Impacts of the Pliocene and Pleistocene glaciations on genetic diversity among New Zealand and Antarctic Arthropods

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Abstract

Geological events such as glaciation and crustal uplift can impact on species' distributions and genetic diversity through the formation of dispersal barriers and fragmentation of habitat. This thesis investigates the effects of the Pliocene and Pleistocene glaciations on intra- and interspecific population diversity in a New Zealand aquatic invertebrate and an Antarctic terrestrial invertebrate.

Caddisflies (Insecta: Trichoptera) are an aquatic invertebrate that are widespread and common throughout both islands of New Zealand. Species are used in stream health studies and as a measure of diversity, but knowledge of their genetic diversity is limited. Mitochondrial DNA (COI) sequence variability was used to examine levels of divergence among closely-related species of caddisflies collected from throughout New Zealand. Based on genetic analysis, seven closely related species pairs were identified, consisting of morphologically distinct species, each restricted to either the North or South Island. Another five species showed similar or greater levels of "intraspecific" divergence, with genetically distinct populations on each island. Sequence divergence between these twelve "species pairs" ranged from 0.41% in *Confluens olingoides / Confluens hamiltoni*, to 9.92% between the North and South Island populations of *Pycnocentria evecta.* Based on molecular clock estimates, divergences for these twelve species pairs were estimated to within the last 5 million years, with most dating to the beginning of the Pleistocene (2 Mya). I conclude that population fragmentation during the Pleistocene glaciations and subsequent closing of the Cook Strait land bridge have both played important roles in the isolation and speciation of the New Zealand caddisflies.

Mitochondrial DNA (COI) analysis was also used to examine the levels of genetic variability within and among populations of three endemic springtail species (Arthropoda: Collembola), collected from the Mackay Glacier region of southern Victoria Land, in the Ross Dependency, Antarctica. I tested the hypothesis that genetic divergences would occur among glacially-isolated habitats within a fragmented landscape. Mitochondrial analysis of 97 individuals showed high levels of genetic divergence at small spatial scales (<15km). High levels of genetic divergence were found among populations for two of the three species. *Gomphiocephalus hodgsoni*, a widespread and common species showed 7.6% sequence divergence on opposite sides of the Mackay Glacier. Similar divergences were also found for *Neocryptopygus nivicolus,* a more range-restricted species showing 4.0% sequence divergence among populations. Based on molecular clock estimates, divergence of these populations occurred in the last 5 Mya. It was suggested that glaciations during the Pliocene (5-2 Mya) and throughout the Pleistocene (2 Mya - 10 Kya) have fragmented springtail populations in this region and isolated them in small, refugial nunataks. I conclude that glaciation has promoted and maintained the levels of diversity observed among populations of springtails and that isolation has occurred on extremely small spatial scales.

Collectively, the two studies presented here suggest that the Pleistocene glaciations are responsible for habitat fragmentation, the genetic signatures for which can be observed in invertebrate taxa in both New Zealand and southern Victoria Land, Antarctica.

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Chapter I

Thesis Introduction

The widespread availability and access to molecular techniques such as DNA sequencing has allowed unprecedented analysis of the genetic diversity that exists within and among natural populations. Through the study of the genetic relationships between organisms, known as phylogenetics, it is now possible to use these data to study the evolution of landscapes using organisms that live within it. The presence of refugial zones, where organisms can survive periods of habitat restrictions will often result in pockets of range-restricted genetic variation and cases of morphologically similar, genetically distinct taxa which indicate cryptic diversity. The determination of such features can be used to build predictive models for the effects of climate change and to identify regions of high species diversity, so called "biodiversity hotspots" (Hogg and Stevens, 2002; Hogg et al., 2006b; McGaughran et al., 2011a).

The coupling of phylogenetics with phylogeography, the study of the geographic distributions of genetic populations, allows for the genetic separation between populations to be used as a means of dating isolating events within a landscape (Brower, 1994; Juan et al., 1996; Quek et al., 2004; McGaughran et al., 2010a). Readily available molecular approaches, (e.g. Hebert et al., 2003; Jinbo et al., 2011), have made it possible to test hypotheses regarding speciation and the possible geological events that may be responsible (e.g. see Trewick, 2001; Stevens and Hogg, 2003a; Knox et al., 2011; McGaughran et al., 2011).

One overarching event that has shaped the arthropod fauna of the Southern Hemisphere is the glaciations of the Pliocene and Pleistocene epochs. The geologic signature of this time period, stretching from ~5 Mya to 10 Kya, indicates a series of glacial events where ice covered much of the land mass within the Southern Hemisphere, punctuated by warmer interglacial periods (Stevens, 1980; Clapperton and Sugden, 1990; Suggate, 1990). This glacial cycling has subsequently left a signature in the population genetics of many taxa found in the affected areas (Trewick, 2001; Ruzzante et al., 2008; McGaughran et al., 2010a). The effects of glaciation on the landscape have been attributed to the fragmentation of once continuous species distributions and to the genetic effects of isolation, survival and recolonisation from refugial zones during interglacial periods.

As mitochondrial DNA is maternally inherited and has relatively slow recombination/mutation rates, it is a widely used genetic tool for the study of phylogeography (e.g. Trewick, 2001; Stevens and Hogg, 2003b). The Cytochrome *c* oxidase subunit I (COI) gene fragment evolves fast enough to identify differences in genetic haplotypes among populations, but slow enough to avoid mutational saturation. Accordingly, this locus was chosen as the focus of the international DNA barcoding barcode of life (iBOL) initiative (Hebert et al., 2003), and is suitable for identifying genetic differences between geographically distinct populations. It has also been shown to effectively identify the presence of high population structuring indicative of cryptic species diversity (Bickford et al., 2007; Knox et al., 2011).

In this thesis, I focussed on the effects of the Pliocene and Pleistocene glacial periods on the phylogeographic distributions of intra- and interspecific diversity across the main islands of New Zealand, and in a small region of southern Victoria Land (Antarctica). The impacts of glaciation and the related environmental changes differ greatly between the two study sites, allowing for the investigation of speciation events in two different habitats, and at two different spatial scales. This thesis consists of two research chapters, dealing with the New Zealand and Antarctic study sites respectively.

The following chapter (Chapter II) focuses on the caddisfly fauna (Trichoptera) of New Zealand. The caddisflies are a species rich, ecologically diverse group whose larvae make up a significant portion of the freshwater ecosystem (Hogg et al., 2009). Larvae and adults are often used as primary indicators of water quality and stream health (e.g. Boulton et al., 1997) but many remain un-described and are often very difficult to identify (Collier and Smith, 1998; Zhou et al., 2007; Johanson et al., 2009). This order is found in a variety of aquatic habitats in New Zealand, including lakes, rivers, seepages and even marine environments (Ward, 1980).

However, limited knowledge exists for caddisfly diversity at the species and genetic levels. This, coupled with limited knowledge on the effects of the Pliocene and Pleistocene on New Zealand's aquatic environments (Trewick, 2001) indicates that the diversity of caddisfly species in New Zealand would benefit from further evaluation. The use of phylogenetics has revealed that the distributions of many taxa show the effects of New Zealand’s dynamic geological past, in particular the impacts of the Pleistocene (e.g. Morgan-Richards, 1995; Trewick, 2001; Hogg et al., 2006b; Knox et al., 2011).

In New Zealand, the Pliocene and Pleistocene caused the formation of many alpine glaciers, mainly in the South Island (Winkworth et al., 2005). These glaciers pushed right out to the sea during peak glacial phases, and likely presented a major dispersal barrier to aquatic invertebrates such as the caddisflies.

The effect of these dispersal barriers on the diversity of the caddisfly fauna has potentially resulted in the North and South Islands being recolonised from separate refugial habitats, resulting in differing diversity between the two islands (Trewick, 2001; Boyer and Giribet, 2009).

The underlying hypotheses of this research were that: 1) genetic divergence occurs among individuals of closely related species occupying each island; and 2) levels of divergence correspond with geological events occurring within the last 5 Mya, such as the Pleistocene glaciations and the closing of the Cook Strait land bridge. Phylogenetic analysis of mtDNA sequences and knowledge of New Zealand's geologic past allowed the timing of population divergences to be dated, thus giving an insight into the geologic events that likely drove these divergences. The status of several putative species was also able to be examined based on the high genetic structuring between populations.

The second research chapter (Chapter III) examines levels of genetic variability that can occur on relatively small spatial scales in southern Victoria Land, Antarctica. The environment here is very harsh, with over 99% of the continent permanently covered in ice and annual temperatures of around -20 (Stevens and Hogg, 2006). This is reflected in the extremely limited faunal diversity in non-coastal regions. The largest year round organisms in this environment are the springtails (Collembola), three species of which are endemic to southern Victoria Land. One species, *G. hodgsoni*, is common throughout the Dry Valley region, and has subsequently been the focus of many studies into the phylogeography and diversity of southern Victoria Land (e.g. Stevens and Hogg, 2003a; Nolan et al., 2006; McGaughran et al., 2008). Earlier studies have revealed the presence of two other, range restricted springtail species, *N. nivicolus* and *A. monoculata,* in small, ice-free regions to the north of the Dry Valleys near the Mackay Glacier (Gressitt et al., 1963).

The Pliocene and Pleistocene periods saw many fluctuations in glacial coverage in this region of Antarctica, and several authors have suggested that the terrestrial organisms of the region, particularly mites and springtails, have survived these cycles in fragmented refugial zones (Janetschek, 1967a; Stevens and Hogg, 2003b; Convey et al., 2009). Accordingly, much of the genetic structure observed among populations of *G. hodgsoni* has therefore been attributed to the re-colonisation of available habitat that has come available in the last 17 Ky (McGaughran et al., 2011a).

The high diversity of taxa in the vicinity of the Mackay Glacier, including lichens, mosses, mites (Acari) and springtails has been used to suggest that this region likely contained several refugial zones during the Pliocene and Pleistocene epochs (Stevens and Hogg, 2003b; Demetras et al., 2010; Green et al., 2011). My study, therefore, focused on the small-scale genetic variability that occurs within this area, in order to investigate the effects of dispersal barriers on geographically close populations as well as factors that may promote and maintain biological diversity.

The underlying hypotheses of this chapter were: 1) that the Mackay Glacier region would support genetically divergent springtail populations among isolated habitats and; 2) that high levels of both genetic variability and genetic divergence would exist, and potentially indicate the presence of refugial zones from the Pliocene and Pleistocene glaciations.

The final chapter of the thesis (Chapter IV) provides an overall summary and suggestions for future research.

Literature Cited

Bickford D., Lohman D.J., Sodhi N.S., Ng P.K.L., Meier R., Winker K., Ingram K.K., Das I., 2007. Cryptic species as a window on diversity and conservation. Trends in Ecology & Evolution. 22, 148–55.

Boulton A.J., Scarsbrook M.R., Quinn J.M., Burrell G.P., 1997. Land-use effects on the hyporheic ecology of five small streams near Hamilton, New Zealand. New Zealand Journal of Marine and Freshwater Research. 31, 609–622.

Boyer S.L., Giribet G., 2009. Welcome back New Zealand: regional biogeography and Gondwanan origin of three endemic genera of mite harvestmen (Arachnida, Opiliones, Cyphophthalmi). Journal of Biogeography. 36, 1084–1099.

Brower A., 1994. Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. Proceedings of the National Academy of Sciences of the United States of America. 91, 6491–5.

Clapperton C.M., Sugden D., 1990. Late Cenozoic glacial history of the Ross Embayment, Antarctica. Quaternary Science Reviews. 9, 253–272.

Collier K.J., Smith B.J., 1998. Dispersal of adult caddisflies (Trichoptera) into forests alongside three New Zealand streams. Hydrobiologia. 361, 53–65.

Convey P., Stevens M.I., Hodgson D.A., Smellie J.L., Hillenbrand C.-D., Barnes D.K. A., Clarke A., Pugh P.J. A., Linse K., Cary S.C., 2009. Exploring biological constraints on the glacial history of Antarctica. Quaternary Science Reviews. 28, 3035–3048.

Demetras N.J., Hogg I.D., Banks J.C., Adams B.J., 2010. Latitudinal distribution and mitochondrial DNA (COI) variability of *Stereotydeus* spp. (Acari: Prostigmata) in Victoria Land and the central Transantarctic Mountains. Antarctic Science. 22, 749–756.

Green T.G.A., Sancho L.G., Türk R., Seppelt R.D., Hogg I.D., 2011. High diversity of lichens at 84°S, Queen Maud Mountains, suggests preglacial survival of species in the Ross Sea region, Antarctica. Polar Biology. 34, 1211–1220.

Gressitt J., Leech R., Wise K.A.J., 1963. Entomological investigations in Antarctica. Pacific Insects. 5, 287–304.

Hebert P.D.N., Cywinska A., Ball S.L., deWaard J.R., 2003. Biological identifications through DNA barcodes. Proceedings of the Royal Society: Biology. 270, 313–21.

Hogg I.D, Smith B.J., Banks J.C., deWaard J.R., Hebert P.D.N., 2009. Testing use of mitochondrial COI sequences for the identification and phylogenetic analysis of New Zealand caddisflies (Trichoptera). New Zealand Journal of Marine and Freshwater Research. 43, 1137–1146.

Hogg I.D., Stevens M.I., Schnabel K.E., Ann Chapman M., 2006. Deeply divergent lineages of the widespread New Zealand amphipod *Paracalliope fluviatilis* revealed using allozyme and mitochondrial DNA analyses. Freshwater Biology. 51, 236–248.

Hogg I.D., Stevens M.I., 2002. Soil Fauna of Antarctic Coastal Landscapes. Ecological Studies. 154, 265–282.

Janetschek H., 1967. Arthropod ecology of south Victoria Land. Antarctic Research Series. 10, 205–293.

Jinbo U., Kato T., Ito M., 2011. Current progress in DNA barcoding and future implications for entomology. Entomological Science. 14, 107–124.

Johanson K.A., Kjer K., Malm T., 2009. Testing the monophyly of the New Zealand and Australian endemic family Conoesucidae Ross based on combined molecular and morphological data (Insecta: Trichoptera: Sericostomatoidea). Zoologica Scripta. 38, 563–573.

Juan C., Oromi P., Hewitt G.M., 1996. Phylogeny of the genus *Hegeter* (Tenebrionidae, Coleoptera) and its colonization of the Canary Islands deduced from Cytochrome Oxidase I mitochondrial DNA sequences. Heredity. 76, 392–403.

Knox M.A., Hogg I.D., Pilditch C.A., 2011. The role of vicariance and dispersal on New Zealand’s estuarine biodiversity: the case of *Paracorophium* (Crustacea: Amphipoda). Biological Journal of the Linnean Society. 103, 863–874.

McCulloch G.A., Wallis G.P., Waters J.M., 2010. Onset of glaciation drove simultaneous vicariant isolation of Alpine insects in New Zealand. Evolution. 64, 2033–43.

McGaughran A., Hogg I.D., Convey P., 2011. Extended ecophysiological analysis of *Gomphiocephalus hodgsoni* (Collembola): flexibility in life history strategy and population response. Polar Biology. 34, 1713–1725.

McGaughran A., Hogg I.D., Stevens M.I., 2008. Patterns of population genetic structure for springtails and mites in southern Victoria Land, Antarctica. Molecular Phylogenetics and Evolution. 46, 606–18.

McGaughran A., Stevens M.I., Holland B.R., 2010. Biogeography of circum-Antarctic springtails. Molecular Phylogenetics and Evolution. 57, 48–58.

Morgan-Richards M., 1995. A new species of tree weta from the North Island of New Zealand (Hemideina: Stenopelmatidae: Orthoptera). New Zealand Entomologist. 18, 15–23.

Nolan L., Hogg I.D., Stevens M.I., Haase M., 2006. Fine scale distribution of mtDNA haplotypes for the springtail *Gomphiocephalus hodgsoni* (Collembola) corresponds to an ancient shoreline in Taylor Valley, continental Antarctica. Polar Biology. 29, 813–819.

Quek S., Davies S., Ilino T., Pierce N., 2004. Codiversification in an ant-plant mutualism: stem testure and the evolution of host use in *Crematogaster* (Formicidae: Myrmicinae) inhabitants of *Macaranga* (Euphorbiaceae). Evolution. 58, 554–570.

Ruzzante D.E., Walde S.J., Gosse J.C., Cussac V.E., Habit E., Zemlak T.S., Adams E.D.M., 2008. Climate control on ancestral population dynamics: insight from Patagonian fish phylogeography. Molecular ecology. 17, 2234–44.

Stevens G., 1980. New Zealand Adrift. A.H and A.W Reed, Wellington.

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

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

Suggate R.P., 1990. Late Pliocene and Quaternary glaciations of New Zealand. Quaternary Science Reviews. 9, 175–197.

Trewick S.A., 2001. Scree weta phylogeography: Surviving glaciation and implications for Pleistocene biogeography in New Zealand. New Zealand Journal of Zoology. 28, 291–298.

Ward J.B., 1980. The New Zealand marine caddisflies (Trichoptera). Ecological Entomology. 18–20.

Winkworth R., Wagstaff S., Glenny D., Lockhart P., 2005. Evolution of the New Zealand mountain flora: Origins, diversification and dispersal. Organisms Diversity & Evolution. 5, 237–247.

Zhou X., Kjer K.M., Morse J.C., 2007. Associating larvae and adults of Chinese Hydropsychidae caddisflies (Insecta:Trichoptera) using DNA sequences. Journal of the North American Benthological Society. 26, 719–742.

Chapter II

Mitochondrial DNA variability among closely-related species of caddisflies (Trichoptera): Evidence for Pleistocene vicariance among populations on the North and South Islands of New Zealand[*]

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Abstract

We used mitochondrial DNA (COI) sequence variability to examine levels of divergence among closely-related species of caddisflies (Insecta: Trichoptera), collected from throughout New Zealand. Based on an analysis of 509 individuals covering a putative 130 species, seven closely related species pairs were identified, consisting of morphologically distinct species, each restricted to either the North or South Island. Another five species showed similar or greater levels of "intraspecific" divergence, with genetically distinct populations on each island. Sequence divergence between theses twelve "species pairs" ranged from 0.4% in *Confluens olingoides / Confluens hamiltoni*, to 9.9% between the North and South Island populations of *Pycnocentria evecta*. Based on molecular clock estimates, divergence of these twelve species pairs has occurred within the last 5 million years, with most dating to the beginning of the Pleistocene (2 Mya). We conclude that: 1) population fragmentation during the Pleistocene glaciations and subsequent closing of the Cook Strait land bridge have played important roles in the isolation and speciation of the New Zealand Caddisflies; and 2) the taxonomy of the New Zealand Caddisflies, and likely other aquatic taxa requires re-evaluation using a combined morphological and molecular approach.

Introduction

The biogeography of New Zealand aquatic invertebrates is often a reflection of a dynamic geological past (e.g. Knox et al. 2011). While the geological origins of the separate New Zealand landmass dates back 75 million years (My), current species assemblages are believed to have originated only during the last 5 My (Winkworth et al., 2005) encompassing events from both the Pliocene (~5 - 2 Mya) and the Pleistocene (~2 Mya - 10,000 Ya) (Stevens, 1980; Suggate, 1990). During the early Pliocene (~5 Mya) there was an increase in crustal uplift activity (Stevens, 1980) and the resulting axial mountain ranges would have generated new alpine habitats. This likely facilitated numerous allopatric speciation events, particularly in the South Island (Trewick, 2001; Winkworth et al., 2005). Uplift in the North Island was less extreme, and the highest present-day peaks have their origins in the volcanic activity of the Late Pleistocene (1 - 0.5 Mya; Suggate, 1990; Winkworth et al., 2005).

Throughout the Pliocene, a steady decrease in air temperatures occurred, culminating in the Late Pliocene and Early Pleistocene with the first major glaciation of the Pleistocene Ice Age (2.6-2.4 Mya; Stevens, 1980; Suggate, 1990). During this period, large glaciers formed in many of New Zealand's mountain ranges which, due to uplift, were now high enough to support glacier formation on the South Island at least (Stevens, 1980; Winkworth et al., 2005). A second glaciation between 2.2 and 2.1 Mya is thought to have affected the north-west of the South Island particularly severely (Suggate, 1990). Two later glacial periods are also known from 350 Kya and 150 Kya (Stevens, 1980).

A series of up to 20 glacial and interglacial cycles occurred during the Pleistocene (Stevens, 1980), and these glacial periods saw extension of ice flows, some down to sea level, particularly on the west coast of the South Island. Much of the North Island remained ice free, largely due to its lower topography and drier climate. Glaciers pushed out to the seas carving new valleys, altering catchments and water ways and depositing vast volumes of moraine. During interglacials, forests previously restricted to fragmented refugia in the South Island, and to the far north in the North Island, were now able to expand, often covering much of the available landscape (Stevens, 1980). During drier periods, grasslands dominated most of both islands. New habitat repeatedly became available and was then inundated along the coast lines as global sea levels changed. These areas may have served as temporary refuges for those taxa that could readily disperse as sea levels changed (Stevens, 1980; Trewick, 2001; Boyer and Giribet, 2009).

