.3	13.8	0.4	0.7	13.5	21.1	15.3
	Vege	0.3	0.2	0.0	0.0	12.7	1.3	0.0	0.5	8.9	4.0	2.8
	Soil	2.5	1.3	0.0	0.0	5.2	3.7	0.0	3.9	6.3	0.2	2.3

B. collinus	Annual mean temp.	26.5	21.5	25.5	1.1	26.2	12.1	9.2	17.4	8.4	11.5	15.9
	Mean diurnal range	21.0	67.2	64.2	98.9	22.9	19.8	7.6	45.1	57.6	42.9	44.7
	Isothermality	0.0	0.6	0.0	0.0	2.9	9.3	0.9	1.7	3.0	3.0	2.1
	Mean temp. wettest 1/4	4.3	1.4	0.0	0.0	9.7	9.6	43.2	0.3	6.4	18.5	9.3
	Mean temp. driest 1/4	20.4	0.4	0.0	0.0	24.7	15.4	0.0	0.0	3.9	0.0	6.5
	Precipitation driest month	8.3	6.2	0.0	0.0	5.5	14.8	5.5	0.0	3.7	14.1	5.8
	Precipitation seasonality	15.0	0.9	0.0	0.0	1.6	17.1	1.5	24.4	4.8	4.2	6.9
	Vege	4.3	1.2	10.3	0.0	4.8	0.9	32.2	5.7	10.6	1.8	7.2
	Soil	0.2	0.7	0.0	0.0	1.7	1.1	0.0	5.4	1.6	4.0	1.5
B. nivalis (All)	Annual mean temp.	2.0	8.3	0.0	18.6	30.6	12.4	10.7	3.9	9.2	9.8	10.6
	Mean diurnal range	2.8	3.6	0.0	4.5	4.0	7.4	7.5	15.6	3.2	5.8	5.4
	Isothermality	39.6	24.7	66.3	24.7	0.8	5.7	40.4	34.3	17.2	27.7	28.1
	Mean temp. wettest 1/5	6.3	3.7	9.7	12.1	12.0	6.2	8.2	1.9	14.7	13.8	8.9
	Mean temp. driest 1/5	25.6	37.3	24.0	24.7	27.0	27.2	26.8	16.1	24.1	19.4	25.2
	Precipitation driest month	5.5	5.9	0.0	3.0	10.4	8.1	0.9	9.8	16.4	3.6	6.4
	Precipitation seasonality	18.1	3.4	0.0	4.8	4.9	10.3	1.6	0.0	8.0	4.1	5.5
	Vege	0.0	10.4	0.0	7.7	4.5	10.7	4.0	18.4	3.8	13.2	7.3
	Soil	0.2	2.6	0.0	0.0	5.6	12.0	0.0	0.0	3.3	2.6	2.6
B. nivalis (SA)	Annual mean temp.	21.9	13.3	33.1	25.7	22.7	7.4	25.8	31.5	19.5	33.9	23.5
	Mean diurnal range	31.7	49.4	29.8	64.5	9.6	20.3	31.1	19.6	34.5	21.3	31.2
	Isothermality	1.1	0.3	0.0	0.0	2.0	3.1	4.2	0.0	0.9	0.1	1.2
	Mean temp. wettest 1/5	5.4	11.6	0.0	0.0	10.4	11.1	2.7	3.9	13.7	2.4	6.1
	Mean temp. driest 1/5	26.3	7.2	37.0	9.8	20.5	17.7	19.6	15.2	7.1	7.3	16.8
	Precipitation driest month	10.9	1.8	0.0	0.0	12.2	9.4	6.8	8.7	9.7	4.1	6.4
	Precipitation seasonality	1.1	8.3	0.0	0.0	2.1	20.1	6.3	18.9	4.9	24.4	8.6
	Vege	0.2	1.4	0.0	0.0	10.7	3.2	0.0	1.0	4.4	2.7	2.4
	Soil	1.4	6.7	0.0	0.0	9.7	7.6	3.4	1.1	5.2	3.8	3.9

B. nivalis (FP)	Annual mean temp.	11.9	3.4	3.8	12.2	13.2	9.7	7.0	12.2	3.8	11.0	8.8
	Mean diurnal range	38.1	46.1	23.6	38.2	17.2	30.5	45.1	37.0	26.4	34.9	33.7
	Isothermality	21.4	8.1	56.9	10.1	6.1	4.1	19.6	28.2	13.7	23.6	19.2
	Mean temp. wettest 1/6	8.3	1.2	4.8	0.0	10.2	4.2	6.8	5.8	6.6	10.2	5.8
	Mean temp. driest 1/6	14.6	27.1	6.7	21.5	15.3	19.4	18.5	13.8	14.8	10.4	16.2
	Precipitation driest month	0.0	3.5	3.9	4.1	12.5	4.3	0.0	2.6	10.9	0.6	4.2
	Precipitation seasonality	1.8	1.3	0.3	2.1	10.5	6.9	0.0	0.0	18.1	3.1	4.4
	Vege	1.3	6.3	0.0	9.7	4.6	11.5	3.0	0.4	2.5	3.5	4.3
	Soil	2.6	2.9	0.0	2.1	10.3	9.5	0.0	0.0	3.3	2.6	3.3
P. dugdali	Annual mean temp.	11.7	9.1	0.0	16.2	14.6	19.2	0.4	25.7	25.9	6.3	12.9
	Mean diurnal range	17.7	17.1	4.2	7.8	6.3	17.1	6.6	1.8	3.5	12.1	9.4
	Isothermality	1.7	0.1	0.0	0.0	3.6	3.1	0.0	0.0	3.0	0.8	1.2
	Mean temp. wettest 1/6	6.7	9.2	17.9	8.1	15.1	10.0	13.5	26.8	18.9	2.3	12.8
	Mean temp. driest 1/6	21.2	27.3	5.4	26.6	15.6	17.4	33.7	4.3	17.2	29.5	19.8
	Precipitation driest month	23.3	26.7	4.6	16.8	19.2	20.1	18.1	23.1	11.6	20.6	18.4
	Precipitation seasonality	10.1	8.7	67.9	17.4	11.9	9.8	27.7	13.5	16.0	26.4	20.9
	Vege	4.8	1.3	0.0	7.2	8.3	0.2	0.0	0.9	1.7	0.4	2.5
	Soil	2.7	0.5	0.0	0.0	5.5	3.1	0.0	4.0	2.2	1.6	1.9
P. nitidus	Annual mean temp.	14.6	25.5	42.7	30.2	23.8	9.3	25.4	27.7	18.8	25.0	24.3
	Mean diurnal range	11.6	24.3	13.8	26.5	6.7	10.8	14.1	4.4	16.4	7.4	13.6
	Isothermality	23.2	2.0	3.3	0.0	0.3	9.2	7.4	13.8	7.6	14.3	8.1
	Mean temp. wettest 1/7	0.7	3.4	0.0	0.0	12.5	13.9	0.0	0.8	9.0	6.9	4.7
	Mean temp. driest 1/7	33.3	32.3	40.2	42.9	28.3	21.6	36.0	25.0	32.2	17.8	31.0
	Precipitation driest month	2.3	1.6	0.0	0.0	13.2	11.1	4.7	9.3	5.3	3.1	5.1
	Precipitation seasonality	13.6	9.7	0.0	0.4	7.2	17.4	10.1	18.7	5.0	21.3	10.4
	Vege	0.2	0.9	0.0	0.0	4.8	4.4	0.0	0.0	1.1	1.8	1.3
	Soil	0.5	0.3	0.0	0.0	3.2	2.3	2.3	0.3	4.6	2.4	1.6

S. australis	Annual mean temp.	42.3	33.2	69.0	25.6	23.7	10.5	61.7	42.3	29.6	11.3	34.9
	Mean diurnal range	36.5	20.1	17.9	0.0	9.0	22.3	11.3	21.7	15.9	31.3	18.6
	Isothermality	9.0	1.6	2.1	0.0	0.5	11.7	0.3	6.2	2.3	8.1	4.2
	Mean temp. wettest 1/7	0.0	0.8	0.0	2.3	15.0	5.1	7.4	6.4	4.7	0.7	4.3
	Mean temp. driest 1/7	11.9	27.7	8.0	37.6	25.0	23.7	9.2	11.7	20.7	10.0	18.6
	Precipitation driest month	0.0	3.1	0.0	0.0	7.4	7.9	4.8	1.4	8.9	16.2	5.0
	Precipitation seasonality	0.0	11.6	0.0	27.9	4.5	6.8	0.0	4.3	11.7	3.6	7.1
	Vege	0.3	1.4	3.0	4.0	8.8	1.8	5.4	3.9	3.8	16.8	4.9
	Soil	0.0	0.5	0.0	2.5	5.9	10.1	0.0	2.1	2.4	1.8	2.5
S. campestris	Annual mean temp.	11.1	8.8	0.0	9.6	14.8	12.5	20.7	23.1	12.0	18.8	13.1
	Mean diurnal range	25.5	19.3	19.6	28.1	7.1	18.9	13.6	11.8	13.4	13.2	17.1
	Isothermality	2.9	0.6	22.7	0.6	1.5	6.2	9.1	8.9	2.6	5.1	6.0
	Mean temp. wettest 1/8	0.5	2.6	1.9	5.8	17.3	10.8	15.1	7.6	13.6	8.0	8.3
	Mean temp. driest 1/8	4.0	4.2	1.1	10.0	15.2	11.3	1.8	2.9	10.6	7.9	6.9
	Precipitation driest month	26.6	25.0	7.9	12.8	15.8	9.1	20.4	27.7	16.4	18.5	18.0
	Precipitation seasonality	3.4	2.6	8.1	2.7	3.6	4.5	9.4	3.5	9.3	6.9	5.4
	Vege	25.0	36.1	37.3	30.4	18.8	16.4	9.9	14.6	20.1	19.2	22.8
	Soil	0.8	1.0	1.4	0.0	5.8	10.3	0.0	0.0	2.0	2.4	2.4
S. childi	Annual mean temp.	18.2	0.0	0.0	0.0	9.6	10.8	0.1	0.0	0.8	0.1	4.0
	Mean diurnal range	27.1	22.9	11.4	30.7	8.7	14.5	54.2	43.6	16.7	16.2	24.6
	Isothermality	4.2	0.0	0.0	0.0	0.0	11.3	0.0	0.0	0.0	0.0	1.6
	Mean temp. wettest 1/8	9.5	0.7	0.0	0.0	24.8	15.2	44.5	14.5	11.9	11.2	13.2
	Mean temp. driest 1/8	2.7	0.1	31.5	0.0	18.0	11.2	0.0	0.0	6.1	5.9	7.5
	Precipitation driest month	34.0	75.8	57.1	69.3	29.9	17.2	0.3	41.7	43.1	36.5	40.5
	Precipitation seasonality	0.0	0.1	0.0	0.0	2.2	15.3	0.9	0.0	6.5	5.9	3.1
	Vege	0.7	0.0	0.0	0.0	2.0	1.2	0.0	0.1	2.9	20.7	2.8
	Soil	3.6	0.4	0.0	0.0	4.7	3.3	0.1	0.0	11.9	3.5	2.8

S. minutus	Annual mean temp.	0.0	3.3	2.0	1.1	12.4	11.7	12.8	1.0	5.4	5.7	5.5
	Mean diurnal range	36.1	49.5	1.5	47.9	10.1	21.6	16.1	29.1	29.6	35.1	27.7
	Isothermality	0.0	0.6	0.0	0.0	2.5	9.0	0.0	13.1	0.3	0.1	2.5
	Mean temp. wettest 1/9	5.2	0.2	0.8	0.0	11.8	8.8	8.1	0.0	6.1	2.7	4.4
	Mean temp. driest 1/9	16.2	11.7	1.6	1.6	11.3	8.5	15.0	14.5	11.4	8.0	10.0
	Precipitation driest month	12.3	25.3	55.7	48.6	19.5	14.4	33.1	22.5	17.0	16.5	26.5
	Precipitation seasonality	17.1	3.9	37.2	0.7	14.2	10.5	7.7	7.4	23.2	22.3	14.4
	Vege	9.5	0.9	0.7	0.1	9.5	9.1	4.4	12.3	4.2	3.5	5.4
	Soil	3.6	4.6	0.6	0.0	8.6	6.6	2.8	0.0	2.8	5.9	3.6
S. piliferus (All)	Annual mean temp.	33.1	2.2	N/A	10.4	39.3	6.3	31.1	34.4	3.6	12.4	19.2
	Mean diurnal range	0.5	0.1	N/A	0.0	2.3	17.1	1.7	0.0	3.0	1.0	2.9
	Isothermality	0.8	13.6	N/A	11.8	0.5	10.4	5.4	4.3	6.1	16.7	7.7
	Mean temp. wettest 1/9	26.3	18.2	N/A	17.9	30.2	9.1	17.4	22.0	20.7	15.2	19.7
	Mean temp. driest 1/9	21.0	25.3	N/A	21.8	6.1	20.4	0.0	5.4	16.1	4.3	13.4
	Precipitation driest month	9.0	21.7	N/A	15.2	9.5	15.3	23.3	13.3	17.4	16.0	15.7
	Precipitation seasonality	8.8	18.7	N/A	22.9	7.1	17.2	19.5	18.4	31.1	18.0	18.0
	Vege	0.6	0.1	N/A	0.0	1.7	2.5	1.3	0.2	0.7	10.5	1.9
	Soil	0.0	0.0	N/A	0.0	3.4	1.7	0.3	2.0	1.2	5.9	1.6
S. piliferus (LW)	Annual mean temp.	31.2	0.6	N/A	0.0	28.2	5.9	33.3	28.8	3.6	13.2	16.1
	Mean diurnal range	0.0	10.5	N/A	12.8	2.7	21.2	3.2	11.0	3.0	9.5	8.2
	Isothermality	3.3	0.5	N/A	0.0	2.6	6.7	6.6	4.1	7.0	16.0	5.2
	Mean temp. wettest 1/10	25.9	16.2	N/A	0.0	21.2	7.9	17.7	14.1	21.6	15.6	15.6
	Mean temp. driest 1/10	24.1	33.9	N/A	37.0	17.8	18.2	4.9	8.7	27.8	3.2	19.5
	Precipitation driest month	13.3	27.0	N/A	31.8	15.8	13.5	27.7	22.4	21.3	10.4	20.4
	Precipitation seasonality	1.6	2.5	N/A	0.0	1.2	14.2	4.4	10.1	12.6	11.6	6.5
	Vege	0.2	8.9	N/A	18.3	5.2	8.7	1.3	0.7	1.8	10.6	6.2
	Soil	0.3	0.0	N/A	0.0	5.5	3.6	0.8	0.0	1.3	9.8	2.4

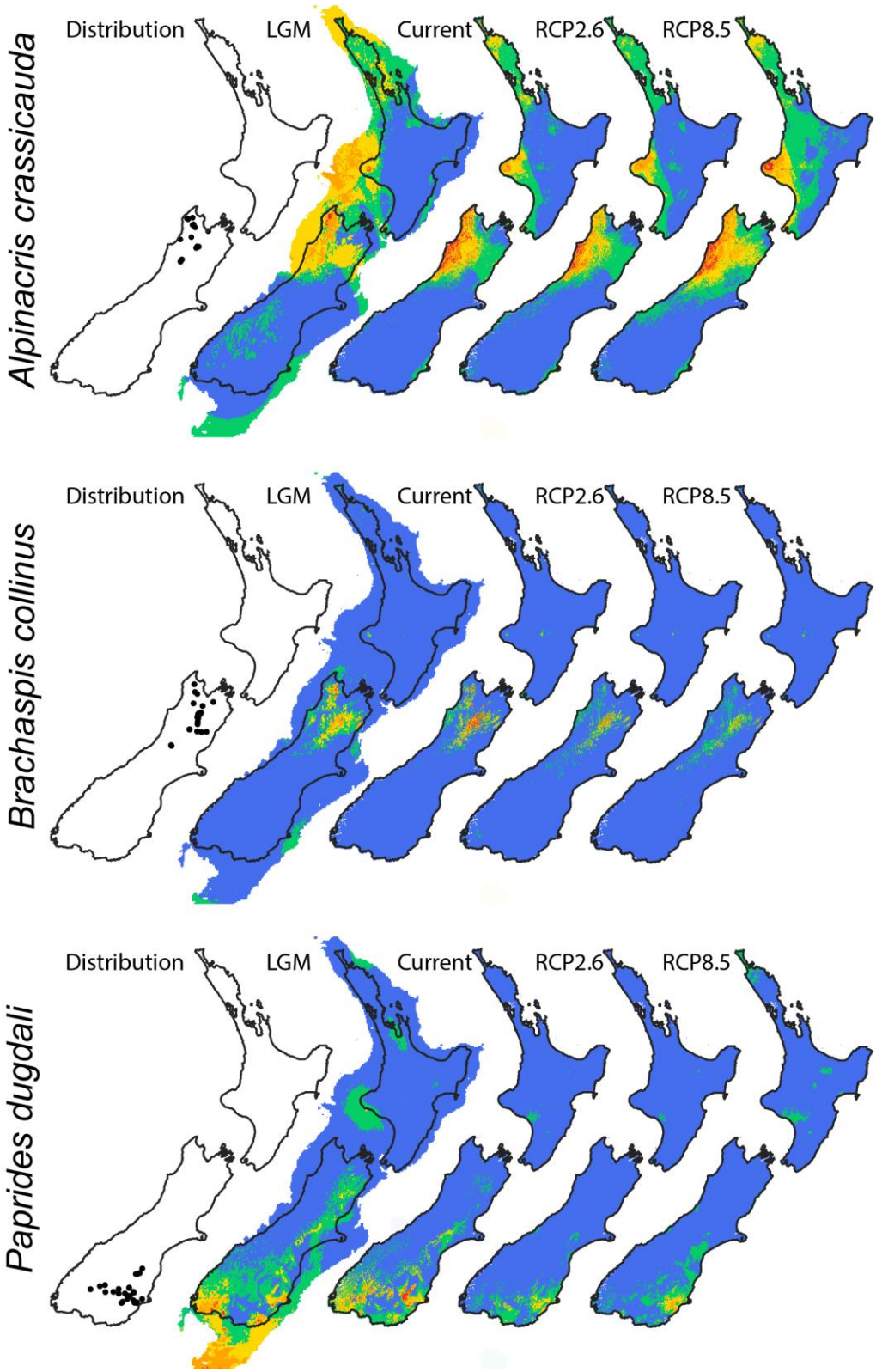
S. piliferus (MH)	Annual mean temp.	22.3	9.3	N/A	18.9	6.5	4.5	6.8	17.5	9.4	5.4	11.2
	Mean diurnal range	25.3	9.9	N/A	36.5	12.6	18.5	4.0	17.3	6.3	11.3	15.7
	Isothermality	1.6	0.3	N/A	0.0	2.2	5.9	0.0	0.0	2.6	1.4	1.6
	Mean temp. wettest 1/10	25.6	6.0	N/A	0.0	17.3	6.6	3.4	0.0	15.3	9.0	9.3
	Mean temp. driest 1/10	0.0	3.5	N/A	0.0	17.5	16.0	10.0	0.0	6.0	0.0	5.9
	Precipitation driest month	0.0	4.9	N/A	0.0	13.7	11.1	4.8	23.4	4.0	17.4	8.8
	Precipitation seasonality	25.3	64.5	N/A	44.6	17.8	18.0	68.9	41.4	48.2	38.3	40.8
	Vege	0.0	1.2	N/A	0.0	9.4	18.1	1.6	0.0	7.5	13.5	5.7
	Soil	0.0	0.5	N/A	0.0	3.0	1.3	0.5	0.3	0.7	3.8	1.1

S. villosus	Annual mean temp.	26.4	68.5	45.0	74.5	29.1	13.8	85.1	37.5	44.9	49.6	47.4
	Mean diurnal range	11.4	9.6	0.0	0.0	8.6	11.9	0.0	10.0	7.6	0.3	5.9
	Isothermality	0.8	0.0	0.0	0.0	0.4	14.8	0.0	1.3	7.3	2.4	2.7
	Mean temp. wettest 1/11	13.6	1.0	6.9	0.0	12.0	12.2	0.0	12.2	9.0	4.6	7.2
	Mean temp. driest 1/11	17.5	13.3	23.2	0.0	15.7	16.8	0.0	5.3	15.6	11.8	11.9
	Precipitation driest month	5.1	4.6	0.0	0.0	17.2	15.3	3.1	9.3	2.8	1.1	5.8
	Precipitation seasonality	20.0	1.3	17.5	25.5	4.1	6.7	11.8	22.7	8.6	23.5	14.2
	Vege	0.7	0.1	0.6	0.0	6.2	1.9	0.0	0.0	1.1	1.4	1.2
	Soil	4.5	1.6	6.8	0.0	6.7	6.5	0.0	1.8	3.1	5.3	3.6

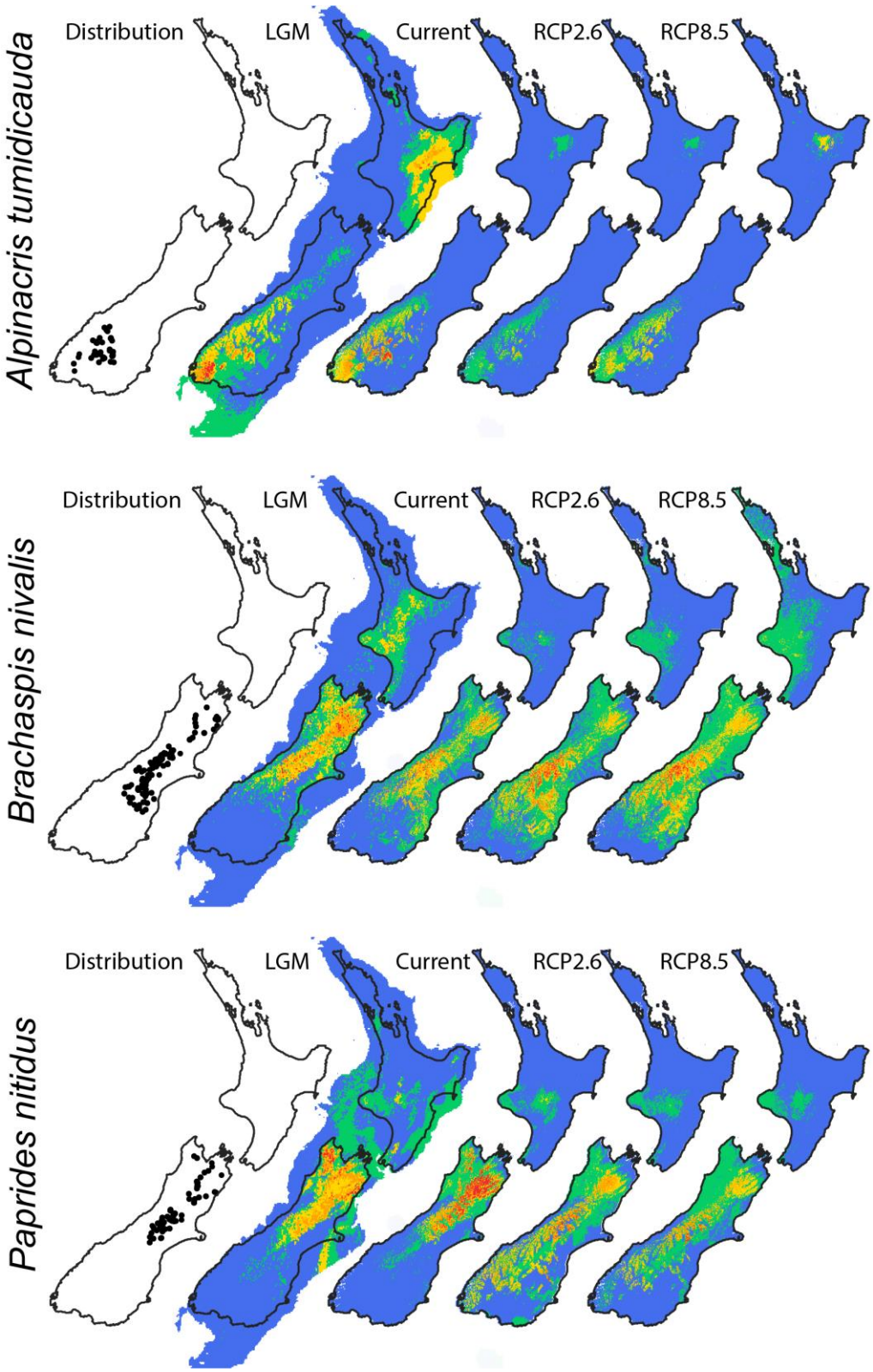
Supplementary Table 4.2 Predicted changes in suitable habitat for the two future scenarios when compared to area of suitable habitat available in current models.. Range changes with dispersal allowed includes “gained” pixels, whilst range change with no dispersal allowed does not.

Species	RCP Scenario	Lost	Never Occupied	Always Occupied	Gained	Current Range Size	Future Range Size No Dispersal	Future Range Size Full Dispersal	Range Change No Dispersal	Range Change With Dispersal
	RCP2.6	24118	370064	15453	7290	39571	15453	22743	-61	-43
A. crassicauda	RCP8.5	4247	290718	35324	86636	39571	35324	121960	-11	208
	RCP2.6	23654	384774	8419	78	32073	8419	8497	-74	-74
A. tumidicauda	RCP8.5	11936	384273	20137	579	32073	20137	20716	-37	-35
	RCP2.6	8951	407552	374	48	9325	374	422	-96	-95
B. collinus	RCP8.5	8261	405502	1064	2098	9325	1064	3162	-89	-66
	RCP2.6	36338	311140	37314	32133	73652	37314	69447	-49	-6
*B. nivalis (*All)	RCP8.5	22900	301521	50752	41752	73652	50752	92504	-31	26
	RCP2.6	48735	348840	5791	13559	54526	5791	19350	-89	-65
B. nivalis (SA)	RCP8.5	47677	36031	6849	2087	54526	6849	8936	-87	-84
	RCP2.6	1536	333398	47283	34708	48819	47283	81991	-3	68
B. nivalis (FP)	RCP8.5	2294	323429	46525	44677	48819	46525	91202	-5	87
	RCP2.6	26368	342111	16790	31656	43158	16790	48446	-61	12
P. dugdali	RCP8.5	14964	351320	28194	22447	43158	28194	50641	-35	17
	RCP2.6	24619	384713	5396	2197	30015	5396	7593	-82	-75
P. nitidus	RCP8.5	29056	386910	959	0	30015	959	959	-97	-97
	RCP2.6	29688	376981	10141	115	39829	10141	10256	-75	-74
S. australis	RCP8.5	37115	377095	2714	1	39829	2714	2715	-93	-93
	RCP2.6	69446	262105	84501	873	153947	84501	85374	-45	-45
S. campestris	RCP8.5	103564	262484	50383	494	153947	50383	50877	-67	-67
	RCP2.6	1589	414492	0	844	1589	0	844	-100	-47
S. childi	RCP8.5	1409	411483	180	3853	1589	180	4033	-89	154
	RCP2.6	6641	401673	1654	6957	8295	1654	8611	-80	4
S. minutus	RCP8.5	3682	389078	4613	19552	8295	4613	24165	-44	191
	RCP2.6	56070	254879	69183	36793	125253	69183	105976	-45	-15
S. piliferus (All)	RCP8.5	96065	268747	29188	22925	125253	29188	52113	-77	-58
	RCP2.6	39540	150924	125604	100857	165144	125604	226461	-24	37
S. piliferus (LW)	RCP8.5	120199	164916	44945	86865	165144	44945	131810	-73	-20
	RCP2.6	2268	367033	742	46882	3010	742	47624	-75	1482
S. piliferus (MH)	RCP8.5	424	385459	2586	28456	3010	2586	31042	-14	931
	RCP2.6	1221	399397	4855	11452	6076	4855	16307	-20	168
S. villosus	RCP8.5	5736	409782	340	1067	6076	340	1407	-94	-77

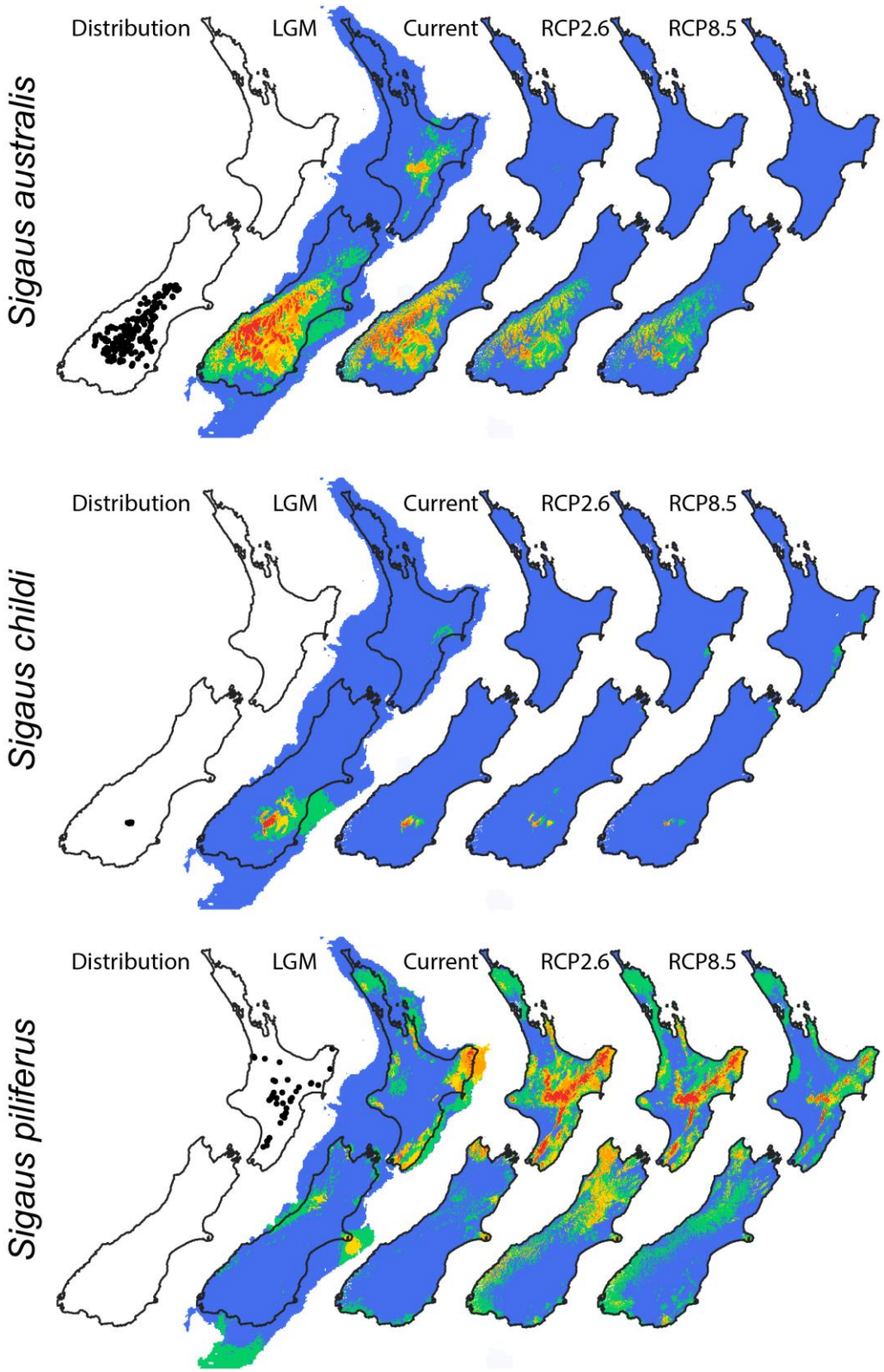
Supplementary Figure 4.1 The realised niche spaces of *Alpinacris crassicauda*, *Brachaspis collinus* and *Paprides dugdali* alongside their predicted potential niche spaces, generated through Ecological Niche Modelling. Ensemble Model Mean Weights models were used to generate these predictions. Maps from left to right: the first map indicates where each species is currently distributed and the presence data input into the model; predicted suitable niche space during the Last Glacial Maximum (LGM); predicted suitable niche space for current climate predictions; and the predicted suitable niche space for two future climate change scenarios based on RCP2.6 and RCP8.5 trajectories, respectively. Hot colours indicate a high probability of suitable habitat availability, cool colours indicate a low probability of suitable habitat availability.



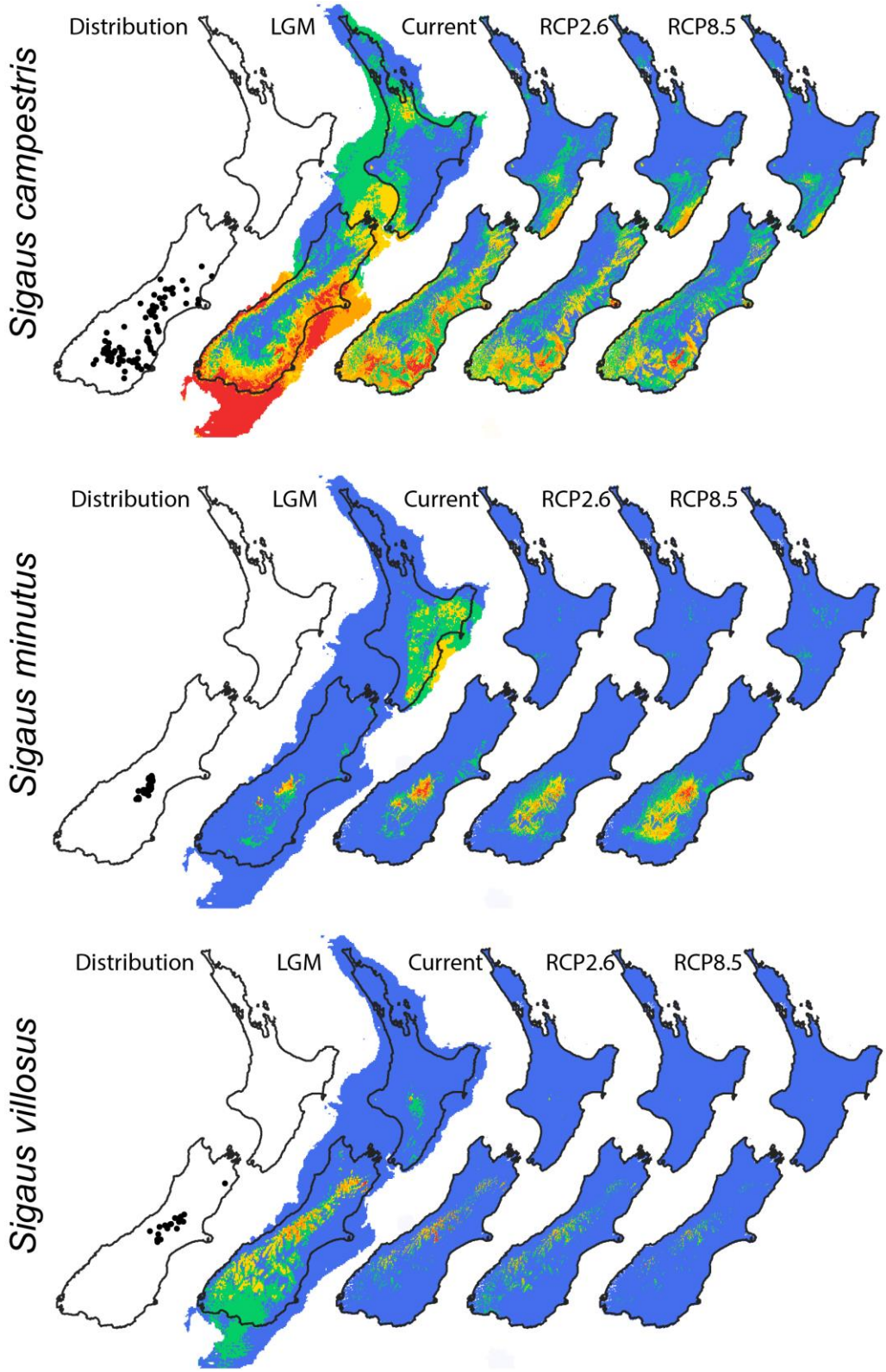
Supplementary Figure 4.2 The realised niche spaces of *Alpinacris tumidicauda*, *Brachaspis nivalis* (All) and *Paprides nitidus* alongside their predicted potential niche spaces, generated through Ecological Niche Modelling. Ensemble Model Mean Weights models were used to generate these predictions. Maps from left to right: the first map indicates where each species is currently distributed and the presence data input into the model; predicted suitable niche space during the Last Glacial Maximum (LGM); predicted suitable niche space for current climate predictions; and the predicted suitable niche space for two future climate change scenarios based on RCP2.6 and RCP8.5 trajectories, respectively. Hot colours indicate a high probability of suitable habitat availability, cool colours indicate a low probability of suitable habitat availability.