Understanding the effects of this geologic history on New Zealand insect taxa may provide a better understanding of patterns of species diversity, as well as helping to identify areas which may be biodiversity “hotspots”. New Zealand has many examples of either cryptic or previously unknown species (e.g. Morgan-Richards, 1995; Trewick, 2001; Hogg et al., 2006b; McCulloch et al., 2010), particularly those which are restricted to either the North or South Islands of New Zealand. Here, we focused on the caddisflies (Insecta: Trichoptera), a species-rich, ecologically diverse group of aquatic insects whose larvae are significant components of freshwater ecosystem (Wiggins 2004). Due to the diverse range, and geographic separation, of aquatic habitats used by caddisflies (e.g. small seepages, streams, lakes), and potentially limited dispersal (Pauls et al., 2006) populations may have an even greater propensity to become reproductively isolated relative to other taxa (e.g. Pauls et al., 2008; Smith and Smith, 2009) . To address these issues, we examined mitochondrial (COI) sequence divergences for New Zealand caddisflies from the North and South Islands of New Zealand. Specifically, we tested the hypothesis that levels of divergence between closely related species occupying each island correspond with geological events occurring within the last 5 My, including the closing of the Cook Strait land bridge.

Methods

Sample collection and specimen identification:

Specimens were obtained from existing collections at Auckland Museum and opportunistically from on-going sampling programmes covering a range of geographic locations in the North and South Islands of New Zealand (Fig. 1). Auckland Museum specimens where obtained either "wet" (stored in 70% ethanol and room temperatures) or "dry" (pinned adult specimens). Samples from on-going sampling programmes were stored in 95% ethanol and kept at 4ºC until needed for DNA extraction. These specimens are stored either at the National Institute of Water and Atmospheric Research, Hamilton (NIWA), or the University of Waikato.

Specimens were identified morphologically or, in the case of museum specimens confirmed, to species level where possible using available taxonomic keys (e.g. (Mosely and Kimmins D, 1953; Wise, 1958; Smith, 2013). A single rear leg was removed from each individual and placed in an individually labelled vial for DNA extraction.

Genetic Analyses:

Total genomic DNA was extracted from a total of 509 specimens, using Red Extract n Amp (Sigma-Aldrich) at a 90% reduction in the suggested volume, due to the small amount of tissue available. Polymerase Chain Reactions (PCRs) were carried out in a 15µL reaction, which contained 5.7µL MQH_2O, 7.5µl of PRC master mix solution (i-Taq, Intron Biotechnology), 0.4µL of each primer and 1µL of template DNA. A 658bp fragment of the mitochondrial CO1 gene was amplified using predominantly the primers LepR1 (sequence: 5'-TAAACTTCTGGATGTCCAAAAAATCA-3') and LepF1 (sequence: 5'-ATTCAACCAATCATAAAGATATTGG-3') (Ratnasingham and Hebert, 2007), or HCO2198 (sequence: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3') and LCO1490 (sequence: 5'-GGTCAACAAATCATAAAGATATTGG-3') (Folmer et al., 1994). Primers were used at 1.0mM concentration. PCR conditions included: initial denaturing phase of 94ºC for 5 min, followed by 5 cycles at 94ºC for 60 s, 48ºC for 90 s and 72 ºC for 60 s, followed by 36 cycles of 94ºC for 60 s, 52ºC for 90 s and 72ºC for 60 s. Final elongation was at 72ºC for 5 min (Folmer et al., 1994). We also employed a variant on this program which omitted the 5 cycles (94ºC for 60 s, 48ºC for 90 s and 72 ºC for 60 s), to remove the lower annealing temperature.

For problematic samples (e.g. older museum specimens not returning a satisfactory PCR product), we diluted the template DNA 1:5 with MQH$_2$O water in an attempt to reduce the concentration of any PCR inhibitors that may have been present in the extraction solution. If full length amplifications were still unsuccessful, specimens were then amplified using the primers MLEPF1 and MLEPR1, following (Hajibabaei et al., 2006). These primers, when paired with LepR1 or LepF1 respectively, would amplify either the first half or last half of the CO1 sequence. The resulting fragments overlapped and following successful sequencing allowed assembly of a full 685bp fragment.

PCR products were visualized on 1% agarose TBE gels stained with ethidium bromide. Successful PCR products were purified using 0.2 µl ExonucleaseI (EXO) and 0.1 µl Shrimp Alkaline Phosphate (SAP) with 2.7 µl MQH$_2$O following manufacturer's instructions (Global Science & Tech Ltd.) and sequenced in both directions on an ABI3130 sequencer using the same primers for amplification. Sequences were aligned using Geneious, ver 5.4.2 and confirmed as Trichoptera using the GenBank BLASTn algorithm and using the BOLD ver. 3 search engine. All new sequences were uploaded to the BOLD project Caddisflies of New Zealand II (NZTRI). We also used existing species records from the public project Caddisflies of New Zealand (NZCAD).

Selection of species pairs:

A 658 bp (219 codons) sequence was obtained for 509 specimens. No stop codons were detected. To remove ambiguous base pair assignments which could not be resolved, sequences were trimmed from both ends to 600bp (199 codons) and aligned for all further analysis. A Neighbour Joining (NJ) tree was then constructed using Geneious, ver 5.4.2. Collection locations for each specimen were overlaid and the tree searched visually for species with closely related (~5% sequence divergence or less) sibling pairs or with deep intraspecific divergences. This produced 14 species (seven species pairs) with North and South Island restricted pairs and another five species that were found to have similar or greater levels of intraspecific sequence divergence which were also treated as separate "species", giving a total of 12 species pairs and 132 individual sequences (Table 1, Fig. 1).

Phylogenetic Analyses

An available CO1 sequence for *Deleatidium sp.* (Ephemeroptera) (BOLD accession number NZMAY021-12) was selected for use as an outgroup taxon.

Chi-square tests (X^2) as implemented in PAUP* 4.0 (Swofford, 2002) were used to determine whether the assumption of equal base frequencies among sites was violated on all sites and on third codon positions only. JModel Test 0.1.1 (Posada, 2008) was used to determine the most appropriate substitution model for Maximum Likelihood (ML). Settings were as follows: 11 substitution schemes (88 models), base frequencies +F, rate variation +I, +G, set to BioNJ. The model selected was GTR + I + G (-lnL = 6,397.238). Maximum Likelihood heuristic searches were conducted using this model in MEGA 5.10 with 1000 bootstrap replications. NJ and MP analyses were also performed using MEGA 5.10 using 1000 bootstrap replicates. For NJ we used Kimura-2-Parameter (K2P), as rates among sites were assumed to be uniform, and substitutions included transitions and transversions. Other settings were set to default options in MEGA 5.10.

MrBayes (Huelsenbeck and Ronquist, 2001) was used to conduct a Bayesian Inference analysis. A general time reversal model (GTR +I +G) was used, with a log normal relaxed clock model and speciation yule process as the tree prior. The Markov chain Monte Carlo (MCMC) was 50,000,000 generations, sampling trees every 5,000. A burn in of 1,000 trees was determined by plotting log-likelihood values against generation time in TRACER (Rambaut and Drummond, 2007). The majority rule tree was acquired from the 9,000 trees sampled after the burn in period. The tree was then visualized in Tree Annotator.

Pair-wise genetic distances between COI sequences for the 12 'species pairs' were calculated in MEGA 5.10. Approximate geological ages between each of the species pairs were estimated using a molecular clock calibration of 2 - 2.3% sequence divergence per million years, determined using invertebrate mitochondrial data (as per: Juan et al., 1996; Roslin, 2001; Trewick and Morgan-Richards, 2005). However, we caution that comparisons of log likelihood scores using chi-square tests implemented in PAUP* for trees with and without a molecular clock enforced, indicated that these sequences may not be evolving in a clock like manner. The GTR model without a molecular clock enforced gave a -log likelihood score of 7410.39726. When a molecular clock was enforced the -log likelihood value was 7470.41430. Comparison of these values using a chi-square test implemented in PAUP* suggested that they were not significantly different (X^2=120.1; p= 0.723;d.f = 130).

Table 1: Closely related species of caddisflies found on the North or South Island of New Zealand. Number of sites sampled in the vicinity of a particular coordinate is provided in parentheses. Exact sampling locations are provided on the Barcode of Life Datasystems (BOLD) database under project NZTRI.

Pair #	**Species Pair**	**Collection Coodinates**
1	*Confluens olingoides* SI	-44.72 168.20
	Confluens hamiltoni NI	-37.80 175.40
2	*Tiphobiosis* indet SI	-42.28 171.47
		-42.35 171.37
	Tiphobiosis cowei NI	-37.79 175.06
3	*Hydrobiosis* nr *spatulata* SI	-41.12 174.09
	Hydrobiosis spatulata NI	-38.98 174.84
		-39.16 175.46
4	*Polyplectropus puerilis* SI	-43.70 172.42
		-41.12 174.09
	Polyplectropus altera NI	-39.15 176.84
		-38.33 175.08
		-37.71 175.12
5	*Psilochorema tautoru* SI	-41.12 174.09
	Psilochorema mimicum NI	-37.71 175.12
		-39.46 176.28 (6)
6	*Psilochorema embersoni* SI	-41.65 171.91

	Psilochorema macroharpax NI	-39.46 176.24
7	*Neurochorema* sp. A SI	-41.65 171.90
	Neurochorema armstrongi NI	-39.16 174.20
8	*Triplectides dolichos* SI	-41.12 174.09 (2)
	Triplectides dolichos NI	-39.16 174.20
9	*Pseudoeconesus bistirpis* SI	-41.12 174.09
	Pseudoeconesus bistirpis NI	-37.80 175.40
		-37.99 176.35
		-37.79 175.06
10	*Oeconesus maori* SI	-41.12 174.09
	Oeconesus maori NI	-37.99 176.35
		-37.71 175.12
		-37.80 175.40 (2)
11	*Hydropsyche raruraru* SI	-40.85 172.77
	Hydropsyche raruraru NI	-39.16 174.20
12	*Pycnocentria evecta* SI	-41.12 174.09
	Pycnocentria evecta NI	-38.33 175.08
		-38.67 174.86 (2)

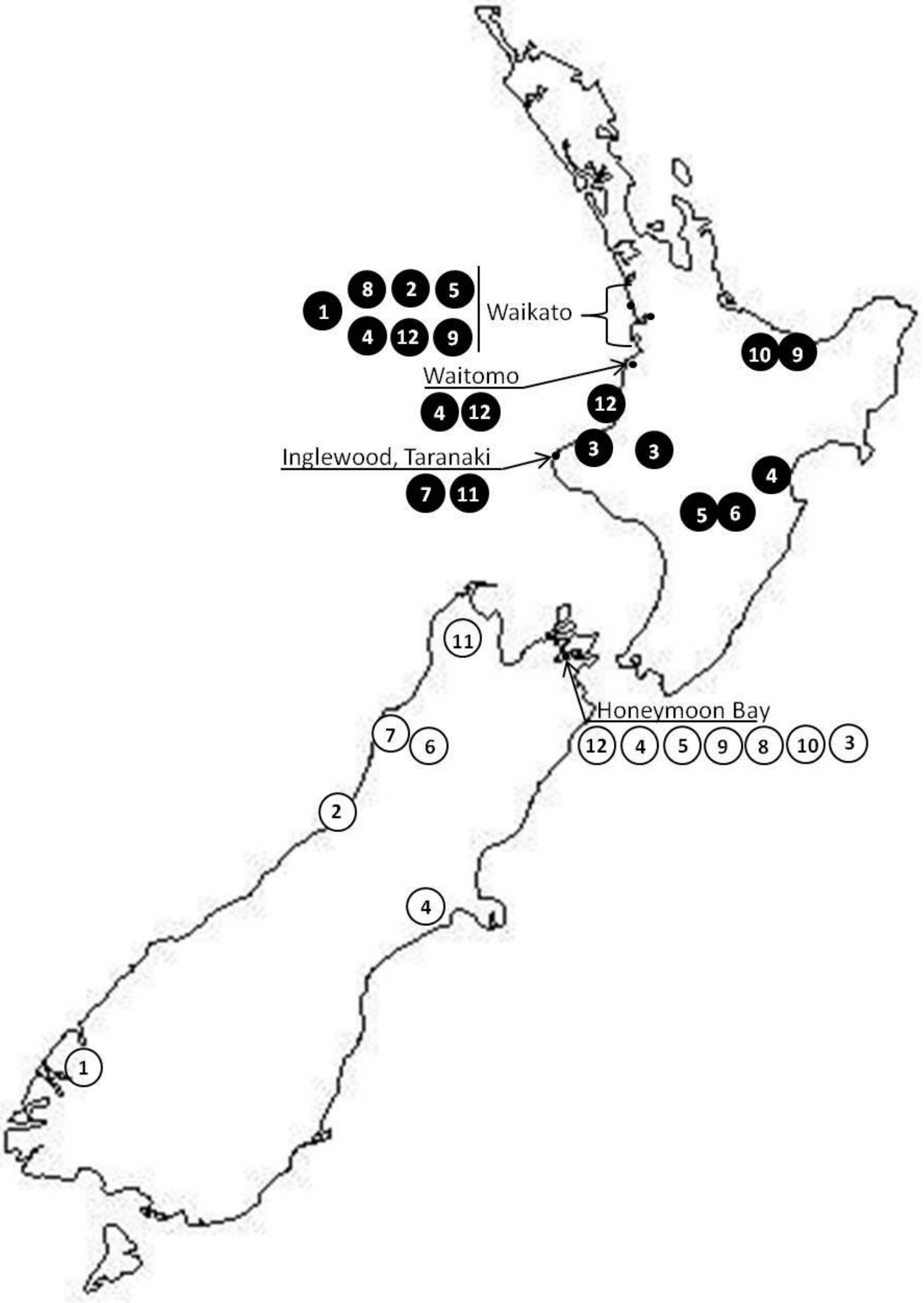


Figure 1: Collection locations for the 12 'species pairs'. Numbers correspond to species pairs in Table A. Black circles correspond to North Island samples and clear circles represent South Island samples for species pairs.

Results

Of the 600 bp analysed, 273 characters were constant, 312 were parsimony informative, and the remaining 15 were parsimony-uninformative. The nucleotide composition averaged across all sequences showed an A-T bias of 68.9% (A = 30.7%, T = 38.2%, C = 16.9%, G = 14.2%). Base pair frequencies were homologous among sequences when all codon positions were included (X^2 = 305.891, p = 0.9997, d.f = 396). However, homology of base pair frequencies was rejected when considering the third codon position only (X^2 = 677.269, p = 0.000, d.f = 396).

A Maximum Likelihood (ML) tree is shown in Figure 2. All other tree constructions (MP, NJ) showed similar topology (data not shown). Species-pair linking nodes in the ML and Neighbour Joining trees all had 99% bootstrap support. For the Maximum Parsimony, 11 of the 12 clusters had >96% bootstrap support. The exception was the *Pseudoeconesus bistirpis* NI/SI pair which had 90% bootstrap support. A Bayesian Inference tree is shown in Figure 3. Posterior probabilities from the BI tree were 1 for 9 of the species pairs. The *Psilochorema mimicum* / *tautoru* pair had a posterior probability of 0.9978, the *Psilochorema embersoni* / *macroharpax* pair had a posterior probability of 0.8155 and the *Pseudoeconesus bistirpis* pair had a posterior probability of 0.4238. This latter 'species pair' received 90-99% bootstrap support from the ML, MP and NJ trees. The topology of these trees confirmed the North Island-South Island distributions of these 'species pairs'.

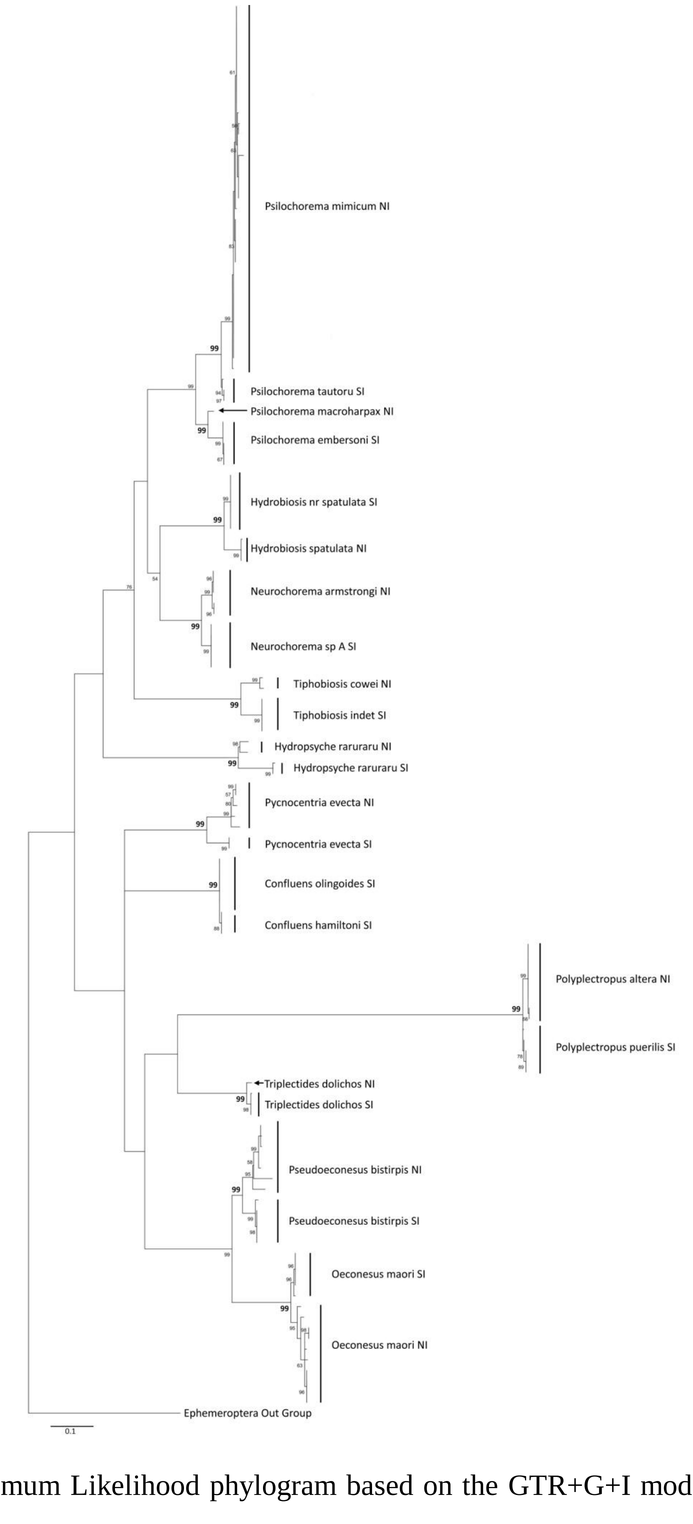


Figure 2: Maximum Likelihood phylogram based on the GTR+G+I model derived from jModelTest, using a 600bp fragment of CO1 mtDNA from 132 individual sequences. Bootstrap values for species pair nodes are in bold. NI or SI identifies species groups as being collected from the North Island or the South Island, respectively.

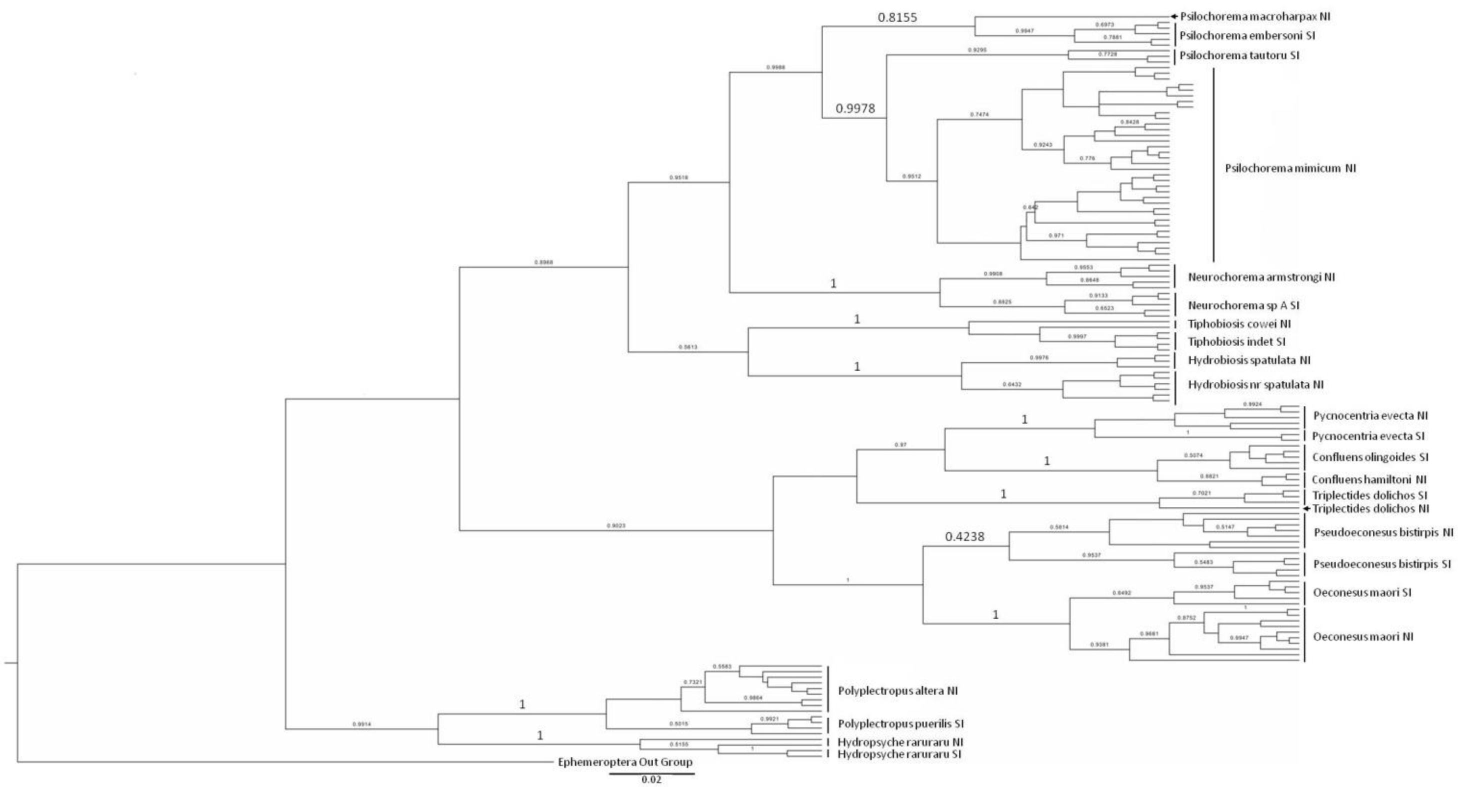


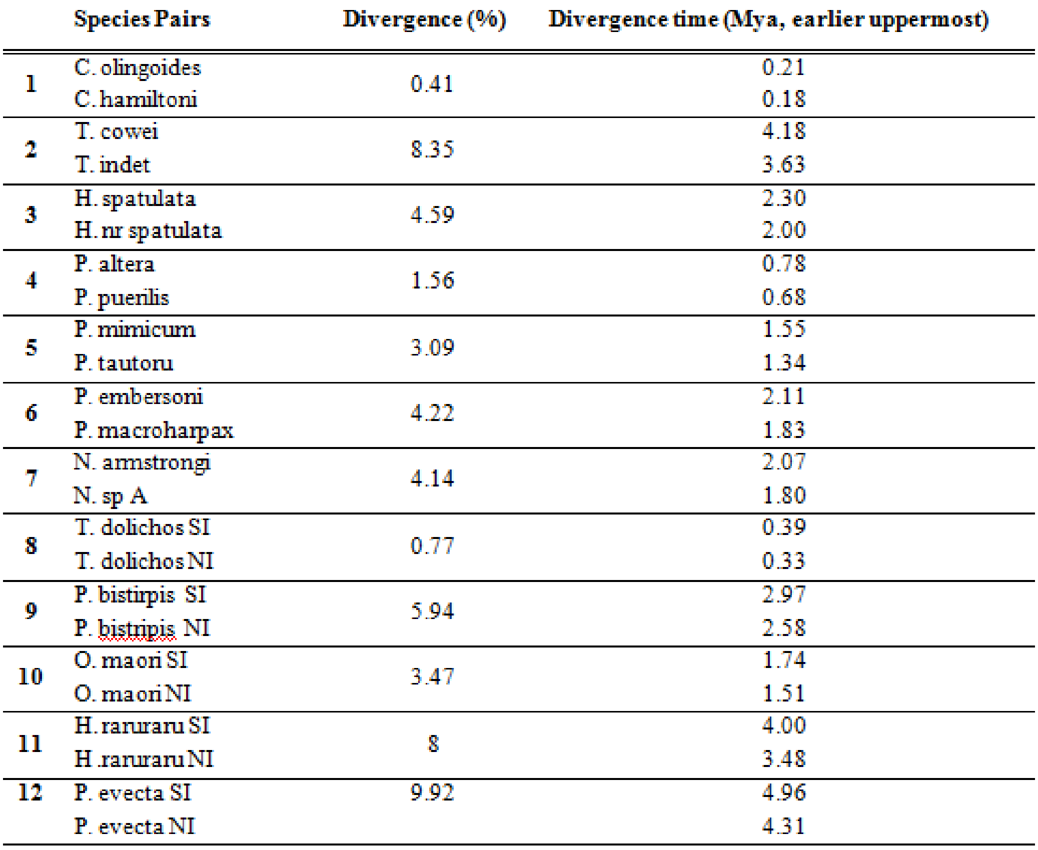
Figure 3: Bayesian Inference phylogram based on the GTR+G+I model derived from jModeltest using a 600bp fragment of CO1 mtDNA from 132 individuals. Posterior probabilities for species pair nodes are shown in bold. NI or SI identifies species groups as being collected from the North Island or the South Island, respectively.

COI sequence divergences and molecular clock estimates

Six of the species pairs showed divergences of between 3 and 6%. The remainder exhibited either higher (n=3) or lower (n=3) inter-'species' divergences (Table 2). For example, *Confluens olingoides* / *hamiltoni* and *Triplectides dolichos* showed less than 1% divergence between species/populations and *Polyplectropus altera* / *puerilis* showed 1.6% sequence divergence. In contrast, *Pycnocentria evecta, Hydropsyche raruraru* and *Pseudoeconesus bistirpis* showed 9.9%, 8% and 5.9% sequence divergences, respectively between populations on the North and South Island.

Based on a molecular clock rate of 2-2.3% per million years, all species pairs diverged within the last 5 MY (Table 2, Fig. 4), with divergence times estimated between 4.96 and 0.18 million years ago. The oldest divergence estimated was associated with the *P. evecta* group, at between 4.96 and 4.31 Mya and the most recent (*C. olingoides / hamiltoni*), dated to between 0.21 and 0.18 Mya. Several of the 'species pairs' overlapped each other in their possible divergence times. These were *T. cowie* / *T.* indet and *H. raruraru* (Group 1), *H. spatulata* / *nr spatulata, P. embersoni* / *macroharpax* and *N. armstrongi* / *sp* A (Group 2), and *O. maori* and *P. mimicum* / *tautoru* (Group 3).

Table 2: Percent sequence divergence and estimated molecular clock divergence times (millions of years) for 12 species- pairs of caddisfly collected from the North (NI) and South Islands (SI) of New Zealand.



	Species Pairs	**Divergence (%)**	**Divergence time (Mya, earlier uppermost)**
1	C. olingoides C. hamiltoni	0.41	0.21 0.18
2	T. cowei T. indet	8.35	4.18 3.63
3	H. spatulata H. nr spatulata	4.59	2.30 2.00
4	P. altera P. puerilis	1.56	0.78 0.68
5	P. mimicum P. tautoru	3.09	1.55 1.34
6	P. embersoni P. macroharpax	4.22	2.11 1.83
7	N. armstrongi N. sp A	4.14	2.07 1.80
8	T. dolichos SI T. dolichos NI	0.77	0.39 0.33
9	P. bistirpis SI P. bistripis NI	5.94	2.97 2.58
10	O. maori SI O. maori NI	3.47	1.74 1.51
11	H. raruraru SI H raruraru NI	8	4.00 3.48
12	P. evecta SI P. evecta NI	9.92	4.96 4.31

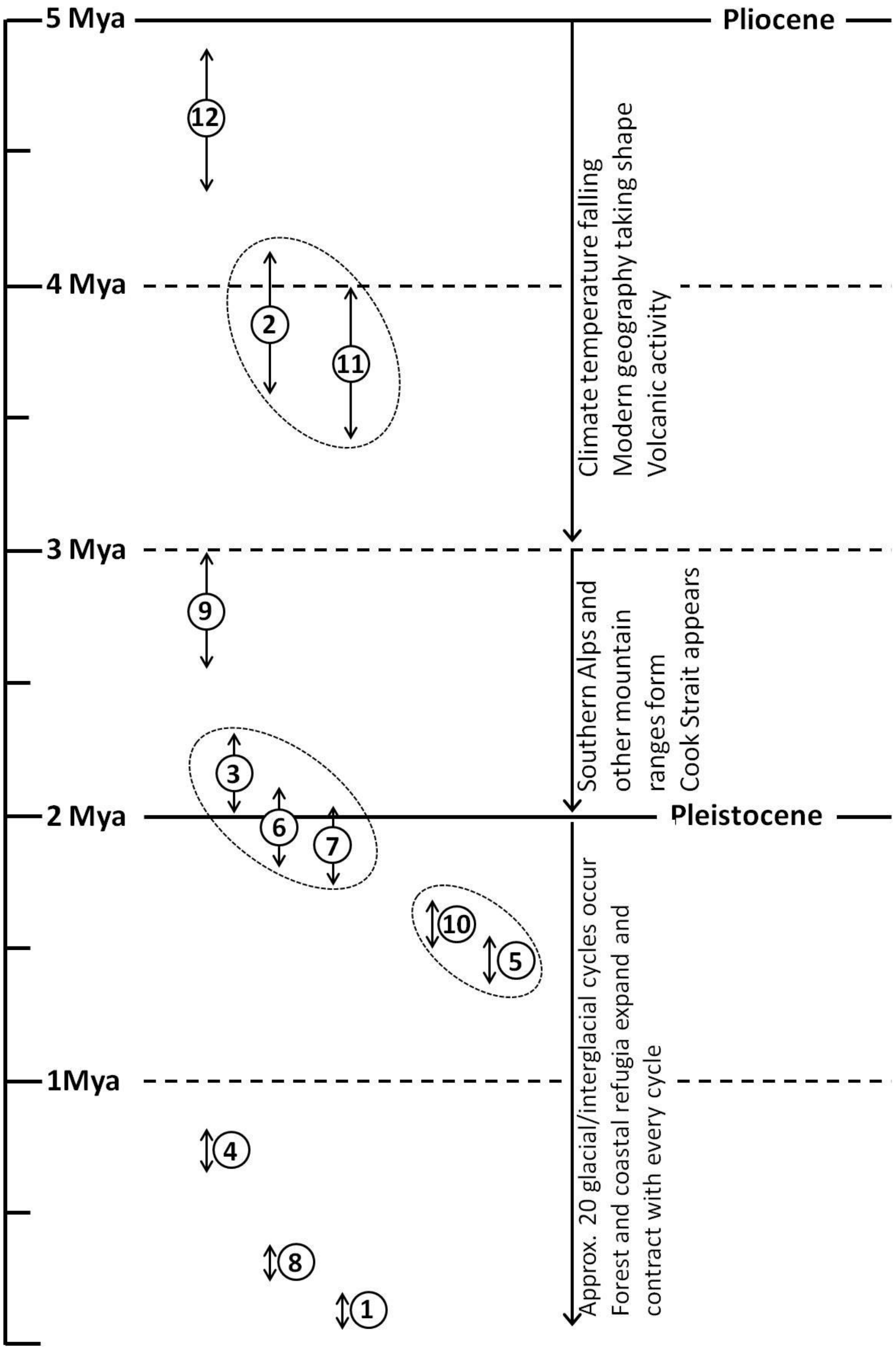


Figure 4: Estimated divergence times for the 12 'species-pairs' of caddisfly. The timeline on the left is in millions of years. Circled numbers correspond to species pairs as used in Table 1. Dotted circles indicate species-pairs with over lapping divergence dates. Overarching geologic events are presented in the appropriate time zones.

Discussion

Our mitochondrial DNA (COI) analyses of 509 caddisflies from 130 species found 12 pairs of putative species with genetic structuring associated with the North or South Islands of New Zealand. This is consistent with previous studies of New Zealand aquatic invertebrate taxa, which have previously found species-level genetic differentiation among populations inhabiting the two islands (e.g. Stevens and Hogg, 2004; Hogg et al., 2009; Knox et al., 2011). Using a molecular clock calibration of 2 - 2.3% sequence divergence per million years (Juan et al., 1996; Roslin, 2001; Trewick and Morgan-Richards, 2005), these species pairs were all estimated to have diverged within the last 5 million years. It is probable that most of these differences can be attributed to isolation and allopatric speciation events occurring during the Pleistocene glaciations. Interestingly, the New Zealand invertebrate fauna does not appear to have any 'cold adapted' taxa (Dumbleton, 1970), and thus, it is likely that the population divergences we observed can be related to survival in glacial refugia during the Pleistocene period.

Existing studies on the dispersal abilities of caddisflies have suggested that larvae are restricted and any dispersal is generally through downstream drift (Smith and Smith, 2009). Similarly, adult caddisflies are capable of dispersing several kilometres, although dispersal among catchments is relatively rare (Smith and Smith, 2009). Accordingly, it is likely that changes within catchments due to glacier formation would have severely restricted dispersal of once widespread species and resulted in isolated populations (e.g. Winkworth et al., 2005).

We estimated that 9 of the 12 species-pairs we examined were likely to have diverged either in the Late Pliocene, as New Zealand's climate began to cool, or during the Pleistocene. For example, species pairs from Group 2 (*H. spatulata* / *nr spatulata, P. embersoni* / *macroharpax* and *N. armstrongi* / sp A) and Group 3 (*O. maori* NI/SI and *P. mimicum / tautoru*) were estimated to have diverged between 2.3 and 1.34 Mya, likely relating to a time of greatest environmental change for freshwater systems, and during the height of the Pleistocene glaciations. Survival in glacial refugia would potentially necessitate species being able adapt to new environments, which in turn would result in changes in the genetic composition of populations through genetic drift and selective pressures. Glaciers would have provided considerable barriers to the dispersal of caddisflies, and given the magnitude of divergences for some species pairs (e.g. *P. evecta*), it is possible that populations may have remained isolated during interglacial periods as well. Fluctuations in the formation and recession of the Cook Strait land bridge may have further restricted gene flow between the North and South Islands during interglacials.

The most closely related species pair, *Confluens olingoides / C. hamiltoni,* was estimated to have diverged only 210,000 to 180,000 years ago. These populations may have been split by the final reforming of Cook Strait which occurred towards the end of the Pleistocene Ice Age (~350,000; Stevens, 1980). In contrast, the largest divergences were found between North Island and South Island populations of *P. evecta*, and species pairs in Group 1 (*T. cowei /* indet and *H. raruraru* SI/ *H. raruraru* NI). These divergences were estimated to have occurred between 4.96 and 3.48 Mya. During this time, the axial mountains of the South

Island were beginning to form, and may have isolated the populations of *P. evecta* and other Group 1 taxa (*sensu* (McCulloch et al., 2010).

Two of the species examined (*P. evecta* and *H. raruraru*) revealed high levels of intraspecific divergence with values ≥8%. Hogg et al. (2009) also found high levels of divergence for these species, finding 8.5 % divergence between three specimens of *P. evecta* and 2.7% between two specimens of *H. raruraru* (which was then *Aoteapsyche raruraru*) and suggested that the divergence between the *P. evecta* specimens may indicate cryptic species. We suggest that both *P. evecta* and *H. raruraru* be further evaluated for the possibility of cryptic species. Hogg et al. (2009) found an average intraspecific sequence divergence of 0.7% among their 105 pairwise comparisons of New Zealand caddisflies. Assuming this value represents general levels of intraspecific genetic variation within the New Zealand caddisfly taxa, two further species could also merit further attention; *O. maori* and *P. bistirpis* showed average divergences of 5.9% and 3.7%, respectively among individuals from the North and South Islands. These divergence values are also similar to the average congeneric species divergences of 6.5% found by (Hebert et al., 2004).

The incidence of cryptic species may be greater among taxa that employ non-visual communication methods (Bickford et al., 2007). This has already been suggested for audial communicators such as cicadas (Henry, 1994), where a minor change in mate selection songs causes an immediate separation within a population. Studies of caddisflies by Wood and Resh (1984) and Bjostad et al. (1996) indicated the use of sex pheromones in mate attraction. Small, non-phenotypic mutations in these pheromones may have the ability to swiftly create populations of the same taxonomic species that do not recognise, and so do not breed with, members outside their own population. This has also been observed in amphipods, where higher levels of divergence between populations corresponded to differences in pheromones resulting in differing mate selection (Sutherland et al., 2010). Accordingly, caddisfly populations have the potential to become reproductively isolated from their parent populations in the few tens of thousands of years that occurred between the interglacial periods of the Pleistocene.

The levels of diversity we found suggest that the Pleistocene glaciation period and subsequent sea level changes have caused the diversification of once potentially more widespread populations of caddisflies. Among this diversity are potentially cryptic, species level groups that have been over looked, and may add to inventories of freshwater biodiversity throughout New Zealand. Uncovering cryptic and overlooked species diversity has been a theme of molecular based studies in New Zealand, with mitochondrial DNA or allozyme analyses uncovering new biodiversity in several divergent taxa over the last decade (e.g. Patterson and Daugherty, 1990; Baker et al., 1995; Morgan-Richards, 1995; Hogg et al., 2006b). Given range of New Zealand taxa with cryptic diversity, it is far from surprising that the caddisfly taxa also exhibit examples of hidden species diversity.

We conclude that the COI sequence divergence that we observed among closely related New Zealand caddisfly species are likely to be a consequence of isolation and range restrictions occurring in the last 5 million years. The molecular clock estimates, coupled with the known geologic history of this time period suggest that the Pleistocene glacial period and subsequent sea level changes are responsible for the diversification of the New Zealand caddisfly fauna. We highlight the potential for species diversity to be underestimated among New Zealand's aquatic taxa, through high genetic structuring of populations and the existence of cryptic species. We suggest that the caddisfly fauna and likely other aquatic invertebrates of New Zealand would benefit from a taxonomic re-evaluation using a combined morphological and molecular approach.

Acknowledgements

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Literature Cited

Allibone R.M., Crowl T.A., Holmes J.M., King T.M., Mcdowall R.M., Townsend C.R., Wallis G.P., 1996. Isozyme analysis of Galaxias species (Teleostei : Galaxiidae) from the Taieri River, South Island, New Zealand: a species complex revealed. Biological Journal of the Linnean Society. 57, 107-127.

Baker A.J., Daugherty C.H., Colbourne R., McLennan J.L., 1995. Flightless brown kiwis of New Zealand possess extremely subdivided population structure and cryptic species like small mammals. Proceedings of the National Academy of Sciences of the United States of America. 92, 8254-8.

Bell B.D., 1994. A review of the status of New Zealand *Leiopelma* species (Anura: Leiopelmatidae), including a summary of demographic studies in Coromandel and on Maud Island. New Zealand Journal of Zoology. 21, 341-349.

Bickford D., Lohman D.J., Sodhi N.S., Ng P.K.L., Meier R., Winker K., Ingram K.K., Das I., 2007. Cryptic species as a window on diversity and conservation. Trends in Ecology & Evolution. 22, 148-55.

Bjostad L., Jewett D., Brigham D., 1996. Sex pheromones of caddisfly *Hesperophylaxa occidentalis* (Banks)(Trichoptera: Limnephilidae). Journal of Chemical Ecology. 22.

Boyer S.L., Giribet G., 2009. Welcome back New Zealand: regional biogeography and Gondwanan origin of three endemic genera of mite harvestmen (Arachnida, Opiliones, Cyphophthalmi). Journal of Biogeography. 36, 1084-1099.

Dumbleton L.J., 1970. Pleistocene climates and insect distributions. New Zealand Entomologist. 4, 3-23.

Folmer O., Black M., Hoeh W., Lutz R., Vrijenhoek R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology. 3, 294-9.

Gleeson D.M., Howitt R.L., Ling N., 1999. Genetic variation, population structure and cryptic species within the black mudfish, *Neochanna diversus,* an endemic galaxiid from New Zealand. Molecular Ecology. 8, 47-57.

Hajibabaei M., Janzen D.H., Burns J.M., Hallwachs W., Hebert P.D.N., 2006. DNA barcodes distinguish species of tropical Lepidoptera. Proceedings of the National Academy of Sciences of the United States of America. 103, 968-71.

Hebert P.D.N., Penton E.H., Burns J.M., Janzen D.H., Hallwachs W., 2004. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterflyes *Astrapt fulgerator*. Proceedings of the National Academy of Sciences of the United States of America. 101, 14812-7.

Henry C.S., 1994. Singing and cryptic speciation in insects. TREE. 9, 388-392.

Hogg I.D., Stevens M.I., Schnabel K.E., Chapman A.M., 2006. Deeply divergent lineages of the widespread New Zealand amphipod Paracalliope fluviatilis revealed using allozyme and mitochondrial DNA analyses. Freshwater Biology. 51, 236–248.

Hogg I., Smith B.J., Banks J.C., Dewaard J.R., Hebert P.D.N., 2009. Testing use of mitochondrial COI sequences for the identification and phylogenetic analysis of New Zealand caddisflies (Trichoptera). New Zealand Journal of Marine and Freshwater Research. 43, 1137-1146.

Huelsenbeck J., Ronquist F., 2001. MRBAYES: Bayesian Inference of Phylogeny. Bioinformatics. 17, 754-755.

Juan C., Oromi P., Hewitt G.M., 1996. Phylogeny of the genus *Hegeter* (Tenebrionidae, Coleoptera) and its colonization of the Canary Islands deduced from Cytochrome Oxidase I mitochondrial DNA sequences. Heredity. 76, 392-403.

Knox M.A., Hogg I.D., Pilditch C.A., 2011. The role of vicariance and dispersal on New Zealand’s estuarine biodiversity: the case of *Paracorophium* (Crustacea: Amphipoda). Biological Journal of the Linnean Society. 103, 863-874.

McCulloch G.A., Wallis G.P., Waters J.M., 2010. Onset of glaciation drove simultaneous vicariant isolation of Alpine insects in New Zealand. Evolution. 64, 2033-43.

Morgan-Richards M., 1995. A new species of tree weta from the North Island of New Zealand (Hemideina: Stenopelmatidae: Orthoptera). New Zealand Entomologist. 18, 15-23.

Mosely M., Kimmins D, 1953. The Trichoptera of Australia and New Zealand. British Museum of Natural History, London. 550.