Supplementary Figure 4.3 The realised niche spaces of *Sigaus australis*, *Sigaus childi* and *Sigaus piliferus* (All) alongside their predicted potential niche spaces, generated through Ecological Niche Modelling. Ensemble Model Mean Weights models were used to generate these predictions. Maps from left to right: the first map indicates where each species is currently distributed and the presence data input into the model; predicted suitable niche space during the Last Glacial Maximum (LGM); predicted suitable niche space for current climate predictions; and the predicted suitable niche space for two future climate change scenarios based on RCP2.6 and RCP8.5 trajectories, respectively. Hot colours indicate a high probability of suitable habitat availability, cool colours indicate a low probability of suitable habitat availability.



Supplementary Figure 4.4 The realised niche spaces of *Sigaus campestris*, *Sigaus minutus* and *Sigaus villosus* alongside their predicted potential niche spaces, generated through Ecological Niche Modelling. Ensemble Model Mean Weights models were used to generate these predictions. Maps from left to right: the first map indicates where each species is currently distributed and the presence data input into the model; predicted suitable niche space during the Last Glacial Maximum (LGM); predicted suitable niche space for current climate predictions; and the predicted suitable niche space for two future climate change scenarios based on RCP2.6 and RCP8.5 trajectories, respectively. Hot colours indicate a high probability of suitable habitat availability, cool colours indicate a low probability of suitable habitat availability.



Rainbow Ski Field, St Arnaud Range, South Island

Chapter Five

Niche differentiation and population dynamics in the alpine zone; a case study of New Zealand's endemic alpine grasshoppers

Introduction

Biogeography is the study of species and ecosystem distributions and the processes behind how distributions change in space and through time. Since the 18th century, developments in biogeographical thinking have shaped our understanding of the biological world (comte de Buffon 1791, von Humboldt 1848). It was partly through observation and study of biogeographical patterns that Charles Darwin and Alfred Russell Wallace independently developed the theory of evolution by natural selection (Darwin 1859, Darwin & Wallace 1858) and Robert McArthur and Edward Osborne Wilson established the theory of island biogeography (MacArthur & Wilson 1967). A modern approach to biogeography is phylogeography, which makes explicit predictions about the distribution of lineage diversity (Avise *et al*. 1987, 2000).

Islands in the sky

When considering the climate warming of the Anthropocene, it is predicted that species' ranges will shift towards higher elevations and higher latitudes to match the environmental envelope they are suited (Galbreath *et al.* 2009, Walther *et al*. 2002). In addition, the lifting of habitat in elevation is predicted to increase habitat fragmentation, which could isolate previously connected species to 'sky islands' on the top of mountains, unless dispersal rates are high (Galbreath *et al.* 2009, Gifford & Kozak 2012, Walther *et al*. 2002). Given the smaller area of suitable land available, these fragmented populations will tend to have smaller population sizes, leading to reduced

genetic diversity than the former, more continuous, population they came from (Avise 2000, Charlesworth & Charlesworth 1987, Excoffier *et al*. 2009). Small isolated populations further lose genetic diversity through genetic drift and increasing homozygosity that can ultimately lead to inbreeding depression (Charlesworth & Charlesworth 1987, Excoffier *et al*. 2009, Frankham 2003, Frankham *et al*. 2002). Isolated populations can be at a higher risk of extinction due to this reduction in genetic diversity and their subsequently diminished adaptive capability (Frankham 1997, 2005, Frankham *et al*. 2002, Newman & Pilson 1997). Isolation, however, can be a factor in lineage diversification. If isolation of a population does not result in extinction, it may enable ecological adaptation to local conditions (Knowles 2000, Pepper *et al*. 2011, Shepard & Burbrink 2008, Smith & Farrell 2005, Whitaker *et al*. 2003).

To predict how species will respond to anthropogenic climate change, we can look at their current genetic diversity and its distribution across a landscape; this can reveal how species/populations may have responded to previous climatic events (Galbreath *et al.* 2009, Gifford & Kozak 2012, Hewitt 2004). For example, high genetic diversity exhibited by species with a confined range could be used to infer range contraction; whereas populations that have a wide range and relatively low genetic diversity can indicate recent range expansion (Hewitt 1999, 2004; Sivyer *et al.* 2018, Trewick *et al.* 2011). For alpine species, past glacial cycling would have connected and isolated populations repeatedly by expanding and contracting populations up an elevation gradient (Galbreath *et al.* 2009, Gifford & Kozak 2012, Hewitt 2004). Furthermore, populations that have regularly reconnected, allowing gene flow to occur, will have shared haplotypes and greater genetic diversity. Whilst populations that have remained isolated, hampering gene flow, will likely have more genetic structure as populations accumulate their own unique haplotypes with time (Gugger *et al.* 2010, Hewitt 2004, Knowles *et al*. 2007, Trewick *et al.* 2000, Trewick & Morris 2008, Winkworth *et al*. 2005). Knowing how populations of New Zealand alpine species have responded in the past as their habitat has expanded and contracted, will help us predict how they will respond to future climate warming.

New Zealand's alpine Acrididae

New Zealand has a diverse fauna of endemic alpine grasshoppers (Bigelow 1967). Ten of thirteen total species are adapted to living above the tree line in the alpine zone. The lineages of these endemic species have been in New Zealand for ~17–19My (Chapter Three); and were well established prior to the glacial cycling of the Pliocene and Pleistocene. Ecological Niche Modelling (ENM) predicts that the majority of these Acridids will lose substantial areas of suitable environmental niche space with future climate change (Chapter Four). But ENMs do not consider the genetic diversity, and therefore adaptability of the populations at hand. Past range expansion during the last glacial cycle might leave a signature in the pattern of genetic variation among populations, and more recent shifts in distribution since the LGM would lead to low diversity at leading edges (Excoffier *et al*. 2009). Thus, an examination of genetic diversity of species within this group could aid our understanding of how to apply niche models (Forester 2013, Gugger *et al.* 2010, Jakob *et al.* 2007, Knowles *et al*. 2007, Morales *et al*. 2015). Such an integrative approach could reveal which populations may be particularly vulnerable with future climate change (Forester 2013, Gugger *et al.* 2010, Morales *et al*. 2015).

Ecological partitioning

Many New Zealand endemic grasshopper species are sympatric with one another, with as many as five species being known to occupy Fox Peak along the Sherwood Range (Bigelow 1967)(Appendix One). Little is known about the interaction of these species in sympatry, or how their niches are partitioned at a finer scale. By understanding what restricts species when they occur in sympatry (e.g. substrate type, elevation, vegetation), it may be possible to understand what restricts a species' range, and thus its genetic diversity (Avise 2000). The alpine zone is a complex ecosystem to inhabit, the climate is extreme and changeable, and slope provides sharp changes in vegetation, geology and climate. Future climate change will likely see many of these alpine specialists shift their ranges to higher elevations (Sala *et al*. 2000, Walther *et al*. 2002), but an increase in elevation may also reduce available habitat; competition between alpine species might therefore be heightened. For New Zealand's endemic grasshoppers to survive in these conditions and to adapt rapidly to change, their best prospect will be to come from

populations that are genetically diverse (Ehlers *et al*. 2008, Pauls *et al*. 2013, Reusch *et al*. 2005).

Predictions for population genetics

The population genetics of three endemic grasshopper species (*Brachaspis nivalis*, *Paprides nitidus* and *Sigaus australis*) will be investigated, and predictions about the population dynamics of these species will be made. All three species have large current ranges, extending over half the length of the South Island; and they are predicted to have large population sizes and overall high genetic diversity (Avise 2000, Charlesworth & Charlesworth 1987, Excoffier *et al*. 2009). All three species are predominantly found inhabiting the alpine zone, and their alpine habitat has contracted in size since the LGM (Chapter Four) (Galbreath *et al.* 2009, Walther *et al*. 2002). It is predicted that their ranges have shrunk in the last ~15,000 years, along with their populations, and that populations will be less connected in lowland habitats than they used to be (Clark *et al*. 2009, Galbreath *et al.* 2009, Gifford & Kozak 2012, Hewitt 2004). Populations that have been isolated to remaining alpine refugia in the mountains for ~15,000 years are expected to have population structure tightly constrained to the mountain that each population inhabits, especially if there has been reduced or no gene flow between mountain populations. If there has been any gene flow between populations, we can predict that it will most likely have occurred between neighbouring mountains. As all species have been subject to the same climatic pressures, we could expect that each species may demonstrate similar patterns in population structure across the four mountains they inhabit. Furthermore, by examining populations along a latitudinal gradient, we may be able to determine if there has been a shift by any species towards predicted higher latitudes (Galbreath *et al.* 2009, Walther *et al*. 2002).

Aims

This study will investigate how representatives of New Zealand's endemic alpine grasshoppers are partitioned when they occur in sympatry. Populations of these species were sampled from five mountains along the length of the South Island of New Zealand. Elevation transects and habitat information of sample sites will be used to model how species are ecologically partitioned. In equilibrium, larger populations have more genetic diversity than smaller populations (Charlesworth 2009). Thus comparing

genetic diversity of population samples of sympatric species can be used to infer relative population size. Changes in population size can also be inferred from patterns of genetic diversity (Excoffier *et al.* 2009) so I will look for evidence of recent expansion (population size and range). To do this, mitochondrial sequence data was gathered from three co-distributed species: *Brachaspis nivalis*, *Paprides nitidus* and *Sigaus australis* sampled across the same five mountains, and used to estimate pairwise site diversity per sample and infer population genetic structure. Consequent comparison with prior Ecological Niche Models can indicate population dynamics of these species since the LGM and help us to predict the future of these species in light of future climate change.

Materials and Methods

Quantifying extent of sympatry

To quantify the extent of sympatry between New Zealand's endemic alpine grasshoppers, location data was collected for 12 endemic grasshopper species: *Alpinacris crassicauda*, *Alpinacris tumidicauda*, *Brachaspis collinus*, *Brachaspis nivalis*, *Paprides dugdali*, *Paprides nitidus*, *Sigaus australis*, *Sigaus campestris*, *Sigaus childi*, *Sigaus minutus*, *Sigaus piliferus* and *Sigaus villosus*, from the literature, Crown Pastoral Lease Tenure Reviews (CPLTR) and from the Phoenix Lab Group collection at Massey University (Appendix One). Minimum convex hull polygons were generated from the location data of each species in QGIS v 2.16.1 (QGIS Development Team 2017), and clipped to the extent of the New Zealand land surface. Polygons of species were overlaid with all other species in turn, and the area of their intersect was estimated in R v1.1.383 (R Core Team 2013) using the 'raster' v 2.6-7 (Hijmans 2017) and 'rgdal' v 1.2-18 (Bivand *et al*. 2014) packages.

Field sampling and model development

In February 2015 and March 2016, field sampling was carried out in the South Island of New Zealand. Five mountains were targeted: St Arnaud Range, Mt Olympus, Mt Hutt, Fox Peak and Cardrona (Figure 5.1). Sampling on each mountain took place along a transect divided into 100 meters above sea level (m.a.s.l.) intervals. Transect lengths varied by mountain, and extended as far as the terrain allowed. Where possible, sampling extended from 1100 to 2000m.a.s.l. At each elevation interval, three different habitat types were sampled: rock/scree, herb and tussock. Each habitat type was sampled twice at each interval. These habitat patches were only sampled if they exceeded $30m^2$, and we avoided sampling patches (of any habitat type) next to each other. Sampling at each patch consisted of walking around a pre-determined $15m^2$ area (leaving a 20m border between different habitat types), and disturbing the grasshoppers, which were then collected by hand. Grasshoppers of all species, sizes, sexes and colour were collected indiscriminately. No more than 20 individuals were collected at each patch, and sampling did not exceed 30min, regardless of whether 20 individuals had been captured. Habitat type, elevation, longitude and latitude, wind velocity and temperature at each site were recorded. Habitat variables of sites from which no

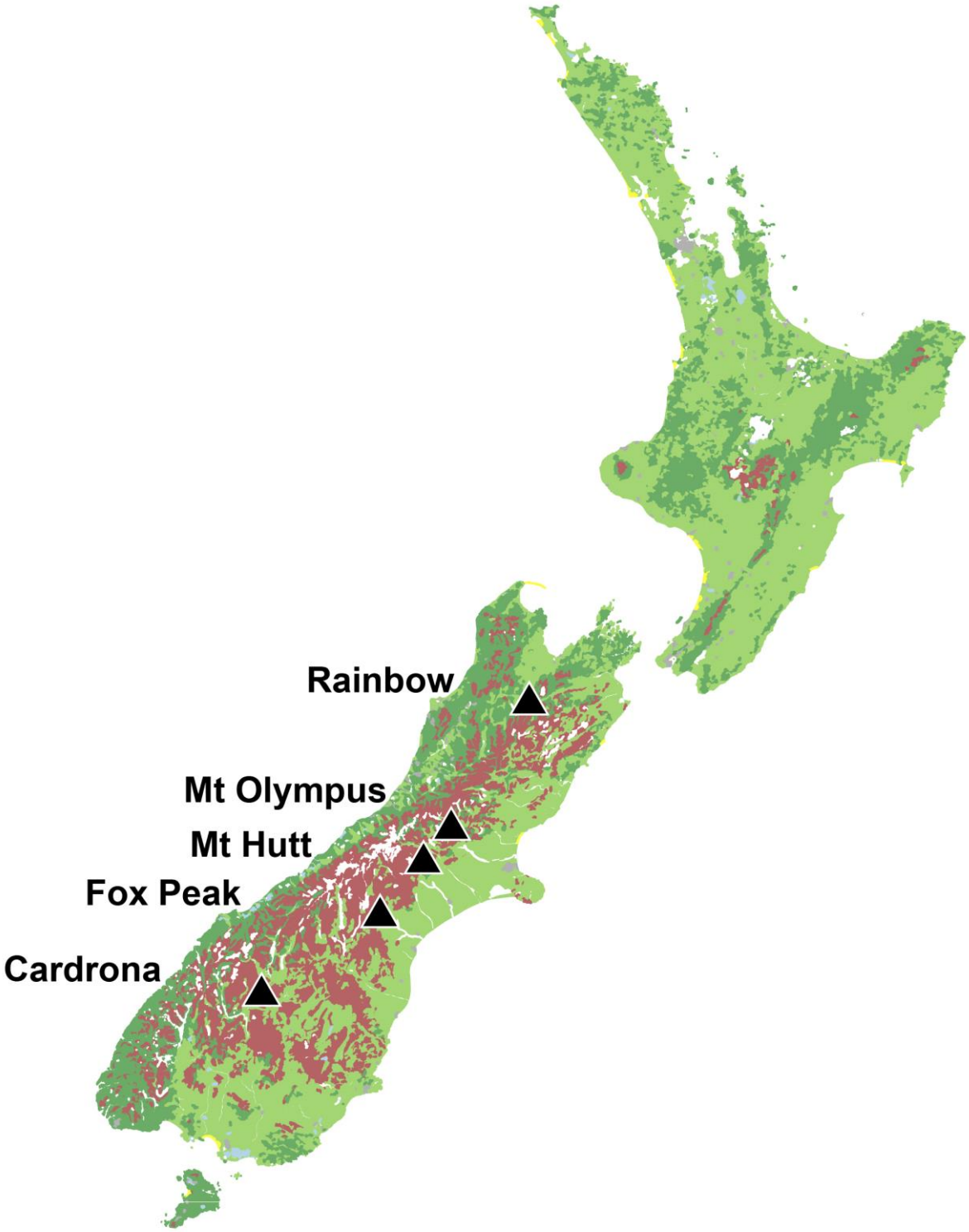


Figure 5.1 The five New Zealand mountains from which grasshoppers were sampled to examine use of microhabitats and genetic diversity.

grasshoppers were collected were also recorded. Any mating pairs collected were treated as two individuals. Grasshoppers were frozen before being preserved in 100% ethanol.

In the laboratory, each individual was sexed, and identified to species using Bigelow (1967). Using the variables recorded at each site, a Poisson General Linear Model (GLM) was developed using the R package 'MASS' v7.3-49 (Venables & Ripley 2002), using count data of each species, at each site, as the response variable. This was in order to determine if ecological partitioning could be detected among sympatric species sampled across the five mountains.

Taxon sampling

From the field analysis, three species of alpine grasshopper were chosen for the population genetics analysis: *Brachaspis nivalis*, *Paprides nitidus* and *Sigaus australis*. All three species are predominantly found inhabiting the alpine zone, have large distributions extending to over half the length of the South Island, and occur at four of the five mountains sampled in the field analysis. *Brachaspis nivalis* and *P. nitidus* were sampled from Rainbow, Mt Olympus, Mt Hutt and Fox Peak, whilst *S. australis* was sampled from Mt Olympus, Mt Hutt, Fox Peak and Cardrona. Representatives of each species, from each mountain, were chosen for DNA extraction (Table 5.1). *Sigaus villosus* individuals, also collected in the field, were chosen as a suitable outgroup for the phylogenetic analysis (Chapter Two).

DNA extraction, quantification and sequencing

The middle femora on the right side of each specimen were removed. A sterile scalpel blade was used to finely dice up the leg and its muscle tissue. Whole genomic DNA was extracted from this leg tissue using the solvent-free Proteinase K and salting-out method (Sunnucks & Hales 1996). Leg tissue was incubated at 55^{o}C overnight in a 1.5mL microfuge tube containing 600µL of TNES (2.5ml 1M Tris, 4ml 5M NaCl, 2ml 500mM EDTA and 5 SDS) and 10µL of Proteinase K. Proteins were precipitated from this mix using 170µL of 5M NaCl. The supernatant was transferred to a fresh sample tube and

Table 5.1 Grasshoppers used to investigate population genetics structure, sampled from five South Island New Zealand mountains. * indicates gene was successfully extracted and used in the study, F = female, M = male.

Species	Location	Specimen code	Sex	NAD2	COI	NAD2 & COI concatenated
Brachaspis nivalis	**Fox Peak**	GH1861	F	*	*	*
		GH1863	M	*	*	*
		GH1868	M	*	*	*
		GH1870	F	*		
		GH1874	M	*		
		GH1875	F	*		
		GH1978	F	*		
		GH1994	F	*	*	*
		GH1997	F	*	*	*
		GH2003	M	*	*	*
		GH2005	M	*	*	*
		GH2006	M	*	*	*
		GH2007	M	*	*	*
		GH2008	M	*		
		GH2016	F	*	*	*
		GH2017	F	*	*	*
	Mt Hutt	GH2180	F	*	*	*
		GH2182	F	*	*	*
		GH2183	M	*		
		GH2184	F	*	*	*
		GH2185	F	*	*	*
		GH2186	M	*	*	*
		GH2187	M	*		
		GH2188	M	*		
		GH2225	F	*		
		GH2231	F	*	*	*
		GH2234	F	*		
		GH2174	F			
		GH2175	M		*	
	Mt Olympus	GH0964	M	*		
		GH0972	M	*	*	*
		GH0973	M	*		
		GH1000	M	*	*	*
		GH1003	M	*	*	*
		GH1004	M	*	*	*
		GH1022	M	*	*	*
		GH1024	M	*	*	*
		GH1025	M	*	*	*
		GH1027	M	*		
	Rainbow	GH2341	F	*	*	*
		GH2381	M	*	*	*
		GH2386	M	*	*	*
		GH2398	F	*	*	*

		GH2401	F	*	*	*
		GH2402	M	*	*	*
Paprides nitidus	**Fox Peak**	GH1111	M	*	*	*
		GH1113	F	*	*	*
		GH1114	M	*	*	*
		GH1366	M	*		
		GH1367	F	*		
		GH1895	F	*	*	*
		GH1904	M	*		
		GH1908	M	*	*	*
		GH1911	F	*	*	*
		GH1917	M	*	*	*
		GH1918	M	*	*	*
		GH2022	F	*	*	*
		GH2023	M	*	*	*
		GH2027	F	*	*	*
		GH2028	M	*	*	*
		GH2031	F	*	*	*
		GH2079	F	*	*	*
	Mt Hutt	GH1394	M	*	*	*
		GH1395	M	*	*	*
		GH1397	F	*	*	*
		GH1398	M	*	*	*
		GH2165	F	*	*	*
		GH2168	F	*	*	*
		GH2196	F	*	*	*
		GH2197	M	*	*	*
		GH2198	M	*	*	*
		GH2200	F	*	*	*
		GH2203	M	*	*	*
		GH2204	F	*	*	*
		GH2205	M	*	*	*
		GH2208	M	*	*	*
		GH2224	F	*	*	*
		GH2275	M	*	*	*
		GH2284	M	*		
		GH2285	F	*	*	*
	Mt Olympus	GH0940	F	*		
		GH0941	F	*		
		GH0942	M	*	*	*
		GH0943	M	*	*	*
		GH0944	M	*		
		GH0946	F	*	*	*
		GH0948	F	*		
		GH0955	M	*	*	*
		GH0959	M	*		
		GH0960	M	*		
		GH0962	M	*		
		GH0997	F	*	*	*

		GH0998	M	*	*	*
		GH1001	F	*	*	*
		GH1007	F	*		
		GH1008	M	*	*	*
		GH1013	F	*	*	*
		GH1014	F	*	*	*
		GH1020	M	*	*	*
		GH1023	M	*		
		GH1031	M	*	*	*
	Rainbow	GH2411	M	*	*	*
		GH2415	M	*	*	*
		GH2428	M	*	*	*
		GH2444	F	*	*	*
		GH2445	F	*	*	*
		GH2468	F	*	*	*
		GH2469	F	*		
		GH2470	M	*		
		GH2471	M	*	*	*
		GH2559	F	*		
		GH2560	F	*		
		GH2561	F	*	*	*
		GH2562	F	*	*	*
		GH2565	M	*	*	*
		GH2569	F	*	*	*
		GH2584	M	*		
		GH2588	M	*		
		GH2589	M	*		
		GH2599	M	*		
Sigaus australis	**Cardrona**	GH1118	F	*	*	*
		GH1119	F	*	*	*
		GH1120	F	*	*	*
		GH1122	F	*	*	*
		GH1127	M	*	*	*
		GH1128	M	*	*	*
		GH1130	M	*		
		GH1132	M	*	*	*
		GH1133	M	*	*	*
		GH1303	M	*	*	*
		GH1306	M	*	*	*
		GH1316	M	*	*	*
		GH1317	F	*		
		GH1318	F	*	*	*
		GH1321	F	*	*	*
		GH1322	F	*	*	*
		GH1323	F	*	*	*
		GH1325	F	*	*	*
		GH1329	M	*	*	*
		GH1121	F		*	
	Fox Peak	GH1112	F	*	*	*

		GH1361	M	*		
		GH1894	F	*	*	*
		GH1898	M	*		
		GH1901	M	*	*	*
		GH1902	M	*		
		GH1914	F	*	*	*
		GH1923	F	*	*	*
		GH1924	F	*	*	*
		GH1925	F	*	*	*
		GH1980	M	*		
		GH1984	M	*		
		GH1990	M	*		
		GH1991	M	*	*	*
		GH1993	F	*	*	*
		GH1996	F	*	*	*
		GH1998	F	*	*	*
		GH1999	F	*	*	*
		GH2000	F	*	*	*
	Mt Hutt	GH1386	F	*	*	*
		GH1387	M	*	*	*
		GH1399	F	*	*	*
		GH2169	M	*	*	*
		GH2189	F	*	*	*
		GH2190	M	*	*	*
		GH2199	F	*	*	*
		GH2201	M	*	*	*
		GH2202	F	*	*	*
		GH2206	M	*	*	*
		GH2226	M	*	*	*
		GH2228	F	*	*	*
		GH2256	F	*	*	*
		GH2264	F	*		
		GH2280	M	*	*	*
	Mt Olympus	GH0947	F	*	*	*
		GH0954	M	*	*	*
		GH0967	F	*		
		GH0968	F	*	*	*
		GH1010	M	*	*	*
		GH1011	M	*	*	*
		GH1012	M	*	*	*
		GH1015	M	*	*	*
		GH1016	M	*	*	*
		GH1019	F	*	*	*
		GH1021	M	*	*	*
		GH1029	M	*		
		GH1030	M	*	*	*
Sigaus villosus	**Fox Peak**	GH1374	F	*	*	*
		GH1950	F	*	*	*

780µL of ice cold 95 ethanol added. After gentle mixing, tubes were centrifuged for 30 minutes at 14000 rpm and the ethanol discarded. Samples were rinsed with 400µL of 70 ethanol which was discarded after spinning for a further 5 minutes at 14000rpm. Dry DNA was re-suspended in 50µL of Milli-Q water.

Polymerase chain reaction (PCR) primers HopND2_147F (5' TGACCAACAACTCTACAAAACTTCT '3) and HopND2_1286R (5' TCAATAATGATTCTAGACTGCAATTCT '3) were used to target the mitochondrial DNA (mtDNA) gene NADH dehydrogenase 2 (ND2); TL2-N-3014 (5' TCCAATGCACTAATCTGCCATATTA '3) (Simon *et al*. 1994) and Hopp_1490 (5' TTTCAACAAACCATAAGGACATTGG 3'), modified from LCO1490 (Folmer *et al*. 1994), were used to target the mtDNA gene cytochrome oxidase subunit 1 (COI). Primer development took place using the 'Primer 3' v 4.1.0 (Untergasser *et al*. 2012) in Geneious R9 v9.1.4 (Kearse *et al*. 2012). PCR was performed in 20µl volumes, containing 0.15µl of DreamTaq™ Hot Start DNA Polymerase (Thermo Fisher Scientific, Waltham, MA, USA), 0.125µl of 10x DreamTaq™ Green Buffer (Thermo Fisher Scientific, Waltham, MA, USA), 0.4µl of 2mM dNTPs, 0.5µl of each primer, and 1µl of genomic DNA. Thermocycling conditions were 95°C for 90sec, 94°C for 15sec, 52°C for 15sec and 72°C for 90sec, repeated 38 times. Amplification products were checked on 1% TAE agarose gels and sequenced using BigDye v3.1 chemistry and an ABI3730 analyzer.

Population genetic analysis

Sequences were edited and aligned in Geneious. Two alignments were generated for each species. One consisted of ND2 sequences, and the other consisted of concatenated ND2 and COI sequences (Table 5.1). Median-joining haplotype networks (Bandelt *et al*. 1999) were generated for each species in PopART (Leigh & Bryant 2015), using the ND2 alignments. DnaSP v5.10 (Librado & Rozas 2009) was used to calculate the haplotype diversity (*h*), nucleotide site diversity (π) and the average number of nucleotide differences (*k*) of the four populations within each of the three species. Additionally, Ramos-Onsins and Rozas R_2 (Ramos-Onsins and Rozas 2002), mismatch distributions (Rogers & Harpending 1992) and Harpending's ruggedness index (Harpending *et al*. 1993) were also generated for each population of each species using

Arlequin v 3.5.22 (Excoffier & Lischer 2010). To investigate phylogenetic relationships of the populations within each species, alignments of the concatenated ND2 and COI genes were analysed using Bayesian Inference (BI) (MrBayes v 3.2.2 (Ronquist & Huelsenbeck 2014)) in Geneious. BI analyses were run using a GTR substitution model combined with the proportion of invariable sites model Invgamma; models were run for five million generations with four MCMC chains in each, and a burnin of 500,000 generations.

Results

Quantifying extent of sympatry

Minimum convex hulls (MCH) were generated for each of the 12 grasshopper species and used to generate estimates of range area. Inferred areas differed by more than an order of magnitude from 125.6 km^2 (*S. childi*) to 76,179.9km^2 (*S. campestris*) (Table 5.2). *Sigaus piliferus* was the only species not in sympatry with any other alpine grasshopper species, as it is the sole representative in the North Island of New Zealand. Otherwise, the minimum convex hulls of each species overlapped with at least three other species to varying extents. Predictably, species with the largest ranges: *B. nivalis*, *S. australis* and *S. campestris*, had ranges (minimum convex hulls) that overlaped with the most other species. *Brachaspis nivalis* overlaped with eight species, and *S. australis* and *S. campestris* each overlaped with nine. For four species, the entirety of their minimum convex hulls was enveloped in that of another species. For example, 100% of *P. nitidus*' range was enveloped by *B. collinus*, 100% of *S. childi's* range was enveloped by *P. dugdali*, *S. australis* and *S. campestris*, 100% of *S. minutus'* range was enveloped by the ranges of *S. australis*, and 100% of *S. villosus'* range was enveloped by *B. nivalis.* By overlaying all minimum convex hulls of all species onto a map of New Zealand, it was determined that central Otago in the lower half of the South Island has the highest species richness, with a total of seven species at a latitude of -45°S (Figure 5.2).

Field sampling

In total, 1222 grasshoppers (782 adults and 440 juveniles) were collected from 90 samping sites across the five focal mountains, representing eight different species (Table 5.3) (Supplementary Table 5.1). At three mountains (Cardrona, Fox Peak, Rainbow), all species that were expected to be present were collected, whilst at two mountains (Mt Hutt, Mt Olympus), two species that were expected to be present (*S. campestris* and *S. villosus*) were not collected. This is likely due to not sampling a wide enough elevational range on these two mountains (Supplementary Figure 5.1). On two mountains (Fox Peak, Rainbow), at 14% of sites sampled there were no grasshoppers present (Table 5.4). On Cardrona, at most sites only one species was present, but at 27% of sample sites, two species were

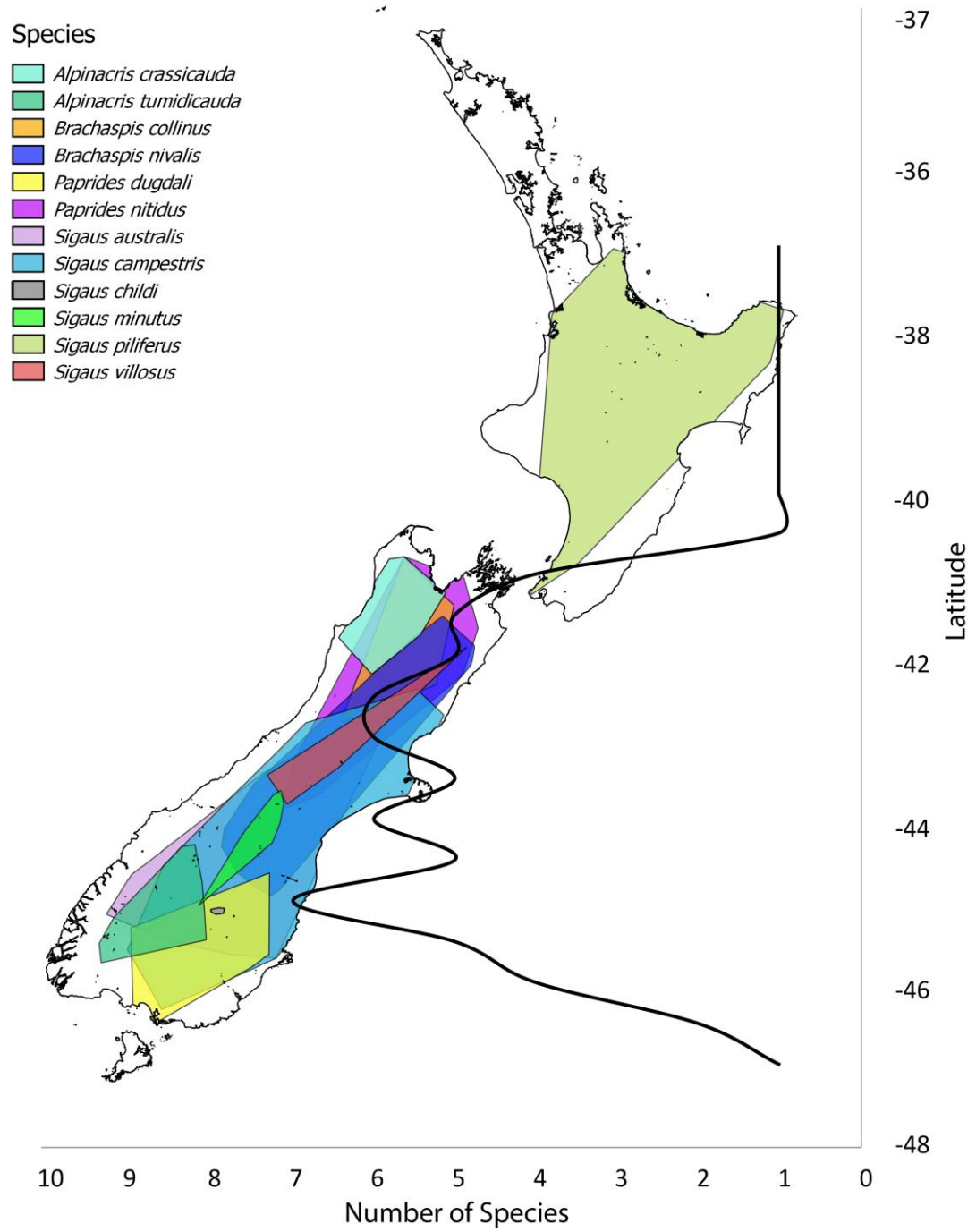


Figure 5.2 Minimum convex hull polygons of 12 endemic New Zealand alpine grasshoppers, with number of species found by latitude.

Table 5.2 The percentage of minimum convex hull overlap between each of the twelve species. The bottom matrix is the percentage of overlap experienced by the minimum convex hull of species in the left column with the minimum convex hull of species along the top row. The top matrix is the opposite. Dark purple of the heat map indicates a high percentage of overlap, white indicates very little or no percentage of overlap between species.