Patterson G.B., Daugherty C.H., 1990. Four new species and one new subspecies of skinks, genus *Leiolopisma* (Reptilia: Lacertilia: Scincidae) from New Zealand. Journal of the Royal Society of New Zealand. 20, 65-84.

Pauls S., Lumbsch H.T., Haase P., 2008. Glacial refugia of the montane caddisfly *Drusus discolor* (Rambur, 1842). Proceedings of the first conference on faunistics and zoogeography of European Trichoptera. 80-84.

Pauls S.U., Lumbsch H.T., Haase P., 2006. Phylogeography of the montane caddisfly *Drusus discolor*: evidence for multiple refugia and periglacial survival. Molecular Ecology. 15, 2153-69.

Posada D., 2008. jModelTest: phylogenetic model averaging. Molecular Biology and Evolution. 25, 1253-6.

Rambaut A., Drummond A., 2007. Tracer v1.4, Available from http://beast.bio.ed.ac.uk/Tracer. .

Ratnasingham S., Hebert P.D.N., 2007. BOLD:The Barcode of Life Data System (www.barcodinglife.org). Molecular Ecology Notes. 7, 355-364.

Roslin T., 2001. Spatial population structure in a patchily distributed beetle. Molecular Ecology. 10, 823-37.

Smith P.J., Smith B.J., 2009. Small scale population genetic differentiation in the New Zealand caddisfly *Orthopsyche fimbriata* and the crayfish *Paranephrops planifrons*. New Zealand Journal of Marine and Freshwater Research. 43, 723-734.

Stevens G., 1980. New Zealand Adrift. , A.H and A.W Reed, Wellington.

Stevens M., Hogg I.D., 2004. Population genetic structure of New Zealand’s endemic corophiid amphipods: evidence for allopatric speciation. Biological Journal of the Linnean Society. 81, 119-133.

Suggate R.P., 1990. Late Pliocene and Quaternary glaciations of New Zealand. Quaternary Science Reviews. 9, 175-197.

Sutherland D.L., Hogg I.D., Waas J.R., 2010. Phylogeography and species discrimination in the *Paracalliope fluviatilis* species complex (Crustacea: Amphipoda): can morphologically similar heterospecifics identify compatible mates? Biological Journal of the Linnean Society. 99, 196-205.

Swofford D., 2002. PAUP*: Phylogenetic Analysis Using Parsimony, version 4.0b10 for Macintosh.

Trewick S.A., 2001. Scree weta phylogeography: Surviving glaciation and implications for Pleistocene biogeography in New Zealand. New Zealand Journal of Zoology. 28, 291-298.

Trewick S.A., Morgan-Richards M., 2005. After the deluge: mitochondrial DNA indicates Miocene radiation and Pliocene adaptation of tree and giant weta (Orthoptera: Anostostomatidae). Journal of Biogeography. 32, 295-309.

Wiggins G.B., 2004. Caddisflies: The underwater architects. University of Toronto Press.

Winkworth R., Wagstaff S., Glenny D., Lockhart P., 2005. Evolution of the New Zealand mountain flora: Origins, diversification and dispersal. Organisms Diversity & Evolution. 5, 237-247.

Wise K.A.J., 1958. Trichoptera of New Zealand: A catalogue of the Auckland Museum collection with descriptions of new genera and new species. Records of the Auckland Institute and Museum. 5, 49-63.

Wood J.R., Resh V.H., 1984. Demonstration of sex pheromones in caddisflies (Trichoptera). Journal of Chemical Ecology. 10, 171-175.

Chapter III

High levels of intra-specific genetic divergences revealed for two species of Antarctic springtails: evidence for small-scale isolation following Pleistocene glaciation[*]

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Abstract

We used mitochondrial DNA (COI) sequences to examine the levels of genetic variability within and among populations of three endemic springtail species (Arthropoda: Collembola), collected from the Mackay Glacier region of southern Victoria Land, in the Ross Dependency. We tested the hypothesis that genetic divergences would occur among glacially-isolated habitats within a small-scale fragmented landscape. Mitochondrial analysis of 97 individuals showed high levels of genetic divergence at small spatial scales (<15km). High levels of genetic divergence were found among populations for two of the three species. *Gomphiocephalus hodgsoni*, a widespread and common species showed 7.6% sequence divergence on opposite sides of the Mackay Glacier. Similar divergences were also found for *Neocryptopygus nivicolus*, a more range-restricted species showing 4.0% sequence divergence among populations. A third species, *Antarcticinella monoculata*, was found at a single site only and consisted of two similar haplotypes. Based on molecular clock estimates, divergence of these populations has occurred within the last 5 Mya. We suggest that glaciations during the Pliocene (5 Mya) and throughout the Pleistocene (2 Mya - 10 Kya) have fragmented springtail populations in this region and isolated them in small, refugial nunataks. We conclude that glaciation has promoted and maintained the levels of diversity we observed among populations of springtails and that isolation has occurred on relatively small spatial scales. Conservation efforts should be directed towards protecting and preserving the biotic integrity of fragmented landscapes along the Transantarctic Mountains.

Introduction

With only 0.34% (46,200 km²) of the total 14 million km² ice free and even marginally habitable, the Antarctic continent represents one of the most extreme environments for terrestrial life (Convey et al., 2009; Hogg and Wall, 2012). The majority of the ice-free areas lie within the Dry Valleys and Transantarctic Mountains of the Ross Dependency (Janetschek, 1967a). Even here, exposed ground is often highly fragmented and comprised of small, rocky outcrops separated by permanent snow fields and glaciers. Suitable habitat is then further restricted by the availability of liquid water necessary to support life (Hogg et al., 2006a). This latter requirement is particularly relevant for the soil arthropod fauna, all of which lack a desiccation-resistant life stage.

The terrestrial arthropods are represented primarily by springtails (Collembola) and mites (Acari) and are the largest year-round taxa on the continent (Gressitt, 1967; Hogg and Stevens, 2002; Adams et al., 2006). These taxa, which lack survival and dispersal strategies possessed by other invertebrate groups such as nematodes (Nkem et al., 2006), have been restricted to these fragmented, ice-free zones since the Middle Miocene (14-11 Mya; Kennett, 1977; Stevens, 1980). At this time, glaciation of the whole continent reached its fullest extent and the polar ice cap overflowed the Transantarctic Mountains (Janetschek, 1967a). Small oases of ice-free ground existed around the edge of the polar cap, the largest of which (the Dry Valleys) is still located within the Transantarctic Mountain on the western edge of the Ross Ice Shelf (Clapperton and Sugden, 1990). Since then, the East Antarctic Ice Sheet (EAIS) has undergone numerous glacial cycles, with the last glacial maximum ending 17 Kya (Suggate, 1990). This extensive glacial history has resulted in extremely low species richness for the Antarctic fauna, with many habitats containing at most one or two arthropod taxa (Janetschek, 1967a). Species are also rarely shared between regions (Gressitt, 1967; Wise, 1971; Sinclair and Stevens, 2006), suggesting limited inter-habitat dispersal. Consequently, the current arthropod taxa are likely to be long-term inhabitants and remnants of, once, much more widespread species (Convey et al., 2009). Even within regions, most species show high levels of genetic divergence across their distributional ranges suggesting the effects of long-term isolation and/or survival in glacial refugia (Frati et al., 2001; Stevens and Hogg, 2003a; McGaughran et al., 2008b; Hawes, 2011). Here, our aim was to extend these studies by focussing on small-scale differences that might occur within faunally-diverse, yet heavily fragmented, landscapes.

Ten species of springtail are currently known from the Ross Dependency, four in northern Victoria Land, three in southern Victoria Land and three in the southern Transantarctic Mountains. All species are range-restricted. Species from southern Victoria Land, the focus of our study, consist of three species covering a 3^{o} latitudinal range. Within this region *Gomphiocephalus hodgsoni* is the only relatively widespread species and is relatively common throughout southern Victoria Land (McGaughran et al., 2011b). Two additional species, *Neocryptopygus nivicolus* and *Antarcticinella monoculata* are extremely range-restricted and known only from one or two locations near the Mackay Glacier to the north of the Dry Valleys (Fig. 1) suggesting the possibility of a glacial refugium. Recent studies of lichens and mosses also near the Mackay Glacier (Greena et al., 2011), as well as haplotype diversity for springtail (*G. hodgsoni*) and mite (*S. mollis*) taxa have further suggested this area as a likely refugial zone

(Stevens and Hogg, 2003; Nolan et al., 2006; McGaughran et al., 2008; Demetras et al., 2010).

In order to determine the geographic scales on which genetic diversity may have been promoted and/or maintained, we focused on small-scale genetic variability in a region of comparatively high species diversity (Mackay Glacier, southern Victoria Land). This glacier is one of only a few outlet glaciers that connect the EAIS with the Ross Ice Shelf in southern Victoria Land (Clapperton and Sugden, 1990). Accordingly, we tested the hypothesis that this region would support genetically divergent springtail populations among the isolated habitats. We predicted that high levels of both genetic variability and genetic divergence would exist among these habitats, potentially indicating refugial zones from the Pliocene and Pleistocene glaciations.

Methods

Study sites and sample collection:

Samples were collected from St John's Ranges near Victoria Valley and on the northern and southern sides of the Mackay Glacier in the northern Dry Valleys region of the Ross Dependency (Fig. 1). Specimens were collected from the undersides of rocks using modified aspirators (Hogg and Stevens, 2002). Soil samples were also taken from each site and invertebrates were removed from soil samples through flotation in a 10% sucrose solution. All specimens were stored in 95% ethanol and returned to the University of Waikato for further processing. All specimens were morphologically identified to species level using (Gressitt et al., 1963) and (Salmon, 1965).

Genetic analyses:

Genetic analyses were jointly carried out at the University of Waikato and at the Canadian Centre for DNA Barcoding (CCDB) at the University of Guelph. Total genomic DNA was extracted from the tissue of entire specimens using a Glassfiber Plate DNA Extractions (AcroPrep) method (Ivanova et al., 2006) at CCDB, and using Red Extract n Amp (Sigma-Aldrich) at the University of Waikato using the following protocol: 10 µl extraction solution and 2.5 µl tissue prep, following manufacture's protocol. Polymerase Chain Reactions (PCR's) carried out at the University of Waikato were comprised of a 15 µl reaction containing 5.7 µl MQH$_2$0, 7.5 µl PCR Master Mix Solution (i-Taq, Intron Biotechnology), 0.4 µl of each primer and 1 µl of template DNA. A 658 bp fragment of the mitochondrial CO1 gene was amplified using the primers

HCO2198 (sequence 5'-TAAACTTCAGGGTGACCAAAAAATCA-3') and an altered LCO1490 (sequence: 5'- AGTTCTAATCATTAARGATATYGG-3') (Folmer et al., 1994) for the *G. hodgsoni* specimens. HCO and LepF1 (sequence: 5'-ATTCAACCAATCATAAAGATATTGG-3') (Hajibabaei et al., 2006) for the *N. nivicolus* and *A. monoculata* specimens. The standard LCO 1490 (sequence: 5'-GGTCAACAAATCATAAAGATATTGG-3') was used for both species (in place of the altered LCO1490 and LepF1) at CCDB. Primers were used at 1.0 mM concentration. PCR conditions at CCDB were: initial denaturing at 94ºC for 1 min; 5 cycles of 94ºC for 1 min, 45ºC for 1.5 min and 72ºC for 1.5 min; 35 cycles of 94ºC for 1 min, 50ºC for 1.5 min and 72ºC for 1 min followed by a final 72ºC for 5 min. PCR conditions at the University of Waikato were: initial denaturing at 94ºC for 5 minutes; 36 cycles of 94ºC for 1 min, 52ºC for 1.5 min and 72ºC for 1 min, followed by a final 72ºC for 5 min.

PCR products were cleaned using Sephadex (CCDB) or 0.2 µl ExonucleaseI (EXO) and 0.1 µl Shrimp Alkaline Phosphate (SAP) with 2.7 µl MQH_20 following manufactures protocol (Global Science & Tech Ltd.) at Waikato. DNA was sequenced in both directions on an ABI3130 sequencer at the University of Waikato DNA sequencing facility using the same primers used for amplification, or on an ABI3730x1 at CCDB. Sequences from the University of Waikato were aligned using Geneious, Ver 5.4.2, and confirmed as the target species using the Barcode of Life Data Systems (BOLD) ver 3 CO1 animal identification searches. Primer sequences were trimmed from sequence fragments before further analysis. All sequences were uploaded to the BOLD project Antarctic Terrestrial Arthropods (ANTSP).

Phylogenetic Analysis

COI sequence fragments of 658 bp (219 codons) were obtained for 67 *G. hodgsoni* specimens and 20 *N. nivicolus* specimens. Approximately 560 bp were obtained from single direction reads (using primer LepF1) for eight *A. monoculata* specimens. No stop codons were detected. Sequences of *G. hodgsoni* were unambiguous at 658 bp (no insertions or deletions). However, sequences of *N. nivicolus* and *A. monoculata* contained ambiguous base pair assignments which could not be easily resolved, so sequences were further trimmed at both ends, resulting in sequence fragments of 547 bp (181 codons) for *N. nivicolus* and 527 (175 codons) for *A. monoculata*. Two additional *N. nivicolus* sequences were also obtained from GenBank (Accession numbers DQ285403 and DQ285404).

Sequences for all species were examined as part of a single Neighbour Joining tree. All duplicate sequences were then removed to include all unique haplotypes for further analyses. An available COI sequence from *Podura aquatica* (Collembola; BOLD accession number ANTSP210-13) was selected for use as an outgroup taxon. Chi-square tests (X^2) as implemented in PAUP* 4.0 (Swofford, 2002) were used to determine whether the assumption of equal base frequencies among sites was violated on all sites and on third codon positions only. JModel test 2.1.2 (Posada, 2008) was used to determine the most appropriate substitution model for Maximum Likelihood (ML). Settings were as follows: 11 substitution schemes (88 models), base frequencies +F, rate variation +I, +G, set to BioNJ. The model selected for the data set was GTR + I + G (-lnL = 1,590.9). Maximum Likelihood heuristic searches were conducted using this model in MEGA 5.10 using 1000 bootstrap replicates. Maximum Parsimony (MP) analyses were also performed in MEGA 5.10 using 1000 bootstrap replicates. Other settings were set to default options in MEGA 5.10.

MrBayes (Huelsenbeck and Ronquist, 2001) was used to conduct a Bayesian Inference analysis. A general time reversal model (GTR +I +G) was used, with a log normal relaxed clock model and speciation yule process as the tree prior. The Markov chain Monte Carlo (MCMC) was 50,000,000 generations, sampling trees every 5,000. A burn in of 1,000 trees was determined by plotting log-likelihood values against generation time in TRACER (Rambaut and Drummond, 2007). The majority rule tree was acquired from the 9,000 trees sampled after the burn in period. The tree was then visualized in Tree Annotator.

Sequences for *G. hodgsoni* and *N. nivicolus* were split into separate data sets for analysis in the program TCS 1.21 (Clement et al., 2000) and to construct networks of sequence haploytpes. The *A. monoculata* sequences were not included in these analyses as they were only collected from a single site and consisted of only two similar haplotypes.

Pair-wise genetic distances between COI sequences for populations at different locations were also calculated for the *G. hodgsoni* and *N. nivicolus* data sets in MEGA 5.10. Comparisons of log likelihood scores (using X^2 tests) for trees with and without a molecular clock enforced indicated that the sequences of both data sets were evolving in a 'clock-like' manner (*G. hodgsoni*: X^2= 113.06; p<0.001; d.f=14. *N. nivicolus*: X^2=141.15; p=<0.001; d.f=10). Approximate geological ages between each of the populations were estimated using a molecular clock calibration of 1.5 - 2.3% sequences divergence per million years, determined using invertebrate mitochondrial data (as per: Brower, 1994; Juan et al., 1996; Quek et al., 2004; McGaughran et al., 2010b).

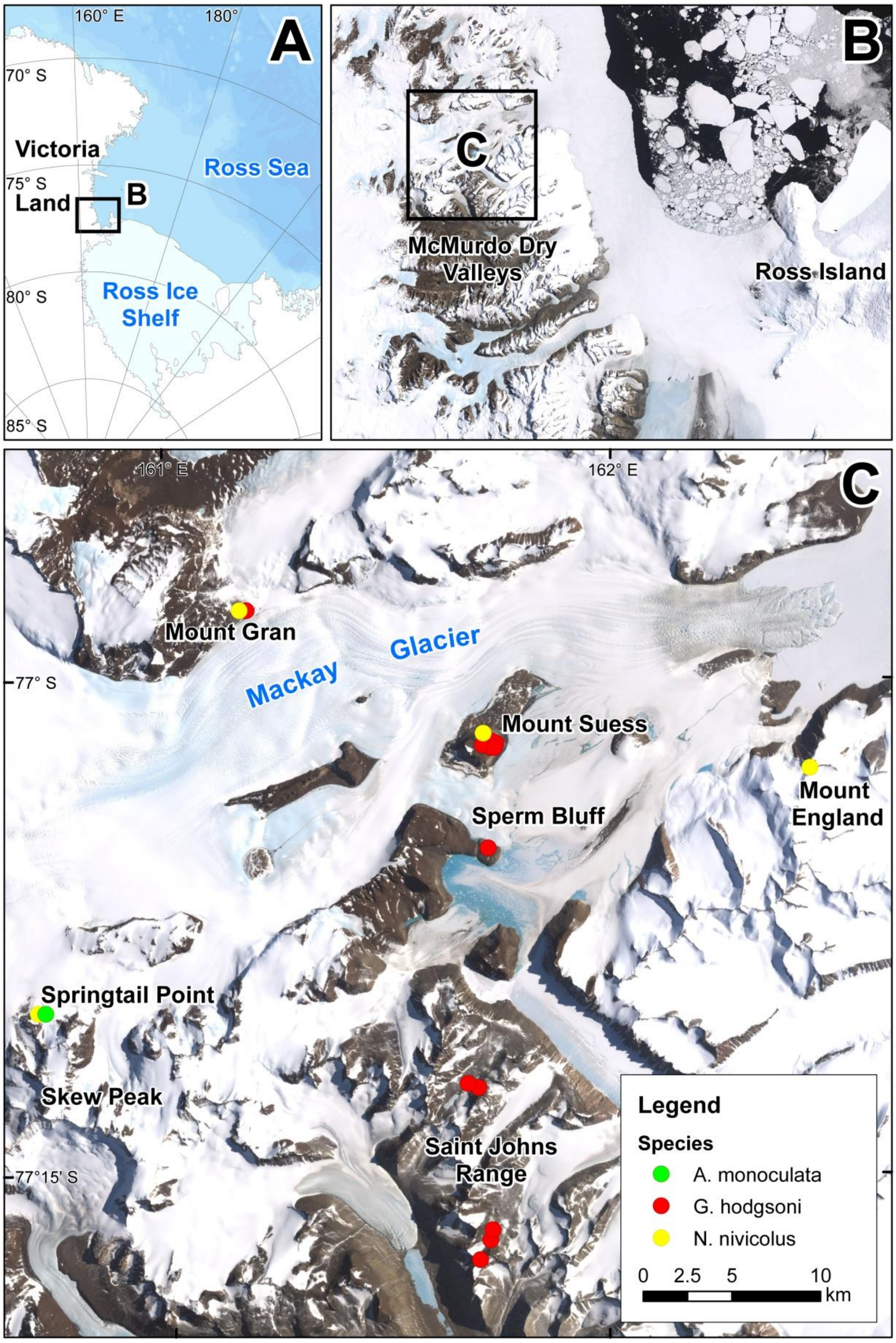

Figure 1: Sampling sites and Collembola species' locations in the Mackay Glacier vicinity. Two *N. nivicolus* specimens were taken from GenBank and were collected from Mt England in 2005. Map adapted from the SCAR Antarctic Digital Database and the Landsat Image Mosaic of Antarctica (LIMA) project.

Results

Of the 658 bp analysed for *G. hodgsoni*, 515 characters were constant, 22 were parsimony informative and the remaining 121 were parsimony uninformative. The nucleotide composition averaged across all sequences showed an A-T bias of 64.0% (A = 27.7%, T = 36.7%, C = 19.3%, G = 16.7%). Base pair frequencies were homologous among sequences for all sites ($X^2 = 2.19$, $p = 1.0$, d.f = 48) and for third codon positions only ($X^2 = 7.18$, $p = 1.0$, d.f = 48). Of the 549 bp analysed for *N. nivicolus*, 433 characters were constant, 22 were parsimony informative and the remaining 94 were parsimony uninformative. The nucleotide composition averaged across all sequences showed an A-T bias of 61.4% (A = 25.8%, T = 35.6%, C = 20.4%, G = 18.2%). Base pair frequencies were homologous among sequences for all sites ($X^2 = 1.41$, $p = 1.0$, d.f = 36) and for third codon positions only ($X^2 = 5.77$, $p =1.0$, d.f = 36). Of the 527 bp (175 codons) analysed for *A. monoculata*, 408 characters were constant, 1 was parsimony informative and the remaining 118 were parsimony uninformative. The nucleotide composition averaged across all sequences showed an A-T bias of 59.0% (A = 23.9%, T = 35.1%, C = 22.3%, G = 18.7%). Base pairs were homologous among sequences for all sites ($X^2 = 3.39$, $p = 1.0$, d.f = 21) and for third codon positions only ($X^2 = 11.55$, $p = 0.95$, d.f = 21).