	Total min. convex hull area (km^2)	*A. crassicauda*	*A. tumidicauda*	*B. collinus*	*B. nivalis*	*P. dugdali*	*P. nitidus*	*S. australis*	*S. campestris*	*S. childi*	*S. minutus*	*S. piliferus*	*S. villosus*
A. crassicauda	11,851.1		0	65	1	0	79	0	0	0	0	0	0
A. tumidicauda	13,393.2	0		0	0	36	0	75	73	0	0	0	0
B. collinus	16,998.7	45	0		49	0	100	0	6	0	0	0	7
B. nivalis	44,953.4	0	0	19		1	51	58	76	0	8	0	21
P. dugdali	23,532.5	0	20	0	2		0	50	91	1	0	0	0
P. nitidus	38,071.9	25	0	45	61	0		21	33	0	0	0	22
S. australis	56,706.1	0	18	0	46	21	14		93	0	8	0	12
S. campestris	76,179.9	0	13	1	45	28	17	69		0	6	0	11
S. childi	125.6	0	0	0	0	100	0	100	100		0	0	0
S. minutus	4,255.6	0	0	0	81	1	0	100	100	0		0	0
S. piliferus	70,542.7	0	0	0	0	0	0	0	0	0	0		0
S. villosus	9,242.4	0	0	13	100	0	90	77	90	0	0	0	

Table 5.3 Summary table of the grasshopper individuals collected in the field, tabulated by species and by mountain. The number of species expected to be present on each mountain was determined by looking at location records of species (Appendix One). The total column at the far right of the table is the sum of all adults and juveniles of all species collected on each mountain. The total row at the bottom of the table is the sum of all individuals of each species collected across all five mountains. – indicates that the species' range does not include that mountain, whereas 0 indicates that the species range does include that mountain but was not collected at that mountain.

Mountain	Number of individuals collected of each species								Species expected	Species collected	Adults	Nymphs	Total
	A. crassicauda	*A.tumidicauda*	*B. collinus*	*B. nivalis*	*P. nitidus*	*S. australis*	*S. campestris*	*S. villosus*					
Cardrona	–	17	–	–	–	142	–	–	2	2	159	48	207
Fox Peak	–	–	–	47	26	124	3	7	5	5	207	214	421
Mt Hutt	–	–	–	40	77	18	0	0	5	3	135	64	199
Mt Olympus	–	–	–	13	42	32	0	0	5	3	87	3	90
Rainbow	26	–	65	35	68	–	–	–	4	4	194	111	305
Total	26	17	65	135	212	317	3	7	8	8	782	440	1222

Table 5.4 A summary of the number of sites sampled at each mountain, and how many species were found occurring together across these sites. Where there are percentages in the 0 column, this indicates the percentage of instances where sites were sampled but there were no grasshoppers present.

Mountain	Number of species present	Number of sites sampled	Total number of individuals collected	Number of species occurring together as a percentage of total collected					
				0	1	2	3	4	5
Cardrona	2	11	207	0%	73%	27%	0%	0%	0%
Fox Peak	5	37	421	14%	46%	32%	8%	0%	0%
Mt Hutt	3	12	199	0%	33%	50%	17%	0%	0%
Mt Olympus	3	8	90	0%	0%	38%	63%	0%	0%
Rainbow	4	22	305	14%	36%	45%	5%	0%	0%

present. On Fox Peak only one species was found present at almost half (46%) of the sites sampled. However, at 32% of sites two species occurred together and at 8% of sites, three species were found together. On Mt Hutt, two species were found occupying the same habitat at 50% of sample sites, whilst at 17% of sites, three species were found inhabiting the same site. On Mt Olympus, 100% of sites had at least two species co-occurring, and at Rainbow Ski Area, 50% of sites were found to have at least two species occurring together. On each mountain species were collected at different frequencies, suggesting they occur at higher densities. *Sigaus australis* was the most commonly collected species on Mt Cardrona and Fox Peak, whilst *Paprides nitidus* was the most commonly collected species at the three other mountains (Table 5.3). However, the range of elevations sampled at each mountain needs to be taken into account when considering this, as some species may occur more commonly at higher or lower elevation than was sampled in this field study (Supplementary Figure 5.1). Grasshoppers across all five mountains were most commonly collected when there was no wind, at temperatures between 18-28°C, and at 1600m.a.s.l..

Ecological partitioning

Four variables recorded at each field site were used in a Poisson GLM: Species, Habitat, Elevation and Location; species counts (including zero counts) at each site were used as the response variable. Temperature and wind were omitted from the models, as their variation was dependent on the time of sampling. A model was achieved using a stepwise algorithm with model selection based on AIC. Species, Habitat, Elevation, and the interactions of Species:Elevation, Species:Habitat and Habitat:Elevation were all found to be significant predictors by the GLM, although investigation of the residual deviance suggests that the model could not fully explain the data (Table 5.5). The interaction of Habitat:Elevation was not able to be fitted for all combinations in the model, thus the simpler model without this interaction is preferred for presenting results for this study.

The model was able to predict the preferred elevation range and habitat type of each species (Table 5.6, 5.7). *Alpinacris crassicauda* was only sampled at 1700m.a.s.l., as this was where the model predicts it to be found, and most commonly in herb habitats.

Table 5.5 Summary statistics of final GLM.

	DF	Deviance	Residual DF	Residual Dev.	Pr(>Chi)
NULL			487	2161.18	
Species	7	645.92	480	1515.26	< 2.2e-16 ***
Alt	7	61.45	473	1453.81	7.740e-11 ***
Habitat	5	33.5	468	1420.31	2.993e-06 ***
Species:Habitat	35	521.28	433	899.02	< 2.2e-16 ***
Species:Alt	49	185.29	384	713.74	< 2.2e-16 ***
Alt:Habitat	11	49.43	373	664.31	7.925e-07 ***

Table 5.6 Proportions of expected values generated by a Poisson GLM model. High numbers (dark purple) indicate at what elevation a species is predicted to be found inhabiting based on field sampling data. Low numbers (white) indicate at what elevation species are least likely to be found. Expected values can be found in Supplementary Table 5.2.

Species	Elevation (masl)							
	1100	1200	1300	1400	1500	1600	1700	1800
A. crassicauda	0	0	0	0	0	0	1.00	0
A. tumidicauda	0.04	0.13	0	0	0.17	0.41	0.15	0.10
B. collinus	0	0.08	0	0.04	0.26	0.15	0.23	0.24
B. nivalis	0	0	0	0.18	0.04	0.12	0.30	0.35
P. nitidus	0	0.10	0.11	0.18	0.03	0.33	0.17	0.08
S. australis	0.17	0.09	0.09	0.04	0.11	0.18	0.13	0.18
S. campestris	0.15	0	0	0.85	0	0	0	0
S. villosus	0	0	0	0	0	0	0	0

Table 5.7 Proportions of expected values generated by a Poisson GLM model. High numbers (dark purple) indicate what habitat type a species is predicted to be found inhabiting based on field sampling data. Low numbers (white) indicate at what habitat type species are least likely to be found on. Expected values can be found in Supplementary Table 5.3.

Species	Habitat types					
	Grass	Herb	Herb/rock	Herb/tuss	Rock	Tussock
A. crassicauda	0.20	0.60	0.14	0.06	0	0
A. tumidicauda	0	0.80	0.20	0	0	0
B. collinus	0	0.08	0	0	0.92	0
B. nivalis	0	0.12	0	0	0.88	0
P. nitidus	0.09	0.13	0.70	0	0	0.08
S. australis	0.38	0.11	0.26	0.14	0.01	0.10
S. campestris	0.50	0	0.06	0.11	0	0.33
S. villosus	0	0	0	0	0	0

Alpinacris tumidicauda is also predicted to be most commonly found in herb habitats, but at 1600m.a.s.l.. *Brachaspis collinus* was predicted to be found in the 1500 – 1800m.a.s.l. range, and in rocky habitats. Rocky habitat is also predicted to be where *B. nivalis* is found most frequently, although they are predicted to be in the zone between 1700 and 1800m.a.s.l.. *Paprides nitidus* and *S. australis* are both predicted to inhabit a wide range of elevations, with *P. nitidus* preferring mixed herb/rock habitats, and *S. australis* preferring grassy and herb/rock mixed habitats. *Sigaus campestris* is predicted to be found in grassy habitats at 1400m.a.s.l.. Sampling of *S.villosus* was too low for statistically valid models.

Population genetics analysis

Brachaspis nivalis

The 747bp alignment of ND2 sequences from 44 *B. nivalis* individuals contained 29 unique mtDNA haplotypes and 127 variable nucleotides sites (Table 5.7). A median-joining haplotype network revealed that these haplotypes were specific to each of the mountain populations, and there was no sharing of haplotypes among the populations from these mountains (Figure 5.3). This was further confirmed by the BI phylogenetic investigation of 31 *B. nivalis* alignments of concatenated ND2 and COI sequences (1497bp); the four populations form four distinct clades, all supported with posterior probabilities of 1.0 (Figure 5.4). ϕst pairwise comparisons of genetic differences between all population samples were high and differed significantly from zero (Table 5.8). Mt Hutt and Mt Olympus populations were genetically most similar, whilst Fox Peak and Rainbow populations were the two most genetically dissimilar (Table 5.8). Haplotype diversity was also investigated (Table 5.7). Combined, the populations had relatively high genetic diversity ($\pi = 0.063$); *B. nivalis* had the highest genetic diversity across species. However, genetic diversity was lower among populations of *B. nivalis*. Of the four *B. nivalis* mountain populations sampled, Fox Peak had both the highest number of samples and haplotypes ($n = 16$, $N_{haps} = 10$), but Mt Olympus had the highest haplotype diversity ($h = 0.93$), followed by Mt Hutt ($h = 0.89$). Mt Hutt also had the highest nucleotide site diversity ($\pi = 0.011$).

Table 5.8 Descriptive statistics for each species and mountain populations within each species based on mitochondrial ND2 sequence data. Bp = alignment length, *n* = sample size, N_{haps} = number of haplotypes, S = number of polymorphic sites, *h* = haplotype diversity, *k* = number of nucleotide differences, π = nucleotide diversity, R_2 = Ramos-Onsins and Roza's R_2, HRI = Harpending's raggedness index.

Species	Alignment length (bp)	Population	*n*	N_{haps}	S	*h*	*k*	π	R_2	HRI
B. nivalis	747	Rainbow	6	3	3	0.6	1.0	0.0013	0.2546	0.0622
		Mt Olympus	10	8	21	0.93	4.82	0.0065	0.1685	0.0894
		Mt Hutt	12	8	27	0.89	8.14	0.011	0.1393	0.0521
		Fox Peak	16	10	15	0.87	2.89	0.0039	0.0887	0.0251
		Total	44	29	127	0.96	47.07	0.063	0.1823	0.011
P. nitidus	733	Rainbow	19	8	9	0.67	1.22	0.0017	0.0699	0.057
		Mt Olympus	21	10	42	0.9	20.04	0.027	0.2375	0.044
		Mt Hutt	19	13	17	0.95	3.36	0.0046	0.0835	0.063
		Fox Peak	17	7	8	0.84	1.51	0.002	0.1101	0.121
		Total	76	38	109	0.67	37.38	0.051	0.1702	0.007
S. australis	728	Mt Olympus	12	7	12	0.91	3.79	0.0052	0.1526	0.092
		Mt Hutt	13	4	3	0.72	0.97	0.0013	0.0955	0.061
		Fox Peak	19	8	8	0.83	1.73	0.0024	0.0918	0.062
		Cardrona	19	12	14	0.95	3.03	0.0041	0.1819	0.125
		Total	63	31	104	0.96	35.93	0.049	0.1691	0.010

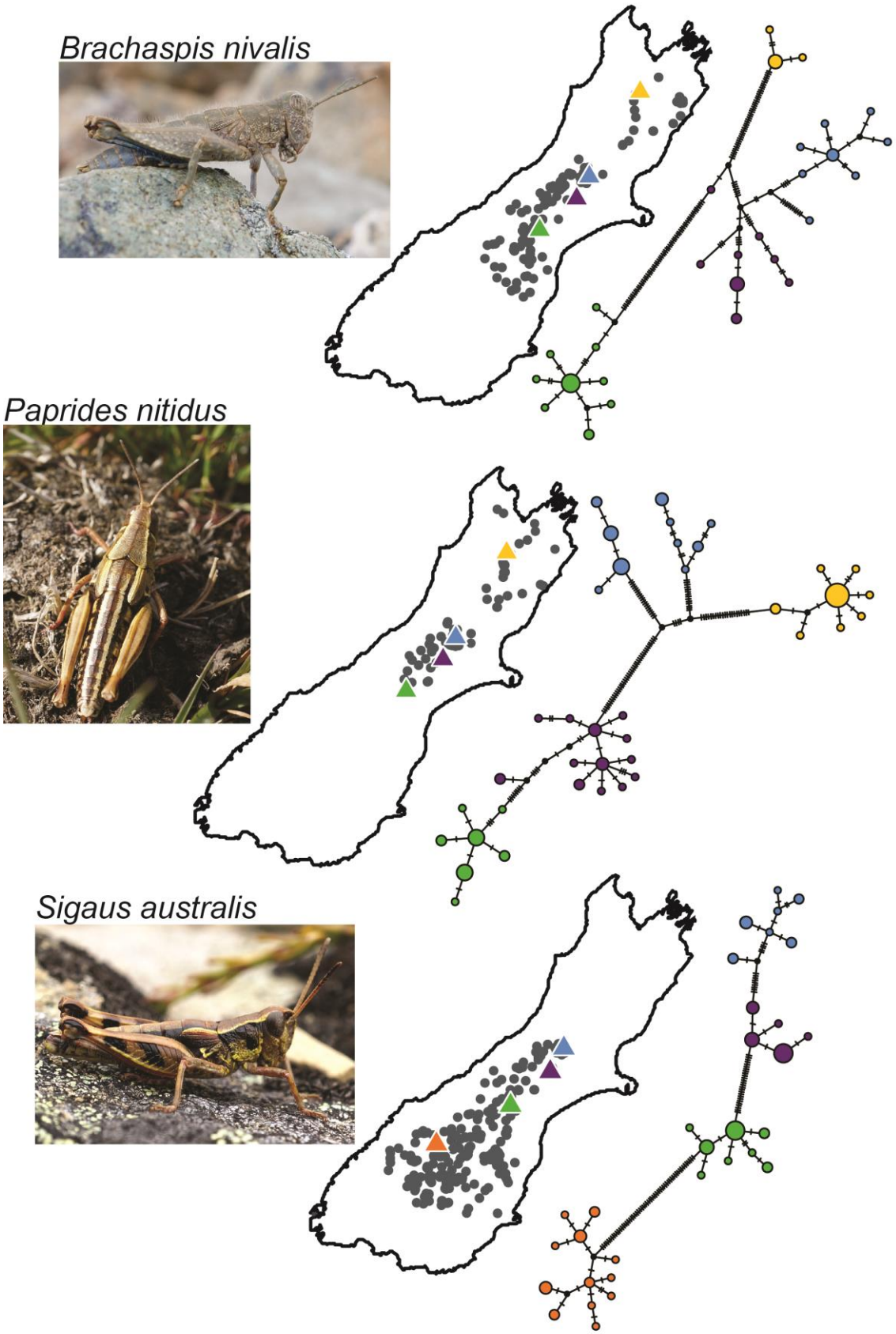


Figure 5.3 The distribution of mtDNA haplotype diversity of *Brachaspis nivalis*, *Paprides nitidus* and *Sigaus australis* across five sampling locations. Grey dots indicate known locations of each species. Colours of triangles on each map and on the network represent the four mountain populations individuals were sampled from. Yellow = Rainbow, blue = Mt Olympus, purple = Mt Hutt, green = Fox Peak and orange = Cardrona. Size of the network haplotypes is proportional to the number of individuals that share that haplotype.

Table 5.9 ϕst pairwise comparisons between mountain populations of *Brachaspis nivalis*, *Paprides nitidus* and *Sigaus australis*. All comparisons between populations of each species showed significant (*P*<0.00) departure from zero.

B. nivalis	**Mt Olympus**	**Fox Peak**	**Mt Hutt**	**Rainbow**
Mt Olympus	0.00000			
Fox Peak	0.95245	0.00000		
Mt Hutt	0.53434	0.93269	0.00000	
Rainbow	0.93527	0.97079	0.88971	0.00000
P. nitidus	**Mt Olympus**	**Fox Peak**	**Mt Hutt**	**Rainbow**
Mt Olympus	0.00000			
Fox Peak	0.81501	0.00000		
Mt Hutt	0.78809	0.82673	0.00000	
Rainbow	0.73999	0.97460	0.95265	0.00000
S. australis	**Mt Olympus**	**Fox Peak**	**Cardrona**	**Mt Hutt**
Mt Olympus	0.00000			
Fox Peak	0.92630	0.00000		
Cardrona	0.94927	0.96012	0.00000	
Mt Hutt	0.85835	0.94806	0.96564	0.00000

Paprides nitidus

The 733bp alignment of ND2 sequences from 76 *P. nitidus* individuals contained 76 unique mtDNA haplotypes and 109 variable nucleotides sites (Table 5.7). As with *B. nivalis*, a median-joining haplotype network revealed that these haplotypes formed clusters specific to each of the mountain populations, and that there was no sharing of haplotypes between the populations on these mountains (Figure 5.3). This was further confirmed by the BI phylogenetic investigation of 55 *P. nitidus* alignments of concatenated ND2 and COI sequences (1393bp); these four populations formed four distinct clades, supported with high posterior probabilities (Figure 5.4). ϕst pairwise estimates of genetic differences between these four populations were high and all differed significantly from zero (Table 5.8). Rainbow and Mt Olympus population samples of *P. nitidus* were the most genetically similar (as seen in *B. nivalis)*, Fox Peak and Rainbow populations were the two most genetically dissimilar (Table 5.8). Similarly to *B. nivalis*, overall genetic diversity was relatively high when all populations were combined (π = 0. 051) (Table 5.7). Of the four *P. nitidus* mountain populations samples, Mt Olympus had the highest number of sample (*n = 21*), but Mt Hutt had the highest number of haplotypes (N_{haps} = 13). Mt Hutt and Mt Olympus both had high haplotype diversity *h* = 0.95 and *h* = 0.9, respectively and Mt Olympus had the highest nucleotide site diversity (π = 0.027) of any of the grasshopper population samples.

Sigaus australis

The 728bp alignment of ND2 sequences from 66 *S. australis* individuals contained only 30 unique mtDNA haplotypes and 102 variable nucleotides sites (Table 5.7). As with *B. nivalis* and *P. nitidus*, a median-joining haplotype network revealed that these haplotypes were specific to each of the mountain populations, and there was no sharing of haplotypes between the populations on these mountains (Figure 5.3). The BI phylogenetic investigation of 55 *S. australis* concatenated ND2 and COI sequences (1393bp) demonstrates how these four populations are related, with nodes supported with high posterior probabilities (Figure 5.4). Genetic differences estimated with pairwise ϕst were high (all differed significantly from zero) (Table 5.8). Comparisons revealed that Mt Hutt and Mt Olympus populations were the most genetically similar, whilst Mt Hutt and Cardrona populations were the two most genetically dissimilar populations sampled for this species (Table 5.8). Despite ample variation, genetic

diversity for *S. australis* ($\pi = 0.049$) was the lowest of the three species examined. Of four *S. australis* mountain populations sampled, Fox Peak and Cardrona had the highest number of samples (*n = 19*), and Cardrona also had the highest number of haplotypes ($N_{haps} = 12$). Cardrona and Mt Olympus both had high haplotype diversity $h = 0.95$ and $h = 0.91$, respectively. Mt Olympus had the highest nucleotide site diversity ($\pi = 0.0052$). Although more *S. australis* individuals were collected in the field than any of the other species (n=317) (Table 5.3), combined population diversity, and all population sample estimates of π for *S. australis* were lower than other species from the same locations (Table 5.7).

Population demographics

Mismatch distributions of the three grasshopper species and their respective mountain populations were estimated based on mtDNA ND2 haplotype data, in order to infer past population size changes (Figure 5.5, 5.6). The mismatch distributions of *B. nivalis, P. nitidus* and *S. australis* were all multimodal, which is consistent with these species having large population sizes at demographic equilibrium (Rogers & Harpending 1992) (Figure 5.5). For *P. nitidus* this was statistically supported by non-significant sum of squared deviation (SSD) and Harpending's raggedness index (HRI) values, indicating there was no departure from the estimated demographic model (Rogers & Harpending 1992). SSD values were significant for both the *B. nivalis* and *S. australis* mismatch distribution analyses, and could be indicative of population substructuring, stable populations that have consequently expanded, mutation rate heterogeneity, or a recent bottleneck (Aris-Brosou & Excoffier 1996, Maltagliati *et al*. 2010, Marjoram & Donnelly 1994). The same analysis was repeated for each population within each species (Figure 5.6). Sample sizes were too small to draw major conclusions at a population level; however mismatch distributions across species and mountains do appear to indicate similar demographic signal. For example the populations of all species at Fox Peak have unimodal mismatch distributions, whereas at Mt Olympus, they all have multimodal mismatch distribution, and both *B. nivalis* and *P. nitidus* Rainbow populations have unimodal mismatch distributions. This suggests that these populations across species have undergone the same processes; genetic sampling of individuals across populations would further reveal if this were the case.

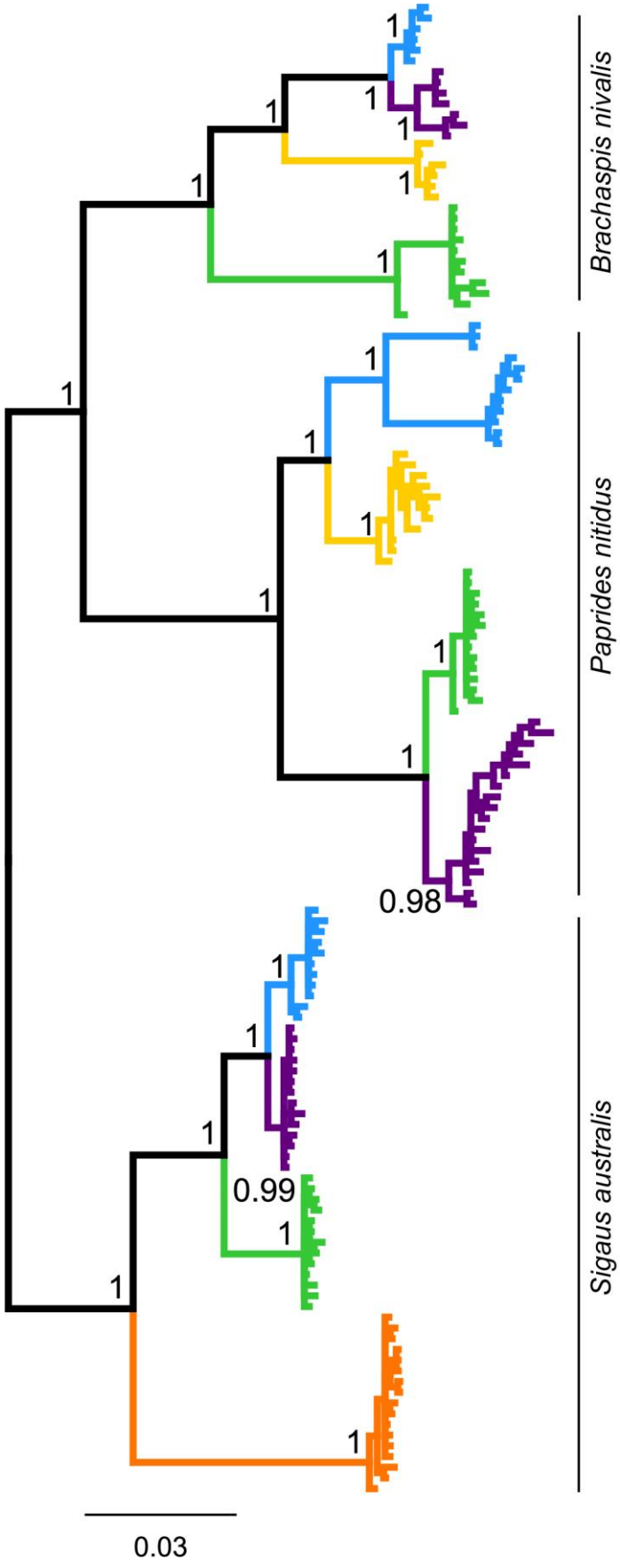


Figure 5.4 Phylogenetic hypothesis of populations within *Brachaspis nivalis*, *Paprides nitidus* and *Sigaus australis* based on a 1375bp alignment of concatenated mtDNA COI and ND2 sequences. Two *Sigaus villosus* sequences were enforced as the outgroup. Bayesian Inference posterior probabilities are displayed at the nodes. Blue = Mt Olympus, purple = Mt Hutt, green = Fox Peak,yellow = Rainbow, orange = Cardrona.

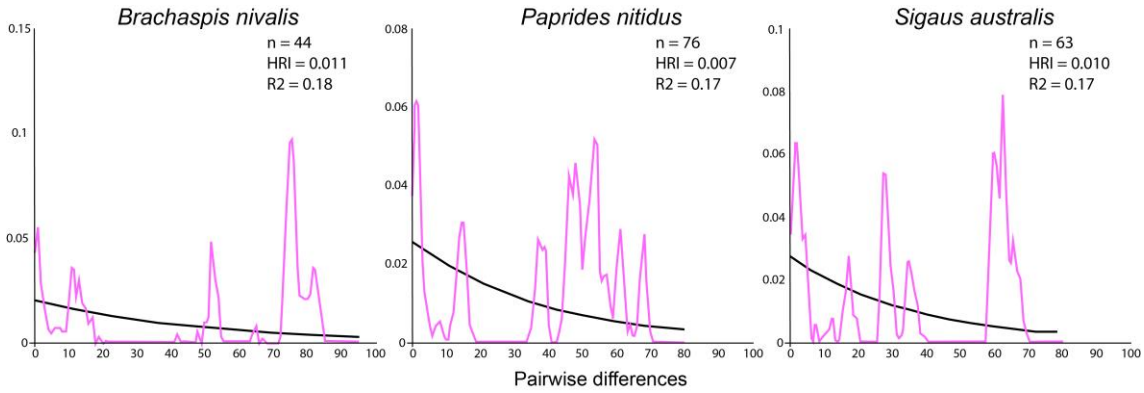


Figure 5.5 Mismatch distribution plots for *Brachaspis nivalis*, *Paprides nitidus* and *Sigaus australis,* all demonstrating multimodal distributions. n = sample size, HRI = Harpending's raggedness index, R_2 = Ramos-Onsins and Roza's R_2.

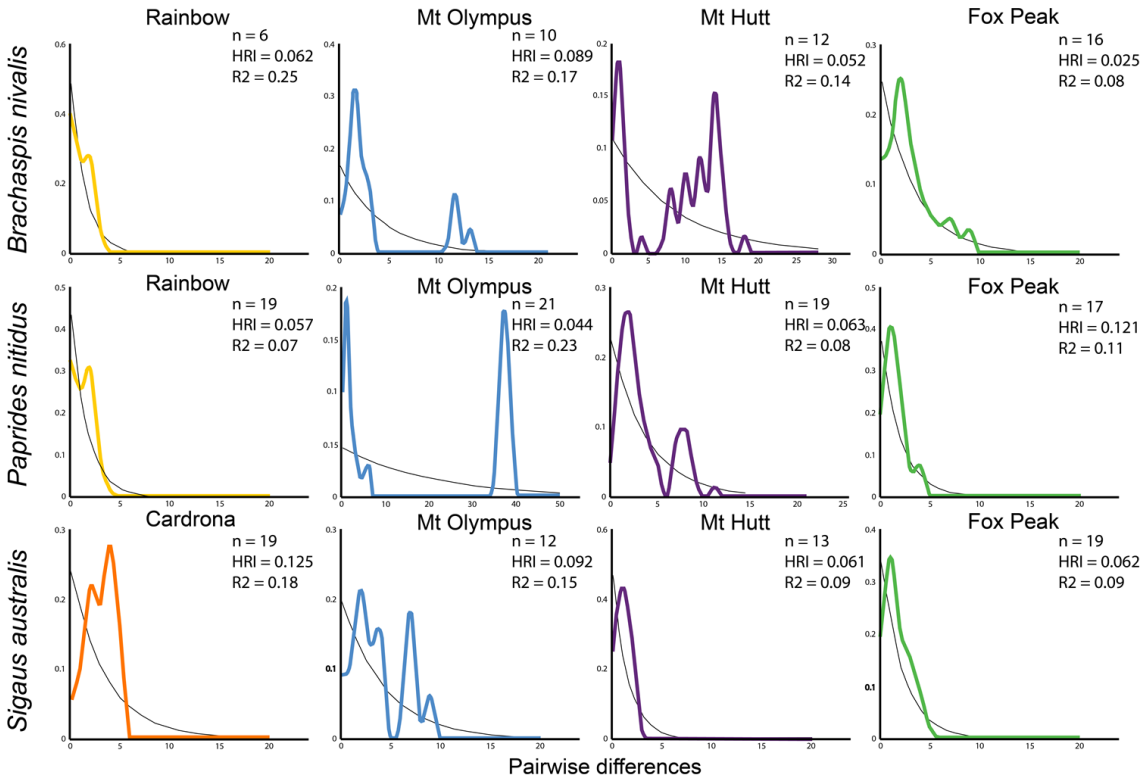


Figure 5.6 Mismatch distribution plots for each of the four populations of *Brachaspis nivalis*, *Paprides nitidus* and *Sigaus australis*. n = sample size, HRI = Harpending's raggedness index, R_2 = Ramos-Onsins and Roza's R_2.

Discussion

Ecological partitioning

Prior Ecological Niche Models of New Zealand's endemic alpine grasshoppers have revealed the distinct potential niche spaces the species inhabit (Chapter Four). However, the geographic ranges of New Zealand's endemic alpine grasshoppers are not disjunct, with many species occurring in sympatry (Table 5.4) (Figure 5.2). Apart from *Sigaus piliferus*, the geographic ranges of the remaining species overlap with at least three others, and up to nine. At the scale of habitat patches (mountain tops), as many as five species occur together. Where species occur in sympatry across five South Island mountains, there is distinct and predictable ecological partitioning. Simple measures of habitat type and elevation were adequate in predicting the distributions of species on these mountains. Knowledge of habitat preferences, makes it possible to infer what factor limits particular species on mountains. For example, some species may occur at higher or lower densities because of the presence or absence of a particular habitat type on a mountain. Such correlations could be further investigated through quantification using high resolution orthoimagery (Fritz *et al*. 2018, Marcinkowska-Ochtyra *et al*.2017). Additionally, if particular habitat types are more vulnerable than others with future climate change, we can predict what species will be more sensitive to warming. For example, if environments suitable for herbs were to decline with future climate change, this would greatly impact *A. crassicauda* and *A. tumidicauda* as this is their preferred habitat type. Quantifying these habitat types is the next step in determining persistence of species on these mountains.

Population genetics

The ability of species to adapt to change is enhanced if they have high genetic diversity (Ehlers *et al*. 2008, Pauls *et al*. 2013, Reusch *et al*. 2005). Four mountain populations of three species, *Brachaspis nivalis*, *Paprides nitidus* and *Sigaus australis* were examined to estimate their genetic diversity and population structure. The genetic diversity of these three species was relatively high, as expected for species with large ranges and large population sizes (Avise 2000, Charlesworth & Charlesworth 1987, Excoffier *et al*. 2009). However, the most common grasshopper species collected in the field (*Sigaus australis*) had the lowest genetic diversity. This suggests it may have been more restricted in the past (perhaps due to microhabitat preferences) compared to the other

species. Within the population samples of each species, genetic diversity was low. Furthermore definitive population structuring was apparent between populations of all species, with each mountain population having its own set of distinct haplotypes. Such strong population structuring likely reflects persistence during and between glacial periods of the Pleistocene (Trewick *et al*. 2000, Trewick 2001a, Trewick *et al*. 2011). Habitat contraction since the LGM, has emphasised segregation of populations into areas of alpine refugia since ~15,000 years ago (Clark *et al*. 2009, Galbreath *et al.* 2009, Gifford & Kozak 2012, Hewitt 2004). This reiterates findings in Chapter Four, that these species are inherently alpine and their distribution have been influenced by past climate cycling. With future climate change, it is predicted that these populations will remain isolated and that genetic divergence will increase, whilst genetic diversity with each population may decrease. Although there are populations of lowland *B. nivalis* and *S. australis*, these do not appear to have facilitated gene flow between mountain populations (Dowle *et al*. 2014). Lowland populations of *B. nivalis* have a tendency to be more closely related to their neighbouring mountain populations than to other lowland populations (Trewick 2001b, Trewick & Morris 2008); suggesting that isolation of populations is not restricted to high elevation populations.

Brachaspis nivalis and *Paprides nitidus* have very similar ranges and both are found on four of the five mountains studied. It might be expected that the intraspecific relationships of mtDNA from these population samples would be similar; however, this is not the case. Mt Hutt and Mt Olympus populations of *B. nivalis* form a clade sister to the Rainbow population, whereas the Mt Olympus population of *P. nitidus* forms a clade with the Rainbow population, which is sister to a clade consisting of the Fox Peak and Mt Hutt populations. This mismatch in population structure between species could indicate regional differences in the connectedness of ancestral populations and/or where they were distributed throughout glacial cycling of the Plio-Pliestocene.

Integrating niche models and population genetics

With increase in global temperatures it is predicted that species will shift their ranges towards higher latitudes (Galbreath *et al.* 2009). This could mean the loss of unique haplotypes or lineages from the northern ends of Southern Hemisphere species distributions (Bulgarella *et al*. 2015). Predictions of suitable niche space in the future

(established in Chapter Four), indicates that there will be a southern shift in suitable niche for *B. nivalis* and *P. nitidus* (Figure 5.7). This could result in a loss of Rainbow haplotypes or the entire Rainbow lineage for both *B. nivalis* and *P. nitidus*. The middle of their range is predicted to be maintained under two future climate change scenarios, and will therefore likely retain high genetic diversity, whilst we can expect expansion of both species distributions southwards. For *S. australis*, its distribution is predicted to decrease, and will likely result in the loss of its northern haplotypes or entire lineages (e.g. Mt Olympus, Mt Hutt and potentially Fox Peak). Niche space is predicted to be maintained in the lower half of the South Island, and thus genetic diversity may also be retained here. However, unlike the former species, *S. australis* is not predicted to expand and/or shift southwards. An increase in global temperatures is expected to result in the loss of populations and genetic diversity of these species.

Conclusion

Many of New Zealand's endemic grasshoppers have distributions that overlap other grasshopper species. However, on a local scale the abundance of each species is determined by interactions between abiotic factors (such as elevation) and biotic factors (such as vegetation and other grasshoppers). Thus, range changes cannot simply be modelled by climate but should consider vegetation, and competitors. The population genetic structure of New Zealand's endemic alpine grasshoppers indicates a shift in population distributions upwards in elevation since the LGM, resulting in fragmented populations retaining much of the past genetic diversity of a widespread species. This is in contrast to lowland New Zealand species where populations have expanded south from refugia since the LGM (Trewick & Wallis 2001, Trewick *et al.* 2011). It is likely that the alpine grasshopper populations will remain isolated with future climate change, without gene flow to increase genetic diversity; each population will therefore have reduced adaptive capabilities and thus reduced resilience in light of future climate change.