Phylogenetic Analysis

A Maximum Likelihood (ML) tree is shown in Figure 2. Tree constructions for Maximum Parsimony and Neighbour Joining (data not shown) showed similar topology and node support (Fig. 3). Linking nodes between the haplotype G16 and the rest of the *G. hodgsoni* haplotypes had 99% bootstrap in the ML and MP trees. The linking node between the *N. nivicolus* haplotypes at Springtail Point and at Mt Gran also received 99% bootstrap support in the ML and MP trees. Bootstrap support for the Mt England *N. nivicolus* haplotypes varied, with 83% from the ML tree, and 90% from the MP tree. The MP tree also demonstrated slightly different topology compared to the other trees (Fig. 3). The topology of the BI tree was also similar to all other trees for both *N. nivicolus* and *A. monoculata.* Posterior probability values between *N. nivicolus* haplotypes at Springtail Point and at Mt Gran was 1.0, and for the Mt England group was 0.76 (Fig. 4). The topology for the *G. hodgsoni* haplotypes differed from the other trees. Two clusters were apparent, with 0.99 posterior probability support for the node. Collection locations of haplotypes were mixed between both clusters. The topology and node support of these trees supports the presence of high genetic structuring across the Mackay Glacier.

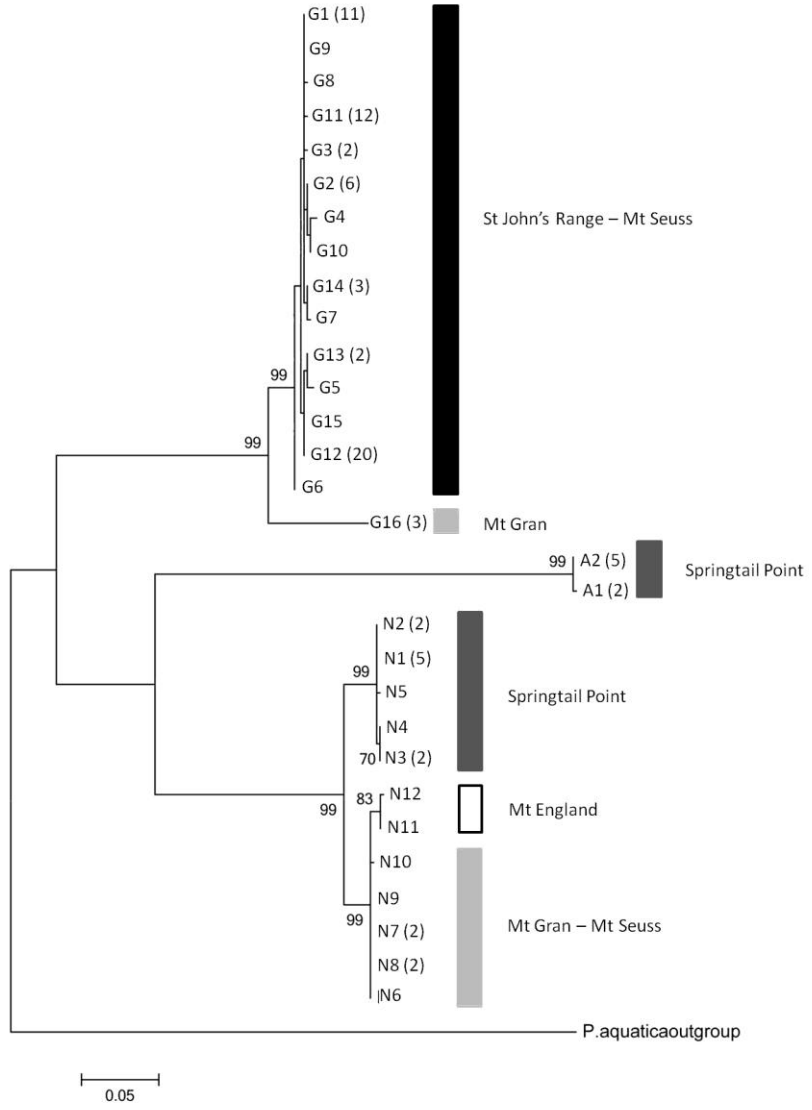

Figure 2: Maximum Likelihood phylogram based on the GTR+G+I model derived from jModelTest, using 97 individual COI sequences reduced to unique haplotypes. The number of individuals for each haplotype are indicated in parentheses. Bootstrap values greater than 50 are shown. Tree is drawn to scale and branch lengths are the number of substitutions per site. Collection locations are indicated for genetically distinct groups.

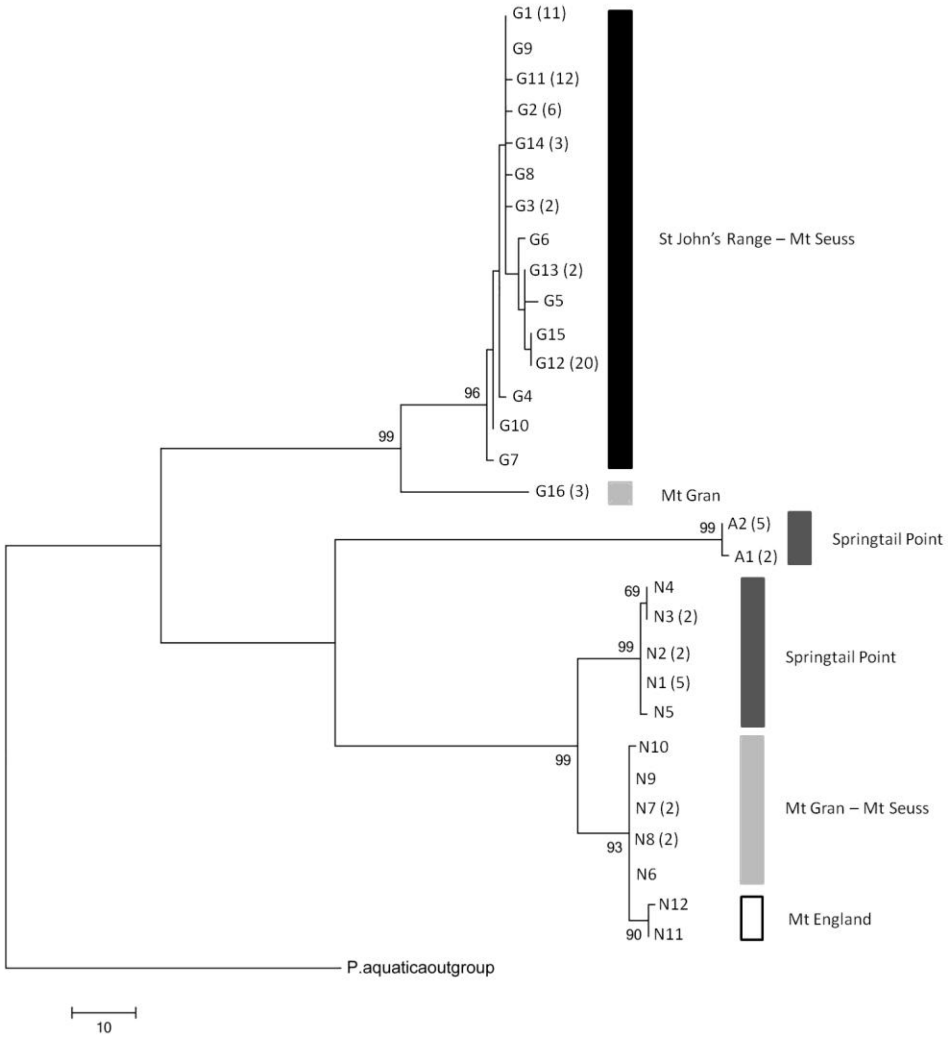

Figure 3: Maximum Parsimony Phylogram constructed in MEGA 5.10, using 97 individual COI sequences reduced to unique haplotypes. The number of individuals for each haplotype are indicated in parentheses. Bootstrap values greater than 50 are shown. Tree is drawn to scale and branch lengths are the number of changes over the whole sequence. Collection locations are indicated for genetically distinct groups.

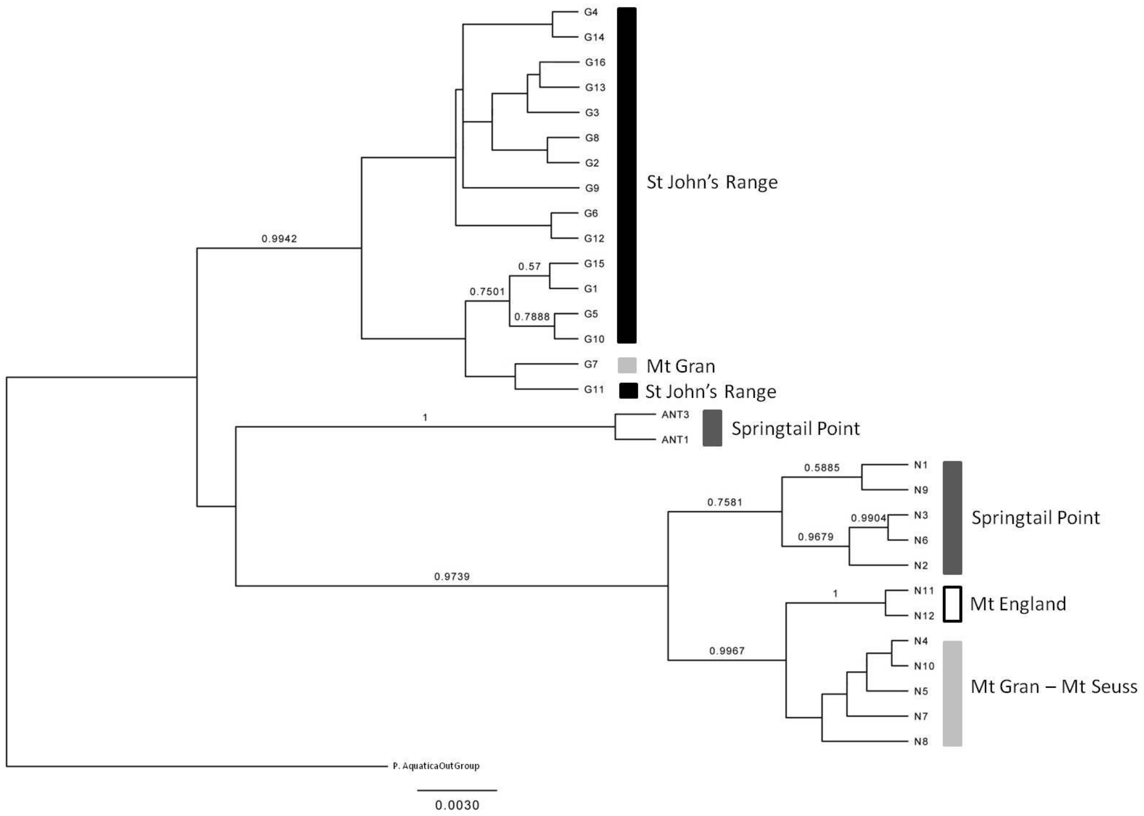

Figure 4: Bayesian Inference Phylogram constructed in MrBayes based on the GTR+G+I model derived from jModelTest, using 97 individual COI sequences and reduced to unique haplotypes. The number of individuals for each haplotype are indicated in parentheses. Posterior probabilities for haplotype group nodes are presented above 0.5. Tree is drawn to scale and branch lengths are measured in the number of changes per site. Collection locations are indicated for genetically distinct groups.

Haplotype networks

The geographic distribution of sequence haplotypes for *G. hodgsoni* and *N. nivicolus* was investigated using haplotype joining networks. Subsequent haplotype assignments and their collection locations are shown in table 1. 16 haplotypes were found from 67 *G. hodgsoni* sequences. Maximum connection steps were fixed at 40 in order to connect haplotype G16 to the rest of the haplotypes (Fig. 5). This network revealed 10 1-step haplotypes, three 2-step haplotypes, two 3-step haplotypes and one 35-step haplotype. The most divergent haplotype shown by this analysis was G16, representing three individuals from Mt Gran. This difference was also supported by divergence values and phylogenetic trees (Fig. 2). The remainder of the network which included haplotypes from the St John's Range and Mt Seuss did not show high geographic structure, similar to that observed in the tree-based approaches (Fig. 2).

Twelve haplotypes were found from 22 *N. nivicolus* sequences. Maximum connection steps were fixed at 30 in order to connect the Mt Gran and Mt England haplotypes to the Springtail point haplotypes (Fig. 6). This network revealed nine 1-step haplotypes, two 3-step haplotypes and one 16-step haplotype. This network analysis showed two groups of haplotypes that were connected by 16 missing mutational steps. These two groups corresponded to populations at Springtail Point on the south edge of Mackay Glacier, and Mt Gran and Mt Seuss to the north and in the centre of the glacier respectively. This difference was supported by divergence values and phylogenetic trees. The 2-step link to haplotypes at Mt England was also supported by divergence values and phylogenetic trees.

COI sequence divergence and molecular clock estimates

Genetic distances ranged from 0.0-0.1% for *G. hodgsoni* and 0.00-0.04% for *N. nivicolus* (Table 2). Greatest differences were found between haplotype G16 at Mt Gran and the remainder of the *G. hodgsoni* haplotypes, and the genetic distance between *N. nivicolus* haplotypes at Mt Gran and Mt England, and those at Springtail Point. The St John's Range and Mt Seuss *G. hodgsoni* haplotypes showed an average divergence of 0.6% within the group (Table 2). The single haplotype, G16, at Mt Gran showed an average of 7.6% sequence divergence from the other haplotypes.

The average sequence divergences among *N. nivicolus* haplotypes within each location were 0.1% at Mt Gran, 0.2% at Springtail Point and 0.2% at Mt England. Sequence divergences between locations showed the haplotypes at Mt Gran to be an average of 4.0% divergent from haplotypes at Mt England. Similarly, Springtail Point haplotypes were an average of 3.8% divergent from those found at Mt Gran. The Mt Gran and Mt England haplotypes were the most similar, with 0.8% sequence divergence between them.

Based on a molecular clock rate of 1.5-2.3% sequence divergence per million years, these populations are all likely to have diverged within the last 5 My (Table 2, Fig. 7). The oldest estimated divergence dated the genetic separation of *G. hodgsoni* haplotypes at Mt Gran (G16) and those in the St John's Range and at Mt Seuss were estimated at 5.1 - 3.3 Mya. Divergence dates between the three *N. nivicolus* populations suggested that the Springtail Point haplotypes diverged from the Mt Gran - Mt Seuss population, and Mt England 2.5-1.6 Mya and 2.7-1.7 Mya respectively. The relationship between haplotypes in the Mt Gran - Mt Seuss population, and those at Mt England is much more recent by comparison, estimated at 0.5-0.3 Mya.

Table 1: Haplotypes, collection locations, coordinates and sequences (BOLD Process Id) associated with each haplotype for three species of Antarctic springtail. Two Mt England *N. nivicolus* sequences (N11, N12) were retrieved from GenBank.

Haplotype #	**Location**	**Co-ordinates**	**Process Id's**
G. hodgsoni			
G1	St John's Range	-77.280 161.731	ANTSP131 ANTSP134 ANTSP136 ANTSP137 ANTSP138 ANTSP140 ANTSP141 ANTSP143 ANTSP129 ANTSP193 ANTSP151
G2			ANTSP133 ANTSP135 ANTSP139 ANTSP211 ANTSP212 ANTSP132
G3		-77.208 161.700	ANTSP213 ANTSP215
G4		-77.285 161.726	ANTSP150
G5			ANTSP142
G6			ANTSP146
G7		-77.208 161.700	ANTSP209
G8			ANTSP210
G9			ANTSP216
G10		-77.285 161.726	ANTSP217
G11		-77.280 161.731	ANTSP144 ANTSP145 ANTSP147 ANTSP148 ANTSP149 ANTSP191 ANTSP192 ANTSP207 ANTSP219 ANTSP214 ANTSP128 ANTSP218
	Mt Seuss	-77.034 161.731	
G12			ANTSP154 ANTSP157 ANTSP158 ANTSP159 ANTSP160 ANTSP163 ANTSP164 ANTSP165 ANTSP168 ANTSP169 ANTSP172 ANTSP174 ANTSP175 ANTSP220 ANTSP222 ANTSP221 ANTSP223 ANTSP224 ANTSP152 ANTSP225
G13			ANTSP162 ANTSP173

G14				ANTSP166 ANTSP153 ANTSP167
G15		77.034	161.731	ANTSP161
G16	Mt Gran	-76.966	161.179	ANTSP201 ANTSP202 ANTSP200

N. nivicolus

N1	Springtail Point	-77.167	160.710	ANTSP121 ANTSP188 ANTSP190 ANTSP230 ANTSP119
N2				ANTSP2234 ANTSP228
N3				ANTSP231 ANTSP226
N4				ANTSP227
N5				ANTSP118
N6	Mt Gran	-76.966	161.179	ANTSP233
N7				ANTSP199 ANTSP197
N8	Mt Seuss	-77.034	161.731	ANTSP156 ANTSP124
N9				ANTSP155
N10				ANTSP170
N11	M England	-77.046	162.450	DQ285403
N12				DQ285404

A. monoculata

A1	Springtail Point	-77.168	160.710	ANTSP196 ANTSP235
A2				ANTSP204 ANTSP205 ANTSP194 ANTSP195 ANTSP203

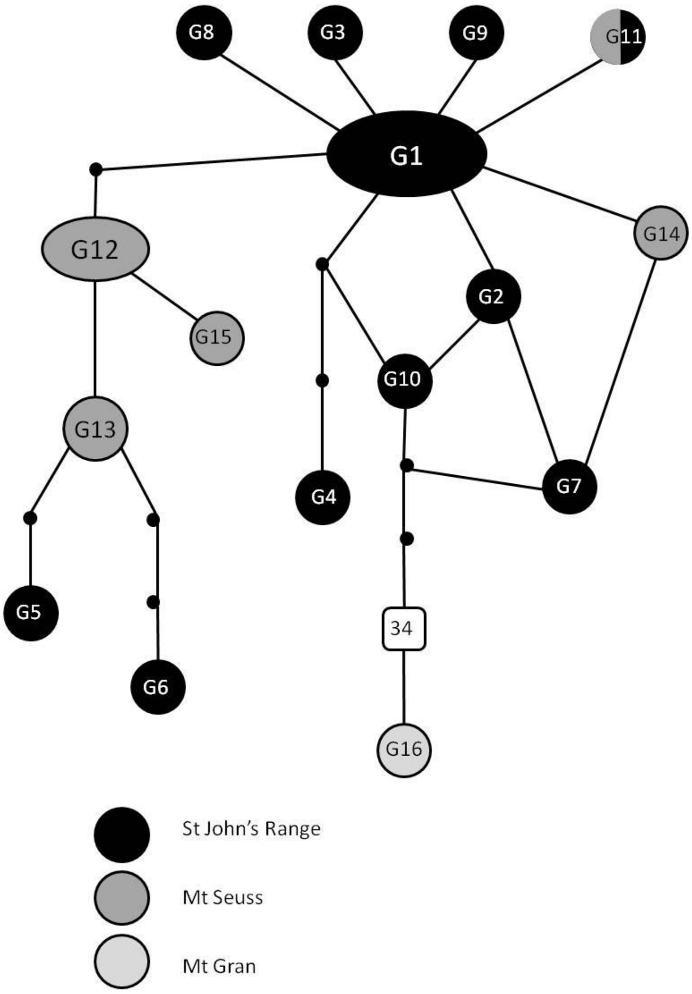

Figure 5: Haplotype network analysis for 16 haplotypes from 67 individuals of *G. hodgsoni*. Haplotypes are indicated by their codes as referred to in Table 1. Missing haplotypes or mutational steps are indicated by black dots, or are collapsed into a count of missing steps as in the single white square.

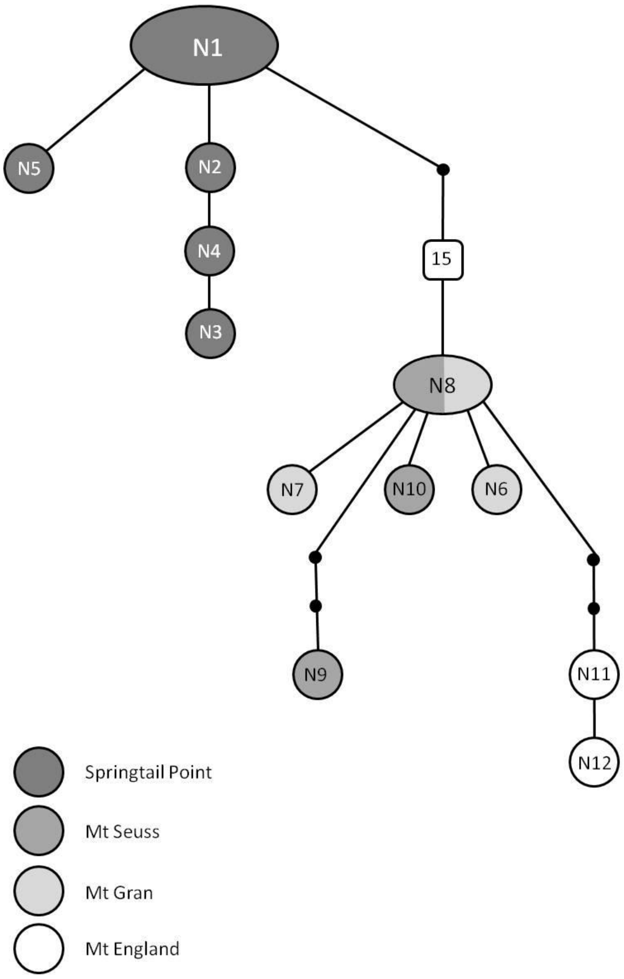

Figure 6: Haplotype network analysis for 12 haplotypes from 22 individuals of *N. nivicolus*. Haplotypes are indicated by their codes as referred to in Table 1. Missing haplotypes or mutational steps are indicated by black dots, or are collapsed into a count of missing steps as in the white square.