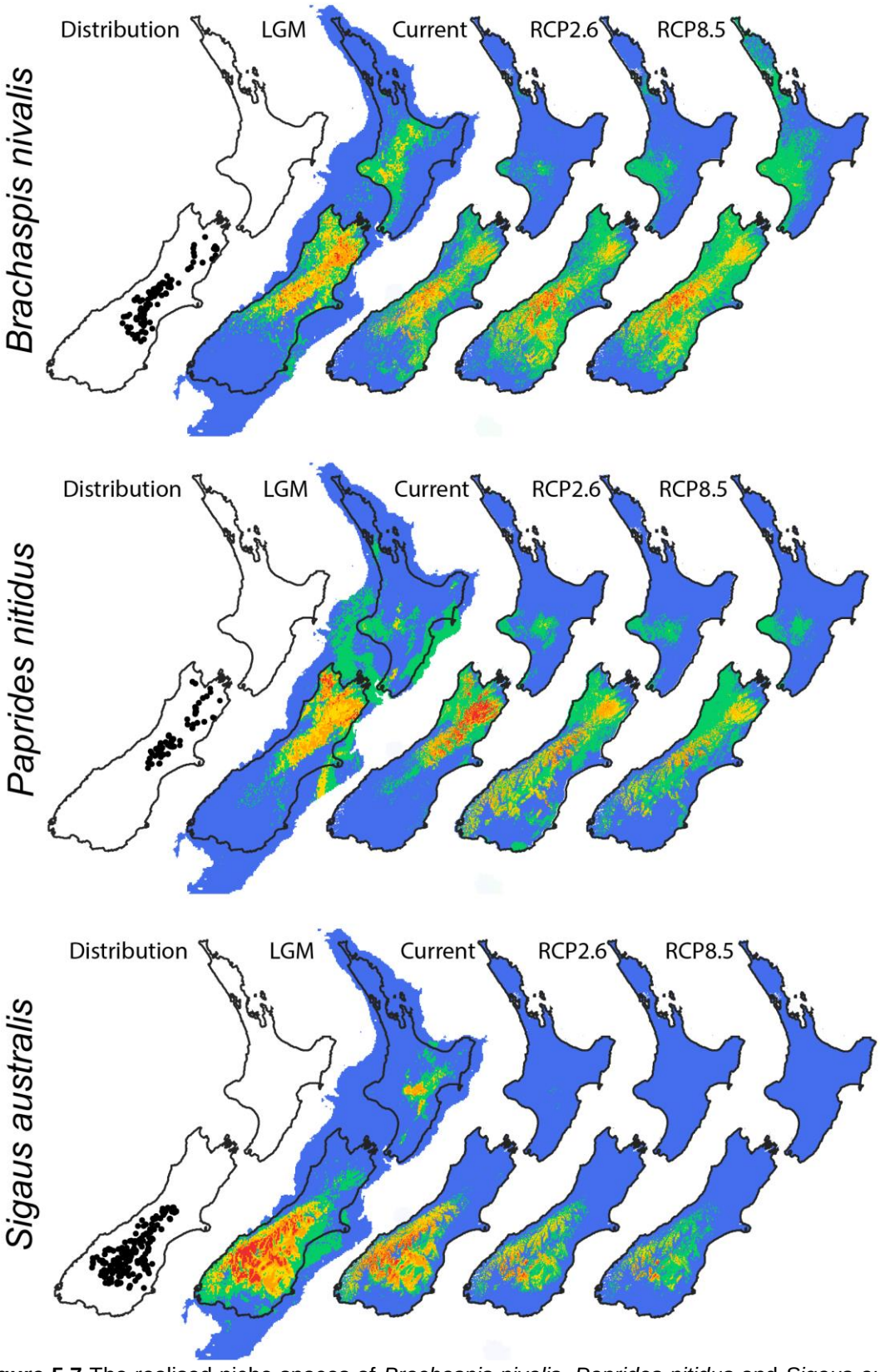


Figure 5.7 The realised niche spaces of *Brachaspis nivalis*, *Paprides nitidus* and *Sigaus australis* as modelled in Chapter Four, alongside their predicted potential niche spaces, generated through Ecological Niche Modelling. Ensemble Model Mean Weights models were used to generate these predictions. Maps from left to right: the first map indicates where each species is currently distributed and the presence data input into the model; predicted suitable niche space during the Last Glacial Maximum (LGM); predicted suitable niche space for current climate predictions; and the predicted suitable niche space for two future climate change scenarios based on RCP2.6 and RCP8.5 trajectories, respectively. Hot colours indicate a high probability of suitable habitat availability, cool colours indicate a low probability of suitable habitat availability.

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Supplementary Material for Chapter Five

Supplementary Table 5.1 Sampling sites used in ecological partitioning analysis, and the variables recorded at each. – indicates variable was not recorded at that site.

Site	Location	Latitude	Longitude	Elevation	Temperature (°C)	Wind speed	Substrate	# Species collected	# individuals collected
1	Cardrona	-44.86	168.95	1890	14.0	0.0	Herb	1	19
2	Cardrona	-44.86	168.95	1840	10.4	0.0	Herb	1	20
3	Cardrona	-44.86	168.95	1790	10.9	0.0	Tussock	1	18
4	Cardrona	-44.86	168.95	1740	11.0	1.3	Tussock	1	20
5	Cardrona	-44.86	168.95	1690	11.6	0.1	Tussock	1	20
6	Cardrona	-44.86	168.96	1640	12.8	0.0	Herb/tuss	2	20
7	Cardrona	-44.88	168.95	1540	23.7	1.2	Herb/rock	1	20
8	Cardrona	-44.88	168.96	1440	15.4	0.0	Herb	2	20
9	Cardrona	-44.88	168.96	1340	17.2	0.0	Herb	2	20
10	Cardrona	-44.87	168.97	1240	19.7	1.5	Herb	1	20
11	Cardrona	-44.87	168.97	1190	22.0	1.3	Herb	1	10
12	Fox Peak	-43.86	170.81	1480	20.1	2.5	Grass	3	10
13	Fox Peak	-43.85	170.81	1540	15.0	1.2	Herb/rock	2	11
14	Fox Peak	-43.85	170.81	1585	12.2	1.8	Herb	1	1
15	Fox Peak	-43.86	170.82	1340	-	-	Tussock	2	12
16	Fox Peak	-43.84	170.79	2010	-	-	Rock	1	3
17	Fox Peak	-43.86	170.80	1500	32.5	1.8	Tussock	2	9
18	Fox Peak	-43.86	170.81	1400	22.3	1.6	Herb	2	18

19	Fox Peak	-43.86	170.81	1400	19.3	1.0	Rock	3	20
20	Fox Peak	-43.86	170.81	1400	19.0	0.7	Rock	0	0
21	Fox Peak	-43.86	170.81	1400	18.1	0.0	Herb	1	13
22	Fox Peak	-43.86	170.81	1300	24.8	0.0	Tussock	2	10
23	Fox Peak	-43.86	170.81	1300	27.9	0.0	Herb	2	19
24	Fox Peak	-43.86	170.81	1300	23.3	1.8	Tussock	1	4
25	Fox Peak	-43.86	170.81	1300	22.7	0.0	Tussock	1	20
26	Fox Peak	-43.86	170.82	1300	19.7	0.0	Tussock	0	0
27	Fox Peak	-43.86	170.82	1200	19.1	0.0	Tussock	0	0
28	Fox Peak	-43.86	170.80	1500	27.0	1.0	Tussock	1	14
29	Fox Peak	-43.86	170.82	1200	-	0.0	Grass	0	0
30	Fox Peak	-43.86	170.82	1100	-	0.0	Grass	0	0
31	Fox Peak	-43.85	170.80	1800	23.3	-	Rock	1	2
32	Fox Peak	-43.85	170.80	1800	23.0	-	Rock	1	5
33	Fox Peak	-43.85	170.81	1700	26.4	-	Rock	1	4
34	Fox Peak	-43.85	170.80	1700	23.4	0.0	Herb	2	20
35	Fox Peak	-43.85	170.80	1700	24.5	1.4	Herb	2	20
36	Fox Peak	-43.85	170.80	1700	26.7	1.3	Rock	1	17
37	Fox Peak	-43.85	170.81	1600	26.3	1.4	Tussock	2	14
38	Fox Peak	-43.85	170.81	1600	23.7	1.3	Herb	1	18
39	Fox Peak	-43.86	170.80	1500	26.3	2.0	Herb	1	20
40	Fox Peak	-43.85	170.81	1600	19.8	0.0	Herb	1	20
41	Fox Peak	-43.85	170.81	1600	22.7	2.0	Tussock	2	16
42	Fox Peak	-43.85	170.81	1600	21.4	2.5	Rock	1	17
43	Fox Peak	-43.85	170.81	1600	20.9	0.8	Rock	2	17

44	Fox Peak	-43.86	170.80	1500	31.2	0.0	Herb	3	20
45	Fox Peak	-43.86	170.80	1500	26.9	1.7	Rock	1	1
46	Fox Peak	-43.86	170.81	1400	21.9	1.4	Tussock	2	19
47	Fox Peak	-43.86	170.81	1400	20.2	0.0	Tussock	1	17
48	Fox Peak	-43.86	170.81	1400	27.6	0.0	Tussock	1	10
49	Mt Hutt	-43.52	171.55	1400	-	-	Tussock	2	6
50	Mt Hutt	-43.50	171.54	1600	-	-	Tuss/rock	3	20
51	Mt Hutt	-43.50	171.54	1622	-	0.0	Tussock	1	18
52	Mt Hutt	-43.50	171.54	1625	-	0.8	Rock	1	14
53	Mt Hutt	-43.49	171.54	1625	-	0.0	Herb	2	19
54	Mt Hutt	-43.49	171.54	1625	-	1.5	Rock	1	17
55	Mt Hutt	-43.49	171.54	1625	-	1.0	Herb	2	20
56	Mt Hutt	-43.50	171.57	1592	-	0.0	Tussock	2	15
57	Mt Hutt	-43.51	171.55	1460	-	1.8	Herb	3	13
58	Mt Hutt	-43.51	171.55	1460	-	1.8	Tussock	2	20
59	Mt Hutt	-43.51	171.54	1460	-	0.0	Tussock	1	18
60	Mt Hutt	-43.51	171.55	1450	-	0.0	Herb	2	19
61	Mt Olympus	-43.19	171.60	1840	18.9	1.5	Tussock	2	5
62	Mt Olympus	-43.19	171.61	1825	25.8	2.0	Rock	3	18
63	Mt Olympus	-43.19	171.61	1815	27.0	1.7	Rock	3	20
64	Mt Olympus	-43.19	171.60	1790	26.0	1.6	Herb	2	12
65	Mt Olympus	-43.19	171.60	1740	31.5	1.2	Tuss/rock	3	11
66	Mt Olympus	-43.19	171.60	1720	28.0	1.2	Tussock	2	11
67	Mt Olympus	-43.19	171.61	1690	25.7	1.0	Herb/rock	3	10

68	Mt Olympus	-43.19	171.61	1660	25.7	1.8	Herb	2	3
69	Rainbow	-41.88	172.86	1477	-	-	-	2	9
70	Rainbow	-41.87	172.86	1727	18.1	0.8	Rock	2	13
71	Rainbow	-41.87	172.86	1720	18.0	1.6	Herb	2	20
72	Rainbow	-41.87	172.87	1711	17.9	2.8	Rock	2	17
73	Rainbow	-41.87	172.87	1720	20.8	2.6	Herb	2	20
74	Rainbow	-41.88	172.85	1866	17.6	1.0	Rock	2	18
75	Rainbow	-41.88	172.85	1834	18.8	1.2	Rock	2	17
76	Rainbow	-41.88	172.85	1830	20.3	2.2	Herb	3	20
77	Rainbow	-41.88	172.85	1723	22.3	1.8	Herb	1	20
78	Rainbow	-41.88	172.85	1725	22.6	0.0	Rock	2	17
79	Rainbow	-41.87	172.86	1620	20.1	2.8	Rock	0	3
80	Rainbow	-41.87	172.86	1625	18.9	1.0	Tussock	1	9
81	Rainbow	-41.87	172.86	1612	15.0	0.0	Rock	1	14
82	Rainbow	-41.87	172.86	1620	17.8	0.0	Tussock	1	20
83	Rainbow	-41.88	172.86	1504	18.9	3.2	Rock	1	14
84	Rainbow	-41.88	172.86	1494	18.8	3.2	Herb	2	10
85	Rainbow	-41.88	172.86	1462	21.7	1.0	Herb	1	20
86	Rainbow	-41.88	172.86	1405	23.3	0.8	Herb	1	20
87	Rainbow	-41.88	172.86	1392	22.9	1.2	Rock	0	0
88	Rainbow	-41.88	172.86	1411	23.2	-	Rock	0	3
89	Rainbow	-41.88	172.86	1286	26.9	0.0	Herb	2	20
90	Rainbow	-41.88	172.86	1288	24.0	1.0	Rock	1	1

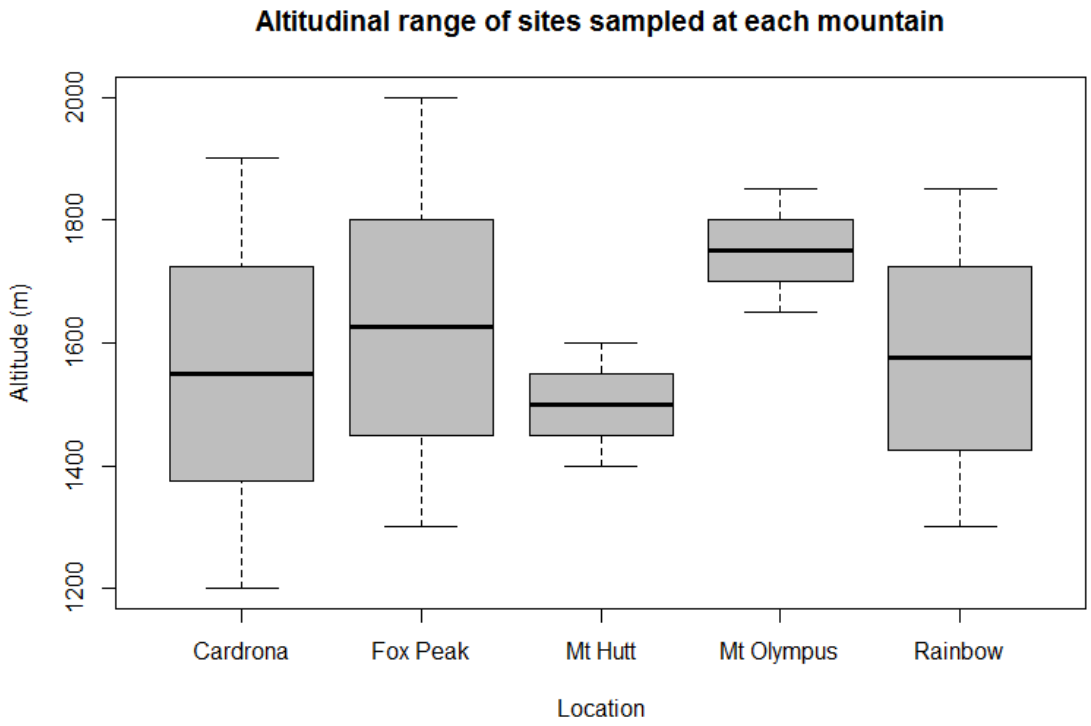
Supplementary Table 5.2 Expected values for each species generated via a Poisson GLM model. High numbers (dark purple) indicate at what elevation(s) a species is predicted to be found inhabiting based on field sampling data. Low numbers (white) indicate at what elevation species are least likely to be found.

Species	Elevation							
	1100	**1200**	**1300**	**1400**	**1500**	**1600**	**1700**	**1800**
A. crassicauda	0	0	0	0	0	0	1.45	0
A. tumidicauda	0.13	0.43	0	0	0.53	1.33	0.48	0.31
B. collinus	0	0.31	0	0.14	1.04	0.61	0.90	0.96
B. nivalis	0	0	0	0.73	0.16	0.49	1.21	1.41
P. nitidus	0	1.93	2.32	3.71	0.60	6.56	3.41	1.65
S. australis	11.85	6.61	6.46	3.07	7.67	12.73	9.20	12.90
S. campestris	0.06	0	0	0.34	0	0	0	0
S. villosus	0	0	0	0	0	0	0	0

Supplementary Table 5.3 Expected values for each species generated via a Poisson GLM model. High numbers (dark purple) indicate what habitat type a species is predicted to be found inhabiting based on field sampling data. Low numbers (white) indicate at what habitat type species are least likely to be found.

Species	Habitat types					
	Grass	Herb	Herb/rock	Herb/tuss	Rock	Tussock
A. crassicauda	0.22	0.65	0.15	0.06	0	0
A. tumidicauda	0	0.23	0.06	0	0	0
B. collinus	0	0.23	0	0	2.75	0
B. nivalis	0	0.37	0	0	2.63	0
P. nitidus	1.36	2.01	10.55	0	0.04	1.16
S. australis	20.10	5.95	13.78	7.61	0.30	5.13
S. campestris	0.16	0	0.02	0.04	0	0.11
S. villosus	0	0	0	0	0	0

Supplementary Figure 5.1 Elevational ranges of sites sampled at each mountain.



Fox Peak Ski Area, Two Thumb Range, South Island

Chapter Six

Thesis discussion

Thesis conclusions

Climate change is predicted to have a detrimental impact on New Zealand's endemic alpine Acrididae (Chapter Four). The species within this group are adapted to alpine living, and their diversity and distributions have been influenced by past climate cycling. Climatic warming since the LGM and contraction of alpine habitat has consequently reduced the suitable niche space of these species (Chapter Four). This has subsequently resulted in the isolation of populations within some species (Chapter Five). Suitable niche space for all species is predicted to further reduce over the next 50+ years, and it is predicted that the isolation of populations within species will persist. Extended periods of isolation of intraspecific populations have prevented gene flow, resulting in populations accumulating unique haplotypes; it is possible that this will have a negative impact on the resilience of these populations in the future. Habitat fragements supporting small populations are vulnerable to local extinction (Frankham 1997). If greenhouse gas emissions are not reduced in the coming century, it is predicted that a number of these grasshopper species will lose suitable habitat, haplotype diversity and distinct genetic lineages, with the worst case scenario being the extinction of species. From predictions made for New Zealand's alpine grasshoppers, it can be inferred that associated alpine fauna will also be negatively impacted by future climate change, particularly species that are poor dispersers.

Implications for alpine fauna

With the arrival of humans to New Zealand in ~1280 A.D., a period of rapid change began. Hunting, the introduction of pests, anthropogenic fires and deforestation led to the destruction of various ecosystems for agricultural systems and settlements; beginning the demise of New Zealand's endemic flora and fauna (Higham *et al.* 1999,

Holdaway & Jacomb 2000, McGlone & Wilmshurst 1999, Wilmshurst *et al.* 2008, Wilmshurst & Higham 2004). This has continued through to the 21st century, with landuse change and introduced predators being the main threat to species persistence; ~2788 species of flora and fauna are at risk of extinction in New Zealand, with a further >3000 data deficient species also likely to be at risk (Hitchmough *et al*. 2007). Future climate change will put species under further pressure as species ranges are altered, extreme weather events become more common, ecosystem composition is altered and mismatch in phenology alters life cycles and food source availability.

New Zealand's alpine zone is home to an array of alpine fauna, a number of which are already classified as 'At risk' or 'Threatened'. Along with the alpine grasshoppers mentioned in this thesis, New Zealand's alpine zone is home to approximately 15 bird species, of which the Kea (*Nestor notabilis*), Rock Wren (*Xenicus gilviventris*) and Takahe (*Porphyrio hochstetteri*) are classified as Nationally Critical or Nationally Endangered (NZ Birds Online Database: *nzbirdsonline.org.nz*); approximately 33 species of skink and gecko (NZ Lizards Database: http://*nzlizards.landcareresearch.co.nz*); approximately 12 species of native fish that inhabit meltwater and glacier fed streams (NIWA Atlas of Freshwater Fishes: *https://www.niwa.co.nz/freshwater-and-estuaries/nzffd/NIWA-fish-atlas*); approximately seven species of endemic cockroach (Chinn & Gemmell 2004); >194 species of endemic Coleoptera (Barret *et al*. 1997, Craw 1999, Holloway 2007, Klimaszewski & Watt 1997; Kuschel 2003, Larochelle & Lariviere 2001, 2005, 2007, 2013, McColl 1982, Townsend 2010, Watt 1992); >187 species of Hemiptera, including the diverse *Kikihia* and *Maoricicada* genera (Cox 1987, Henderson 2011, Lariviere 1995, 1999, Lariviere & Larochelle 2004, Lariviere *et al*. 2010, 2011, Morales 1991); >37 species of Hymenoptera (Berry 2007, Donovan 2007, Harris 1987, Naumann 1988, Noyes 1988, Noyess & Valentine 1989); >30 species of endemic Lepidoptera (Donner *et al*. 1989, Dugsdale 1994, Gibbs 2004, Hoare 2005, 2010, Weintraub & Scoble 2004); alongside the alpine Acrididae, there are three other genera of Orthoptera that have at least one alpine species: *Deinacrida*, *Hemideina* and *Hemiandrus* (Sinclair *et al.* 1999); approximately 28 species of Plecoptera and approximately 15 species of Thysanoptera (McLellen 1991, 1993, Mound *et al*. 1986). In addition there are many endemic alpine plant species, and undoubtedly many species are yet to be discovered and/or are waiting

to be classified in collections around the country, all vulnerable to the current loss of alpine habitat.

Islands within islands

Anthropogenic climate change will raise treelines and snowlines globally (Dullinger *et al*. 2004, Grace *et al*. 2002, Halloy & Mark 2003, Kullman 2001). Alpine organisms are predicted to shift their ranges towards higher latitudes and elevations with increases in global temperature, however the height of mountains is finite and the ability for species to shift their ranges upwards is limited (Galbreath *et al*. 2009, Moritz *et al*. 2008, Wilson *et al*. 2005). Migration is an option, however there may not always be a viable habitat to disperse to, and migrating is not always straightforward. Mountains and their alpine zones have been referred to in the literature as "sky islands", "islands in the sky" and "archipelagos of alpine islands", suitably describing how the alpine zone can be an isolated ecosystem to inhabit (Bigelow 1987, Gifford & Kozak 2012, Heald 1951, Knowles 2000, Mayr & Diamond 1976, Smith & Farrell 2005). But what about alpine zones that are islands within islands?

In the same way that New Zealand's alpine grasshoppers are representatives for New Zealand's alpine fauna, New Zealand's alpine zone is a representative of other island alpine zones around the world. Oceanic islands are susceptible to rising sea levels and the increased volatile weather conditions that come with anthropogenic climate change (Mimura *et al*. 2007). Islands with alpine zones include: Iceland, Hawaii, Japan, Madagascar, New Guinea, Taiwan, Tasmania, the United Kingdom, Ireland and New Zealand. Where alpine habitat is available on these islands it is often limited. Depending on an organism's dispersal ability, surrounding bodies of water can hamper migration from such alpine zones; their distance and isolation from continental landmasses can impede successful migration (MacArthur & Wilson 2016). This puts island alpine species in a precarious position; once alpine habitat disappears, their best chance of survival is rapid *in situ* adaptation or niche shifting.

Globally, extinction rates for species under future climate change scenarios are predicted to be relatively high (~18-35%) (Thomas *et al.* 2004). Taking into consideration the vulnerability of the island alpine zone, it could be considered one of

the most at risk ecosystems in the world. But what do we face losing? Madagascar, Sumatra, Borneo, Java, Japan, New Guinea, the Philippines, Hawaii and New Zealand are all considered to be 'hotspots' of biodiversity (Brooks *et al*. 2002, Mittermeier *et al.* 1999). These islands all have high levels of species endemicity and are experiencing a rapid loss of habitat – currently due to landuse change by man; this makes them both important and high conservation priorities. The regions within which these islands reside contain ~10% of the world's endemic plant species and ~6% of the world's endemic vertebrate species; it is likely they contain a high proportion of endemic invertebrate species also (Brooks *et al*. 2002, Mittermeier *et al.* 1999). A loss of alpine area from these islands will inevitably result in a loss of endemic diversity.

Modelling alpine loss

With the knowledge that with every 0.6°C of warming, treelines will shift approximately 100m in elevation (Halloy & Mark 2003, Mark *et al.* 2000, Wardle 1998), it makes it possible to predict the loss of island alpine area under future climate change scenarios (see Supplementary Material for Chapter Six for methods) (Table 6.1) (Figure 6.1). Of eight island alpine zones examined, all were predicted to lose alpine habitat (Table 6.1). For most islands, they were predicted to lose >50% of alpine habitat with 1.8°C; Madagascar will completely lose what little alpine habitat it does have. With 4.0°C of warming, Ireland and Tasmania are predicted to be alpine free; Japan and Taiwan will have less than 15km^2, and Great Britain will have less than 3km^2 of alpine habitat remaining. New Zealand is predicted to lose 50% of its alpine habitat with a temperature increase of 1.8°C, and 86% with a temperature increase of 4.0°C.

Migration of individuals to continental alpine environments or to the poles may be the only way in which species are able to persist (Table 6.2). This could be as far as 5000km for those that migrate to continents (Hawaii to Alaska), or as little as 629km for others (Tasmania to Australia). The distance to the poles from these islands ranges from 801km (Iceland to Greenland) to 5752km (Hawaii to Greenland). For New Zealand, the nearest continental alpine zone is Australia's Mt Kosciuszko (2019km), and its closest pole is Antarctica (3479km). It must be kept in mind, however, that different species have different dispersal abilities, and this will greatly impact their capacity to migrate.

Table 6.1 Alpine area of eight islands and the modelled loss of alpine area with 1.8°C and 4.0°C of warming.

Island	Present		1.8°C warming		4.0°C warming		Total Land Area (km^2)
	Alpine area (km^2)	% of total land area	Alpine area (km^2)	% of total land area	Alpine area (km^2)	% of total land area	
Great Britain	3,816.9	1.6	231.6	0.1	2.3	0.0	233,540.2
Hawaii	950.6	5.7	620.2	3.7	359.1	2.1	16,762.2
Iceland	51,403.0	50.8	23,371.3	23.1	8,326.1	8.2	101,196.5
Ireland	109.2	0.1	0.2	0.0	0	0.0	84,624.8
Japan	594.4	0.2	145.2	0.0	11.1	0.0	374,122.5
Madagascar	19.5	0.0	0	0.0	0	0.0	587,540.0
New Zealand	26,301.7	9.7	13,092.8	4.9	3,653.1	1.4	269,947.9
Taiwan	665.3	1.8	174.3	0.5	14.4	0.0	36,445.4
Tasmania	2,771.0	4.0	126.4	0.2	0	0.0	68,580.8

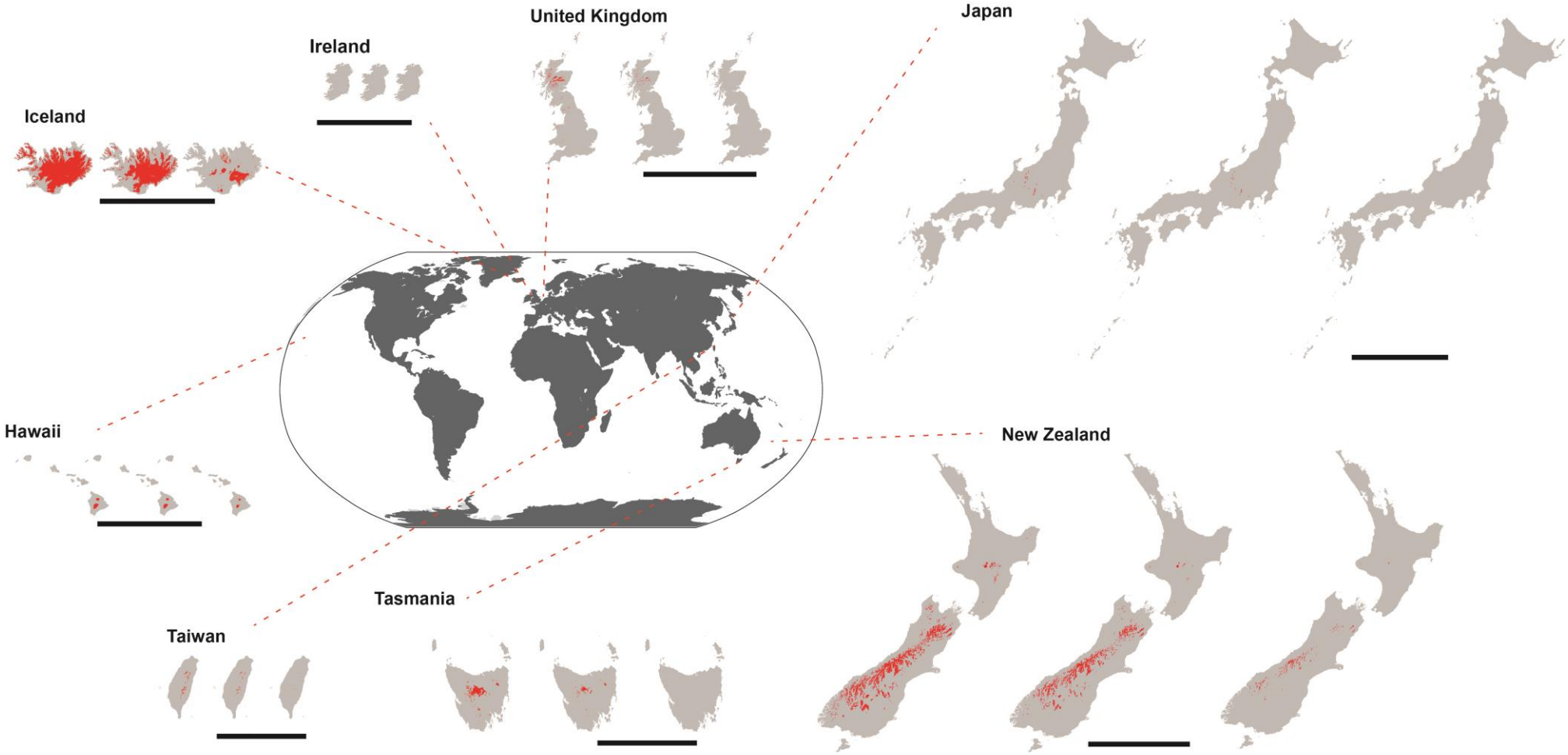


Figure 6.1 Predicted loss of alpine habitat of eight islands. Red = alpine habitat. From left to right, each set of map consists of: alpine area at present, alpine area with 1.8°C of warming, and alpine area with 4.0°C of warming. Black scale bar is 400km.

Table 6.2 The distance (in kilometres (km)) from the highest point of each island to the highest point of the closest continent, and to the closest pole.

Island	Distance to closest & highest continental alpine zone (km)		Distance to closest pole (km)	
Great Britain	2248	Mt Elbrus, Russia	1134	Gunnbjørn Fjeld, Greenland
Hawaii	4817	Denali, Alaska, USA	5752	Gunnbjørn Fjeld, Greenland
Iceland	4311	Mt Elbrus, Russia	801	Gunnbjørn Fjeld, Greenland
Ireland	3943	Mt Elbrus, Russia	1345	Gunnbjørn Fjeld, Greenland
Japan	3060	Mt Everest	5221	Gunnbjørn Fjeld, Greenland
Madagascar	1760	Mt Kilimanjaro	5809	Mt Vinson, Antarctica
New Zealand	2019	Mt Kosciuszko, Australia	3479	Mt Vinson, Antarctica
Taiwan	3440	Mt Everest	5899	Gunnbjørn Fjeld, Greenland
Tasmania	629	Mt Kosciuszko, Australia	3864	Mt Vinson, Antarctica

Resilience in the face of change

Different species are predicted to respond to climate change in different ways, even between closely related taxa, and not all species will be affected negatively (Chen *et al*. 2011, Lenoir *et al*. 2010, Moritz & Agudo 2013, Parmesan & Yohe 2003). This is not the first time that climate has changed. Species, including the grasshoppers in this study, have persisted through climate cycling in the past (Galbreath *et al*. 2009). Particular characteristics may aid in the survival of some organisms, this includes species with: high dispersal ability; high genetic diversity with gene flow between populations; phenotypic plasticity; large distributions; occupy continental landmasses and/or islands that are not too isolated; inhabit a broad niche space; have high reproductive output and success; have relatively short and/or flexible life cycles that are not dependent on climate; and species that play a generalist role in their habitat (Colautti & MacIsaac 2004, Hughes *et al.* 2003, Lee 2002, Moritz & Agudo 2013, Reusch *et al.* 2005, Sakai 2001).

New Zealand's alpine Acrididae

New Zealand's alpine grasshoppers have some characteristics that could help them persist in the coming century. They are capable of long distance dispersal, as their ancestors likely came from Tasmania. It is also likely that when their ancestor arrived in New Zealand, there was no alpine habitat available – suggesting that they could survive without alpine habitat in the future. From what we know, these species are generalist herbivores, and thus are not restricted by the presence or absence of particular plant species in their environment (Watson 1971, White 1975). They have flexible lifestyles and can overwinter at any life stage – their life cycle may not be dependent on overwintering (Batcheler 1967, Hawes 2015, Mason 1971, Sinclair 2001, White & Sedcole 1999). Females of some species are known to lay multiple clutches of eggs if the spring/summer is particularly long (White & Sedcole 1999). Some species also have ranges that extend into lowland habitats, whilst some are purely lowland in distributions (Bigelow 1967). It is possible that shortened winters and reduced snow pack may not have as detrimental impact on New Zealand's endemic grasshopper species as it may for some alpine specialists (Inouye *et al*. 2000, Kausrud *et al*. 2008, Tafani *et al*. 2003).

Future research

This study has provided predictions of how New Zealand's endemic alpine grasshoppers, and thus New Zealand's alpine fauna, may respond to future climate change. However, there are still aspects of New Zealand's endemic alpine grasshopper biology and ecology that we do not understand:

- New Zealand's alpine grasshoppers are unusual in their ability to overwinter at any life stage, and we do not know if this is an ancestral trait or if it evolved multiple times within the alpine grasshopper species. For example, two freeze tolerant New Zealand stick insects have independently converged on their alpine adaptations (Dennis *et al.* 2015, Dunning *et al*. 2014).
- The grasshoppers are all flightless which inhibits dispersal, and also their ability to communicate audibly (as orthoptera often sing with their wings). It is likely the New Zealand grasshoppers communicate using chemical cues; however this is yet to be investigated.
- Colouration of grasshoppers can vary greatly within a species but its role has not been studied. Observation and behaviour studies could inform if cryptic colours aid in predator avoidance, or if they are under sexual selection.
- Hybridization has been observed between some grasshopper species (*Brachaspis collinus* and *Brachaspis nivalis*, *Sigaus australis* and *Sigaus childi*); we do not know if this will help or hinder these species adaptive abilities in light of future climate change (Becker *et al*. 2013).
- From my phylogenetic analysis of New Zealand's grasshopper species it is apparent that reclassification of members within this group is required; further geometric morphometric analysis may reveal more suitable taxonomic groupings.
- Additionally, geometric morphometrics could be used to explore the species delimitation of some species' mitochondrial lineages (e.g. *Alpinacris crassicauda*, *Brachaspis nivalis* and *Sigaus piliferus*).
- With the knowledge that species are ecologically partitioned in alpine environments, we can use satellite imagery and Ecological Niche Modelling to quantify and predict the persistence of these habitats and their inhabitatns in the future. This could be compared with population sizes and genetic diversity of

alpine grasshopper species to infer which populations may be particularly vulnerable with future climate change.