Table 2: Genetic distances based on mitochondrial COI sequences of 97 springtail sequences covering 30 unique haplotype. Haplotype codes refer to those in Table 1. Collection locations for each haplotype are indicated in the bar at the top and side of the table

	Haplotypes	St John's Range										St John's Range – Mt Seuss	Mt Seuss				Mt Gran	Springtail Point					Mt Gran		Mt Gran – Mt Seuss	Mt Seuss		Mt England		Springtail Point
G. hodgsoni	G1																													
	G2	0.002																												
	G3	0.002	0.004																											
	G4	0.004	0.006	0.006																										
	G5	0.010	0.012	0.008	0.014																									
	G6	0.006	0.008	0.008	0.010	0.008																								
	G7	0.004	0.002	0.006	0.008	0.014	0.006																							
	G8	0.002	0.004	0.004	0.006	0.012	0.008	0.006																						
	G9	0.000	0.002	0.002	0.004	0.010	0.006	0.004	0.002																					
	G10	0.004	0.002	0.006	0.004	0.014	0.010	0.004	0.006	0.004																				
	G11	0.002	0.004	0.004	0.006	0.012	0.008	0.006	0.004	0.002	0.006																			
	G12	0.004	0.006	0.006	0.008	0.006	0.006	0.008	0.006	0.004	0.008	0.006																		
	G13	0.006	0.008	0.008	0.010	0.004	0.004	0.010	0.008	0.006	0.010	0.008	0.002																	
	G14	0.002	0.004	0.004	0.006	0.012	0.004	0.002	0.004	0.002	0.006	0.004	0.006	0.008																
	G15	0.004	0.006	0.006	0.008	0.006	0.006	0.008	0.006	0.004	0.008	0.006	0.000	0.002	0.006															
	G16	0.078	0.075	0.075	0.078	0.075	0.071	0.073	0.080	0.078	0.073	0.080	0.078	0.075	0.075	0.078														
N. nivicolus	N1	0.221	0.218	0.218	0.218	0.224	0.224	0.216	0.224	0.221	0.216	0.221	0.227	0.230	0.218	0.227	0.241													
	N2	0.221	0.218	0.218	0.218	0.224	0.224	0.216	0.224	0.221	0.216	0.221	0.227	0.230	0.218	0.227	0.241	0.000												
	N3	0.224	0.221	0.221	0.221	0.227	0.227	0.218	0.227	0.224	0.218	0.224	0.230	0.233	0.221	0.230	0.244	0.002	0.002											
	N4	0.224	0.221	0.221	0.221	0.227	0.227	0.218	0.227	0.224	0.218	0.224	0.230	0.233	0.221	0.230	0.244	0.002	0.002	0.000										
	N5	0.224	0.221	0.221	0.221	0.227	0.227	0.218	0.227	0.224	0.218	0.224	0.230	0.233	0.221	0.230	0.244	0.002	0.002	0.004	0.004									
	N6	0.216	0.213	0.213	0.213	0.219	0.219	0.210	0.219	0.216	0.210	0.216	0.221	0.224	0.213	0.221	0.233	0.036	0.036	0.038	0.038	0.038								
	N7	0.216	0.213	0.213	0.213	0.219	0.219	0.210	0.219	0.216	0.210	0.216	0.221	0.224	0.213	0.221	0.233	0.036	0.036	0.038	0.038	0.038	0.000							
	N8	0.216	0.213	0.213	0.213	0.219	0.219	0.210	0.219	0.216	0.210	0.216	0.221	0.224	0.213	0.221	0.233	0.036	0.036	0.038	0.038	0.038	0.000	0.000						
	N9	0.216	0.213	0.213	0.213	0.219	0.219	0.210	0.219	0.216	0.210	0.216	0.221	0.224	0.213	0.221	0.233	0.036	0.036	0.038	0.038	0.038	0.000	0.000	0.000					
	N10	0.219	0.216	0.216	0.216	0.221	0.221	0.213	0.221	0.219	0.213	0.219	0.224	0.227	0.216	0.224	0.236	0.038	0.038	0.040	0.040	0.040	0.002	0.002	0.002	0.002				
	N11	0.216	0.213	0.213	0.213	0.218	0.218	0.210	0.218	0.216	0.210	0.216	0.221	0.224	0.213	0.221	0.232	0.040	0.040	0.042	0.042	0.042	0.006	0.006	0.006	0.006	0.008			
	N12	0.216	0.213	0.213	0.213	0.218	0.218	0.210	0.218	0.216	0.210	0.216	0.221	0.224	0.213	0.221	0.232	0.038	0.038	0.040	0.040	0.040	0.008	0.008	0.008	0.008	0.010	0.002		
A. monoculata	A1	0.264	0.261	0.261	0.261	0.261	0.267	0.261	0.267	0.264	0.258	0.261	0.264	0.267	0.264	0.264	0.286	0.241	0.241	0.244	0.244	0.243	0.240	0.240	0.240	0.240	0.243	0.243	0.246	
	A2	0.267	0.264	0.264	0.264	0.264	0.270	0.264	0.264	0.267	0.261	0.264	0.267	0.270	0.267	0.267	0.289	0.244	0.244	0.246	0.246	0.246	0.243	0.243	0.243	0.243	0.246	0.246	0.249	0.002
		G. hodgsoni																*N. nivicolus*												*A. monoculata*

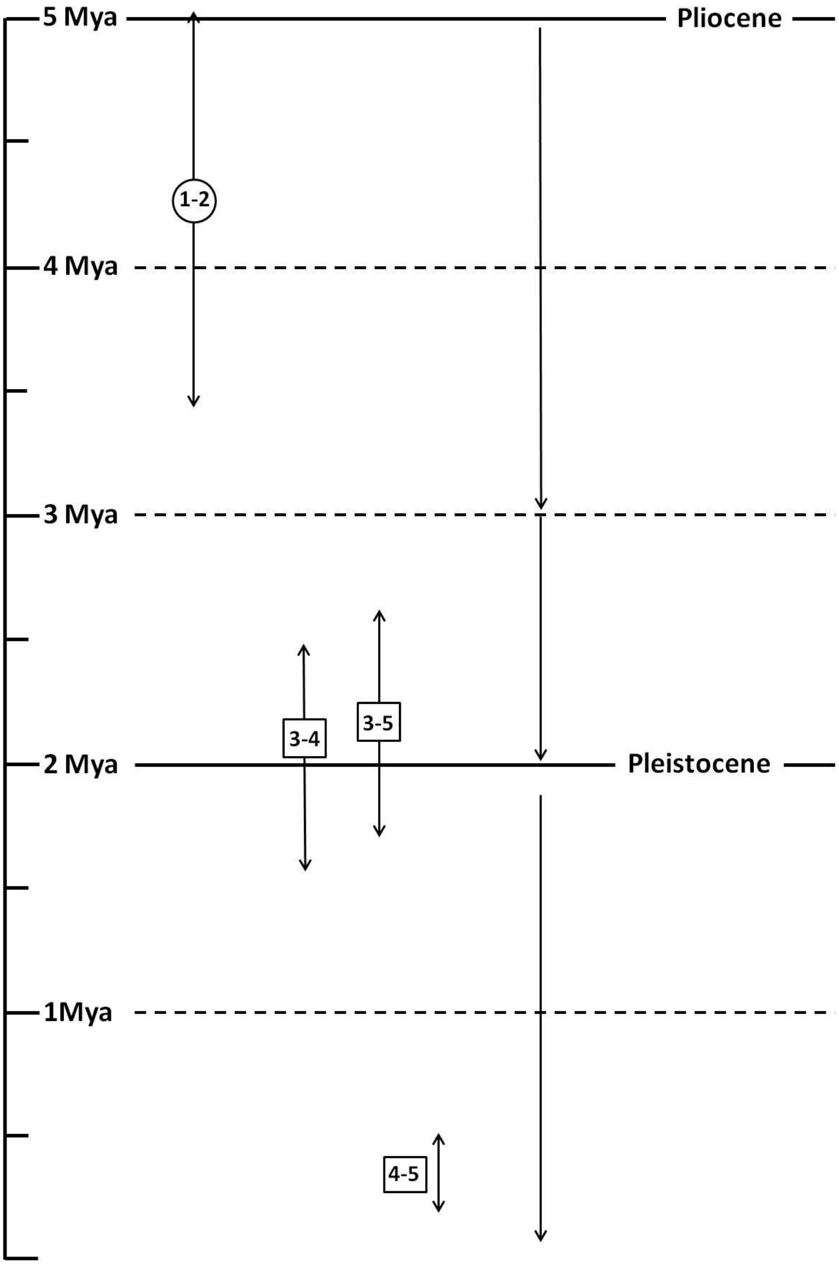

Figure 7: Estimated divergence times for populations of *G. hodgsoni* (circle) and *N. nivicolus* (squares). The timeline on the left is in millions of years. Overarching geologic events are presented in the appropriate time zones. Each bar indicates the divergence range between populations as indicated by the associated number pair. Each number refers to haplotypes from geographic locations as follows: 1 = *G. hodgsoni* haplotypes from the St John's range and Mt Seuss; 2 = the *G. hodgsoni* haplotype at Mt Gran; 3 = *N. nivicolus* haplotypes from Springtail Point; 4 = *N. nivicolus* haplotypes from Mt Gran and Mt Seuss; 5 = *N. nivicolus* haplotypes from Mt England.

Discussion

Our mitochondrial DNA (COI) analysis of 97 Antarctic springtails from three taxonomic species revealed highly divergent populations across 65 km within the Mackay Glacier. Populations of *Gomphiocephalus hodgsoni* and *Neocryptopygus nivicolus* on the lower slopes of Mt Gran were shown to be 7.6% and 3.8% divergent from their nearest neighbours. For *G. hodgsoni*, this represents a considerably greater genetic divergence among populations than the 2.4% divergence previously found for this species (Stevens and Hogg, 2003a; Nolan et al., 2006a; McGaughran et al., 2008b). High genetic diversity, within both putative species, suggests that populations may have survived *in situ* since the Antarctic continent became fully glaciated. Given the elevations of surrounding mountains it is possible that several locations protruded above the advancing Mackay Glacier, and remained so since the early Pliocene (Janetschek, 1967a; Clapperton and Sugden, 1990). In particular, this area is known to contain the highest species diversity of springtails in southern Victoria Land, with *G. hodgsoni*, *N. nivicolus* and *A. monoculata* all known from this area (Gressitt et al., 1963). The species diversity of mites, lichens and mosses have also been shown to be high in the Mackay Glacier region relative to other nearby areas such as the Dry Valleys (Demetras et al., 2010; Green et al., 2011). This suggests that this area has served as a glacial refuge for several different taxa during the last 5 Mya.

We now also highlight the potential for species-level genetic divergences within two springtail taxa for populations on opposite sides of the Mackay Glacier. Our data suggest that the population of *G. hodgsoni* present on the lower slopes of Mt Gran has been isolated from other known *G. hodgsoni* populations since the

Pliocene (5 Mya). Similarly, the population of *N. nivicolus* from the same location has been isolated from a neighbouring population at Springtail Point by as much as 3.8 Mya. The occurrence of *A. monoculata* at Springtail Point, coupled with the highly divergent populations at Mt Gran supports the notion of high arthropod diversity for this area.

The differences in divergence estimates for *G. hodgsoni* (5Mya) and *N. nivicolus* (3.8Mya) may be the result of different evolutionary histories (e.g. later isolation) or possibly differences in mutation rates. For example, Stevens and Hogg (2006) suggested that differing mutation rates may exist between *G. hodgsoni* and the mite *Stereotydeus mollis.* However, little is known about the life history of *N. nivicolus.* The lack of ecological knowledge for *N. nivicolus* also makes it difficult to predict its dispersal abilities. Dispersal events in Antarctica are likely to be rare, and often accidental, making it difficult to attribute the presence of a species to ecological gradients (Janetschek, 1967b; Magalhães et al., 2012). *G. hodgsoni* is known to survive floating on both sea and fresh water, and dispersal events through wind or accidental carriage by birds is also possible (Hogg and Stevens, 2002; Hawes, 2011; McGaughran et al., 2011a, 2011b).

As Mackay Glacier is an outlet glacier for the EAIS, it is unlikely to have retreated during the interglacial periods of the Pleistocene as many of the alpine glaciers did (Clapperton and Sugden, 1990; Sugden et al., 1999). This appears to have isolated the Mt Gran population of *G. hodgsoni* from the populations on Mt Seuss in the centre of the glacier, and those in the St John's Range bordering Victoria Valley. It is possible that the presence of haplotypes from the St John's range in the Mt Seuss population relate to recent dispersal since the last glacial maximum. The sharing of *N. nivicolus* haplotypes between Mt Gran and Mt Seuss also indicates potentially recent dispersal from Mt Gran across the glacier. (Hawes, 2011) suggested that potential dispersal mechanisms may work in concert, whereby individuals could be wind-blown onto glaciers and then moved by glacial surface streams. The ‘accidental’ nature of dispersal events in Antarctica may explain why *G. hodgsoni* has yet to disperse from the Mt Gran population.

One species, *A. monoculata,* was found at only one location in our study area, although another isolated population is known from Mt Murray 150 km to the north (Gressitt et al., 1963). Similarly, haplotypes of *N. nivicolus* present at this site were not found elsewhere in our study area. Springtail Point is in an 'up-glacier' position, making dispersal through temporary melt water to more sea-ward locations possible. However, there was no evidence of water courses being formed by temporary streams in this area, and visual assessment of snow banks that surround the site, indicate they have changed little since a previous visit (Gressitt et al., 1963). Even with surface water, the dispersal mechanisms used by other springtail species such as wind and stream flow may be limited for *A. monoculata*. The loss of pigmentation, limited tolerance of UV light and presence deeper in the soil profile (Janetschek, 1967a) make it less likely that *A. monoculata* would experience accidental dispersal by water or wind movement.

We conclude that the Mackay Glacier has been a sufficient dispersal barrier to promote and maintain high levels of genetic divergence in two Antarctic springtail species endemic to southern Victoria Land. This isolation likely occurred around the beginning of the Pliocene (5 Mya), and has been maintained by on-going glaciations during the Pleistocene. The high genetic diversity, both at the population and species level, suggests that high altitude sites in this region have served as glacial refugia over the past 5 Mya. The isolation of these sites highlights the potential for high genetic diversity to be maintained on a small scale among the fragmented ice-free areas of Antarctica. Accordingly, we suggest that conservation efforts be directed toward maintaining and protecting the integrity of highly fragmented landscapes within the Transantarctic Mountains of the Ross Dependency.

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Literature Cited

Adams B., Bardgett R., Ayres E., Wall D., Aislabie J., Bamforth S., Bargagli R., Cary C., Cavacini P., Connell L., 2006. Diversity and distribution of Victoria Land biota. Soil Biology and Biochemistry. 38, 3003–3018.

Brower A., 1994. Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. Proceedings of the National Academy of Sciences of the United States of America. 91, 6491–5.

Clapperton C.M., Sugden D., 1990. Late Cenozoic glacial history of the Ross Embayment, Antarctica. Quaternary Science Reviews. 9, 253–272.

Clement M., Posada D., Crandall K., 2000. TCS: a computer program to estimate gene genealogies. Molecular Ecology. 9, 1657–1660.

Convey P., Stevens M.I., Hodgson D. A., Smellie J.L., Hillenbrand C.D., Barnes D.K. A., Clarke A., Pugh P.J. A., Linse K., Cary S.C., 2009. Exploring biological constraints on the glacial history of Antarctica. Quaternary Science Reviews. 28, 3035–3048.

Demetras N.J., Hogg I.D., Banks J.C., Adams B.J., 2010. Latitudinal distribution and mitochondrial DNA (COI) variability of *Stereotydeus* spp. (Acari: Prostigmata) in Victoria Land and the central Transantarctic Mountains. Antarctic Science. 22, 749–756.

Folmer O., Black M., Hoeh W., Lutz R., Vrijenhoek R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology. 3, 294–9.

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

Green T.G. A., Sancho L.G., Türk R., Seppelt R.D., Hogg I.D., 2011. High diversity of lichens at 84°S, Queen Maud Mountains, suggests preglacial survival of species in the Ross Sea region, Antarctica. Polar Biology. 34, 1211–1220.

Gressitt J., Leech R., Wise K.A.J., 1963. Entomological investigations in Antarctica. Pacific Insects. 5, 287–304.

Gressitt J., 1967. The Fauna., in: Bushnell V.(Eds.), Terrestrial life of Antarctica: Antarctic map folio series, New York, pp. 17–24.

Hajibabaei M., Janzen D.H., Burns J.M., Hallwachs W., Hebert P.D.N., 2006. DNA barcodes distinguish species of tropical Lepidoptera. Proceedings of the National Academy of Sciences of the United States of America. 103, 968–71.

Hawes T.C., 2011. Rafting in the Antarctic springtail, *Gomphiocephalus hodgsoni*. Antarctic Science. 23, 456–460.

Hogg I.D, Wall D.H, 2012. Extreme Habitats: Polar Deserts., in: Bell E.(Eds.), Life at Extremes: Environments, Organisms and Strategies for Survival, CAB International, Cambridge, UK,

Hogg I.D., Cary C.S., Convey P., Newsham K.K., O'Donnell A.G., Adams B.J., Aislabie J., Frati F., Stevens M.I., Wall D.H., 2006. Biotic interactions in Antarctic terrestrial ecosystems: Are they a factor? Soil Biology and Biochemistry. 38, 3035–3040.

Hogg I.D., Stevens M.I., 2002. Soil Fauna of Antarctic Coastal Landscapes. Ecological Studies. 154, 265–282.

Huelsenbeck J., Ronquist F., 2001. MRBAYES: Bayesian Inference of Phylogeny. Bioinformatics. 17, 754–755.

Janetschek H., 1967a. Arthropod ecology of south Victoria Land. Antarctic Research Series. 10, 205–293.

Janetschek H., 1967b. Growth and maturity of the springtail *Gomphiocephalus hodgsoni* Carpenter, from south Victoria Land and Ross Island. Antarctic Research Series. 10, 295 –305.

Juan C., Oromi P., Hewitt G.M., 1996. Phylogeny of the genus *Hegeter* (Tenebrionidae, Coleoptera) and its colonization of the Canary Islands deduced from Cytochrome Oxidase I mitochondrial DNA sequences. Heredity. 76, 392–403.

Kennett J.P., 1977. Cenozoic evolution of Antarctic glaciation, the Circum-Antarctic ocuean and their impact on global paleoceanography. Journal of Geophysical Research. 82.

Magalhães C., Stevens M.I., Cary S.C., Ball B. A, Storey B.C., Wall D.H., Türk R., Ruprecht U., 2012. At limits of life: multidisciplinary insights reveal environmental constraints on biotic diversity in continental Antarctica. PloS one. 7, e44578.

McGaughran A., Hogg I.D., Convey P., 2011a. Extended ecophysiological analysis of *Gomphiocephalus hodgsoni* (Collembola): flexibility in life history strategy and population response. Polar Biology. 34, 1713–1725.

McGaughran A., Hogg I.D., Stevens M.I., 2008. Patterns of population genetic structure for springtails and mites in southern Victoria Land, Antarctica. Molecular Phylogenetics and Evolution. 46, 606–18.

McGaughran A., Stevens M.I., Hogg I.D., Carapelli A., 2011b. Extreme Glacial Legacies: A Synthesis of the Antarctic Springtail Phylogeographic Record. Insects. 2, 62–82.

McGaughran A., Torricelli G., Carapelli A., Frati F., Stevens M.I., Convey P., Hogg I.D., 2010. Contrasting phylogeographical patterns for springtails reflect different evolutionary histories between the Antarctic Peninsula and continental Antarctica. Journal of Biogeography. 37, 103–119.

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

Nolan L., Hogg I.D., Stevens M.I., Haase M., 2006. Fine scale distribution of mtDNA haplotypes for the springtail *Gomphiocephalus hodgsoni* (Collembola) corresponds to an ancient shoreline in Taylor Valley, continental Antarctica. Polar Biology. 29, 813–819.

Posada D., 2008. jModelTest: phylogenetic model averaging. Molecular Biology and Evolution. 25, 1253–6.

Quek S., Davies S., Ilino T., Pierce N., 2004. Codiversification in an ant-plant mutualism: stem testure and the evolution of host use in *Crematogaster* (Formicidae: Myrmicinae) inhabitants of *Macaranga* (Euphorbiaceae). Evolution. 58, 554–570.

Rambaut A., Drummond A., 2007. Tracer v1.4, Available from http://beast.bio.ed.ac.uk/Tracer. .

Ratnasingham S., Hebert P.D.N., 2007. BOLD:The Barcode of Life Data System (www.barcodinglife.org). Molecular Ecology Notes. 7, 355–364.

Sinclair B.J., Stevens M.I., 2006. Terrestrial microarthropods of Victoria Land and Queen Maud Mountains, Antarctica: Implications of climate change. Soil Biology and Biochemistry. 38, 3158–3170.

Stevens G., 1980. New Zealand Adrift. , A.H and A.W Reed, Wellington.

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

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

Sugden D.E., Summerfield M. A, Denton G.H., Wilch T.I., McIntosh W.C., Marchant D.R., Rutford R.H., 1999. Landscape development in the Royal Society Range, southern Victoria Land, Antarctica: stability since the mid-Miocene. Geomorphology. 28, 181–200.

Suggate R.P., 1990. Late Pliocene and Quaternary glaciations of New Zealand. Quaternary Science Reviews. 9, 175–197.

Swofford D., 2002. PAUP*: Phylogenetic Analysis Using Parsimony, version 4.0b10 for Macintosh.

Wise K.A.J., 1971. The Collembola of antarctica. Pacific Insects Monograph. 25, 57–74.

Chapter IV

Thesis Summary and Conclusions

Geological events often leave discernible signatures in the genetics of populations affected by the event. Changes in climate, habitat size or the formation of dispersal barriers have been shown to cause the fragmentation of once widespread species, creating a mosaic of closely related, genetically divergent populations (Trewick, 2001). The analysis of mtDNA sequences in the study of phylogenetics can provide valuable insight into the impacts of these events, through investigation of genetic diversity across landscapes (Trewick and Morgan-Richards, 2005; Hogg et al., 2006b, 2009). This information can then be used to detect diversity 'hotspots', or the presence of important refugial zones which may be useful in conservation and ecosystem management.

In this thesis, I used mtDNA as a tool to investigate the effect of Pleistocene glaciations on the genetics of New Zealand and Antarctic arthropods. High levels of genetic divergence were found among populations for the Trichoptera (Chapter II) and Collembola (Chapter III). These patterns suggest that limited dispersal and recolonisation from isolated glacial refugia during the Pliocene and Pleistocene are significant factors for both taxa.

In Chapter II, genetic divergence times and patterns among species of the New Zealand caddisflies indicated that populations of these taxa may have been restricted to the North or South Island's during the Pleistocene. Genetic structure between populations located on the two islands is common at both the intra- and interspecific level among many invertebrate taxa (Stevens and Hogg, 2004; Hogg et al., 2006b; Knox et al., 2011) and may relate to the temporal existence of the Cook Strait land bridge at various times during the Pleistocene (Stevens, 1980). The 12 species pairs of caddisflies used in this investigation showed a strong split between the two islands, suggesting that significant dispersal barriers were present during the Pleistocene.

The Pleistocene consisted of a series of up to 20 cycles of cold and warm periods (Stevens, 1980) in New Zealand, and this appears to be reflected in the isolation of species on the North and South Islands. Molecular divergence times estimated for the isolation of each population varied considerably, although all were within the last 5 Mya, indicating that the extent of habitat fragmentation and the restriction of each population to refugia varied through each glacial cycle. The greatest divergence between populations was *P. evecta*, showing 9.9% sequence divergence between North and South Island populations. This suggested that isolating events among aquatic ecosystems may have had origins as early as the Pliocene. The majority of the species pairs examined (nine) were estimated to have likely diverged during either the late Pliocene as New Zealand's climate began to cool, or during the Pleistocene itself.

It has been suggested that New Zealand's invertebrate taxa have no 'cold adapted' representatives, suggesting that survival of the Pleistocene must have been through the presence of refugial zones throughout both islands (Dumbleton, 1970). The allopatric speciation of several other taxa have been linked to such refuges (e.g. Trewick 2001) and the study in Chapter II showed that the caddisflies were similarly affected. Three of the 12 species pairs examined revealed mtDNA divergences well above 3%, indicating the possible presence of cryptic species among the caddisfly fauna. This was supported by Hogg et al. (2009), and it is noted that cryptic speciation in New Zealand has many links with the Pleistocene period (e.g. Allibone et al., 1996; Stevens and Hogg, 2004; Knox et al., 2011). Accordingly, it is likely that population fragmentation and subsequent speciation is a key signature of the Pleistocene in New Zealand. This has many ramifications for the management and conservation of diversity in New Zealand, and highlights the need to readdress diversity measures, as these are often used as indicators of ecosystem health (e.g. Collier et al., 1997; Collier and Smith, 1998).

In Chapter III, I focused on genetic divergences at a small spatial scale, over a 65km stretch of the Mackay Glacier, Antarctica. Dispersal ability and habitat availability (or lack thereof), have had a great influence on the biogeography of arthropods in this region (Stevens and Hogg, 2002; Hawes, 2011; McGaughran et al., 2011a, 2011b). However, close inspection of population genetics using the mitochondrial COI gene has revealed a phylogenetic pattern consistent with Pleistocene survival for many arthropod species (e.g. Stevens and Hogg, 2003b). Springtails, being the largest of the arthropod taxa, have been studied throughout the Dry Valley region of southern Victoria Land. Population divergences were generally low within the common species *G. hodgsoni*, with values commonly less than 2.4% (Stevens and Hogg, 2003b; Nolan et al., 2006; McGaughran et al., 2008). This was taken to suggest that most populations represented relatively recent recolonisations from small refugial zones.

My study found that the Mackay Glacier region, as well has harbouring the largest number of species in southern Victoria Land (three; Gressitt et al., 1963), also contained the highest genetic divergences within an Antarctic springtail population yet recorded. Populations of *G. hodgsoni* were shown to have an average of 7.6% sequence divergences between Mt Gran to the north and St John's

Range to the south of the Mackay Glacier. This represents an isolation time of around 5 million years. Similarly the species *N. nivicolus* exhibited 4.0% sequence divergence at the same location.

The dispersal ability of springtails is thought to be poor in the Antarctic environment. Windblown individuals desiccate over short distances, and while floatation on stream and sea surfaces has been observed, liquid water is a rarity in this landscape (Stevens and Hogg, 2002; Hawes, 2011; McGaughran et al., 2011a). Therefore, the genetic structure observed by this study is likely related to the permanence of the Mackay Glacier, as an outlet of the East Antarctic Ice Sheet (EAIS), after its formation 5 Mya.

The differing divergence times of *G. hodgsoni* and *N. nivicolus* are believed to relate to differences in mutation rate caused by different life histories, as this has been observed in other Antarctic taxa (Stevens and Hogg, 2006). This indicates the importance of understanding the ecology of taxa used to infer the influence of geological events on diversity. Incorrectly estimated divergence times may cause speciation events to be associated with the wrong isolating mechanism, and so provide misinformation. Correctly identifying large scale mechanisms of divergence, such as the effect of the Pleistocene on aquatic and terrestrial arthropods as investigated here, can bring to light environments and ecosystems likely to contain hidden diversity. This has been shown among many New Zealand taxa, including the caddisflies used here, and suggests that both the freshwater environment and species populations on both main islands may contain overlooked or cryptic species diversity (Hogg et al 2006; Trewick 2001; Knox et al 2011). Similarly, the Antarctic environment, particularly in Victoria Land, has been shown to harbour high population divergences, possibly even at the species level, on small spatial scales. This would suggest that, given the right geographical circumstances like the presence of a polar cap outlet glacier such as the Mackay, undiscovered populations of springtails and other arthropods may exist that have been genetically isolated for millions of years. The use of mtDNA and phylogenetics provides tools to investigate and understand such populations.

Future research

Many studies have looked into the diversity and phylogenetics of taxa affected by the Pleistocene (e.g. Trewick, 2001; Hogg et al., 2006a; Stevens and Hogg, 2006). In New Zealand, studies of arthropod taxa tend to focus on the distribution of only a few species, or at small spatial scales (e.g. Trewick, 2001). The evidence presented in Chapter II of this thesis suggests that the pattern of Pleistocene-based diversity in New Zealand is common, among the aquatic taxa at least. Investigation through mtDNA of other aquatic taxa, such as the Plecoptera and Ephemeroptera orders which are used as water quality indicators along with the caddisflies, may identify similar genetic population structures. This may provide insight into locations or habitats of particular environmental or ecological importance. Morphological and ecological investigation of the three species with high genetic divergence identified in Chapter II (*P. evecta, O. maori* and *P. bistirpis*), may also be worthwhile, to determine if species status is appropriate for the populations from either island.

Within southern Victoria Land, Antarctica, many studies have focused on the phylogenetics and distributions of diversity for the springtail species *G. hodgsoni* (Stevens and Hogg, 2003a; Nolan et al., 2006; McGaughran et al., 2008). However, the high genetic diversity exhibited by populations of both *G. hodgsoni* and *N. nivicolus* near the Mackay Glacier shown in Chapter III suggest that while much of the range of both species has been well covered, a systematic investigation of mtDNA COI diversity across all possible populations should be undertaken. This should involve using current knowledge of population diversity and habitat to search for new locations likely to harbour springtail populations.

Greater information of the effects of the Pleistocene on the Antarctic environment could be obtained if entire species distributions and diversity levels are known. Similarly, investigation should be made into the population genetics of *A. monoculata* at Mt George Murray, to compliment information gathered by this study.

Literature Cited

Allibone R.M., Crowl T.A., Holmes J.M., King T.M., Mcdowall R.M., Townsend C.R., Wallis G.P., 1996. Isozyme analysis of *Galaxias* species (Teleostei: Galaxiidae) from the Taieri River, South Island, New Zealand: a species complex revealed. Biological Journal of the Linnean Society. 57, 107-127.

Collier K.J., Smith B.J., 1998. Dispersal of adult caddisflies (Trichoptera) into forests alongside three New Zealand streams. Hydrobiologia. 361, 53-65.

Collier K.J., Smith B.J., Baillie B.R., 1997. Summer light-trap catches of adult Trichoptera in hill-country catchments of contrasting land use, Waikato, New Zealand. New Zealand Journal of Marine and Freshwater Research. 31, 623-634.

Dumbleton L.J., 1970. Pleistocene climates and insect distributions. New Zealand Entomologist. 4, 3-23.

Gressitt J., Leech R., Wise K.A.J., 1963. Entomological investigations in Antarctica. Pacific Insects. 5, 287-304.

Hawes T.C., 2011. Rafting in the Antarctic springtail, *Gomphiocephalus hodgsoni*. Antarctic Science. 23, 456-460.

Hogg I., Smith B.J., Banks J.C., Dewaard J.R., Hebert P.D.N., 2009. Testing use of mitochondrial COI sequences for the identification and phylogenetic analysis of New Zealand caddisflies (Trichoptera). New Zealand Journal of Marine and Freshwater Research. 43, 1137-1146.

Hogg I.D., Stevens M.I., Schnabel K.E., Chapman A.M., 2006. Deeply divergent lineages of the widespread New Zealand amphipod *Paracalliope fluviatilis* revealed using allozyme and mitochondrial DNA analyses. Freshwater Biology. 51, 236-248.

Knox M.A., Hogg I.D., Pilditch C.A., 2011. The role of vicariance and dispersal on New Zealand’s estuarine biodiversity: the case of *Paracorophium* (Crustacea: Amphipoda). Biological Journal of the Linnean Society. 103, 863-874.

McGaughran A., Hogg I.D., Convey P., 2011a. Extended ecophysiological analysis of *Gomphiocephalus hodgsoni* (Collembola): flexibility in life history strategy and population response. Polar Biology. 34, 1713-1725.

McGaughran A., Hogg I.D., Stevens M.I., 2008. Patterns of population genetic structure for springtails and mites in southern Victoria Land, Antarctica. Molecular Phylogenetics and Evolution. 46, 606-18.

McGaughran A., Stevens M.I., Hogg I.D., Carapelli A., 2011b. Extreme Glacial Legacies: A Synthesis of the Antarctic Springtail Phylogeographic Record. Insects. 2, 62-82.

Nolan L., Hogg I.D., Stevens M.I., Haase M., 2006. Fine scale distribution of mtDNA haplotypes for the springtail *Gomphiocephalus hodgsoni* (Collembola) corresponds to an ancient shoreline in Taylor Valley, continental Antarctica. Polar Biology. 29, 813-819.

Stevens G., 1980. New Zealand Adrift. A.H and A.W Reed, Wellington.

Stevens M., Hogg I.D., 2004. Population genetic structure of New Zealand’s endemic corophiid amphipods: evidence for allopatric speciation. Biological Journal of the Linnean Society. 81, 119-133.

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

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

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

Trewick S.A., 2001. Scree weta phylogeography: Surviving glaciation and implications for Pleistocene biogeography in New Zealand. New Zealand Journal of Zoology. 28, 291-298.

Trewick S.A., Morgan-Richards M., 2005. After the deluge: mitochondrial DNA indicates Miocene radiation and Pliocene adaptation of tree and giant weta (Orthoptera: Anostostomatidae). Journal of Biogeography. 32, 295-309.

Appendix I: BOLD sample Id's, genus and species names and collection location information for caddisfly specimens used in the initial data set of this study.

Sample ID	Genus and species name	Collection location			
		Sector	Region	Latitude	Longitude
NZT1	Polyplectropus altera				
NZT2	Polyplectropus sp.				
NZT3	Psilochorema mimicum				
NZT4	Oeconesus maori				
NZT5	Chathamia integripennis				
NZT6	Philanisus plebeius				
NZT7	Triplectidina moselyi				
NZT8	Hydrobiosis spatulata				
NZT9	Hydrobiosis gollanis				
NZT10	Oeconesus maori				
NZT11	Pseudoeconesus bistirpis				
NZT12	Triplectides dolichos				
NZT13	Polyplectropus altera				
NZT14	Hydrobiosis soror				
NZT15	Hydropsyche colonica				
NZT16	Hydropsyche fimbriata				
NZT17	Pycnocentria funerea				
NZT18	Psilochorema mimicum				
NZT19	Edpercivalia thomasoni				
NZT20	Hydrobiosella mixta				
NZT21	Hydrobiosis styracine				
NZT22	Hydrobiosis parumbripennis				
NZT23	Hydrobiosis copis				
NZT24	Oxyethira albiceps				
NZT25	Olinga jeanae				
NZT26	Costachorema hecton				
NZT27	Beraeoptera roria				
NZT28	Costachorema xanthopterum				
NZT29	Polyplectropus aurifuscus				
NZT30	Atrachorema mangu				
NZT31	Zelandoptila moselyi				
NZT32	Hydropsyche thomasi				
NZT33	Hydropsyche thomasi				
NZT34	Diplectrona zealandensis				
NZT35	Hydrochorema tenuicaudatum				
NZT36	Hydrochorema tenuicaudatum				
NZT37	Tiphobiosis kleinpastei				
NZT38	Tiphobiosis kleinpastei				
NZT39	Hydrobiosis johnsi				
NZT40					
NZT41	Pseudoeconesus hudsoni				
NZT42	Hydrobiosis harpidiosa				
NZT43	Edpercivalia cassicola				
NZT44	Hydrobiosis falcis				
NZT45	Costachorema callistum				
NZT46	Rakiura vernale				
NZT47	Hydrobiosis charadraea				

NZT48	Pycnocentrella eruensis
NZT49	Oecetis unicolor
NZT50	Tiphobiosis veniflex
NZT51	Helicopsyche albescens
NZT52	Neurochorema armstrongi
NZT53	Pycnocentria evecta
NZT54	Paroxyethira hendersoni
NZT55	Pycnocentrodes aeris
NZT56	Pycnocentrodes aureolus
NZT57	Neurochorema confusum
NZT58	Hydropsyche raruraru
NZT59	Hydrobiosis budgei
NZT60	Hydrobiosis umbripennis
NZT61	Hydropsyche winterbourni
NZT62	Oeconesus maori
NZT63	Pycnocentria funerea
NZT64	Pseudoeconesus bistirpis
NZT65	Pycnocentria indet
NZT66	Tiphobiosis cowiei
NZT67	Zelandopsyche maclellani
NZT68	Cryptobiosella hastata
NZT69	Cryptobiosella hastata
NZT70	Hydrobiosella tonela
NZT71	Hydrobiosella tonela
NZT72	Tiphobiosis kleinpastei
NZT73	Alloecentrella magnicornis
NZT74	Alloecentrella magnicornis
NZT75	Alloecentrella magnicornis
NZT76	Alloecentrella magnicornis
NZT77	Alloecentrella magnicornis
NZT78	Alloecentrella incisus
NZT79	Alloecentrella incisus
NZT80	Oxyethira ahipara
NZT81	Oxyethira ahipara
NZT82	Oxyethira ahipara
NZT83	Zelolessica meizon
NZT84	Zelolessica cheira
NZT85	Zelolessica cheira
NZT86	Zelolessica cheira
NZT87	Philorheithrus aliciae
NZT88	Philorheithrus aliciae
NZT89	Philorheithrus sp.
NZT90	Philorheithrus sp.
NZT91	Philorheithrus sp.
NZT92	Hydrobiosis harpidiosa
NZT93	Hydrobiosis harpidiosa
NZT94	Hydrobiosis harpidiosa
NZT95	Tarapsyche olis
NZT96	Tarapsyche olis
NZT97	Tarapsyche olis
NZT98	Plectrocnemia maclachlani
NZT99	Olinga feredayi
NZT100	Olinga feredayi
NZT101	Olinga feredayi

NZT102	Olinga feredayi				
NZT103	Hydropsyche fimbriata				
NZT104	Hydropsyche fimbriata				
NZT105	Hydrobiosis soror				
NZT106	Hydrobiosis soror				
NZT107	Hydrobiosis soror				
NZT108	Confluens hamiltoni				
NZT109	Pycnocentria evecta				
NZT110	Pycnocentria evecta				
NZT111	Neurochorema confusum				
NZT112	Neurochorema confusum				
NZT113	Neurochorema armstrongi				
NZT114	Neurochorema armstrongi				
NZT115	Confluens hamiltoni				
NZT116	Confluens hamiltoni				
NZT117	Pycnocentrodes aureolus				
NZT118	Pycnocentrodes aureolus				
NZT119	Hydropsyche winterbourni				
NZT120	Hydropsyche winterbourni				
NZT121	Hydropsyche raruraru				
NZT122	Neurochorema armstrongi				
NZT123	Neurochorema armstrongi				
NZT124	Hydrobiosis copis				
NZT125	Hydrobiosis copis				
NZT126	Hydrobiosis copis				
NZT127	Costachorema xanthopterum				
NZT128	Costachorema xanthopterum				
NZT129	Costachorema xanthopterum				
NZT130	Pseudoeconesus bistirpis				
NZT131	Pseudoeconesus bistirpis				
NZT132	Pseudoeconesus bistirpis				
NZT133	Oeconesus maori				
NZT134	Oeconesus maori				
NZT135	Oeconesus maori				
NZT136	Hydropsyche winterbourni				
NZT137	Hydropsyche thomasi				
NZT138	Hydropsyche fimbriata				
NZT139	Hydropsyche fimbriata				
NZT140	Triplectides obsoletus				
NZ141	Polyplectropus altera	North Island	Te Kuiti	-38.32699966	175.0809937
NZ142	Polyplectropus altera	North Island	Te Kuiti	-38.32699966	175.0809937
NZ143	Polyplectropus altera	North Island	Te Kuiti	-38.32699966	175.0809937
NZ146	Pycnocentrodes aureolus	North Island	Te Kuiti	-38.32699966	175.0809937
NZ147	Pycnocentrodes aureolus	North Island	Te Kuiti	-38.32699966	175.0809937
NZ148	Pycnocentrodes aureolus	North Island	Te Kuiti	-38.32699966	175.0809937
NZ149	Pycnocentrodes aureolus	North Island	Te Kuiti	-38.32699966	175.0809937
NZ150	Pycnocentrodes aureolus	North Island	Te Kuiti	-38.32699966	175.0809937
NZ151	Plectrocnemia maclachlani	North Island	Te Kuiti	-38.32699966	175.0809937
NZ152	Plectrocnemia maclachlani	North Island	Te Kuiti	-38.32699966	175.0809937
NZ153	Plectrocnemia maclachlani	North Island	Te Kuiti	-38.32699966	175.0809937
NZ154	Plectrocnemia maclachlani	North Island	Te Kuiti	-38.32699966	175.0809937
NZ162	Pycnocentria evecta	North Island	Waikato	-38.67100143	174.878006
NZ163	Pycnocentria evecta	North Island	Waikato	-38.67100143	174.878006
NZ164	Pycnocentria evecta	North Island	Waikato	-38.67100143	174.878006