Conclusion

To those who understand the very real implications of unrestricted anthropogenic climate change, the conclusions of this study will not be unexpected. However, quantifying the potential impacts of future climate change, especially of biodiversity loss, is imperative. Such evidence is vital for lawmakers and policy makers, as there needs to be realised consequences to inaction. Preventing the extinction of species is a challenge faced around the globe, with the additional pressure of anthropogenic climate change, the urgency to do so has been amplified. Without the reduction of greenhouse gas emissions in the near future, many ecosystems and the flora and fauna they contain will be at risk of extinction.

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Supplementary Material for Chapter Six

Materials and Methods

Digital Elevation Model (DEM) tiles for eight islands were acquired from ASTER GDEM (Tachikawa *et al*. 2011). Literature was searched for the average elevation of treelines on these islands (Supplementary Table 1). Assuming a 100m increase in treeline elevation with every 0.6°C of warming (Halloy & Mark 2003, Mark *et al.* 2000, Wardle 1998), the total alpine area of each island was modelled for two different time periods (current and 2070), including two future climate change scenarios. Alpine area was quantified using the R v1.1.383 (R Core Team 2013) packages: 'raster' v 2.6-7 (Hijmans 2017), 'rgdal' v 1.2-18 (Bivand *et al*. 2014) and geosphere v 1.3-11 (Hijmans *et al*. 2015).

Supplementary Table 6.1 The average treeline of island alpine zones in meters above sea level (m.a.s.l), under current climate conditions, and for future temperature increases of 1.8°C and 4°C. max = the heightest possible point of an islands alpine zone.

Island	Current (m.a.s.l)	+1.8°C (m.a.s.l)	+4°C (m.a.s.l)	Reference
Great Britain	650	950	1316	Hodkinson *et al*. 2011
Hawaii	2590	2890	3256	Körner & Paulsen 2004
Iceland	380	680	1050	Woll 1980
Ireland	650	950	1316	Hodkinson *et al*. 2011
Japan	2350	2650	3016	Ganser 2004
Madagascar	2600	2876 (max)	2876 (max)	Gade 1996
New Zealand	1200	1500	2166	Halloy & Mark 2003,
Taiwan	2900	3200	3566	Greenwood *et al*. 2014

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Appendix One

Species Locations

Locations for all of New Zealand's endemic alpine grasshopper species. Locations were used throughout thesis for mapping distributions, inputting into Ecological Niche Models and generating minimum convex polygons. Phoenix Collection is held at Massey University, Palmerston North. Tenure Review = Crown Pastoral Lease Tenure Reviews, available from: https://www.linz.govt.nz/crown-property/crown-pastoral-land/status-and-location-crown-pastoral-land.

Species	Location name	Latitude	Longitude	Synonym	Reference
Alpinacris crassicauda	Gouland Downs	-40.92	172.34		Bigelow 1967
	Lake Sylvester	-41.11	172.62		Bigelow 1967
	Lead Hills, Boulder Lake	-40.89	172.58		Bigelow 1967
	Mt Arthur	-41.21	172.70		Bigelow 1967
	Mt Glasgow, Seddonville	-41.61	172.06		Bigelow 1967
	Mt Goul	-40.94	172.38		Bigelow 1967
	Mt Haast	-42.34	172.08		Bigelow 1967
	Mt Owen	-41.53	172.54		Bigelow 1967
	Mt Peel	-41.13	172.58		Bigelow 1967
	Mt Peel Cobb	-41.13	172.58		Phoenix collection
	Mt Roberts, St Arnaud	-41.83	172.81		Phoenix collection
	Nelson	-41.32	173.27		Bigelow 1967
	Paparoa Range	-41.89	171.53		Bigelow 1967
	Speargrass Creek	-41.88	172.76		White 1975
	St Arnaud Range	-41.84	172.87		Bigelow 1967
	Travers Range	-41.89	172.76		Bigelow 1967
	Victoria Range	-42.27	172.14		Bigelow 1967
Alpinacris tumidicauda	Allandale Greenvale	-45.32	168.63		Tenure Review
	Bold Peak, Lake Hawea	-44.85	168.31		Bigelow 1967
	Borland Rd Manapouri	-45.59	167.38		Phoenix collection
	Cardrona	-44.86	168.95		Phoenix collection
	Cardrona	-44.86	168.96		Phoenix collection
	Cardrona	-44.88	168.96		Phoenix collection
	Cardrona	-44.88	168.96		Phoenix collection
	Cardrona	-44.87	168.97		Phoenix collection
	Carrick Range	-45.16	169.08		Bigelow 1967
	Carrick Range, Nevis Rd	-45.17	169.08		Phoenix collection
	Cleughearn	-45.83	167.40		Bigelow 1967
	Coronet Peak	-44.93	168.74		Tenure Review
	Courthill, Old Man Range	-45.35	169.22		Tenure Review

	Gem Lake	-45.57	169.10	Dickinson *et al.* 1998
	Gorge Creek, Old Man Range	-45.39	169.20	Tenure Review
	Halfway Bay Station	-45.25	168.65	Tenure Review
	Happy Valley	-45.20	169.07	Tenure Review
	Hector Mts	-45.41	168.77	Bigelow 1967
	Jane Peak	-45.33	168.34	Mark *et al.* 1989
	Mataura Valley Snowbanks	-45.33	168.45	Tenure Review
	Mid Dome	-45.58	168.56	Bigelow 1967, Dickinson *et al.* 1998
	Minaret Station	-44.42	169.09	Tenure Review
	Mt Alta	-44.52	168.95	White 1975
	Mt Aurum	-44.77	168.63	Bigelow 1967
	Mt Benger	-45.59	169.25	Dickinson *et al.* 1998
	Mt Creighton, Luna Basin	-44.92	168.48	Tenure Review
	Mt Creighton, Luna Creek Flats	-44.94	168.49	Tenure Review
	Mt Creighton, Minor Peak	-44.93	168.46	Tenure Review
	Mt Creighton, Wire Creek	-44.90	168.47	Tenure Review
	Mt Eostre, Mt Aspiring Station	-44.44	168.86	Tenure Review
	Mt Luxmore	-45.39	167.61	Bigelow 1967
	Mt Pisa	-44.88	169.20	Bigelow 1967
	Mt Tennyson	-45.45	168.83	Dickinson *et al.* 1998
	Nokomai Range	-45.47	168.75	Dickinson *et al.* 1998
	Obelisk, Old Man Range	-45.37	169.21	Phoenix collection
	Obelisk, Old Man Range	-45.28	169.21	Phoenix collection
	Obelisk, Old Man Range	-45.32	169.21	Phoenix collection
	Obelisk, Old Man Range	-45.24	169.23	Phoenix collection
	Old Man Range	-45.38	169.18	Bigelow 1967
	Slate Basin	-45.31	168.37	Mark *et al.* 1989
	Snow Farm	-44.88	169.09	Phoenix collection
	Southern Old Man Range	-45.43	168.20	Dickinson *et al.* 1998
	Symmetry Peaks	-45.29	168.58	Mark *et al.* 1989
	Takahe Valley	-45.28	167.66	Bigelow 1967
	The Larches	-44.81	169.15	Tenure Review
	The Larches, Gold Diggings	-44.78	169.15	Tenure Review
	The Larches, Luggate Creek	-44.80	169.13	Tenure Review
	Walter Peak White Burn	-45.22	168.39	Tenure Review
	West Wanaka	-44.56	169.00	Tenure Review
Brachaspis collinus	Arthurs Pass National Park	-42.93	171.60	Bigelow 1967
	Boulder Lake	-40.90	172.57	Bigelow 1967
	Cupola Basin A	-41.97	172.73	White 1975
	Cupola Basin B	-41.97	172.73	White 1975
	Golden Downs	-41.61	172.95	Bigelow 1967
	Lake Tennyson	-42.21	172.74	Bigelow 1967
	Lewis Pass	-42.38	172.40	Bigelow 1967

	Lewis Pass	-42.38	172.38		Trewick 2001
	Mt Arthur	-41.21	172.70		Bigelow 1967
	Mt Cupola, Dry Face	-42.01	172.77		Batcheler 1967
	Mt Cupola, Hut Face	-41.99	172.73		Batcheler 1967
	Mt Lyford	-42.45	173.15		Trewick 2001
	Mt Owen	-41.53	172.54		Bigelow 1967
	Mt Peel	-41.13	172.59		Bigelow 1967
	Mt Peel Cobb	-41.15	172.60		Phoenix collection
	Mt Percival	-42.47	172.93		Bigelow 1967
	Mt Robert, St Arnaud	-41.84	172.80		Trewick 2001
	Mt Robert, St Arnaud	-41.85	172.79		Phoenix collection
	Mt St Patrick	-42.44	172.73		Bigelow 1967
	Rainbow Skifield	-41.88	172.86		Phoenix collection
	Rainbow Skifield	-41.87	172.86		Phoenix collection
	Rainbow Skifield	-41.87	172.86		Phoenix collection
	Richmond Range	-41.47	173.41		Bigelow 1967
	Six Mile Creek	-41.87	172.85		White 1975
	Speargrass Creek	-41.88	172.76		White 1975
	St Arnaud Range	-41.84	172.87		Bigelow 1967
	St Arnaud Range	-41.83	172.88		White 1975
	Temple Basin	-42.91	171.58		Mason 1971
	Travers Range	-41.89	172.76		Bigelow 1967
	Turk Ridge	-42.13	172.75		White 1975
***Brachaspis nivalis* (FP)**	Ahuriri River	-44.24	169.60	*Brachaspis robustus*	Bigelow 1967
	Awakino	-44.74	170.42	*Brachaspis robustus*	Phoenix collection
	Balmoral	-44.03	170.37	*Brachaspis robustus*	Tenure Review
	Black Forest Innes Burn	-44.47	170.40		Tenure Review
	Curraghmore, Ballon Stream	-44.34	170.54	*Brachaspis* "hunter"	Tenure Review
	Curraghmore, Stony River	-44.39	170.54	*Brachaspis* "hunter"	Tenure Review
	Erewhon, Cloudy Stream	-43.44	170.76		Tenure Review
	Ewe Range	-44.60	169.92		Patrick 1994
	Eweburn	-44.96	170.12		Tenure Review
	Fork Stream, Balmoral	-43.97	170.40	*Brachaspis robustus*	White 1994; Tenure Review
	Fox Peak	-43.85	170.81		Bigelow 1967
	Fox Peak	-43.85	170.80		Phoenix collection
	Fox Peak	-43.85	170.81		Phoenix collection
	Glenmore	-43.74	170.35	*Brachaspis* "hunter"	Tenure Review
	Glenrock Holbrook Rollesby Station	-44.04	170.58		Tenure Review
	Glenrock Station, Sawdon Flats	-44.10	170.54	*Brachaspis robustus*	Tenure Review
	Godley Peak, Hall Range	-43.70	170.47	*Brachaspis* "hunter"	Tenure Review
	Grays Hills	-44.27	170.33	*Brachaspis robustus*	White 1994

Hawkdun Range	-44.83	170.00		Patrick 1994
Hopkins Valley, Lake Ohau	-44.17	169.88		Bigelow 1967
Hunter Hills	-44.64	170.91		Trewick & Morris 2008
Ida Range	-44.94	170.18		Patrick 1994
Invercroy	-44.48	170.57	*Brachaspis* "hunter"	Tenure Review
Kakanui Mountains	-45.06	170.38		Patrick 1991
Kirkliston, Basin Stream Headwaters	-44.48	170.42		Tenure Review
Kirkliston, Hay Stream Headwaters	-44.47	170.53		Tenure Review
Kurow	-44.73	170.47	*Brachaspis robustus*	Phoenix Collection
Lake Tekapo	-44.02	170.58		Trewick 2001
Mackenzie River	-44.18	170.52	*Brachaspis robustus*	White 1994
Maitland Stream	-44.23	169.72		White 1975
Malte Brun Range	-43.61	170.24		White 1975
Maryburn, Tekapo Terraces	-44.17	170.37	*Brachaspis robustus*	Tenure Review
Mesopotamia Mt Toby	-43.68	170.72		Tenure Review
Mesopotamia, Bush Stream	-43.63	170.85	*Brachaspis* "lowland"	Tenure Review
Mt Buster	-44.93	170.22		Patrick 1994
Mt Cook	-43.71	170.11		Phoenix collection
Mt Cook	-43.69	170.11		Bigelow 1967
Mt Dobson	-43.97	170.67		Trewick 2001
Mt Hay, Two Thumbs Range	-43.96	170.61	*Brachaspis* "hunter"	Tenure Review
Mt Ida Syndicate	-44.84	170.23		Tenure Review
Mt Kirkliston	-44.55	170.52		Bigelow 1967
Mt Melina Ridge	-44.45	169.55		Bigelow 1967
Mt Nimrod	-44.44	170.80	*Brachaspis* "hunter"	Phoenix collection
Mt St Bathans	-44.76	169.80		Trewick 2001
Mt Sutton	-44.23	169.78		Trewick 2001
Mt Wakefield	-43.70	170.13		Bigelow 1967
Ohau River	-44.32	170.18	*Brachaspis robustus*	Trewick 2001
Ohau River	-44.34	170.19	*Brachaspis robustus*	White 1994
Pisgah Downs	-44.99	170.53		Tenure Review
Pukaki River	-44.28	170.19	*Brachaspis robustus*	White 1994
Rangitata Headwaters	-43.51	170.82		Bigelow 1967
Richmond, Richmond Range	-43.85	170.64		Tenure Review
Rocky Top	-44.78	170.60		Trewick 2001
Sawdon Stream	-44.13	170.52	*Brachaspis robustus*	White 1994
Sawdon Stream	-44.12	170.52	*Brachaspis robustus*	White 1994
Sawdon Stream	-44.12	170.52	*Brachaspis robustus*	White 1994
Snow River	-44.22	170.50	*Brachaspis robustus*	White 1994
Tekapo Canal	-44.03	170.45	*Brachaspis robustus*	White 1994
Tekapo Delta	-44.34	170.20	*Brachaspis*	Trewick 2001

				robustus	
	Tekapo Powerhouse	-44.01	170.46	*Brachaspis robustus*	White 1994
	Tekapo River	-44.17	170.40	*Brachaspis robustus*	White 1994
	The Grampians	-44.27	170.51	*Brachaspis* "hunter"	Tenure Review
	The Grampians, Black Rocks	-44.33	170.54	*Brachaspis* "hunter"	Tenure Review
	The Grampians, Mackenzie River	-44.18	170.54	*Brachaspis robustus*	Tenure Review
	The Grampians, Snow River	-44.22	170.50	*Brachaspis robustus*	Tenure Review
	The Wolds, Nth Tekapo Canal	-44.07	170.40	*Brachaspis robustus*	Tenure Review
	The Wolds, Tekapo River Terraces	-44.08	170.43	*Brachaspis robustus*	Tenure Review
	Twin Peaks Wether Range	-44.58	169.76		Tenure Review
***Brachaspis nivalis* (SA)**	Arrowsmith	-43.37	171.01		Trewick 2001
	Arthurs Pass	-42.91	171.58		Bigelow 1967
	Blackbirch Marlborough	-41.90	173.68		Bigelow 1967
	Cameron Valley, Mt Arrowsmith	-43.34	170.97		Bigelow 1967
	Camp Stream Saddle, Craigieburn	-43.13	171.70		White 1974; 1991
	Cass	-43.03	171.78		Bigelow 1967
	Castle Hill	-43.23	171.72	*Brachaspis* "lowland"	Tenure Review
	Clarence Valley, Lake Tennyson	-42.24	172.77		Bigelow 1967
	Cora Lynn, Pylon Gully	-43.04	171.71	*Brachaspis* "lowland"	Tenure Review
	Craigieburn	-43.12	171.68		Mason 1971
	Craigieburn	-43.12	171.70		Trewick 2001
	Craigieburn Range	-43.19	171.63		Bigelow 1967
	Craigieburn, Broken River Headwaters	-43.13	171.67		Watson 1970
	Dee Stream	-42.00	173.70		Bigelow 1967
	Dee Stream, Bluff Station	-42.00	173.76		Trewick & Morris 2008
	Double Hill	-43.32	171.29		Tenure Review
	Erewhon Park, Mt Potts Range	-43.50	170.90	*Brachaspis* "hunter"	Tenure Review
	Erewhon Park, Potts River	-43.58	170.94	*Brachaspis* "lowland"	Tenure Review
	Erewhon, Lizard Valley	-43.48	170.89		Tenure Review
	Foggy Peak	-43.28	171.75		Trewick 2001
	Glenfalloch, Charlie Stream	-43.38	171.17	*Brachaspis* "lowland"	Tenure Review
	Glenfalloch, Lake Stream	-43.38	171.15	*Brachaspis* "lowland"	Tenure Review
	Glenfalloch, Neil Stream	-43.39	171.18	*Brachaspis* "lowland"	Tenure Review
	Glenfalloch, Palmer Range	-43.35	171.24		Tenure Review
	Glenthorne, Birdwood Range	-43.14	171.44		Tenure Review
	Glenthorne, Boundary Stream	-43.16	171.42	*Brachaspis* "lowland"	Tenure Review
	Glenthorne, Fanghill Stream	-43.13	171.37	*Brachaspis* "lowland"	Tenure Review
	Hakatere, Craddock Stream	-43.57	171.01	*Brachaspis* "lowland"	Tenure Review
	Hodder Valley, Tapuanuka	-41.98	173.63		Bigelow 1967

	Island Hills Mt Skedaddle	-42.68	172.51		Tenure Review
	Island Hills Mt Skedaddle	-42.68	172.51		Tenure Review
	Island Hills Mt Skedaddle	-42.67	172.51		Tenure Review
	Jagged Stream, Rakaia Valley	-43.33	171.04		Bigelow 1967
	Kowhai River	-42.32	173.57		Bigelow 1967
	Lochaber Mt Peel	-43.85	171.15	*Brachaspis* "hunter"	Tenure Review
	Manuka Point	-43.26	171.19		Tenure Review
	Manuka Point, Rakaia Valley	-43.28	171.18	*Brachaspis* "lowland"	Tenure Review
	Mt Arrowsmtih	-43.49	171.11		Tenure Review
	Mt Cupola	-41.99	172.72		Batcheler 1967
	Mt Hutt	-43.47	171.53		Bigelow 1967
	Mt Hutt	-43.50	171.54		Phoenix collection
	Mt Lyford	-42.45	173.15		Trewick 2001
	Mt Olympus	-43.19	171.60		Phoenix collection
	Mt Olympus	-43.19	171.61		Phoenix collection
	Mt Olympus	-43.19	171.61		Phoenix collection
	Mt Olympus	-43.19	171.61		Phoenix collection
	Mt Patriarch, Richmond Range	-41.61	173.23		Phoenix collection
	Mt St Patrick	-42.44	172.74		Bigelow 1967
	Mt Storm, Puteteraki Range	-43.14	172.10		Bigelow 1967
	Mt Terako	-42.45	173.15		Bigelow 1967
	Porter Heights	-43.27	171.63		Mason 1971
	Porter River	-43.25	171.73	*Brachaspis* "lowland"	Phoenix collection
	Porter River	-43.29	171.66		Trewick & Morris 2008
	Rainbow Skifield	-41.87	172.86		Phoenix collection
	Red Spur, Mead Stream	-41.96	173.76		Trewick & Morris 2008
	Rolleston Range	-43.19	171.25		Bigelow 1967
	Seaward Kaikouras	-42.20	173.72		Bigelow 1967
	Shale Peak, Mt Tekoa	-42.61	172.64		Bigelow 1967
	Speargrass Creek, Travers Range	-41.87	172.76		Bigelow 1967
	Torlesse Range Canterbury	-43.28	171.75		Bigelow 1967
	Turk Ridge	-42.13	172.75		White 1975
	Winterslow	-43.55	171.34		Tenure Review
Paprides dugdali	Awakino	-44.78	170.36		Tenure Review
	Blue Mountains, nr Clutha	-45.90	169.37		Bigelow 1967
	Blue Mountains, nr Clutha	-45.86	169.43		Phoenix collection
	Castle Dent Run Block	-45.77	169.65		Tenure Review
	Ida Range	-44.96	170.13		Patrick 1994
	Lake Mahinerangi	-45.82	169.93		Bigelow 1967
	Lammerlaw Top Lake Onslow	-45.66	169.64		Phoenix collection
	Lammermoor Range	-45.67	169.77		Bigelow 1967
	Little Mt Ida	-44.96	170.06		Tenure Review

	Lochaber Mt peel	-43.85	171.15	Tenure Review
	Loganburn Reservoir	-45.54	169.94	Barratt & Patrick 1987
	Manuka Point	-43.26	171.19	Tenure Review
	Maungatua, Dunedin	-45.89	170.10	Phoenix collection
	Mt Anglem, Stewart Is.	-46.74	167.92	Bigelow 1967
	Mt Benger	-45.59	169.25	Dickinson *et al.* 1998
	Mt Buster	-44.93	170.24	Patrick 1994
	Mt Maungatua	-45.91	170.09	Bigelow 1967
	Mt Prospect	-45.42	167.94	Tenure Review
	Mt Teviot	-45.59	169.54	Phoenix collection
	Rock Pillar Range	-45.57	169.96	Phoenix collection
	Silver Peaks	-45.78	170.34	Anonymous 1986
	Slate Basin	-45.33	168.38	Mark *et al.* 1989
	Slate Range	-45.53	168.63	Dickinson *et al.* 1998
	South Rough Ridge	-45.40	169.74	Bigelow 1967
	South Rough Ridge	-45.41	169.74	Patrick 1989
	Southern Old Man Range	-45.44	169.25	Dickinson *et al.* 1998
	Waikaia Bush	-45.53	169.00	Dickinson *et al.* 1998
	Waipora Falls	-45.90	169.98	Bigelow 1967
	Way Spur	-45.30	168.62	Mark *et al.* 1989
Paprides nitidus	Arthurs Pass	-42.94	171.57	Bigelow 1967
	Arthurs Pass	-42.93	171.57	Peterson 1968
	Ashburton Gorge	-43.63	171.22	Bigelow 1967
	Blackbirch Marlborough	-41.74	173.81	Bigelow 1967
	Cameron Glacier	-43.36	171.01	Peterson 1968
	Cameron Valley, Wild Mans Range	-43.36	171.01	Bigelow 1967
	Camp Stream Saddle, Craigieburn	-43.13	171.70	White 1974; 1991
	Cass	-43.03	171.78	Bigelow 1967
	Cora Lynn, Mt Horrible	-43.03	171.71	Tenure Review
	Craigieburn	-43.12	171.70	Mason 1971
	Craigieburn Range	-43.28	171.68	Bigelow 1967
	Craigieburn, Broken River Headwaters	-43.12	171.69	Watson 1970
	Double Hill	-43.32	171.29	Tenure Review
	Dun Mountain	-41.35	173.37	Bigelow 1967
	Erewhon Park, Grasslands	-43.59	170.96	Tenure Review
	Erewhon Park, Mt Potts Range	-43.50	170.90	Tenure Review
	Erewhon, Cloudy Stream	-43.44	170.76	Tenure Review
	Foggy Peak, Christchurch	-43.28	171.75	Phoenix collection
	Fox Peak	-43.86	170.81	Peterson 1968
	Fox Peak	-43.86	170.81	Phoenix collection
	Fox Peak	-43.85	170.81	Phoenix collection
	Fox Peak	-43.86	170.82	Phoenix collection
	Garnet Peak	-42.55	172.41	Peterson 1968

Glenfalloch, Lagoon Peak	-43.38	171.24	Tenure Review
Glenfalloch, Palmer Range	-43.35	171.24	Tenure Review
Glenthorne, Birdwood Range	-43.14	171.44	Tenure Review
Hamilton Peak	-43.12	171.70	Peterson 1968
Hanmer Springs	-42.49	172.84	Bigelow 1967
Island Hills, Mt Skedaddle	-42.67	172.51	Tenure Review
Kaikouras	-42.30	173.53	Bigelow 1967
Lake Pukaki	-44.16	170.22	Phoenix collection
Lake Tennyson	-42.20	172.74	Peterson 1968
Lead Hills	-40.90	172.57	Peterson 1968
Lead Hills, Boulder Lake	-40.89	172.58	Bigelow 1967
Lewis Pass	-42.38	172.38	Bigelow 1967
Lewis Pass	-42.38	172.38	Peterson 1968
Lewis Pass	-42.38	172.39	Phoenix collection
Lindis Valley	-44.75	169.51	Phoenix collection
Manuka Point	-43.26	171.19	Tenure Review
Mesopotamia	-43.68	170.72	Tenure Review
Mt Arrowsmith	-43.49	171.11	Tenure Review
Mt Arthur	-41.20	172.72	Bigelow 1967
Mt Chrome	-41.67	173.06	Peterson 1968
Mt Cupola, Dry Face	-42.01	172.77	Batcheler 1967
Mt Cupola, Hut Face	-42.00	172.74	Batcheler 1967
Mt Fitzwilliam	-43.17	171.53	Phoenix collection
Mt Fitzwilliam	-43.17	171.52	Phoenix collection
Mt Fyffe	-42.32	173.59	Peterson 1968
Mt Fyffe	-42.32	173.59	Phoenix collection
Mt Hutt	-43.53	171.54	Bigelow 1967
Mt Hutt	-43.53	171.54	Peterson 1968
Mt Hutt	-43.50	171.54	Phoenix collection
Mt Hutt	-43.52	171.55	Phoenix collection
Mt Hutt	-42.12	172.78	White 1975
Mt Ida, Craigieburn	-43.23	171.54	Phoenix collection
Mt Lyford	-42.45	173.15	Phoenix collection
Mt Olympus	-43.19	171.60	Phoenix collection
Mt Olympus	-43.19	171.61	Phoenix collection
Mt Olympus	-43.19	171.61	Phoenix collection
Mt Olympus	-43.19	171.60	Phoenix collection
Mt Olympus	-43.19	171.60	Phoenix collection
Mt Olympus	-43.19	171.60	Phoenix collection
Mt Olympus	-43.19	171.61	Phoenix collection
Mt Olympus	-43.19	171.61	Phoenix collection
Mt Patriarch	-41.61	173.23	Phoenix collection
Mt Peel	-41.13	172.58	Bigelow 1967
Mt Peel, Canterbury	-43.84	171.17	Bigelow 1967

	Mt Peel, Cobb	-41.14	172.59		Phoenix collection
	Mt Richmond	-41.47	173.39		Bigelow 1967
	Mt Tekoa	-42.67	172.62		Bigelow 1967
	Mt Tekoa	-42.67	172.63		Peterson 1968
	Mt Travers	-42.02	172.75		Peterson 1968
	Oxford	-43.24	172.08		Bigelow 1967
	Porter Heights	-43.27	171.63		Mason 1971
	Porters Pass	-43.29	171.75		Peterson 1968
	Pukaki Dam	-44.19	170.15		Phoenix collection
	Puketeraki Range	-43.09	172.08		Bigelow 1967
	Rainbow Skifield	-41.88	172.86		Phoenix collection
	Rainbow Skifield	-41.87	172.86		Phoenix collection
	Rainbow Skifield	-41.87	172.86		Phoenix collection
	Rangitata Headwaters	-43.51	170.82		Bigelow 1967
	Red Hill Nelson	-41.13	173.56		Bigelow 1967
	Richmond, Camp Stream Coal River	-43.81	170.65		Tenure Review
	Rolleston Range	-43.17	171.21		Bigelow 1967
	St Arnaud Range	-41.83	172.89		White 1975
	Sugar Loaf Cass	-43.04	171.78		Phoenix collection
	Temple Basin	-42.91	171.58		Mason 1971
	Torlesse Range	-43.28	171.82		Bigelow 1967
	Travers, St Arnaud Ranges	-41.97	172.73		Bigelow 1967
	Turk Ridege	-42.13	172.75		White 1975
	Two Thumb Range	-43.99	170.64		Bigelow 1967
	Winterslow	-43.55	171.34		Tenure Review
	Woodbank	-42.55	172.66		Tenure Review
Sigaus australis	Alexandra Airport	-45.21	169.36		Phoenix collection
	Alexandra Tailings	-45.25	169.37	*Sigaus* sp. A.	Morris 2002
	Awakino	-44.83	170.37		Tenure Review
	Awakino Ski Field	-44.78	170.31		Phoenix collection
	Bannockburn Nevis Rd	-45.16	169.10		Phoenix collection
	Beaumont	-45.70	169.69		Tenure Review
	Ben Ledi	-44.99	170.45		Tenure Review
	Ben Ledi	-45.05	170.39		Tenure Review
	Ben Ledi	-45.00	170.44		Tenure Review
	Ben Nevis	-45.18	168.90		Tenure Review
	Ben Ohau, Dobson Valley	-43.99	169.99		Bigelow 1967
	Black Forest, Lake Benmore	-44.42	170.26		Tenure Review
	Black Forest, Stony River	-44.40	170.50		Tenure Review
	Branch Creek Mt Cardrona	-44.86	168.96	*Sigaus obelisci*	Tenure Review
	Caithness	-45.25	170.52		Tenure Review
	Cambrian Hills	-44.86	169.70		Tenure Review
	Cameron Valley, Mt Arrowsmith	-43.42	170.92		Bigelow 1967

Camp Stream Saddle, Craigieburn	-43.13	171.70		White 1974; 1991
Cardrona	-44.86	168.95		Phoenix collection
Cardrona	-44.86	168.95		Phoenix collection
Cardrona	-44.86	168.95		Phoenix collection
Cardrona	-44.86	168.95		Phoenix collection
Cardrona	-44.86	168.95		Phoenix collection
Cardrona	-44.86	168.96		Phoenix collection
Cardrona	-44.88	168.95		Phoenix collection
Cardrona	-44.88	168.96		Phoenix collection
Cardrona	-44.88	168.96		Phoenix collection
Cardrona	-44.87	168.97		Phoenix collection
Cardrona	-44.87	168.97		Phoenix collection
Castle Dent, Run Block	-45.77	169.65		Tenure Review
Chetwynd, Albury Range	-44.16	170.73		Tenure Review
Chetwynd, Upper Tramway Stream	-44.15	170.72		Tenure Review
Cloudy Peak	-44.91	169.54		Tenure Review
Cloudy Peak, Dry Creek	-44.92	169.53		Tenure Review
Cluden Station, Cluden Pass	-44.80	169.71		Tenure Review
Cluden Station, Dunstan Mountains	-44.86	169.60		Tenure Review
Conical Hill, Routeburn Track	-44.72	168.17		Phoenix collection
Conroys Dam	-45.28	169.32		Trewick 2008
Courthill	-45.37	169.25		Tenure Review
Courthill Old Man Range	-45.35	169.22	*Sigaus obelisci*	Tenure Review
Craigieburn	-43.14	171.70		Mason 1971
Craigieburn Range	-43.20	171.63		Bigelow 1967
Craigieburn Range	-43.16	171.67		Trewick 2008
Craigieburn, Broken River Headwaters	-43.13	171.67		Watson 1970
Crawford Hills	-45.22	169.57		Trewick 2008
Dana Peaks	-45.22	167.63	*Sigaus takahe*	Morris 2003
Dana Peaks	-45.23	167.61	*Sigaus takahe*	Morris 2003
Dana Peaks	-45.24	167.60	*Sigaus takahe*	Morris 2003
Dana Peaks	-45.22	167.61	*Sigaus takahe*	Morris 2003
Dansey District	-45.04	170.48		Patrick 1991
Danseys Pass	-44.95	170.38		Bigelow 1967
Danseys Pass	-44.95	170.37		Phoenix collection
Danseys Pass	-44.95	170.37		Trewick 2008
Deep Creek, Camp Creek	-44.71	169.41		Tenure Review
Domett Downs	-44.87	170.40		Tenure Review
Double Hill	-43.32	171.29		Tenure Review
Duffers Saddle, Nevis Rd	-45.17	169.18		Phoenix collection
Dunstan Range	-45.09	169.34		Trewick 2008
Earl Mountains	-44.95	167.99	*Sigaus homerensis*	Trewick 2008
Earncleugh	-45.24	169.35	*Sigaus* sp. A.	Trewick 2008

Earnscleugh Tailings	-45.24	169.36	*Sigaus* sp. A.	Morris 2002
East Dome	-45.61	168.69	*Sigaus obelisci*	Dickinson *et al.* 1998
Erewhon Park, Mt Potts Range	-43.50	170.90		Tenure Review
Erewhon, Cloudy Stream	-43.50	170.77		Tenure Review
Erewhon, Lizard Gully	-43.48	170.89		Tenure Review
Erewhon, The Point	-43.52	170.83		Tenure Review
Ewe Range	-44.60	169.92		Patrick 1994
Flagstaff Hill	-45.83	170.46		Trewick 2008
Foggy Peak	-43.28	171.75		Trewick 2008
Fox Peak	-43.85	170.81		Bigelow 1967
Fox Peak	-43.86	170.81		Phoenix collection
Fox Peak	-43.85	170.81		Phoenix collection
Fox Peak	-43.86	170.82		Phoenix collection
Fox Peak	-43.85	170.81		Phoenix collection
Galloway Station	-45.21	169.49	*Sigaus* sp. A.	Morris 2002
Gem Lake	-45.57	169.11	*Sigaus obelisci*	Dickinson *et al.* 1998
Gem Lake	-45.57	169.10	*Sigaus obelisci*	Tenure Review
Glen Dene	-44.49	169.19		Tenure Review
Glenaray Station, Dome Hut Ridge	-45.57	168.82		Tenure Review
Glenaray Station, Garvie Mountains	-45.52	168.79		Tenure Review
Glenfalloch, Lagoon Peak	-43.38	171.24		Tenure Review
Glenfalloch, Palmer Range	-43.35	171.24		Tenure Review
Glenfellan	-45.45	168.76		Tenure Review
Glenmore	-43.74	170.35		Tenure Review
Glenorchy, Lake Wakatipu	-44.85	168.44		Bigelow 1967
Glenrock	-44.11	170.58		Tenure Review
Glenthorne, Birdwood Range	-43.14	171.44		Tenure Review
Godley Peak, Hall Range	-43.69	170.45		Tenure Review
Gorge Creek	-45.38	169.27		Tenure Review
Gorge Creek, Old Man Range	-45.39	169.20		Tenure Review
Halfway Bay Station	-45.19	168.65		Tenure Review
Happy Valley	-45.20	169.07		Tenure Review
Happy Valley	-45.17	169.09		Tenure Review
Harris Saddle	-44.73	168.17		Trewick 2008
Hawkdun Range	-44.89	170.05		Patrick 1994
Headlong Peak	-44.52	168.58		Watt 1980
Homer Tunnel	-44.77	167.99	*Sigaus homerensis*	Morris 2003
Homer Tunnel	-44.77	167.99	*Sigaus homerensis*	Morris 2003
Homer Tunnel	-44.76	167.99	*Sigaus homerensis*	Morris 2003
Homer Tunnel	-44.76	167.99	*Sigaus homerensis*	Phoenix collection
Hunter Valley	-44.27	169.49		Bigelow 1967
Hut Creek	-44.89	167.99	*Sigaus homerensis*	Morris 2003

Hut Creek	-44.88	167.99	*Sigaus homerensis*	Morris 2003
Hut Creek	-44.88	167.99	*Sigaus homerensis*	Morris 2003
Ida Range	-44.94	170.19		Patrick 1994
Invercroy	-44.56	170.55	*S.* "Undescribed"	Tenure Review
Invercroy	-44.48	170.57		Tenure Review
Invercroy Bog	-44.47	170.56		Tenure Review
Irishman Creek	-44.04	170.34		Tenure Review
Islay Downs	-45.24	170.47		Tenure Review
Jane Peak	-45.33	168.34	*Sigaus obelisci*	Mark *et al.* 1989
Kakanui Mountains	-45.19	170.52		Trewick 2008
Kawarau Gorge Mining Centre	-45.04	169.12		Phoenix collection
Kye Burn Stream	-44.95	170.37	*Sigaus obelisci*	Poulin 1995
Kye Burn Stream	-44.95	170.37		Poulin 1995
Kyeburn Fraters, Hut Creek	-44.84	170.28		Tenure Review
Kyeburn, Green Gully Catchment	-44.88	170.33		Tenure Review
Lake Dunstan	-44.97	169.27		Phoenix collection
Lake Harris, Routeburn	-44.73	168.18		Phoenix collection
Lake Hawea	-44.56	169.37		Tenure Review
Lake Hawea	-44.56	169.36		Tenure Review
Lake Hawea	-44.56	169.40		Tenure Review
Lake Hawea	-44.55	169.44		Tenure Review
Lake Hawea	-44.55	169.44		Tenure Review
Lake Ohau, Kettle Holes	-44.31	169.92		Patrick 1992
Lake Pukaki	-44.16	170.22		Phoenix collection
Lake Te Anau	-45.07	167.94		Bigelow 1967
Lake Tekapo	-43.94	170.61		Bigelow 1967
Lake Waitaki	-44.66	170.42		Bigelow 1967
Lake Wakitipu	-44.84	168.31		Bigelow 1967
Lauder Donald Stuarts Creek	-44.88	169.67		Tenure Review
Lindis Pass	-44.59	169.65		Bigelow 1967
Lindis Pass	-44.58	169.64		Phoenix collection
Lindis Pass	-44.61	169.55		Trewick 2008
Lindis Valley	-44.75	169.51		Phoenix collection
Little Mt Ida	-44.96	170.06		Tenure Review
Little Valley Rd	-45.28	169.43	*S.* "Undescribed"	Trewick 2008
Little Valley Rd	-45.28	169.43		Trewick 2008
Loch Linnhe Whittens, Sproules Face	-45.28	168.88		Tenure Review
Loch Linnhe, James Peak	-45.25	168.85	*Sigaus obelisci*	Tenure Review
Loch Linnhe, Sproules Creek	-45.24	168.84	*Sigaus obelisci*	Tenure Review
Loch Linnhe, Staircase Creek	-45.19	168.82	*Sigaus obelisci*	Tenure Review
Lochaber, Mt Peel	-43.85	171.15		Tenure Review
Loganburn Reservoir	-45.54	169.94	*Sigaus obelisci*	Barratt & Patrick 1987

Long Gully	-44.77	169.38		Tenure Review
Long Gully, Luggate-Tarras Rd	-44.77	169.37		Tenure Review
Longlands Station	-45.16	170.39		Tenure Review
Maitland Stream	-44.23	169.72		White 1975
Malte Brun Range	-43.55	170.28		White 1975
Manor Burn	-45.25	169.56		Phoenix collection
Manorburn District	-45.37	169.64		Patrick 1989
Manuka Point	-43.26	171.19		Tenure Review
Mataura Valley	-45.40	168.51	*Sigaus obelisci*	Tenure Review
Mataura Valley	-45.38	168.52		Tenure Review
Mesopotamia	-43.68	170.72		Tenure Review
Mid Dome	-45.59	168.54	*Sigaus obelisci*	Bigelow 1967; Dickinson *et al.* 1998
Monkey Creek	-44.80	168.00	*Sigaus homerensis*	Morris 2003
Mt Algidus	-43.25	171.35		Bigelow 1967
Mt Alta	-44.54	168.95		White 1975
Mt Arrowsmith	-43.49	171.11		Tenure Review
Mt Aspiring	-44.49	168.70		Bigelow 1967
Mt Aspiring Station, Rob Roy Flats	-44.51	168.75		Tenure Review
Mt Aspiring Station, Brides Veil Flat	-44.50	168.69		Tenure Review
Mt Aspiring Station, Glenfinnan Face	-44.50	168.82		Tenure Review
Mt Benger	-45.60	169.25	*Sigaus obelisci*	Dickinson *et al.* 1998
Mt Buster	-44.94	170.24		Patrick 1994
Mt Cook	-43.74	170.09		Bigelow 1967
Mt Creighto,n Craigellachie	-44.93	168.58	*Sigaus obelisci*	Tenure Review
Mt Creighton, Ben More	-44.95	168.52	*Sigaus obelisci*	Tenure Review
Mt Creighton, Ben More	-44.95	168.52		Tenure Review
Mt Creighton, Dead Horse Basin	-44.94	168.55	*Sigaus obelisci*	Tenure Review
Mt Creighton, Luna Basin	-44.92	168.48		Tenure Review
Mt Creighton, Minor Peak	-44.93	168.45		Tenure Review
Mt Creighton, Wire Creek	-44.90	168.47	*Sigaus obelisci*	Tenure Review
Mt Creighton, Wire Creek	-44.90	168.47		Tenure Review
Mt Dasher	-45.18	170.50		Tenure Review
Mt Dobson	-43.95	170.66		Trewick 2008
Mt Grand	-44.63	169.34		Tenure Review
Mt Hutt	-43.50	171.54		Phoenix collection
Mt Hutt	-43.52	171.55		Phoenix collection
Mt Hutt	-43.50	171.53		White 1975
Mt Ida Syndicate	-44.84	170.23		Tenure Review
Mt John	-43.99	170.46		Trewick 2008
Mt Kirkliston	-44.53	170.53		Bigelow 1967
Mt Melina	-44.45	169.56		Bigelow 1967
Mt Olympus	-43.19	171.60		Phoenix collection
Mt Olympus	-43.19	171.61		Phoenix collection

Mt Olympus	-43.19	171.61		Phoenix collection
Mt Olympus	-43.19	171.60		Phoenix collection
Mt Olympus	-43.19	171.60		Phoenix collection
Mt Olympus	-43.19	171.60		Phoenix collection
Mt Olympus	-43.19	171.61		Phoenix collection
Mt Olympus	-43.19	171.61		Phoenix collection
Mt Peel, Canterbury	-43.85	171.16		Bigelow 1967
Mt Scott	-44.99	168.93		Trewick 2008
Mt St Bathans	-44.73	169.75		Bigelow 1967
Mt St Bathans	-44.73	169.76		Trewick 2008
Mt St Bathans, Dunstan Creek	-44.75	169.72		Tenure Review
Mt St Bathans, Sawtooth Creek	-44.80	169.71		Tenure Review
Mt Sutton	-44.22	169.78		Trewick 2008
Mt Tennyson	-45.46	168.82	*Sigaus obelisci*	Dickinson *et al.* 1998
Mt Torlesse, Canterbury	-43.27	171.81		Bigelow 1967
Mutton Town Tailings	-45.22	169.35	*Sigaus* sp. A.	Morris 2002
Nokomai Range	-45.50	168.72		Dickinson *et al.* 1998
North Dunstan	-45.00	169.48		Phoenix collection
Obelisk	-45.28	169.21		Phoenix collection
Obelisk, Old Man Range	-45.34	169.21	*Sigaus obelisci*	Tenure Review
Obelisk, Old Man Range	-45.24	169.23	*Sigaus obelisci*	Trewick 2008
Obelisk, Old Man Range	-45.32	169.21	*Sigaus obelisci*	Phoenix collection
Old Man Range	-45.38	169.18	*Sigaus obelisci*	Bigelow 1967
Old Woman Range	-45.18	169.07		Trewick 2008
Omahau Downs	-44.33	170.19		Tenure Review
Omahau Hills	-44.23	169.95		Tenure Review
Omarama	-44.47	169.90		Phoenix collection
Omarama, Baldy Knob	-44.61	169.97		Tenure Review
Omarama, Mt St Cuthbert	-44.55	169.99		Tenure Review
Pisa Range	-44.88	169.20		Bigelow 1967
Pisgah Downs	-44.99	170.73		Tenure Review
Plateau Creek	-45.24	167.53	*Sigaus takahe*	Morris 2003
Porter Heights	-43.27	171.63		Mason 1971
Porters Pass	-43.30	171.74		Phoenix collection
Pukaki Dam	-44.19	170.15		Phoenix collection
Pukaki Dam	-44.19	170.15		Phoenix collection
Queenstown Area	-45.05	168.79		Bigelow 1967
Raggedy Range Rd	-45.12	169.65		Phoenix collection
Rangitata	-43.71	170.97		Bigelow 1967
Rastus Burn Basin	-45.04	168.82	*Sigaus obelisci*	Patrick *et al.* 1992
Rastus Burn Basin	-45.04	168.82		Patrick *et al.* 1992
Rastus Burn Reserve	-45.07	168.82		Phoenix collection
Rees Valley, Stn Mt Ferguson	-44.65	168.55	*Sigaus* "Queenstown"	Tenure Review

Remarkables	-45.05	168.81		Trewick 2008
Remarkables Ski Field	-45.03	168.80		Phoenix collection
Remarkables Ski Field	-45.06	168.81		Phoenix collection
Remarkables Ski Field Rd	-45.03	168.80		Phoenix collection
Richmond Richmond Range	-43.85	170.64		Tenure Review
Rob Roy	-44.47	168.73		Trewick 2008
Robrosa Criffel Range Summit	-44.83	169.12	*Sigaus obelisci*	Tenure Review
Robrosa Pisa Tops	-44.88	169.18	*Sigaus obelisci*	Tenure Review
Rock Pillar Ranges	-45.42	170.07		Trewick 2008
Rocky Top	-44.78	170.30		Trewick 2008
Rolleston Range	-43.17	171.21		Bigelow 1967
Rough Ridge	-45.32	169.83		Bigelow 1967
Sealy Tarns	-43.71	170.08		Trewick 2008
Shag Valley	-45.30	170.58		Tenure Review
Shenley Hakataramea Valley	-44.26	170.64		Tenure Review
Shortlands	-45.00	170.34		Tenure Review
Shortlands	-45.00	170.34		Tenure Review
Shortlands	-45.03	170.33		Tenure Review
Shortlands	-44.99	170.34		Tenure Review
Shortlands	-44.99	170.32		Tenure Review
Silver Hill	-44.18	170.76		Tenure Review
Silver Island	-44.45	169.35		Edwards & Logan 1999
Silver Peaks	-45.78	170.34		Anonymous 1986
Silverbirch Station	-45.61	169.22	*Sigaus obelisci*	Tenure Review
Simons Hill	-44.24	170.30	*Sigaus* "Bigelowi"	Tenure Review
Simons Pass	-44.19	170.28	*Sigaus* sp. A.	Tenure Review
Simons Pass	-44.23	170.32		Phoenix collection
Simons Pass, SW Corner	-44.23	170.21	*Sigaus* sp. A.	Tenure Review
Slate Basin	-45.32	168.38	*Sigaus obelisci*	Mark *et al.* 1989
Snow Farm	-44.88	169.09		Phoenix collection
Soldiers Syndicate, Boundary stream	-44.88	170.16		Tenure Review
Soldiers Syndicate, Guffies Creek	-44.93	170.19		Tenure Review
Soldiers Syndicate, Long Spur	-44.87	170.22		Tenure Review
Symmetry Peaks	-45.29	168.57	*Sigaus obelisci*	Mark *et al.* 1989
Symmetry Peaks	-45.29	168.57		Mark *et al.* 1989
Takahe Valley	-45.28	167.65	*Sigaus takahe*	Morris 2003
The Dasher Jimmys Creek	-45.25	170.55		Tenure Review
The Dasher, Oteporo Spur	-45.21	170.52		Tenure Review
The Dasher, Siberia Hill	-45.16	170.53		Tenure Review
The Dasher, Siberia Hill	-45.16	170.52		Tenure Review
The Grampians	-44.27	170.51		Tenure Review
The Grampians, Black Rocks	-44.33	170.54		Tenure Review

	The Larches	-44.81	169.14		Tenure Review
	The Larches, Gold Diggings	-44.78	169.15		Tenure Review
	The Larches, Luggate Creek	-44.80	169.13		Tenure Review
	The Wandle Rock, Pillar Range	-45.38	170.14		Tenure Review
	The Wolds, West Marys Range	-44.14	170.24	*Sigaus* sp. A.	Tenure Review
	Torlesse Range, Canterbury	-43.25	171.82		Bigelow 1967
	Torlesse Range, Canterbury	-43.26	171.82		Phoenix collection
	Twin Peaks, Unnamed Stream	-44.58	169.80		Tenure Review
	Twin Peaks, Wether Range	-44.58	169.76		Tenure Review
	Walter Peak, McKinlays Creek	-45.16	168.50	*Sigaus* "Queenstown"	Tenure Review
	Walter Peak, White Burn	-45.22	168.39	*Sigaus* "Queenstown"	Tenure Review
	Whitecoomb Range	-45.64	169.09		Dickinson *et al.* 1998
Sigaus campestris	Allandale	-45.32	168.63		Tenure Review
	Bankside Scientific Reserve	-43.73	172.16		Emberson *et al*. 2011
	Bannockburn Nevis Rd	-45.16	169.10		Phoenix collection
	Beaumont, MacKays Creek	-45.65	169.69		Tenure Review
	Beaumont, Northern Block	-45.59	169.77		Tenure Review
	Ben Ledi	-44.98	170.46		Tenure Review
	Ben Lomond	-45.01	168.62		Bigelow 1967
	Blue Mts	-45.95	169.32		Bigelow 1967
	Bold Peak, Lake Hawea	-44.49	169.34		Bigelow 1967
	Castle Dent, Run Block	-45.77	169.95		Tenure Review
	Chetwynd, Albury Range	-44.16	170.73		Tenure Review
	Chetwynd, Coal Stream	-44.14	170.73		Tenure Review
	Chetwynd, upper Tramway Stream	-44.15	170.72		Tenure Review
	Cheviot	-42.82	173.29		Bigelow 1967
	Clent Hills	-43.53	171.19		Tenure Review
	Courthill	-45.37	169.25		Tenure Review
	Dansey District	-45.04	170.47		Patrick 1991
	Danseys Pass	-44.95	170.38		Bigelow 1967
	Danseys Pass	-44.95	170.37		Phoenix collection
	Domett Downs	-44.87	170.40		Tenure Review
	East Dome	-45.61	168.70		Dickinson *et al.* 1998
	Erewhon, Havelock River Faces	-43.45	170.72		Tenure Review
	Erewhon, Park Grasslands	-43.56	170.96		Tenure Review
	Flagstaff Hill	-45.83	170.46		Trewick 2008
	Fox Peak	-43.85	170.81		Bigelow 1967
	Fox Peak	-43.86	170.81		Phoenix collection
	Gem Lake	-45.57	169.10		Dickinson *et al.* 1998
	Glenrock	-44.11	170.58		Tenure Review
	Gorge Creek	-45.38	169.27		Tenure Review

Grange Hill, Hunter Hills	-44.49	170.82	Tenure Review
Hanmer Springs	-42.51	172.83	Bigelow 1967
Hector Mountains	-45.34	168.82	Bigelow 1967
Hermitage	-43.73	170.09	Bigelow 1967
Hooker Valley	-43.70	170.10	Bigelow 1967
Jane Peak	-45.33	168.34	Mark *et al.* 1989
Kaituna	-43.74	172.69	Bigelow 1967
Kaiwarua	-44.58	170.88	Tenure Review
Kinross	-45.28	170.62	Tenure Review
Kye Burn Stream	-44.95	170.37	Poulin 1995
Lake Forsythe	-43.79	172.73	Bigelow 1967
Lake Mahinerangi	-45.82	169.93	Bigelow 1967
Lake Tekapo	-43.94	170.61	Bigelow 1967
Lake Tekapo	-44.00	170.50	Phoenix collection
Lake Waitaki	-44.66	170.42	Bigelow 1967
Lammermoor Range	-45.67	169.85	Bigelow 1967
Loch Linnhe Whittens, Sproules Face	-45.28	168.88	Tenure Review
Lochaber, Mt Peel	-43.85	171.15	Tenure Review
Loganburn Reservoir	-45.54	169.94	Barratt & Patrick 1987
Manuka Point	-43.27	171.23	Tenure Review
Maryburn	-44.20	170.32	Tenure Review
Mataura Valley	-45.39	168.53	Tenure Review
Maungatua, Dunedin	-45.89	170.10	Phoenix collection
Mid Dome	-45.57	168.55	Bigelow 1967; Dickinson *et al.* 1998
Mt Benger	-45.60	169.25	Dickinson *et al.* 1998
Mt Creighton, Minor Peak	-44.93	168.44	Tenure Review
Mt Dasher	-45.18	170.50	Tenure Review
Mt Dobson	-43.99	170.69	Phoenix collection
Mt Gray	-42.98	171.99	Bigelow 1967
Mt Hay, nth branch Edward Stream	-43.96	170.61	Tenure Review
Mt Ida, Craigieburn	-43.23	171.54	Phoenix collection
Mt Maungatua	-45.88	170.12	Bigelow 1967
Mt Nimrod	-44.45	170.82	Tenure Review
Mt Studholm	-44.65	170.92	Phoenix collection
Mt Teviot	-45.59	169.54	Phoenix collection
Nokomai Range	-45.50	168.72	Dickinson *et al.* 1998
Obelisk, Old Man Range	-45.28	169.21	Phoenix collection
Obelisk, Old Man Range	-45.32	169.21	Phoenix collection
Obelisk, Old Man Range	-45.24	169.23	Phoenix collection
Old Man Range	-45.38	169.23	Trewick 2008
Oxford	-43.27	172.17	Bigelow 1967
Parasol Hill	-45.72	169.04	Dickinson *et al.* 1998
Patearoa	-45.32	169.96	Allan & McIntosh 1997

	Port Hills	-43.59	172.71	Bigelow 1967
	Purau	-43.66	172.76	Bigelow 1967
	Rakaia Valley	-43.41	171.56	Bigelow 1967
	Rangitata Valley	-43.73	171.09	Bigelow 1967
	Red Tussock Reserve	-45.55	168.00	Phoenix collection
	Rees Vally Station, Mt Ferguson	-44.67	168.53	Tenure Review
	Richmond Camp Stream Coal River	-43.81	170.65	Tenure Review
	Scotsburn	-43.92	171.21	Tenure Review
	Seaward Moss	-46.43	168.43	Bigelow 1967
	Silver Hill	-44.18	170.76	Tenure Review
	Silver Peaks	-45.78	170.34	Anonymous 1986
	Slate Range	-45.53	168.63	Dickinson *et al.* 1998
	Sumner	-43.60	172.76	Bigelow 1967; Northcroft 1967
	Symmetry Peaks	-45.29	168.57	Mark *et al.* 1989
	Takitimu Mountains	-45.68	167.87	Bigelow 1967
	The Dasher, Jimmys Creek	-45.25	170.55	Tenure Review
	The Dasher, Siberia Hill	-45.16	170.53	Tenure Review
	The Gorge	-43.90	171.19	Tenure Review
	Turinui Razorback	-45.37	170.72	Phoenix collection
	Turinui Razorback	-45.37	170.71	Phoenix collection
	Turinui Razorback	-45.37	170.71	Phoenix collection
	Waikaia Bush	-45.50	169.09	Dickinson *et al.* 1998
	Wairuna	-46.18	169.29	Bigelow 1967
	Walter Peak, Head McKinlays Creek	-45.16	168.50	Tenure Review
	Walter Peak, McKinlays Creek	-45.15	168.58	Tenure Review
	Walter Peak, White Burn	-45.22	168.39	Tenure Review
	Wilberforce Valley	-43.27	171.42	Bigelow 1967
Sigaus childi	Alexandra	-45.25	169.37	Jamieson 1999a & 1999b
	Alexandra Tailings	-45.25	169.37	Morris 2002
	Crawford Hills	-45.21	169.49	Jamieson 1999a, 1999b, 1996, Jamieson & Manly 1997
	Crawford Hills	-45.22	169.56	Jamieson 1999a, 1999b, 1997, Jamieson & Manly 1997
	Crawford Hills	-45.21	169.58	Jamieson 1999a, 1999b, 1998, Jamieson & Manly 1997
	Crawford Hills	-45.21	169.49	Jamieson 1999a, 1999b, 1999, Jamieson & Manly 1997, Morris 2002
	Crawford Hills	-45.21	169.55	Phoenix collection
	Earnscleugh	-45.22	169.34	Jamieson 1998
	Earnscleugh	-45.24	169.34	Jamieson 1998
	Earnscleugh	-45.23	169.35	Jamieson 1998

	Earnscleugh	-45.24	169.35	Jamieson 1998
	Earnscleugh	-45.23	169.36	Jamieson 1998
	Earnscleugh	-45.24	169.36	Jamieson 1998
	Earnscleugh	-45.24	169.35	Jamieson 1998
	Earnscleugh	-45.24	169.36	Jamieson 1998
	Earnscleugh	-45.24	169.35	Jamieson 1998
	Earnscleugh	-45.25	169.35	Jamieson 1998
	Earnscleugh	-45.24	169.35	Jamieson 1999a, 1999b, 1998, Morris 2002
	Earnscleugh	-45.24	169.35	Trewick 2008
	Galloway	-45.20	169.48	Jamieson 1999a, 1999b, 1996, Jamieson & Manly 1997
	Galloway	-45.22	169.47	Patrick 1989
	Graveyard Gully	-45.27	169.41	Bigelow 1967
	Graveyard Gully	-45.26	169.40	Trewick 2008
	Graveyard Gully Ridge	-45.27	169.40	Phoenix collection
	Litle Valley Rd	-45.23	169.48	Jamieson 1999a & 1999b
	Litle Valley Rd	-45.23	169.48	Jamieson 1999a & 1999b
	Litle Valley Rd	-45.24	169.48	Jamieson 1999a & 1999b
	Litle Valley Rd	-45.26	169.49	Jamieson 1999a & 1999b
	Litle Valley Rd	-45.26	169.48	Jamieson 1999a & 1999b
	Litle Valley Rd	-45.27	169.45	Jamieson 1999a & 1999b
	Litle Valley Rd	-45.28	169.44	Jamieson 1999a & 1999b
	Litle Valley Rd	-45.28	169.44	Jamieson 1999a & 1999b
	Litle Valley Rd	-45.25	169.42	Jamieson 1999a, 1999b, 1996, Jamieson & Manly 1997
	Litle Valley Rd	-45.26	169.41	Phoenix collection
	Litle Valley Rd	-45.28	169.43	Trewick 2008
	Lower Manor Burn	-45.24	169.46	Phoenix collection
	Manor Burn	-45.25	169.56	Phoenix collection
	Manor Burn Rd	-45.25	169.51	Jamieson 1999a, 1999b, 1996, Jamieson & Manly 1997
	Manor Burn Rd	-45.27	169.54	Jamieson 1999a, 1999b, 1996, Jamieson & Manly 1997
	Manor Burn Rd	-45.27	169.56	Jamieson 1999a, 1999b, 1996, Jamieson & Manly 1997
	Mutton Town Tailings	-45.22	169.35	Morris 2002
Sigaus minutus	Ahuriri River, Omarama	-44.47	169.94	Phoenix collection
	Black Forest North	-44.41	170.41	Tenure Review
	Blackforest	-44.37	170.37	Phoenix collection
	Cass River	-43.90	170.49	Patrick 1992

Cowans Hill, Tekapo	-44.02	170.49	Phoenix collection
Curraghmore	-44.37	170.37	Tenure Review
Edward Stream	-44.05	170.52	Jamieson 1999a
Edward Stream	-44.06	170.50	Phoenix collection
Glenmore Tarns Moraine	-43.87	170.42	Tenure Review
Glenmore Tarns Moraine	-43.87	170.42	Tenure Review
Glenmore Tarns Moraine	-43.87	170.42	Tenure Review
Glenmore, Cass River Delta	-43.88	170.48	Tenure Review
Glenrock Station, Sawdon Flats	-44.10	170.54	Tenure Review
Godley Peak, Hazard Range	-43.83	170.43	Tenure Review
Grays Hills	-44.28	170.39	Jamieson 1999a
Haldon Rd	-44.34	170.26	Jamieson 1999a
Happy Valley	-45.17	169.13	Tenure Review
Lake Tekapo	-43.94	170.61	Bigelow 1967
Lake Tekapo	-44.01	170.50	Jamieson 1999a
Lake Tekapo	-44.02	170.48	Trewick 2008
Maryburn, Tekapo floodplains	-44.22	170.35	Tenure Review
Mt Gerald, The Island	-43.77	170.56	Tenure Review
Mt Hay	-43.90	170.60	Tenure Review
Omahau Downs	-44.33	170.19	Tenure Review
Omahau Hills	-44.26	169.95	Tenure Review
Omarama	-44.49	169.97	Jamieson 1999a
Omarama, Old Man Creek	-44.53	169.97	Tenure Review
Richmond Coal Creek Washout	-43.80	170.58	Tenure Review
Simons Pass, Pukaki River Terraces	-44.26	170.21	Tenure Review
Simons Pass, Pukaki River Terraces	-44.25	170.21	Tenure Review
Simons Pass, Pukaki River Terraces	-44.25	170.23	Tenure Review
Simons Pass, Pukaki River Terraces	-44.24	170.19	Tenure Review
Simons Pass, Pukaki River Terraces	-44.23	170.20	Tenure Review
Simons Pass, Pukaki River Terraces	-44.23	170.20	Tenure Review
Simons Pass, Pukaki River Terraces	-44.22	170.18	Tenure Review
Tekapo River Terrace	-44.02	170.48	Jamieson 1999a
Tekapo River Terrace	-44.04	170.50	Patrick 1992
Tekapo Riverbed	-44.07	170.43	Patrick 1992
The Grampians Snow River	-44.22	170.50	Tenure Review
The Grampians, Mackenzie River	-44.18	170.54	Tenure Review
The Grampians, Snow River	-44.22	170.50	Tenure Review
The Grampians, Snow River	-44.22	170.50	Tenure Review
The Wolds	-44.08	170.42	Phoenix collection
The Wolds, Tekapo river Terraces	-44.08	170.42	Tenure Review
The Wolds, Tekapo River Terraces	-44.08	170.42	Tenure Review

	Twin Peaks, Ahuriri Outwash	-44.53	169.85	Tenure Review
***Sigaus piliferus* (LW)**	Armstrong Saddle	-39.78	176.17	Phoenix collection
	Awaawaroa Ridge, Waikaremoana	-38.78	177.14	Phoenix collection
	East Kaweka Range	-39.28	176.38	Phoenix collection
	Iwikau Village	-39.24	175.56	Phoenix collection
	Kaimanawa Range	-39.12	175.96	Bigelow 1967
	Kauaeranga Valley, Coromandel	-37.02	175.70	Bigelow 1967
	Kaweka Range	-39.29	176.38	Bigelow 1967
	Kuripapango Hill	-39.40	176.33	Phoenix collection
	Lake Waikaremoana	-38.75	177.16	Brock 1980
	Lake Waikaremoana	-38.75	177.16	Phoenix collection
	Makahu Spur, Kawekas	-39.29	176.40	Phoenix collection
	Maungatautari, Cambridge	-38.03	175.58	Bigelow 1967
	Mt Hikurangi	-38.29	178.22	Bigelow 1967
	Mt Hikurangi	-38.29	178.22	Phoenix collection
	Mt Karioi	-37.86	174.80	Trewick & Morris 2008
	Mt Piorongio	-37.98	175.12	Phoenix collection
	Mt Ruapehu	-39.31	175.53	Phoenix collection
	Mt Ruapehu, near Ohakune	-39.36	175.47	Phoenix collection
	Ohakune	-39.40	175.43	Phoenix collection
	Pirongia Range	-38.01	175.14	Trewick & Morris 2008
	Pohangina Saddle, Ruahines	-39.94	176.11	Bigelow 1967
	Pukahunui Valley	-38.92	176.65	Phoenix collection
	Rangipo Desert	-39.31	175.74	Phoenix collection
	Rangiwahia Hut	-39.89	176.02	Phoenix collection
	Rotorua	-38.12	176.20	Bigelow 1967
	Ruahine Corner Hut	-39.63	176.17	Phoenix collection
	Ruahine Range	-40.12	175.96	Bigelow 1967
	Ruakituri Valley	-38.81	177.45	Phoenix collection
	Smith Russell track	-39.34	176.32	Phoenix collection
	Taihape-Napier Rd	-39.47	176.01	Bigelow 1967
	Takarere Rd	-39.06	176.54	Phoenix collection
	Tauhara Summit Taupo	-38.70	176.16	Bigelow 1967
	Te Araroa	-37.64	178.36	Trewick & Morris 2008
	Tongariro National Park	-39.14	175.60	Bigelow 1967
	Waihaha River, Lake Taupo	-38.74	175.56	Bigelow 1967
	Waiouru	-39.50	175.75	Bigelow 1967
	Waipawa Saddle	-39.81	176.15	Phoenix collection
	Whenuakura	-38.77	175.64	Trewick & Morris 2008
***Sigaus piliferus* (MH)**	between Arete & Pukematawai	-40.75	175.43	Phoenix collection
	Bridge Peak, Tararuas	-40.94	175.27	Phoenix collection
	Mt Hector	-40.95	175.28	Phoenix collection

	Mt Holdsworth	-40.88	175.42	Phoenix collection
	Tararua Forest Park	-40.63	175.52	Bigelow 1967
	Tararua Rd, Arete	-40.75	175.43	Phoenix collection
	Tararua Rd, Dundas	-40.73	175.45	Phoenix collection
	Tararua Rd, Pukematawai	-40.75	175.42	Phoenix collection
Sigaus villosus	Craigieburn	-43.10	171.71	Mason 1971
	Craigieburn	-43.13	171.67	Watson 1970
	Craigieburn Range, Canterbury	-43.15	171.66	Bigelow 1967
	Double Hill	-43.34	171.28	Tenure Review
	Erewhon Cloudy Stream	-43.44	170.76	Tenure Review
	Fox Peak	-43.85	170.81	Bigelow 1967
	Fox Peak	43.85	170.80	Phoenix collection
	Fox Peak	43.84	170.79	Phoenix collection
	Glenfalloch, Palmer Range	-43.35	171.24	Tenure Review
	Glenthorne	-43.15	171.45	Tenure Review
	Hodder Valley, Tapuanuka	-41.98	173.64	Bigelow 1967
	Mesopotamia, Two Thumbs Range	-43.68	170.70	Tenure Review
	Mt Arrowsmith	-43.49	171.11	Tenure Review
	Mt Binser	-43.03	171.86	Bigelow 1967
	Mt Dobson	-43.93	170.66	Phoenix collection
	Mt Dobson	-43.94	170.67	Trewick 2008
	Mt Hamilton	-43.58	170.33	Bigelow 1967
	Mt Hutt	-43.49	171.54	Phoenix collection
	Porter Heights	-43.27	171.63	Mason 1971
	Porter Heights Ski Field	-43.27	171.64	Phoenix collection
	Richmond, Richmond Range	-43.86	170.66	Tenure Review
	Torlesse Range, Canterbury	-43.26	171.83	Bigelow 1967
	Wild Mans Brother Range	-43.39	171.01	Bigelow 1967

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Appendix Two

Anthropogenic cause of range shifts and gene flow between two grasshopper species revealed by environmental modelling, geometric morphometrics and population genetics

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Abstract

1. The range of a species is controlled by biotic and abiotic factors; both could have changed recently due to human activity.
2. We used environmental modelling, morphometric and genetic data to interpret ecological responses at the species boundary of a pair of New Zealand grasshoppers with very different ranges; one widespread (*Phaulacridium marginale*) and one restricted to semi-arid central/southern South Island (*Phaulacridium otagoense*).
3. Climate- and habitat-based distribution models for grasshoppers in the past (last glacial maximum), present and future (2070), in concert with modelling of vegetation patterns imply range and demographic expansion of *P. marginale* and stability of *P. otagoense.*
4. mtDNA sequence revealed four main lineages with pronounced differences in genetic diversity and geographical range. The widespread lineage associated with *P. marginale* revealed a signature of range expansion but regionally restricted lineages were geographically structured at a fine scale. Within the nar- row geographical range of *P. otagoense*, three mtDNA lineages resulted in high diversity, more typical of large stable populations.
5. Geometric analysis of pronotum shape identified individuals from a region of sympatry with mixed characteristics. Mismatch of phenotype, mtDNA lin- eage and nuclear DNA sequence indicates introgression between grasshopper species now in contact. This appears to be accompanied by *P. otagoense* range reduction through ecological competition.
6. Deforestation by people starting ~800 years ago best explains range change and resulting hybridisation of these grasshoppers. Anthropogenic habitat modifi- cation can have indirect consequences on insect biodiversity and conservation by enabling introgression between formerly separate populations and species.

Key words. Climate change, global warming, habitat modification, hybridisation, last glacial maximum, niche models.

Introduction

The major influence of climate on species distribution and abundance has been apparent since the introduction of phylogeographic tools (Avise *et al.*, 1987) that revealed the population genetic signature of expanding and contracting plant and animal ranges (e.g. Taberlet *et al.*, 1998; Hewitt, 2004; Excoffier *et al.*, 2009). More recently, the same approach has been directed at documenting and predicting outcomes of climate change in the Anthropocene (e.g. Robinet & Roques, 2010; Alsos *et al.*, 2012; Andrew *et al.*, 2013; Pauls *et al.*, 2013; Razgour *et al.*, 2015) Recent rapid range shifts may have an overriding effect on the extent and distribution of intra-specific genetic variation (e.g. Excoffier *et al.*, 2009; Garroway *et al.*, 2011; Bubac & Spellman, 2016), that is most often appar- ent when comparing closely related species (e.g. Lewis & Crawford, 1995; Chen *et al.*, 2007; Blair *et al.*, 2014; Krosby *et al.*, 2015). Different histories of range change create different patterns of genetic structure in related species (Hewitt, 2004). Species pairs provide valuable insight on evolutionary ecology because they allow comparison of population responses at the species boundary (e.g. Mathews *et al.*, 2002; Acevedo *et al.*, 2012; Dawson, 2012; Bulgarella *et al.*, 2014). Closely related lineages frequently have adjacent ranges with differing environmental characteristics leading to ecological adaptation and restricted niche breadth (Endler, 1977; Peers *et al.*, 2013), so that an interplay between gene flow and selection on adaptive traits exists unless assortative mating evolves (Dr'es & Mallet, 2002; Emelianov *et al.*, 2004). Shifts in resource availability associated with changing global environmental conditions could drive range changes that alter gene flow equilibria (Lenormand, 2002). In turn, this may redirect the outcome of current competitive interactions (Acevedo *et al.*, 2012; Bulgarella *et al.*, 2014), especially where these are influenced by range overlap (Krosby *et al.*, 2015). The detection of species responses to past and future environmental change is of increasing interest and necessity as the consequences of anthropogenic habi- tat modification becomes better understood (Hellmann *et al.*, 2012; Razgour *et al.*, 2013; Wisz *et al.*, 2013).

Here, we considered the situation for a sister pair of endemic short-horned grasshoppers that are the only representatives of the genus *Phaulacridium* (Key, 1992) in New Zealand. They live primarily in low elevation grassland, with *Phaulacridium marginale* (Walker, 1870) distributed in mesic habitats through New Zealand and many near-shore islands (Westerman & Ritchie, 1984), and *Phaulacridium otagoense* (Westerman & Ritchie, 1984) restricted to a limited semiarid habitat in central South Island. This distribution suggests species ecology linked to environmental conditions associated with cli- mate, and the potential for different responses to climate change and human modification of the landscape. The contrasting distributions of the two species (widespread vs. restricted) furnish a prediction of contrasting genetic diversity due to population size variation and isolation by distance (Wright, 1943; Slatkin, 1993; Charlesworth, 2009), and a mismatch of mtDNA diversity and spatial distribution has been reported in these grasshoppers (Goldberg *et al.*, 2015). If, as it appears from low

genetic diversity, *P. marginale* has recently expanded its spatial range, what change drove this response? Possibly *P. otagoense* formerly had a wider range during the last glacial maximum (LGM; ~20 kya) when drier conditions were more widespread, but has now retracted. If wider range was accompanied by larger population size, as tends to be the case in animals (e.g. Blackburn *et al.*, 2001), this would explain the relatively high diversity reported in *P. otagoense* (Goldberg *et al.*, 2015). We examined this scenario using a combination of spatial, climatic, morphological and phylogeographic data to compare these spe- cies, and infer the processes shaping their range and variation. Ecological modelling of presence/absence data across the spatial and elevational range of New Zealand was used to confirm that the two species have distinct environmental envelopes and to infer the major variables associated with their spatial ranges. We sampled modern populations across the species' ranges and applied new Bayesian assignment tools to traditional morphometric traits to test the two-species hypothesis and classify individuals. With this sample, we then used mtDNA haplotype data to examine in more detail than previously matrilineal diversity and phylogeographic structure of the two species. mtDNA haplotype data together with allelic diversity at the rRNA internal transcribed spacer (ITS) locus, and geometric morphometric analysis of pronotum shape were used to examine whether range change had resulted in interspecific introgression. Finally, we exam-ned the impact of rapid anthropogenic habitat modification by comparing evidence of potential and realised niche space.

Materials and methods

Grasshoppers

Phaulacridium is an Australasian genus of small short-horned grasshoppers (Acrididae) represented in southern Australia and New Zealand by endemic species. Their populations comprise one generation per year with egg diapause broken by cool winter temperatures, and peak adult activity in summer. They require open space for thermoregulation through basking and forage in natural and modified grasslands (Clark, 1967; Harper, 1972; Chapman, 1987; Scott, 1997), eating native herbs and small shrubs and exotic herbs including clover (*Trifolium repens*) and plantain (*Plantago lanceolata*). Native tussock grasses that dominate natural vegetation of open habitat in New Zealand are rarely eaten (Watson, 1970; and unpublished data). The commonest Australian species (*Phaulacridium vittatum*) is reported to have increased since European pasturalisation and is able to survive drought using deep rooted exotic weeds (Clark, 1967; Key, 1992), and although commonly called the wingless grasshopper *P. vittatum* is polymorphic for flight functional wings (Rentz, 1996). In New Zealand, fully winged *P. marginale* are occasionally recorded, but all *P. otagoense* are micropterous. The two New Zealand species differ in shape, size (even when in sympatry), colour pattern and genome size (Westerman & Ritchie, 1984), and preliminary allozyme analysis indicates differences in allele frequencies (Scott, 1997). Where the two New Zealand species meet in Central Otago, *P. otagoense* is reported to favour the driest microhabitats while *P. marginale* uses lusher conditions as it does elsewhere in its range (Westerman &

Ritchie, 1984; Scott, 1997). Inter-species mating was reported by Westerman and Ritchie (1984), but they were unable to detect gene flow.

Environmental distinction of species ranges

To test whether the two New Zealand species of *Phaulacridium* have distinct environmental envelopes, we collated and mapped all available locality records, draw- ing on published material including Tenure Review reports (Appendix S1) prepared for the New Zealand Government's Department of Conservation, and our own observations. We used our database of field survey records spanning New Zealand from coastline to 2000 m above sea level, to generate a presence/absence matrix of New Zealand grasshoppers. This comprised 949 localities and records of 14 native New Zealand grasshopper species including 217 locations where *P. marginale* was present and 26 with *P. otagoense* (Fig. 1). This approach allowed inclusion of the large number of data points spanning potential range across New Zealand. Only mainland loca- tions were used for mapping, despite the presence of *P. marginale* on some near-shore islands and the Chatham archipelago (Goldberg *et al.*, 2015).

In order to define and project the climatic envelopes of *P. marginale* and *P. otagoense*, the 19 current climate variables available from Worldclim (http://www.worldc lim.org/) were downloaded at a resolution of 30 arc seconds (~1 km^2) (Appendix S2). Variables were cropped to the extent of New Zealand (Latitude 49°–32°S; Longitude 165°–180°E) using QGIS v2.16.1 (QGIS Development Team, 2017). We used variance inflation factor (VIF) analysis to refine the variable list to increase parsimony and minimise over-fitting during the modelling process.

The R package 'VIF' (Lin *et al.*, 2015) implements stepwise selection to identify and remove collinear variables using VIF regression (Lin *et al.*, 2011). This reduced the informative and independent variables to seven. As it has previously been reported that soil and vegetation types influence ranges of some orthoptera (e.g. Nattier *et al.*, 2013; Weiss *et al.*, 2013), we added soil and vegetation data layers to our analysis. These were rasterised and scaled from their original files 'Fundamental Soil Layers New Zealand Soil Classification' and 'Vegetation Cover Map of New Zealand' obtained through the Landcare Research New Zealand Limited (https://www.landcare research.co.nz/resources/data/lris) Land Resource Infor- mation System (LRIS) portal (https://lris.scinfo.org.nz/). This brought the total number of predictor variables used in our analysis to nine.

We used the R package 'biomod2' v3.3-7 to individu- ally model the environmental envelopes of the two focal grasshopper species (Thuiller *et al.*, 2016). Ten different modelling methods analysed the presence/absence data against the predictor variables: generalised linear model (GLM), generalised boosting model/boosted regression tree (GBM), generalised additive model (GAM), classifica- tion tree analysis, artificial neural network, surface range envelope (SRE), flexible discriminant analysis (FDA), multiple adaptive

regression splines, random forest (RF) and maximum entropy (MAXENT). Descriptions of each model and their use within 'biomod2' can be found in Thuiller *et al.* (2009). All modelling parameters were kept at default values and 80% of the data were used to cali- brate the models, with the remaining 20% being used to test them. Current climatic conditions were analysed using the nine variables, with each model repeated three times resulting in 30 environmental niche models. Importance values for each variable were calculated by subtracting the

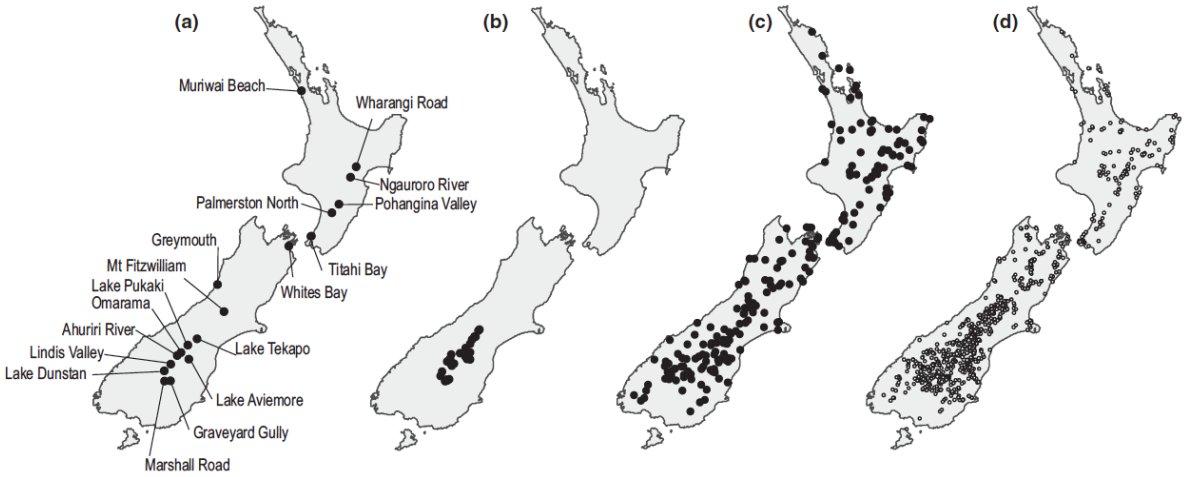

Fig. 1. *Phaulacridium* grasshoppers in New Zealand. (a) Source locations of specimens used for morphological and genetic analyses. (b–d) Spatial occurrence data for 16 species of New Zealand grasshoppers at 949 locations used in environmental modelling: (b) presence of *Phaulacridium otagoense n* = 26, (c) presence of *Phaulacridium marginale n* = 217, and (d) presence of other native grasshopper species pre- dominantly from alpine zones (*Brachaspis*, *Sigaus*, *Paprides*, *Alpinacris*). All sites were searched for all taxa. mean correlation score of each variable from 1, with scores closest to 1 indicating a variable of most impor- tance in the model.

Environmental niche model accuracy was investigated using two different evaluation methods: receiver operating characteristic (ROC) and true skill statistic (Appendix S3). An ensemble model was then created from a subset of these models included if their ROC values were ≥0.9. Spatial plots of potential habitat distribution were produced using the ensemble mean weights model (EMmw). EMmw variable importance was calculated by applying the weights produced in the ensemble model to the associated models in the 30 model data set. Three runs of each model method were averaged, and the EMmw calculated by summing the total of these averages for each predictor variable and dividing by the number of modelling methods used (10). Final scores of variable importance were converted into percentages of total variable importance for each modelling method.

Past and future potential ranges of species were inferred using bioclimatic variables from the MIROC-ESM global climate models (Watanabe *et al.*, 2011) for the LGM and 2070. These were at a resolution of 2.5 arc minutes (~5

km^2) for the LGM and 30 arc seconds (~1 km^2) for 2070. The future predicted climate layers RCP2.6 and RCP8.5 were used separately for the 2070 data set. These are two of the AR5 greenhouse gas concentration trajecto- ries used by the inter-governmental panel on climate change (IPCC) and project an average global temperature increase of 1.0 and 3.7 °C respectively (IPCC, 2014). Using

the ensemble model, species' potential range forecasts were projected for the LGM and the 2070 climate data. The soil and vegetation predictor variables were kept as static layers throughout the analysis as no past or future projections of such data are currently available for New Zealand. Models combining both static and dynamic predictor variables are known to perform as well as, or better than, models where only dynamic variables are included (Stanton *et al.*, 2012). We ran all our analyses with and without the inclusion of data on soil and vegetation.

We also independently mapped natural and modified vegetation patterns of New Zealand as these likely represent important biological constraints on the spatial range of native grasshoppers (realised niche). To do this, we generated vegetation maps representing current and pre- human patterns using the 'Potential Vegetation of New Zealand' and 'Vegetative Cover Map of New Zealand' files obtained from the LRIS portal (https://lris.scinfo.org. nz/) (Newsome, 1987; Leathwick *et al.*, 2012). The Poten- tial Vegetation initiative seeks to provide a model of the distribution of natural vegetation types in New Zealand that would inform conservation and restoration projects and extends previous statistical modelling (Leathwick, 2001) to reconstruct vegetation pattern expected in the absence of human activity (Leathwick *et al.*, 2012). Envi- ronmental data layers were drawn from the most recent iteration of the Land Environments of New Zealand classification (Leathwick *et al.*, 2003) allowing grid resolution of 100 m. During modelling of these layers, particular attention was given to defining the treeline (Leathwick *et al.*, 2012), which marks the boundary between forest and subalpine vegetation (notably *Chionochloa* tussock communities), and is an influential natural biological limi- tation for New Zealand grasshoppers (Bigelow, 1967). The files were edited in QGIS where broad vegetation classifications were regrouped as: indigenous forest; agricultural land; Tussock grassland/subalpine scrub; urban; wetland communities; sand-dune communities and unclassified categories.

Taxonomic identification with morphology

We collected 149 *Phaulacridium* grasshoppers from low- land grassland habitat at 18 locations by hand or sweep net (Fig. 1, Appendix S4). Nine locations were sampled in southern South Island New Zealand where both *Phaulacridium* species have been reported (Westerman & Ritchie, 1984; Key, 1992; Goldberg *et al.*, 2015). We iden- tified this sample of grasshoppers using data collected from adults for the diagnostic characteristics previously reported (Westerman & Ritchie, 1984). Adults were distin- guished from juveniles by the presence of tegmina entirely covering the vestigial wings. Females were distinguished from males by the presence of an ovipositor. Eight non- independent variables were measured, comprising three size differences (hind femur length, hind tibia length

and tegmina length), three ratios (hind femur length/width, pronotum width 3/pronotum length and pronotum width 1/pronotum width 2) and two angles of the pronotum margin in dorsal view (angle 1–PA1 and angle 2–PA2) (Appendix S5). Measurements were made using an Olympus SZX7 stereomicroscope with Olympus SC100 image capture and Olympus cellSens Dimension v1.6 software (Olympus Corporation, Tokyo, Japan).

In order to test the hypothesis of two morphologically distinct species of *Phaulacridium* in our sampling and assess signal in the data, we implemented a parameterised Gaussian hierarchical clustering algorithm to model the most likely number of clusters within the data and assign each specimen to the groups inferred. Model-based clustering used the Mclust package (Fraley & Raftery, 2003) in the R programming environment (R Development Core Team 2014). The Mclust v5.0.2 algorithm (Fraley *et al.*, 2012) is built from a general model where the total data set is considered as a mixture of multivariate normal data sets, with a selection of covariance structures and vectors of expectation (Nanova, 2014). Unlike discriminant analysis, Mclust analysis does not require prior information about specimen identity to classify sample data. The optimal model of variance and number of clusters in the data are selected based on Bayesian information criteria (BIC), using the value of the maximised log likelihood with a penalty for the number of parameters in the model (Fraley & Raftery, 2003; Cordeiro-Estrela *et al.*, 2008; Nanova, 2014). The higher the BIC score, the lower the global average and median classification uncertainty, the better the model fits the data set (Cordeiro-Estrela *et al.*, 2008). Additionally, under each model, the assignment probability of an individual belonging to a cluster can be computed (Fraley & Raftery, 2003).

Model-based clustering applied in this way allows exploration of the data, so we reran analyses varying the number and combination of variables included in order to optimise diagnostic signal. In particular, we sought to minimise non-independence of variables in analyses by examining signal resulting from use of one variable of each type (a linear dimension, a ratio and an angle). This revealed that three variables (hind femur length, hind femur length/width and pronotum angle 2) used in combination were sufficient to identify specimens to species. Additional variables did not alter results. Males and females were best treated separately as the grasshoppers are sexually dimorphic, primarily in size (females larger).

Demographic history of mtDNA lineages

Whole genomic DNA was extracted from foreleg tissue of recently collected or alcohol preserved specimens using a salting-out method (Sunnucks & Hales, 1996; Trewick & Morgan-Richards, 2005). Polymerase chain reaction (PCR) primers L2-N-3014 (Simon *et al.*, 1994) and hopp-1490 (5' TTTCAACAAAC CATAAGGACATTGG 3'), modified from LCO1490 (Folmer *et al.*, 1994), were used to target a 755 bp fragment of the mitochondrial DNA gene cytochrome oxidase subunit I (COI). PCR was performed in 20 μl volumes

containing 0.2 μl of MCLAB Taq (Molecular Cloning Laboratories, South San Fransisco, CA, USA), 2.0 μl of 10x MCLAB buffer (Molecular Cloning Laboratories), 2.0 μl of 2 mM dNTPs, 1.4 μl of 25 mM Mg^{2+}, 0.8 μl of each primer (1 mM) and 2.0 μl of genomic DNA (~5 ng/μl). Thermocycling conditions were 95°C for 90 s; 94°C for 15 s, 52°C for 15 s and 72°C for 90 s repeated 38 times. Amplification products were checked on 1% TAE agarose gels and sequenced using BigDye v3.1 chemistry and an ABI3730 DNA analyzer. Sequences were edited and aligned using Geneious v9.1.4 (Kearse *et al.*, 2012).

A Bayesian phylogenetic analysis using an outgroup of homologous sequence data from the endemic New Zealand *Sigaus australis* (Bigelow, 1967) and representatives of four species of Australian *Phaulacridium*, was used to establish statistical support for distinct clades within New Zealand *Phaulacridium*. A neighbour-joining tree, inferred with the Geneious tree builder, was used to examine the distribution of haplotypic variation within New Zealand *Phaulacridium*. Haplotype diversity (*h*), nucleotide site diversity (π, Nei, 1987) and the average number of nucleotide differences (*k*) within each *Phaulacridium* clade were calculated in DnaSP v5.10 (Librado & Rozas, 2009).

We examined the demographic history of mitochondrial lineages to infer past range changes of this non-recombining, unitary genome. We used mismatch distribution, the frequency distribution of pairwise mutational differences among individuals (Rogers & Harpending, 1992), to explore demographic signature in *Phaulacridium* genetic lineages with DnaSP v5.10 (Librado & Rozas, 2009). The distribution of pairwise differences in stable populations is expected to be multimodal compared to a unimodal distri- bution for populations that have undergone recent demographic expansion (Rogers & Harpending, 1992; Harpending, 1994). Goodness of fit to a unimodal mismatch distribution was tested using the Harpending's raggedness index (Harpending *et al.*, 1993) and the sum of squares deviation (SSD; Schneider & Excoffier, 1999) using Arlequin v3.5 (Excoffier & Lischer, 2010) with 1000 bootstrap resamples. A non-significant result indicates a good fit and support for population expansion.

In addition, three neutrality tests, Tajima's *D* (Tajima, 1989), Fu's F_S (Fu, 1997), and Ramos-Onsins and Rozas's R_2 (Ramos-Onsins & Rozas, 2002), implemented in DnaSP v5.10 (Librado & Rozas, 2009), were used to detect departures from the mutation–drift equilibrium that would be indicative of changes in historical demography and natural selection. We assessed statistical significance with 1000 permutations. Values near zero for Tajima's *D* and Fu's F_S imply constant population size. Significant negative values imply population expansion or purifying selection, while positive values indicate population subdi- vision or recent population bottleneck. Ramos-Onsins and Rozas's R_2 and Fu's F_S are considered the best statistical tests for sensing population growth, with R_2 being better suited to small sample sizes than F_S (Ramos-Onsins & Rozas, 2002). Significant R_2 values close to zero are inter- preted as implying population expansion.

Evidence of introgression

Pronotum shape. We sought evidence of introgression between the two *Phaulacridium* species by examining the match between mtDNA lineage and specimen identifica- tion. In addition, we used geometric morphometrics to analyse fine-scale shape variation, as inter-species gene flow would be expected to yield individuals with a mixed morphological phenotype (e.g. Gόmez *et al.*, 2013; Pentinsaari *et al.*, 2014). We used pronotum shape as this struc- ture is not susceptible to arbitrary changes during preservation (cf. whole body dimensions), and has previ- ously been shown to be amenable to the methods used (e.g. grasshoppers– Dowle *et al.*, 2014; beetles– Ober & Connolloy, 2015). The pronotum of *Phaulacridium* is known to have features that differ between the described species, but this has never been formally analysed in terms of shape. Geometric analysis allows elimination of isometric size variation by superimposition via Procrustes transformation, while capturing all shape variability of the raw landmark data (Bookstein, 1991; Rohlf & Marcus, 1993; Klingenberg, 2016). We included only adults in our study, thereby avoiding allometric effects on shape associated with ontogeny. We obtained data from each grasshopper using twelve landmarks on the perimeter of the dorsal surface of the pronotum using digital images on a Wacom Cintiq 22HD digitising tablet and tspDIG v2.17 software (Rohlf, 2013) (Appendix S5). High-resolution digital images were captured with an Olympus SC100 and Olym- pus SZX7 stereomicroscope using Olympus cellSens Dimension v1.6 software. The landmarks were selected for their ease of identification, homology among grasshopper individuals, and their capacity to capture the major shape features of the structure.

Measurement error was minimised by careful mounting, photography and landmark selection, and measurement error associated with photographing and digitising the images was assessed by reimaging and digitising one grasshopper 10 times. A Procrustes analysis of variance (Procrustes ANOVA) was used to compare variation in landmark location among iterations. The scale of the measurement error was assessed by comparison of the mean squares of pronotum shape variation and confirmed to be significantly lower than biological variation.

A Procrustes fit aligned by principal axes was per- formed to eliminate size differences, and linearly uncorrelated variables were obtained from a principal component analysis (PCA) across all individuals and all landmarks in MORPHOJ (Klingenberg, 2011). We analysed the principal components accounting for most vari- ation, using the model-based clustering approach of Mclust v5.0.2 (Fraley *et al.*, 2012) to identify the optimal number of clusters of shape variation in the data, and their composition.

Nuclear rRNA diversity. Allelic variation at a nuclear locus would confirm gene flow; however, single copy neutral nuclear loci have not been developed for these species. Instead, preliminary evidence of introgression at nuclear loci was sought by comparing 45S ribosomal cassette DNA sequences from three representative

specimens using a genomic approach. Eukaryote genomes contain numerous copies of the 45S rRNA cassette, but minimal variation among copies is normally maintained by concerted evolution (Ganley & Kobayashi, 2007). High copy number makes the 45S locus amenable to next generation sequencing (NGS) approaches, while concerted evolution means any allelic variation encountered is most likely to be the result of recent introgression (e.g. Trewick, 2001; Trewick *et al.*, 2008; Wan *et al.*, 2014). Total genomic DNAs from three individuals were separately processed through massive parallel, high-throughput sequencing (Illumina HiSeq 2500, Illumina, San Diego, CA, USA) for a separate phylogenetic study. Genomic DNA was fragmented, prepared using the ThruPLEX® DNA-seq Kit (Rubicon Genomics, Ann Arbor, MI, USA) and used to generate 100 bp paired-end sequence. The scale of sequencing did not provide sufficient data to assemble single copy nuclear genes as Acrididae have large genomes approximately three times bigger than humans (e.g. the related grasshopper *P. vittatum* has a haploid genome of 10.73 pg); however, it was sufficient for rich coverage of multicopy nuclear markers including the 45S rRNA cassette.

Adapter sequence barcodes and poor-quality data were removed from IIlumina sequence reads using cutadapt (Martin, 2011). Geneious v9.1.4 (Kearse *et al.*, 2012), was used to pair and map sequence reads and to align result- ing consensus sequences. The entire 45S was initially assembled by aligning paired reads to an annotated reference sequence from the New Zealand grasshopper *Paprides nitidus* (unpublished) that belongs to the same subfamily (Cantanopinae). The resulting consensus sequence was used as a reference for iterative remapping of raw read data until gaps were filled by extension with new sequence data. The highly conserved 5.8S locus within 45S was then excised and used for separate, iterative mapping of each set of raw sequence reads in order to assemble the flanking ITS 1 and 2 alleles. Sequence variants within the ITS region (including 5.8S, ITS1 and 2) were identified for each individual by identifying consistent single nucleotide substitutions among the paired reads when compared to the reference sequence. An unrooted network of sequence variants for the three New Zealand *Phaulacridium* was constructed using the median joining algorithm (Bandelt *et al.*, 1999) in PopART (Leigh & Bryant, 2015).

Results

Environmental envelopes

A total of 243 locations for *Phaulacridium* were obtained from our own observations and published records dating from 1959 to the present, and mapped (Fig. 1). *Phaulacridium marginale* was recorded throughout New Zealand and many surrounding islands. In contrast, the distribution of *P. otagoense* was limited to the central/southern South Island.

Analyses with and without the inclusion of soil and vegetation data produced

similar results, implying that climate data provide a proxy for the key attributes dic- tating grasshopper distribution. Natural vegetation at the scale relevant to these grasshoppers (i.e. open grass-and/scrub vs. evergreen forest) is primarily a function of climate. Most environmental models for *P. marginale* found annual mean temperature to have the highest pre- dictive power of the seven climate variables included (Table 1). The ensemble model combined models using four methods (GLM, GBM, GAM, RF, all with ROCs > 0.9) resulting in annual mean temperature (60%), and mean diurnal range (14.59%) the most important variables in predicting where *P. marginale* occurs. Environmental models for *P. otagoense* differed in finding mean diurnal temperature range to be most influential (Table 2). The ensemble model for this spe- cies combined models using four methods (GLM, GBM, FDA, MAXENT).

The potential distribution of *P. marginale* and *P. otagoense* can be inferred for the past and future climate of New Zealand (Fig. 2). For *P. marginale*, suitable climatic conditions were widespread during the LGM with distribution likely in most of northern and western New Zealand. Suitability of southern New Zealand increased to the present and is predicted to further increase in the future (2070 projection), as models with warming of 2.2 °C would increase climatically suitable areas for *P. marginale* (Fig. 2). Climatically suitable habitat for *P. otagoense* during the LGM was inferred as little different from that predicted for current conditions in Central Otago, South Island, that has the lowest moisture levels in New Zealand (Leathwick *et al.*, 2003). In contrast, cold dry winters similar to those currently experienced in Central Otago were also indicated for northeast New Zealand where the species is not recorded. Projections of future climate, suggest some increase in suitable area for *P. otagoense* based on our ensemble environmental model (Fig. 2), and this is consistent with the prediction that eastern dry areas will tend to get drier (Harrington & Renwick, 2014; Renwick *et al.*, 2016). The finding that the region of South Island in which *P. otagoense* occurs today has conditions that are mostly the least suitable for *P. marginale*, confirms that the two taxa are ecologically different.

APPENDIX TWO

Table 1. Predictive power of climate variables in determining the range of the New Zealand grasshopper *Phaulacridium marginale*, using ten modelling methods* and combining the best fitting models (EMmw).

Average%	GLM	GBM	GAM	CTA	ANN	SRE	FDA	MARS	RF	MAXENT	EMmw
Annual mean temperature	67.53	66.76	78.53	78.59	32.28	22.67	73.17	72.44	38.02	71.08	60.11
Mean diurnal temperature range	12.96	10.72	6.39	17.09	5.55	9.03	5.93	13.28	7.62	3.51	9.21
Isothermality	3.42	0.76	1.09	0.00	0.87	18.53	3.08	0.60	2.67	1.14	3.22
Mean temp of wettest quarter	0.00	10.60	0.00	2.10	13.00	24.98	6.25	0.60	13.79	5.26	7.66
Mean temp of driest quarter	9.60	4.20	2.09	0.00	12.30	5.83	0.05	5.38	20.14	13.76	7.34
Precipitation of driest month	0.00	2.28	3.48	0.00	9.97	8.29	10.78	5.25	5.11	0.82	4.60
Precipitation seasonality	4.12	1.69	1.51	0.00	3.07	7.95	0.66	1.49	1.74	1.16	2.34
Soil type	0.62	0.47	2.54	0.00	9.86	1.62	0.00	0.00	1.45	0.24	1.68
Vegetation type	1.76	2.52	4.37	2.22	13.10	1.08	0.08	0.97	9.46	3.03	3.86

Predictive power of each variable under each model was given by the correlation between the post-training standard score and the score from a new prediction made after randomising the given variable. Low correlation indicates strong influence of the variable on the model. Here, predictive power of the variable is proportionated for each model separately (as a percentage); a high value indicates high importance of that variable in the model.

Table 2. Predictive power of climate variables in determining the range of the New Zealand grasshopper *Phaulacridium otagoense*, using ten modelling methods and combining the best fitting models (EMmw).

Average%	GLM	GBM	GAM	CTA	ANN	SRE	FDA	MARS	RF	MAXENT	EMmw
Annual mean temperature	22.36	3.17	0.00	39.66	12.09	12.64	0.00	0.72	6.43	0.00	9.71
Mean diurnal temperature range	50.04	51.15	64.36	60.34	16.37	23.99	72.41	40.26	35.37	63.61	47.79
Isothermality	12.49	0.08	0.00	0.00	3.21	9.64	0.00	3.36	0.38	3.81	3.30
Mean temp of wettest quarter	1.11	0.41	0.00	0.00	8.47	7.52	11.59	5.21	13.34	0.00	4.77
Mean temp of driest quarter	1.52	23.16	0.00	0.00	15.33	13.04	1.41	3.85	5.77	3.70	6.78
Precipitation of driest month	4.41	5.66	0.00	0.00	23.14	13.00	0.00	12.30	17.76	12.72	8.90
Precipitation seasonality	0.94	14.56	0.40	0.00	5.26	9.94	0.00	19.60	9.38	9.05	6.91
Soil type	2.63	0.87	1.44	0.00	7.42	6.61	12.24	3.56	1.67	1.18	3.76
Vegetation type	4.50	0.95	33.80	0.00	8.71	3.61	2.35	11.13	9.90	5.94	8.09

The most influential variable for each model is indicated with grey background (see Table 1 for further details).

Taxonomic identification and range changes in 30 years

We found three morphological traits reported to be diagnostic for the two grasshopper species (hind femur length, hind femur length/width and pronotum angle 2) (Westerman & Ritchie, 1984) were effective for distin- guishing taxa. Bayesian assignment found support for two morphological clusters for each sex (Appendices S6, S7), consistent with our expectations of two *Phaulacridium* species. The model with best statistical support for male *Phaulacridium* (BIC = -537.92) was EEI (diagonal, equal volume and shape) and for female *Phaulacridium* (BIC = -744.65) was EVI (diagonal, equal volume, vary- ing shape). A total of 124 (56 males, 68 females) grasshoppers were determined to be *P. marginale*, and 24 (7 males, 17 females) were *P. otagoense* (Appendix S4). Most (94.6% of the sample) assignment probabilities of individuals to a species were ≥0.9. The northern South Island locations and all of the North Island populations contained only *P. marginale*. *Phaulacridium otagoense* was collected from five southern South Island locations, but at three of these we also collected *P. marginale* (Table 3). We found only *P. marginale* at Omarama and Lake Avie- more, both places where *P. otagoense* has previously been collected (Westerman, 1974, 1983; Westerman & Ritchie, 1984), and we found both species at Graveyard Gully that was formerly occupied only by *P. otagoense.*

Demographic history of mtDNA lineages

The 755 bp alignment of homologous COI sequences from 149 *Phaulacridium* individuals contained 52 unique mtDNA haplotypes, with 99 of these base pair positions being variable (13%) and 71 (9%) being parsimony infor mative sites. Phylogenetic analysis of the haplotypes revealed four lineages (clades) rather than two as expected from sampling two species (Fig. 3, Appendix S8). Distinct clades were confirmed by posterior probabilities of 1.0 from Bayesian phylogenetic analysis of DNA sequences with an outgroup. Lineage I was equivalent to the previously reported *P. marginale* clade, whereas our lineages III and IV correspond to the two clades associated with *P. otagoense* (Gold berg et al., 2015). Lineage II had not been encountered before.

All four the haplotype lineages were found in both grasshopper species (Table 3), but haplotype diversity was spatially structured. Lineage I haplotypes were found in all population samples except Lake Dunstan in the south (Fig. 3). In contrast, within lineages II, III and IV, 25 individuals had 23 haplotypes with an average of 22.1 nucleotide differences ($p = 0.029$; Table 4). Nucleotide diversity within haplotype lineages (Table 4) therefore contrasts with the cur- rent geographical area in which they occur. The low genetic diversity of the widespread lineage I could be the result of recent population expansion. In contrast, the diversity within the Otago lineages II, III and IV suggests a relatively large stable population.

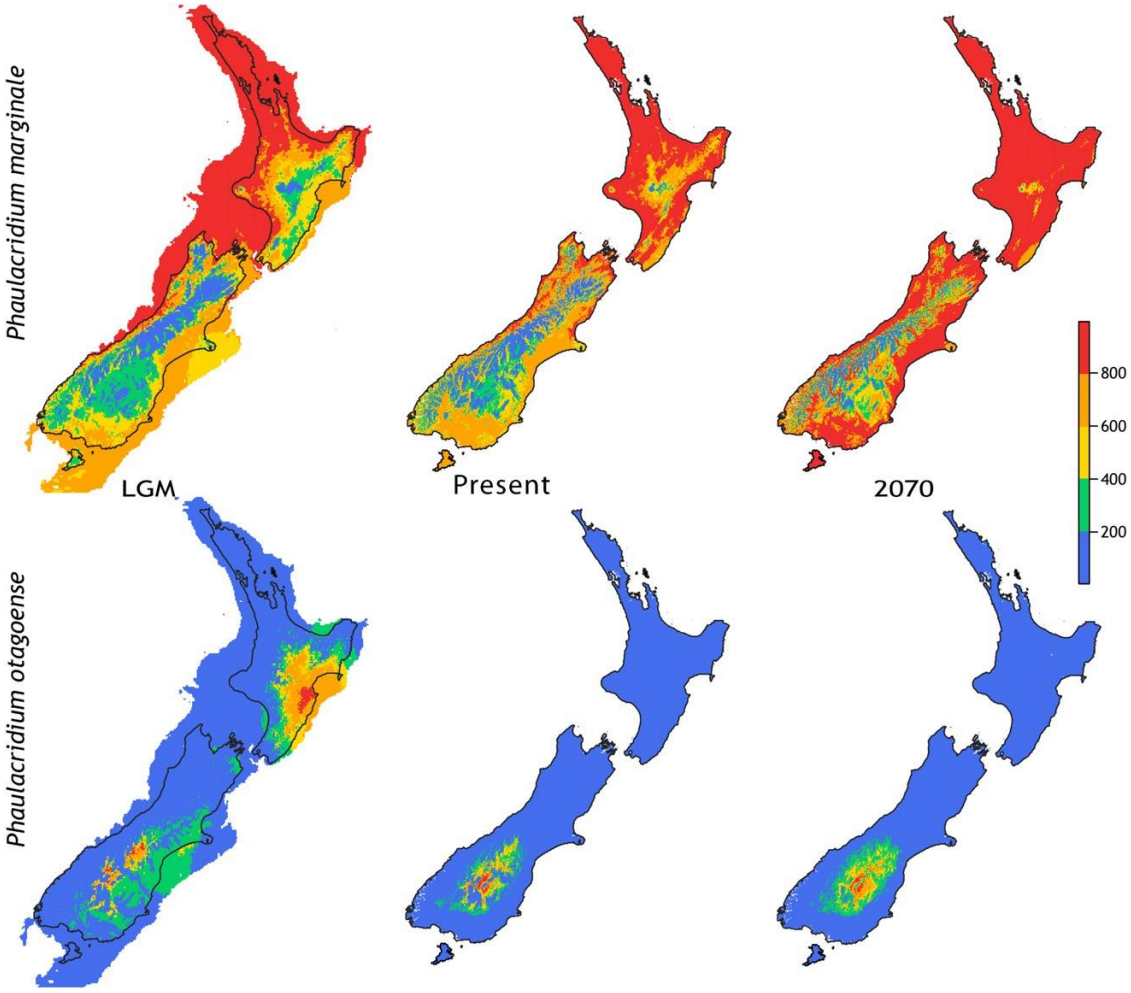

Fig. 2. Contrasting climatically suitable regions of New Zealand inferred for two lowland *Phaulacridium* grasshopper species: Upper row, widespread *P. marginale*; Lower row, restricted *P. otagoense.* The ensemble model mean weights combined the best fitting models from scenarios based on seven climate variable plus vegetation and soil data. Range probabilities were estimated for last glacial maxima; pre- sent; and future (2070; based on predicted global warming of 2.2 °C). Warm colours (e.g. red) equate to high probability of suitable cli- mate, cool colours (e.g. blue) indicate low probability of suitable climate. GLM, generalised linear model.

Table 3. Population samples of Phaulacridium grasshoppers in region of sympatry (central South Island New Zealand) display some mismatch between morphology and mtDNA.

			Pronotum		mtDNA	
Specimen	**Location**	**Species**	**Cluster**	***P***	**Lineage**	**Haplo.**
GH1464	Lake Tekapo	*otagoense*	C	1.00	IV	45
GH1465		juvenile	–		I	29
GH1466		*otagoense*	C	1.00	I	23
GH1467		*otagoense*	C	0.99	I	23
GH1468		*otagoense*	B	0.94	I	29
GH1469		*otagoense*	C	0.99	I	29
GH1474	Lake Pukaki	*marginale*	A	0.64	I	28
GH1475		*marginale*	B	0.95	IV	44
GH1476		*otagoense*	A	0.86	I	28
GH1477		*marginale*	C	0.87	IV	43
GH1479		*marginale*	A	0.84	I	23
GH1480		*marginale*	A	0.76	I	29
GH1484		*marginale*	C	0.93	I	29
GH1485		*marginale*	A	0.95	I	1
GH1494		*marginale*	B	0.94	I	23
GH1499	Lake Aviemor	*marginale*	A	1.00	IV	52
GH1500		*marginale*	A	0.99	I	25
GH1501		*marginale*	A	1.00	I	23
GH1502		*marginale*	A	0.98	I	29
GH1503		*marginale*	A	0.98	I	29
GH1504		*marginale*	B	0.95	IV	51
GH1506		*marginale*	B	0.89	IV	52
GH1507		*marginale*	B	0.91	I	29
GH1512		*marginale*	B	0.98	I	28
GH1513		*marginale*	B	0.93	I	29
GH1519	Omarama	*marginale*	C	0.87	I	29
GH1522		*marginale*	A	1.00	I	29
GH1524		*marginale*	A	0.99	IV	48
GH1525		*marginale*	B	0.97	IV	49
GH1526		*marginale*	A	0.91	I	29
GH1527		*marginale*	A	0.97	IV	47
GH1529		*marginale*	B	0.62	I	29
GH1532		*marginale*	B	0.94	I	28
GH1536	Ahuriri River	*marginale*	A	1.00	I	4
GH1538		*marginale*	A	0.93	I	19
GH1539		*marginale*	A	0.67	IV	50
GH1540		*marginale*	C	0.73	IV	46
GH1541		*marginale*	B	0.72	I	29
GH1542		*marginale*	B	0.98	I	29
GH1543		*marginale*	B	0.98	I	29
GH1544		*marginale*	B	0.98	I	29
GH1548		*marginale*	A	1.00	I	4
GH1553		*marginale*	B	0.67	I	18
GH1556	Lindis Valley	*otagoense*	C	1.00	I	29
GH1557		*otagoense*	C	1.00	II	36
GH1559		*otagoense*	C	0.99	II	35
GH1560		*otagoense*	C	1.00	II	37
GH1561		*otagoense*	C	0.99	I	29
GH1562		*otagoense*	C	1.00	I	29
GH1563		*otagoense*	C	1.00	II	34
GH1566		*otagoense*	C	1.00	II	38
GH1572		*otagoense*	C	1.00	II	37
GH1574	Lake Dunstan	*marginale*	C	0.73	II	32
GH1575		*otagoense*	C	1.00	II	33
GH1576		*otagoense*	C	1.00	II	30

GH1578		*otagoense*	C	1.00	II	31
GH1580	Graveyard Gully	*otagoense*	B	0.58	III	41
GH1581		*marginale*	B	0.88	I	28
GH1582		*marginale*	B	0.82	I	29
GH1583		*marginale*	A	1.00	I	28
GH1584		*otagoense*	C	1.00	I	28
GH1585		*otagoense*	C	0.98	I	28
GH1586		*otagoense*	A	0.63	III	42
GH1588		*otagoense*	C	1.00	I	28
GH1589		*otagoense*	C	1.00	I	29
GH1599	Marshall Road	*marginale*	A	0.98	I	29
GH1600		*marginale*	B	0.97	III	39
GH1601		*marginale*	A	0.99	I	29
GH1602		*marginale*	A	0.99	III	40
GH1604		*marginale*	A	0.99	I	29
GH1605		*marginale*	B	0.98	I	29
GH1606		*marginale*	B	0.97	I	29
GH1607		*marginale*	B	0.96	I	23

We inferred demographic histories of the mitochondrial lineages to estimate past range changes of this maternally inherited genetic marker. Mismatch distribution for lineage I was unimodal and smooth (Fig. 3) consistent with recent demographic expansion. Demographic expansion of lineage I was further supported by a low and statistically insignifi- cant Harpending's raggedness index (0.024, $P > 0.05$) and a low SSD value (0.002, $P < 0.05$). Tajima's D and Fu's F_S were significantly negative (D = -1.963, $P < 0.01$; expansion of mitochondrial lineage I, although a selective sweep could produce a similar effect.

In contrast, by grouping haplotypes unique to the nar- row range of *P. otagoense* (lineages II, III and IV) a more ragged and multimodal distribution is revealed that is typ- ical of population size at demographic equilibrium (Fig. 3). The mismatch distribution goodness of fit tests were not significant (Harpending's raggedness index 0.121, $P > 0.05$; SSD = 0.013, $P > 0.05$), indicating that there was no severe departure from the estimated demographic model. For *P. otagoense* lineages,

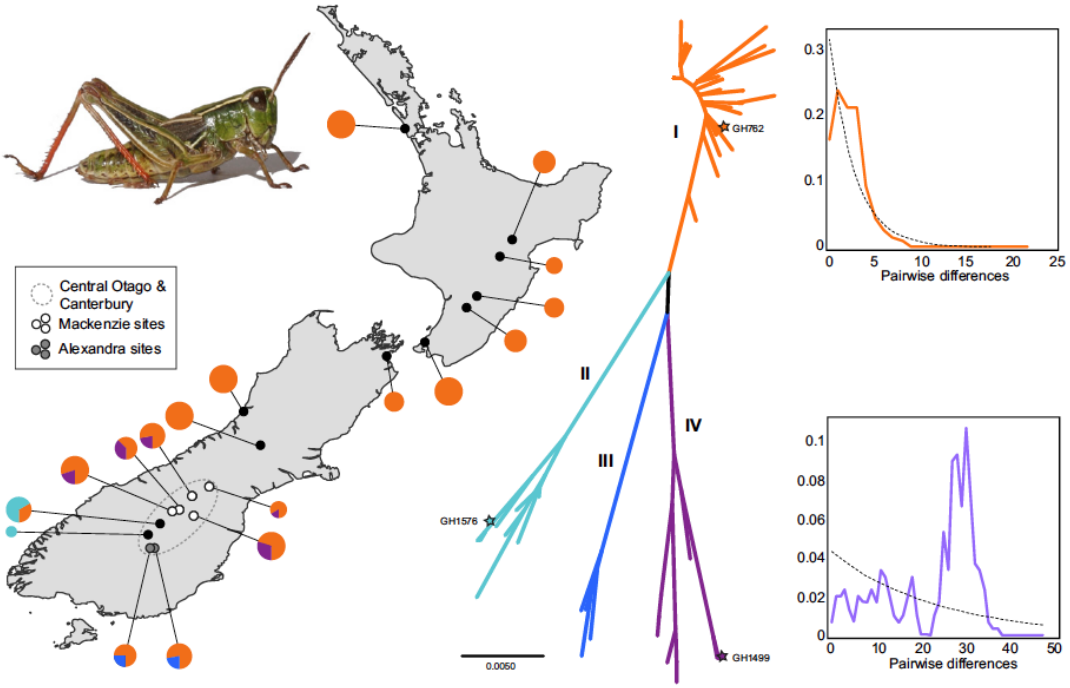

Fig. 3. Mitochondrial DNA cytochrome oxidase subunit I haplotype variation among New Zealand *Phaulacridium*. The relative frequency and spatial distribution among population samples (left) of the four mitochondrial lineages shown in the unrooted neighbour-joining tree (centre). The haplotypes of the three specimens that provided nuclear rRNA internal transcribed spacer sequence data are indicated by stars. Mismatch distribution plots for mtDNA lineage I (top right) and lineages II, III and IV (bottom right) show contrast- ing degrees of raggedness.

Table 4. Descriptive statistics for four mtDNA cytochrome oxidase subunit I sequence lineages observed in two species of New Zealand *Phaulacridium* grasshoppers. Sample size (*n*), number of observed haplotypes (N_{haps}), average number of nucleotide differences (*k*), nucleotide diversity (p), number of polymorphic sites (*S*) and haplotype diversity (*h*). The total number of identified specimens was 148, whereas the total number of specimens sequenced was 149 as it included one juvenile grasshopper without species id.

Lineage	*n*	N_{haps}	*k*	π	*S*	*h*
P. otagoense	24	14	17.043	0.00907	67	0.917
P. marginale	124	41	6.845	0.00907	87	0.886
Lineage I	124	29	2.191	0.00290	34	0.841
Lineage II	10	9	7.489	0.00992	22	0.978
Lineage III	4	4	5.833	0.00773	11	1.000
Lineage IV	11	10	11.891	0.01575	28	0.982
Lineages II, III & IV	25	23	22.140	02932	74	0.993

F_S = -21.109, $P < 0.001$) for lineage I haplotypes, with Ramos-Onsins and Roza's R_2 being significantly low (0.032, $P < 0.05$). These results are indicative of population expansion had not occurred. In contrast, Fu's F_S was significantly negative (-5.623, $P < 0.05$), which could be a result of the low population size or low sample size (Ramos-Onsins & Rozas, 2002).

Detecting introgression

Pronotum shape. Hybridisation between the grasshopper species might lead to individuals with intermediate phenotype, so we examined the shape of adult pronota in more detail. Geometric morphometric analysis of 12 pronotum landmarks found highly significant differences between pronotum shapes (MS pronotum = 0.004148, MS error = 0.000027, $P < 0.0001$) with Procrustes ANOVA. The mean squares for pronotum shape variation exceeded the mean squares for measurement error 157-fold, indicating measurement error would have a negligible influence on analysis of the landmark locations on the pronotum.

Data from males and females were combined as the iso- metric influence of size was removed through superimposi- tion of the raw data. We analysed principal components of pronotum shape variation obtained from MORPHOJ (Klingenberg, 2011) using model-based clustering without priors. The first two principal components explained 75% of the observed morphological variance in the sample (PC1 = 47.81%, PC2 = 27.54%), and were sufficient to reveal three pronotum shape clusters within our sample using BIC in Mclust v5.0.2 [VEI model

(diagonal, equal shape); BIC = 1105.19]. One pronotum shape cluster con- tained most (83%) of the *P. otagoense* grasshoppers in our sample (both sexes). The two other clusters corre- sponded to female and male *P. marginale*, showing that in this species sexes differ in shape as well as size (Fig. 4). Overall, *P. otagoense* had a wider pronotum than *P. marginale*, and had more acute external angles on the lateral carinae.

Nuclear rRNA–ITS diversity. To further test whether recent contact between species has resulted in genetic introgression, we examined allelic diversity at ITS using high-throughput sequencing of whole genomic DNA. This yielded 1.17×10^9–2.34×10^9 bp of sequence (<20% of the genome) from each of three grasshopper individuals. Of this between 9245 and 30 797 reads assembled to the 45S rDNA cassette. After annotation, we extracted align- ments of the ITS regions and identified a total of five ITS sequence variants (830 bp consisting of ITS1-5.8S-ITS2). All three *Phaulacridium* specimens, each representing a different mtDNA haplotype lineage (Fig. 3), had more than one ITS sequence variant. The DNA alignment included a short (5 bp) insertion/deletion region, resulting in some of the difference between the five sequences and preventing resolution of sequence variation via Sanger sequencing of this marker. The *P. otagoense* specimen had four different ITS sequence variants (a, b, c, d; Appendix S9). In contrast, both *P. marginale* grasshoppers each had only two ITS sequences (d, e).

Introgression

The majority of grasshoppers we collected were *P. marginale* with pronotum shape characteristic of that species and mtDNA haplotypes from lineage I (n = 59). In contrast, many *P. marginale* had either a pronotum shape characteristic of *P. otagoense* and/or a haplotype from lineages II, III and IV (n = 30). Likewise, some *P. otagoense* specimens had concordant traits for that spe- cies (n = 10), but more had a pronotum shape or mtDNA haplotype characteristic of *P. marginale* (n = 14). Of the eight population samples with more than one haplotype lineage, five had specimens with mismatch of species and pronotum shape, as expected of hybrids. Where the spe- cies and the mtDNA haplotype did not concur, we inferred it likely that hybridisation had led to mtDNA introgression.

Geometric analysis of pronotum shape for individuals previously classified by Bayesian clustering of traditional morphological characters revealed that five *P. marginale* specimens in our sample had a pronotum shape more typical of *P. otagoense*, and four *P. otagoense* specimens had a pronotum shape nearer that of *P. marginale* (Table 3). Cluster assignment probabilities for each individual revealed that a minority of grasshoppers (28 of 148) were assigned to clusters with a probability <0.9. Most of these came from locations where both species had been col- lected (Fig. 4, Appendix S8). The rRNA cassette surveyed from NGS data revealed abnormally high ITS variation in the specimen of *P. otagoense*, most likely the result of recent introgression from *P. marginale*.

Discussion

The geographical range of a species has been regarded as one of the most significant factors influencing genetic diversity and its distribution (Endler, 1977). Generally, it has been accepted that species with restricted ranges exhibit lower genetic diversity than species that are more widespread. Although population density might vary among taxa, population size at equilibrium is positively correlated with genetic diversity (Charlesworth, 2009) and with range size (e.g. Blackburn *et al.*, 2001). Neither pre- vious surveys (Westerman & Ritchie, 1984) nor our observations suggest that *P. otagoense* exists in significantly denser populations than *P. marginale*, and both species have strictly annual lifecycles. Therefore, the smaller spa- tial range of *P. otagoense* suggests this species has a smaller population size, predicting lower genetic diversity, compared to the more widespread *P. marginale*, which is the reverse of the relative genetic diversity documented here. Similar patterns of contrasting genetic diversity have been observed in other organisms including rare insect and plant species with high diversity compared to wide- spread congeneric species; in these studies, recent human-mediated reduction in population size was inferred (e.g. Chen *et al.*, 2007; Bȧlint *et al.*, 2012).

The observed relative level of genetic diversity in these two grasshopper species was a reversal of expectations based on current species' range size and can be explained by a temporal lag in population genetic structure (Char- lesworth, 2009; Landguth *et al.*, 2010). A temporal lag is supported by our demographic history analyses. We inferred recent population expansion of *P. marginale*, while *P. otagoense* has retained a signature of comparatively large, stable population history. Although high intra-specific haplotype diversity might also result from combination of lineages associated with cryptic taxa (Galtier *et al.*, 2009), our detailed morphological examina- tion and spatial evidence support just one taxon spanning haplotype lineages II, III and IV. Contrary to the widely held idea that species should typically comprise single mitochondrial lineages (e.g. Tavares & Baker, 2008), simulation studies show that large stable populations are expected to have multimodal distributions because the his- tory of coalescent events imposes a substantial correlation on this non-recombining DNA sequence (Slatkin & Hudson, 1991). Similar intra-specific diversity of mtDNA has been demonstrated in other Orthoptera (Morgan-Richards *et al.*, 2017).

Species range and environmental envelope

Contrasting population sizes in the recent past might be the result of *P. otagoense* reduction and/or *P. marginale* increase. Natural climate cycling through the Pleistocene provides a prediction of changing fortunes in related species with different environmental envelopes (Parmesan, 2006; Stewart *et al.*, 2010). This is in line with phylogeographic evidence of range change linked to climate shifts in the Northern Hemisphere (Hewitt, 2004). Available information for New Zealand (e.g. Newnham *et al.*, 2013) predicts retraction of forest and expansion of grasslands associated with cooler, drier conditions during the LGM. Perhaps, *P. otagoense* had a bigger range and thus larger population during the LGM (Vandergoes *et al.*, 2013; Williams *et al.*, 2015).

We investigated the possibility that species' range differences related to natural climate change since the LGM using ecological niche modelling. We found that although the two *Phaulacridium* species in New Zealand have contrasting distributions leading to distinct models of their environmental envelopes, climate modelling did not reveal a significantly larger area of contiguous optimal environment for *P. otagoense* during the LGM (Fig. 2). An apparently suitable area in North Island east coast (Hawkes Bay) might not have been reached due to physical and climatic habitat barriers. The main areas indicated as potentially suitable for *P. marginale* during the LGM (north and west North Island), were probably refugia for native forest vegetation that would have precluded these grasshoppers (Newnham *et al.*, 2013). Since the Holocene, but prior to the arrival of humans about 800 years ago, most of New Zealand (87%; including nearly all lowland habitat) was under native forest vegetation; habitat not suitable for these grasshoppers. Although we determined that a suitable climate envelope (fundamental niche) existed over much of New Zealand for thousands of years, most of this area would have been unavailable to *P. marginale* (Fig. 6).

Phaulacridium marginale appears to have expanded its range into areas of open habitat as forest was replaced with native tussock grassland in eastern South Island and fernland (*Pteridium*) in North Island through human activity (Wardle, 1991; Ausseil *et al.*, 2011; Perry *et al.*, 2014). In contrast to climate-based models (even with vegetation and soil type included), clearing forest during the last 800 years has been the major factor determining grasshopper distribution. Before humans arrived in New Zealand there would have been little overlap between the areas with a climate envelope suitable for *P. marginale* and areas lacking forest vegetation (Fig. 6). Both *Phaulacridium* grasshoppers require open grassland/herbfield/scrub and are not found in forests. Therefore, we can infer that prior to human modification of the vegetation, *P. marginale* would have had a realised niche limited to forest edge habitat including river margins, braided rivers and coastal systems, where it does occur today. In contrast, before human activity, tussock grasslands and subalpine scrub were the only vegetation types in the current *P. otagoense* climatic range (Fig. 6), thus *P. otagoense* could have rea- lised its full distribution based on climatic variables.

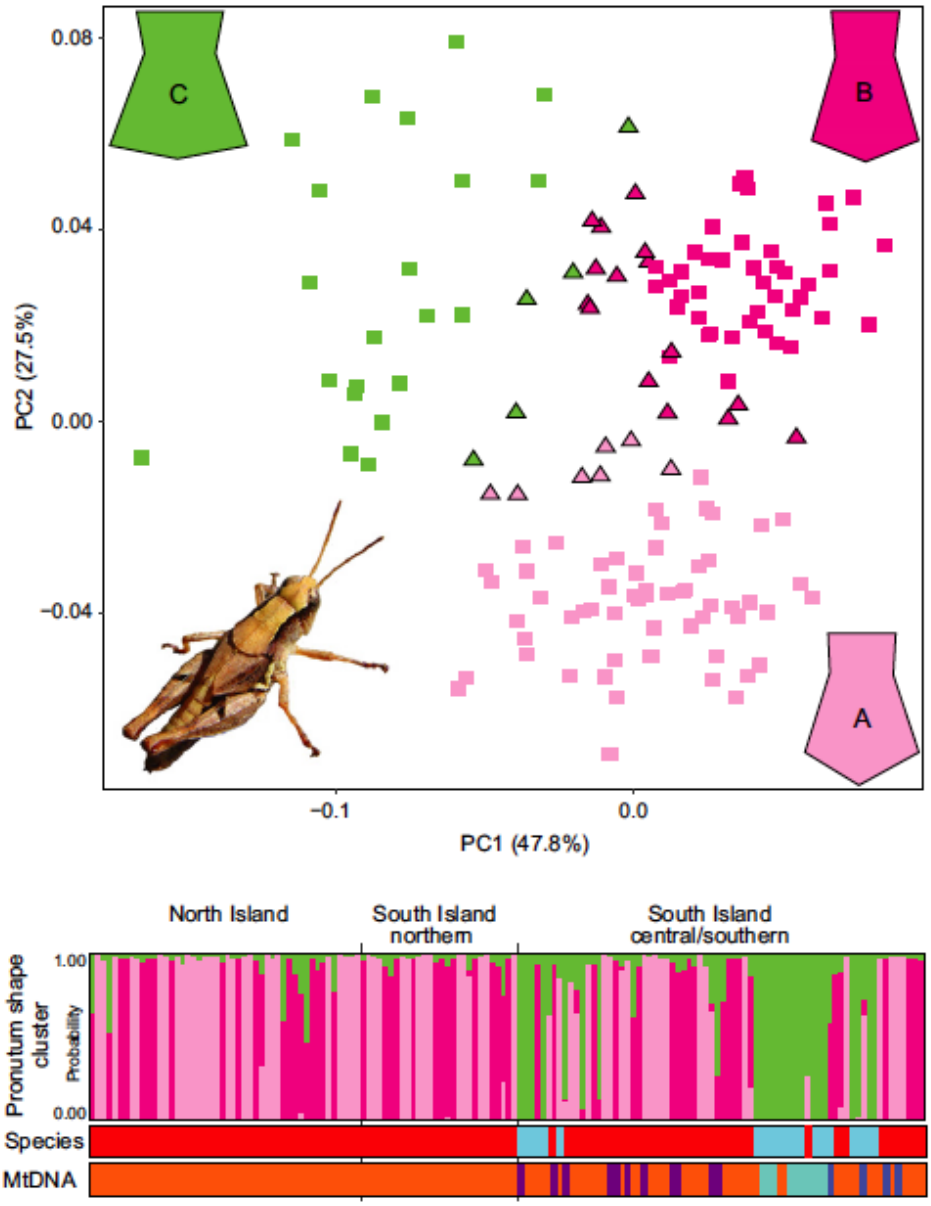

Fig. 4. Geometric morphometric analysis of pronotum shape reveals limited phenotypic introgression of New Zealand *Phaulacridium* grasshopper species. Top: Bayesian assignment of pronotum geometric landmark data (PC1 and PC2), without priors, found most support for a model with three phenotypic clusters. Symbols in the classification plot indicate pronotum shape of female *P. marginale* (cluster A, pink, *n* = 65) and male *P. marginale* (cluster B, magenta, *n* = 57), and *P. otagoense* (cluster C, green, *n* = 26). Squares indicate high assign- ment probability (≥0.9), and triangles show lower assignment probability (<0.9), corresponding to intermediate forms. Representative pronotum shapes of each cluster are shown. Bottom: Bar plot of assignment probabilities of each pronotum to the three model-based clusters, arranged by geographical region. Also shown are species assignments from traditional diagnostic characters (colours as in Appendix S7) (red = *P. marginale*; blue = *P. otagoense*), and mtDNA lineage (colours as in Fig. 3) of each grasshopper.

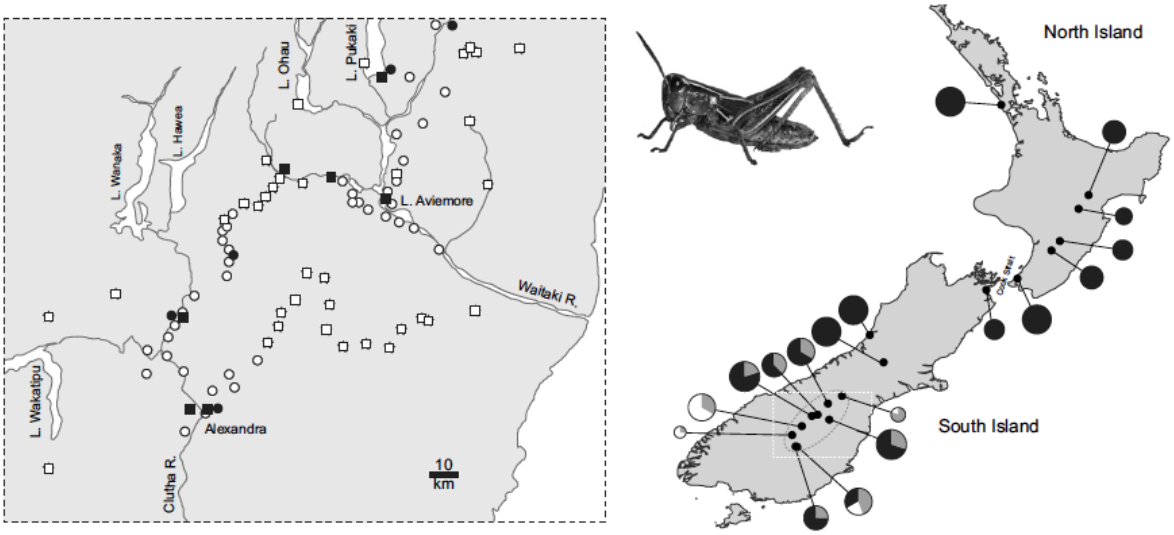

Fig. 5. Geographical distribution of *Phaulacridium* grasshoppers in New Zealand suggests recent range expansion of *P. marginale* and hybridisation in sympatry. Left: Historic distribution of *P. otagoense* (open circles) and *P. marginale* (open squares) in Central Otago and Canterbury, South Island (Westerman & Ritchie, 1984), new records reported in this study (black fill). Right: Proportional composition of population samples comprising *P. marginale* (black), *P. otagoense* (white) and hybrids (grey). Putative hybrids were inferred from mis- match between species identification, pronotum geometry and mtDNA haplotype (see Appendix S8). Intermediate forms exist only in the region of sympatry.

Two species, secondary contact and gene flow

Two morphologically distinct species of grasshoppers as established in their original descriptions (Westerman & Ritchie, 1984; Key, 1992) were present in our sample. Yet, introgression between these two species was apparent from a mismatch between species identification (based on a diagnostic combination of three morphological traits), pronotum shape and mtDNA haplotype distribution (Fig. 3). In addition, evidence that nuclear sequences were common to the two species and diversity of variants within individuals, confirms recent inter-specific gene flow as con- certed evolution normally rapidly homogenises variation among ribosomal DNA repeats (Trewick, 2001; Ganley & Kobayashi, 2007; Trewick *et al.*, 2008). We found that grasshoppers of mixed ancestry were restricted to the area where the ranges of the two species overlap today. Such inter-specific introgression is most likely to occur where taxa that have diverged in isolation make secondary con- tact following environmental change (e.g. Durand *et al.*, 2000; Stemshorn *et al.*, 2011; Potts *et al.*, 2014).

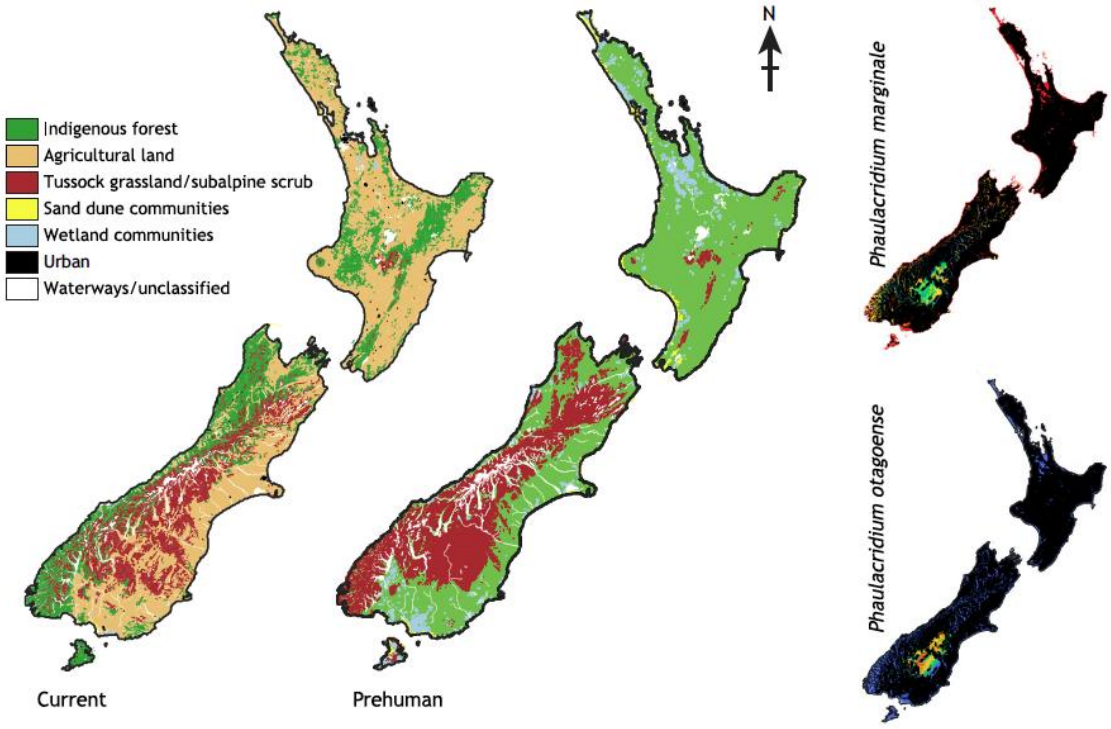

Fig. 6. New Zealand vegetation cover has changed substantially since the arrival of humans ~800 years ago. For *Phaulacridium* grasshoppers, this represents an increase in their realised niche with pre-human native forests cleared and replaced with exotic grasses and herbs. Initial expansion of native tussock grassland in south east South Island following Polynesian fires (Ausseil *et al.*, 2011) has been countered by plantation forestry and agricultural intensification that is also reducing suitability of mixed exotic pastures for these and other native insects. Climate based environmental models for the two species under present conditions (from Fig. 2) were masked (black) to exclude areas naturally unavailable to *Phaulacridium* in pre-human times (Right). The mask encompasses areas with native forest cover, and elevation above 800 m a.s.l., but excludes dunes, river beds and wetlands which might have been used by *Phaulacridium.*

Phaulacridium marginale is currently widespread in New Zealand and sympatric with *P. otagoense* at three locations we surveyed. Our population samples reveal range expansion of *P. marginale* and suggest competitive exclusion within the last 30 years (Fig. 5). In contrast, *P. otagoense* is restricted to inland South Island where diurnal temperature ranges are extreme, and moisture levels low, and is now rare at sites where it was common 30 years ago (Westerman & Ritchie, 1984). Two areas in this region (Otago), Lindis Valley and Lake Dunstan, may be the last strongholds of *P. otagoense* (Fig. 6). Historical records of the distribution of *Phaulacridium* and the results of the current study, suggest that *P. marginale* is replacing *P. otagoense*. This type of advancing replacement (and introgression) has been documented in animal species pairs in various landscapes (e.g. Gill, 1980; Shapiro, 1998). In Otago New Zealand, human activity is implicated in at least three cases of genetic introgression (fish–Esa *et al.*, 2000; lizards–Chapple *et al.*, 2012; grasshoppers–Dowle *et al.*, 2014).

The potential for the exchange of alleles among individuals, populations and higher taxonomic levels is expected in an evolving world (Vaux *et al.*, 2016). However, anthropogenic habitat modification is likely to be leading to elevated rates of secondary contact and inter- specific introgression. Genomic

introgression associated with alien species is expected to be aggravated by global warming (e.g. Muhlfeld *et al.*, 2014), but native species also experience range shifts that increase contact (e.g. Grenouillet & Comte, 2014; Krosby *et al.*, 2015). As the last substantial land environment to be colonised by people (800 years BP cf. 80 000 BP in Europe), New Zealand pro- vides an informative system for exploring evolution of ecological responses to recent human activity. Hybridisation has been documented among New Zealand native and alien species (Morgan-Richards *et al.*, 2009) with many examples linked to human activity. But, we know the interplay between gene flow and natural selection is complex and does not always result in loss of distinct traits or lin- eages (e.g. Dowle *et al.*, 2014). Anthropogenic habitat change is strongly indicated as enabling expansion of the *P. marginale* realised niche into parts of its fundamental niche from which it was formerly excluded by native forest. Although we cannot rule out rapid ecological speciation the current distribution of phenotypic and genetic diversity suggests recent introgression of two previously partitioned species. Replacement of native forest with pasture created from introduced European herbs and grass species has been rapid and widespread in New Zealand. Ongoing agri- cultural 'improvement' threatens the long-term viability of endemic species throughout New Zealand and particularly in the region where *P. marginale* is increasing its range today.

Responses to future climate change are likely to involve not only range shifts and ecological competition among existing genotypes, but also gene flow that could have a major influence on evolutionary change and sustainability of biodiversity.

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Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: doi: 10.1111/ icad.12289:

Appendix S1. Conservation resource reports used for locality records of *Phaulacridium* in New Zealand.

Appendix S2. Nineteen available climatic layers. Appendix S3. Environmental niche model accuracy.

Appendix S4. Population sample size and location details of *Phaulacridium* grasshopper population sampled in New Zealand.

Appendix S5. (a) Morphological features used to char- acterise New Zealand

Phaulacridium grasshoppers. Mor- phometric data were collected for: FW, hind femur width; FL, hind femur length; TL, hind tibia length; PW, prono- tum width; PL, pronotum length; PA, pronotum angle; TeL, tegmina length. (b) Geometric morphometric analy- sis used landmarks around the margin of the pronotum (white circles).

Appendix S6. Variation at eight morphometric charac- ters in female (A) and male (B) New Zealand *Phaulacrid- ium* grasshoppers of two species clustered using Bayesian assignment.

Appendix S7. Classification plots for male and female New Zealand *Phaulacridium* grasshoppers using model- based clustering analysis (Mclust) with three morphomet- ric characters, and no *a priori* identification.

Appendix S8. Individual grasshopper morphological and genetic information.

Appendix S9. Median joining network of nuclear ITS sequence variants (ITS1-5.8S-ITS2) identified from high- throughput genome sequencing of three *Phaulacridium* grasshoppers.

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