NZ165	Pycnocentria evecta	North Island	Waikato	-38.67100143	174.878006
NZ166	Pycnocentria evecta	North Island	Waikato	-38.67100143	174.878006
NZ167	Costachorema xanthopterum	North Island	Waikato	-37.91600037	175.0970001
NZ168	Costachorema xanthopterum	North Island	Waikato	-37.91600037	175.0970001
NZ169	Costachorema xanthopterum	North Island	Waikato	-37.91600037	175.0970001
NZ170	Edpercivalia schistaria	South Island	West Coast	-41.65399933	171.9089966
NZ171	Ecnomina zealandica	North Island	Waikato	-37.77799988	175.0709991
NZ172	Ecnomina zealandica	North Island	Waikato	-37.77799988	175.0709991
NZ173	Ecnomina zealandica	North Island	Waikato	-37.77799988	175.0709991
NZ174	Ecnomina zealandica	North Island	Waikato	-37.77799988	175.0709991
NZ175	Psilochorema embersoni	South Island	West Coast	-41.65399933	171.9089966
NZ176	Psilochorema embersoni	South Island	West Coast	-41.65399933	171.9089966
NZ177	Psilochorema embersoni	South Island	West Coast	-41.65399933	171.9089966
NZ178	Psilochorema embersoni	South Island	West Coast	-41.65399933	171.9089966
NZ179	Psilochorema embersoni	South Island	Westcoast	-41.76599884	171.7949982
NZ180	Psilochorema vomerharpax	South Island	West Coast	-41.65399933	171.9089966
NZ181	Psilochorema vomerharpax	South Island	West Coast	-41.65399933	171.9089966
NZ182	Psilochorema vomerharpax	South Island	West Coast	-41.65399933	171.9089966
NZ183	Psilochorema vomerharpax	South Island	West Coast	-41.65399933	171.9089966
NZ184	Psilochorema vomerharpax	South Island	West Coast	-41.65399933	171.9089966
NZ185	Polyplectropus puerilis				
NZ186	Polyplectropus puerilis				
NZ187	Polyplectropus puerilis				
NZ188	Polyplectropus puerilis				
NZ189	Polyplectropus puerilis				
NZ190	Pycnocentria evecta				
NZ192	Pycnocentrodes aeris	North Island	Te Kuiti	-38.32699966	175.0809937
NZ193	Pycnocentrodes aeris	North Island	Te Kuiti	-38.32699966	175.0809937
NZ194	Pycnocentrodes aeris	North Island	Te Kuiti	-38.32699966	175.0809937
NZ195	Pycnocentrodes aeris	North Island	Te Kuiti	-38.32699966	175.0809937
NZ196	Pycnocentrodes aeris	North Island	Te Kuiti	-38.32699966	175.0809937
NZ198	Pycnocentria funerea	North Island	Waikato	-38.67100143	174.878006
NZ199	Pycnocentria funerea	North Island	Waikato	-38.67100143	174.878006
NZ200	Pycnocentria funerea	North Island	Waikato	-38.67100143	174.878006
NZ201	Pycnocentria funerea	North Island	Waikato	-38.67100143	174.878006
NZ202	Beraeoptera roria	North Island	Waikato	-37.91600037	175.0970001
NZ203	Beraeoptera roria	North Island	Waikato	-37.91600037	175.0970001
NZ204	Beraeoptera roria	North Island	Waikato	-37.91600037	175.0970001
NZ205	Beraeoptera roria	North Island	Waikato	-37.91600037	175.0970001
NZ206	Beraeoptera roria	North Island	Waikato	-37.91600037	175.0970001
NZ207	Polyplectropus impluvii	North Island	Waikato	-38.67100143	174.878006
NZ208	Polyplectropus impluvii	North Island	Waikato	-38.67100143	174.878006
NZ209	Polyplectropus impluvii	North Island	Waikato	-38.67100143	174.878006
NZ210	Polyplectropus impluvii	North Island	Waikato	-38.67100143	174.878006
NZ211	Polyplectropus impluvii	North Island	Waikato	-38.67100143	174.878006
NZ212	Diplectrona bulla	North Island	Waikato	-37.77799988	175.0700073
NZ213	Hydropsyche thomasi	North Island	Waikato	-37.77999878	175.0700073
NZ214	Hydropsyche thomasi	North Island	Waikato	-37.81600189	175.2879944
NZ215	Hydropsyche thomasi	North Island	Waikato	-37.81600189	175.2879944
NZ216	Hydropsyche thomasi	North Island	Waikato	-37.81600189	175.2879944
NZ217	Hydropsyche thomasi				
NZ218	Hydropsyche thomasi				
NZ219	Hydropsyche thomasi				
NZ220	Hydropsyche thomasi				

NZ221	Hydropsyche thomasi				
NZ223	Oecetis unicolor	North Island	Waikato	-38.26100159	175.1029968
NZ224	Oecetis unicolor	North Island	Waikato	-38.26100159	175.1029968
NZ225	Oecetis unicolor	North Island	Waikato	-38.26100159	175.1029968
NZ226	Oecetis unicolor	North Island	Waikato	-38.26100159	175.1029968
NZ227					
NZ228					
NZ229					
NZ230					
NZ231					
NZ232	Hudsonema cf. alienum	North Island	Te Kuiti	-38.32699966	175.0809937
NZ233	Hudsonema cf. alienum	North Island	Te Kuiti	-38.32699966	175.0809937
NZ234	Hudsonema cf. alienum	North Island	Te Kuiti	-38.32699966	175.0809937
NZ235	Hudsonema cf. alienum	North Island	Te Kuiti	-38.32699966	175.0809937
NZ236	Hudsonema cf. alienum	North Island	Te Kuiti	-38.32699966	175.0809937
NZ238	Edpercivalia shandi	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZ239	Edpercivalia shandi	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZ240	Edpercivalia shandi	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZ241	Edpercivalia shandi	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZ242	Edpercivalia shandi	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZ243	Edpercivalia shandi	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZ244	Edpercivalia shandi	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZ245	Edpercivalia shandi	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZ246	Edpercivalia shandi	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZ247	Oeconesus maori	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZ248	Oeconesus maori	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZ249	Oeconesus maori	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZ250	Oeconesus maori	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZ251	Oeconesus maori	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZ252	Pseudoeconesus bistirpis	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZ253	Pseudoeconesus bistirpis	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZ254	Pseudoeconesus bistirpis	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZ255	Pseudoeconesus bistirpis	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZ256	Pseudoeconesus bistirpis	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZ257	Hydrobiosis nr. spatulata	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZ258	Hydrobiosis nr. spatulata	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZ259	Hydrobiosis nr. spatulata	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZ260	Hydrochorema crassicaudatum	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZ261	Hydrochorema crassicaudatum	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZ262	Hydrochorema crassicaudatum	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZ263	Polyplectropus puerilis	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZ264	Polyplectropus aurifuscus	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZ265	Polyplectropus aurifuscus	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZ266	Polyplectropus aurifuscus	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZ267	Polyplectropus aurifuscus	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZ268	Philanisus plebeius	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZ269	Philanisus plebeius	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZ270	Xenobiosella motueka	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZ271	Xenobiosella motueka	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZ272	Xenobiosella motueka	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZ273	Xenobiosella motueka	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZ274	Xenobiosella motueka	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZ275	Hydrobiosella mixta	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZ276	Hydrobiosella mixta	South Island	Marlborough Sounds	-41.12009811	174.0850067

NZ277	Hydrobiosella mixta	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZ278	Hydrobiosella mixta	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZ279	Hydrobiosella mixta	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZ280	Triplectides dolichos	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZ281	Triplectides dolichos	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZ282	Triplectides dolichos	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZ283	Psilochorema tautoru	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZ284	Psilochorema tautoru	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZ285	Psilochorema tautoru	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZ286	Hydrobiosis nr. spatulata	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZ287	Hydrobiosis nr. spatulata	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZ288	Hydrobiosis nr. spatulata	South Island	Marlborough Sounds	-41.12009811	174.0850067
NZCAD293	Hydrobiosis lindsayi	Chatham Islands		-43.99300003	176.5420074
NZCAD294	Edpercivalia cassicola	North Island	Taupo	-39.1570015	175.4640045
NZCAD295	Edpercivalia cassicola	North Island	Taupo	-39.1570015	175.4640045
NZCAD296	Edpercivalia cassicola	North Island	Taupo	-39.1570015	175.4640045
NZCAD298	Edpercivalia cassicola	North Island	Taupo	-39.1570015	175.4640045
NZCAD300	Costachorema callistum	North Island	Taupo	-39.1570015	175.4640045
NZCAD301	Costachorema callistum	North Island	Taupo	-39.1570015	175.4640045
NZCAD302	Hydrobiosis sherleyi	South Island	Fiordland	-45.7480011	167.3860016
NZCAD304	Hydrobiosis harpidiosa	North Island	Taupo	-39.1570015	175.4640045
NZCAD305	Hydrobiosis harpidiosa	North Island	Taupo	-39.1570015	175.4640045
NZCAD306	Hydrobiosis spatulata	North Island	Taranaki	-38.97800064	174.8399963
NZCAD307		South Island	Fiordland	-45.7480011	167.3860016
NZCAD308		South Island	Fiordland	-45.7480011	167.3860016
NZCAD309		South Island	Fiordland	-45.7480011	167.3860016
NZCAD310	Hydrobiosis spatulata	North Island	Taupo	-39.1570015	175.4640045
NZCAD311	Hydropsyche tepoka				
NZCAD312	Hydropsyche tepoka	North Island	Waikato	-37.93000031	175.548996
NZCAD313	Hydropsyche tepoka	North Island	Waikato	-37.93000031	175.548996
NZCAD314	Traillochorema rakiura		Stewart Island	-46.79800034	167.7230072
NZCAD315	Kokiria miharo	South Island		-41.66400146	171.9049988
NZCAD318	Triplectides obsoletus	North Island	Coromandel	-36.72600174	175.8139954
NZCAD319	Chathamia integripennis	North Island	Coromandel	-36.72600174	175.8139954
NZCAD320	Chathamia integripennis	North Island	Coromandel	-36.72600174	175.8139954
NZCAD321	Chathamia integripennis	North Island	Coromandel	-36.72600174	175.8139954
NZCAD322	Chathamia integripennis	North Island	Coromandel	-36.72600174	175.8139954
NZCAD323	Chathamia integripennis	North Island	Coromandel	-36.72600174	175.8139954
NZCAD324	Psilochorema donaldsoni	North Island	Hawke`s Bay	-39.52000046	176.2059937
NZCAD325	Psilochorema mimicum	North Island	Hawke`s Bay	-39.4620018	176.2819977
NZCAD326	Psilochorema mimicum	North Island	Hawke`s Bay	-39.4620018	176.2819977
NZCAD327	Psilochorema donaldsoni	North Island	Hawke`s Bay	-39.4620018	176.2819977
NZCAD328	Psilochorema mimicum	North Island	Hawke`s Bay	-39.4620018	176.2819977
NZCAD329	Psilochorema mimicum	North Island	Hawke`s Bay	-39.4620018	176.2819977
NZCAD330	Psilochorema mimicum	North Island	Hawke`s Bay	-39.4620018	176.2819977
NZCAD331	Psilochorema mimicum	North Island	Hawke`s Bay	-39.4620018	176.2819977
NZCAD332	Psilochorema mimicum	North Island	Hawke`s Bay	-39.46500015	176.2409973
NZCAD333	Psilochorema donaldsoni	North Island	Hawke`s Bay	-39.46500015	176.2409973
NZCAD334	Psilochorema mimicum	North Island	Hawke`s Bay	-39.46500015	176.2409973
NZCAD335	Psilochorema mimicum	North Island	Hawke`s Bay	-39.46500015	176.2409973
NZCAD336	Psilochorema mimicum	North Island	Hawke`s Bay	-39.46500015	176.2409973
NZCAD337	Psilochorema mimicum	North Island	Hawke`s Bay	-39.46500015	176.2409973
NZCAD338	Psilochorema mimicum	North Island	Hawke`s Bay	-39.46500015	176.2409973
NZCAD339	Psilochorema mimicum	North Island	Hawke`s Bay	-39.46500015	176.2409973

NZCAD340	Psilochorema mimicum	North Island	Hawke`s Bay	-39.46500015	176.2409973
NZCAD341	Psilochorema mimicum	North Island	Hawke`s Bay	-39.46500015	176.2409973
NZCAD342	Psilochorema mimicum	North Island	Hawke`s Bay	-39.45600128	176.2400055
NZCAD343	Psilochorema mimicum	North Island	Hawke`s Bay	-39.45600128	176.2400055
NZCAD344	Psilochorema donaldsoni	North Island	Hawke`s Bay	-39.45600128	176.2400055
NZCAD345	Psilochorema mimicum	North Island	Hawke`s Bay	-39.45899963	176.2180023
NZCAD346	Psilochorema mimicum	North Island	Hawke`s Bay	-39.45899963	176.2180023
NZCAD347	Psilochorema mimicum	North Island	Hawke`s Bay	-39.45899963	176.2180023
NZCAD348	Psilochorema mimicum	North Island	Hawke`s Bay	-39.45899963	176.2180023
NZCAD349	Psilochorema donaldsoni	North Island	Hawke`s Bay	-39.45899963	176.2180023
NZCAD350	Psilochorema mimicum	North Island	Hawke`s Bay	-39.45899963	176.2180023
NZCAD351	Psilochorema donaldsoni	North Island	Hawke`s Bay	-39.52000046	176.2059937
NZCAD352	Psilochorema donaldsoni	North Island	Hawke`s Bay	-39.52000046	176.2059937
NZCAD353	Psilochorema donaldsoni	North Island	Hawke`s Bay	-39.52000046	176.2059937
NZCAD354	Psilochorema donaldsoni	North Island	Hawke`s Bay	-39.52000046	176.2059937
NZCAD355	Psilochorema donaldsoni	North Island	Hawke`s Bay	-39.52000046	176.2059937
NZCAD356	Psilochorema donaldsoni	North Island	Hawke`s Bay	-39.52000046	176.2059937
NZCAD357	Psilochorema mimicum	North Island	Hawke`s Bay	-39.46500015	176.2400055
NZCAD358	Psilochorema donaldsoni	North Island	Hawke`s Bay	-39.45600128	176.2400055
NZCAD359	Psilochorema donaldsoni	North Island	Hawke`s Bay	-39.45600128	176.2400055
NZCAD360	Psilochorema donaldsoni	North Island	Hawke`s Bay	-39.45600128	176.2400055
NZCAD361	Psilochorema donaldsoni	North Island	Hawke`s Bay	-39.45600128	176.2400055
NZCAD362	Psilochorema donaldsoni	North Island	Hawke`s Bay	-39.45600128	176.2400055
NZCAD363	Psilochorema donaldsoni	North Island	Hawke`s Bay	-39.45600128	176.2400055
NZCAD364	Psilochorema donaldsoni	North Island	Hawke`s Bay	-39.45600128	176.2400055
NZCAD365	Psilochorema donaldsoni	North Island	Hawke`s Bay	-39.45600128	176.2400055
NZCAD366	Psilochorema mimicum	North Island	Hawke`s Bay	-39.45600128	176.2400055
NZCAD367	Psilochorema donaldsoni	North Island	Hawke`s Bay	-39.45600128	176.2400055
NZCAD368	Psilochorema mimicum	North Island	Hawke`s Bay	-39.45899963	176.2180023
NZCAD369	Psilochorema mimicum	North Island	Hawke`s Bay	-39.52000046	176.2059937
NZCAD370	Psilochorema donaldsoni	North Island	Hawke`s Bay	-39.47000122	176.25
NZCAD371	Psilochorema donaldsoni	North Island	Hawke`s Bay	-39.47000122	176.25
NZCAD372	Psilochorema donaldsoni	North Island	Hawke`s Bay	-39.47000122	176.25
NZCAD373	Psilochorema mimicum	North Island	Hawke`s Bay	-39.47200012	176.2449951
NZCAD374	Psilochorema donaldsoni	North Island	Hawke`s Bay	-39.47200012	176.2449951
NZCAD375	Psilochorema donaldsoni	North Island	Hawke`s Bay	-39.47200012	176.2449951
NZCAD376	Psilochorema donaldsoni	North Island	Hawke`s Bay	-39.47200012	176.2449951
NZCAD377	Psilochorema macroharpax	North Island	Hawke`s Bay	-39.46500015	176.2409973
NZCAD378	Psilochorema leptoharpax	North Island	Hawke`s Bay	-39.52000046	176.2059937
NZCAD379	Psilochorema leptoharpax	North Island	Hawke`s Bay	-39.52000046	176.2059937
NZCAD380	Psilochorema leptoharpax	North Island	Hawke`s Bay	-39.52000046	176.2059937
NZCAD381	Psilochorema leptoharpax	North Island	Hawke`s Bay	-39.48099899	176.2489929
NZCAD382	Psilochorema leptoharpax	North Island	Hawke`s Bay	-39.48099899	176.2489929
NZCAD384	Tiphobiosis kleinpastei	North Island	Waikato	-37.79000092	175.0610046
NZCAD385		South Island	West Coast	-41.5320015	172.0650024
NZCAD386		South Island	West Coast	-41.5320015	172.0650024
NZCAD388		South Island	West Coast	-41.5320015	172.0650024
NZCAD389		South Island	West Coast	-41.5320015	172.0650024
NZCAD390		South Island	West Coast	-41.5320015	172.0650024
NZCAD394		South Island	West Coast	-41.5320015	172.0650024
NZCAD395		South Island	West Coast	-41.5320015	172.0650024
NZCAD400	Costachorema hecton	North Island	Waikato	-37.79000092	175.0610046
NZCAD404	Hydrobiosis centralis	North Island	Waikato	-37.7820015	175.0740051
NZCAD405	Pycnocentria forcipata	North Island	Hawke`s Bay		

NZCAD407	Hudsonema alienum	North Island	Waikato	-37.78499985	175.0690002
NZCAD408	Triplectides cephalotes	North Island	Hawke's Bay		
NZCAD409	Hudsonema amabile	North Island	Hawke`s Bay		
NZCAD410	Hudsonema amabile	North Island	Hawke`s Bay		
NZCAD413	Tiphobiosis cowiei	North Island	Waikato	-37.79000092	175.0610046
NZCAD414	Hydrobiosis umbripennis	North Island	Hawke`s Bay	-39.14500046	176.8359985
NZCAD415	Oecetis unicolor	North Island	Waikato		
NZCAD416	Oecetis unicolor	North Island	Waikato		
NZCAD420	Synchorema tillyardi	South Island	West Coast	-40.80799866	172.522995
NZCAD422		North Island	Waikato	-37.77999878	175.0700073
NZCAD424	Hydrochorema crassicaudatum	North Island	Waikato	-37.79000092	175.0610046
NZCAD426	Oxyethira cf. albiceps	North Island	Manawatu-Wanganui	-39.13399887	175.6569977
NZCAD427	Hydropsyche colonica	North Island	Waikato	-37.77999878	175.0700073
NZCAD428	Pseudoeconesus bistirpis	North Island	Waikato	-37.77999878	175.0700073
NZCAD429	Oeconesus maori	North Island	Waikato	-37.77999878	175.0700073
NZCAD430	Zepsyche acinaces	North Island	Waikato	-37.77999878	175.0700073
NZCAD431	Zelandopsyche maclellani	South Island	West Coast	-42.26399994	171.5910034
NZCAD433	Tiphobiosis plicosta	North Island	North Island	-39.46500015	176.2409973
NZCAD434	Hydrobiosella stenocerca	South Island	Mid Cantebury	-43.02000046	171.7050018
NZCAD439	Triplectides cephalotes	North Island	Hawke`s Bay	-39.14500046	176.8359985
NZCAD443					
NZCAD444	Hydropsyche raruraru	South Island	North-west Nelson	-40.84500122	172.772995
NZCAD445	Hydropsyche raruraru	South Island	North-west Nelson	-40.84500122	172.772995
NZCAD447	Pycnocentrodes aureolus	South Island	North West Nelson	-40.84500122	172.772995
NZCAD451	Pycnocentria nr. sp.	North Island	Hawke`s Bay	-39.46500015	176.2409973
NZCAD452	Pycnocentria nr. sp.	North Island	Hawke`s Bay	-39.46500015	176.2409973
NZCAD453	Pycnocentria forcipata	North Island	Bay of Plenty	-38.21099854	176.3650055
NZCAD454	Oeconesus maori	North Island	Bay of Plenty	-38.31800079	176.6380005
NZCAD455	Triplectides obsoletus	North Island	Hawke`s Bay	-39.14500046	176.8359985
NZCAD456	Polyplectropus aurifusca	North Island	Hawke`s Bay	-39.14500046	176.8359985
NZCAD457	Hydrobiosella stenocerca	South Island		-46.44300079	169.4689941
NZCAD458	Hydrobiosella stenocerca	South Island		-46.44300079	169.4689941
NZCAD459	Triplectides dolichos	South Island	Marlborough Sounds	-41.15000153	174.1589966
NZCAD460	Pycnocentria nr. sylvestris	South Island	Upper Buller Gorge	-41.76900101	172.1529999
NZCAD464		South Island	Westcoast	-42.35100174	171.3679962
NZCAD465		South Island	Westcoast	-42.35100174	171.3679962
NZCAD466		South Island	Westcoast	-42.35100174	171.3679962
NZCAD467	Polyplectropus altera	North Island	Hawke`s Bay	-39.14500046	176.8359985
NZCAD468	Polyplectropus altera	North Island	Hawke`s Bay	-39.14500046	176.8359985
NZCAD475	Olinga feredayi	North Island	Waikato	-37.77999878	175.0700073
NZCAD476	Olinga feredayi	North Island	Waikato	-37.77999878	175.0700073
NZCAD477	Olinga feredayi	North Island	Waikato	-37.77999878	175.0700073
NZCAD478	Olinga feredayi	North Island	Waikato	-37.77999878	175.0700073
NZCAD480	Olinga feredayi	North Island	Waikato	-37.77999878	175.0700073
NZCAD481	Olinga feredayi	North Island	Waikato	-37.77999878	175.0700073
NZCAD482	Olinga feredayi	North Island	Waikato	-37.77999878	175.0700073
NZCAD483	Olinga feredayi	North Island	Waikato	-37.77999878	175.0700073
NZCAD484	Olinga feredayi	North Island	Waikato	-37.77999878	175.0700073
NZCAD485	Olinga feredayi	North Island	Waikato	-37.77999878	175.0700073
NZCAD486	Olinga feredayi	North Island	Waikato	-37.77999878	175.0700073
NZCAD487	Olinga jeanae	North Island	Waikato	-37.79000092	175.0610046
NZCAD488	Olinga jeanae	North Island	Waikato	-37.79000092	175.0610046
NZCAD489	Olinga jeanae	North Island	Waikato	-37.79000092	175.0610046
NZCAD490	Olinga jeanae	North Island	Waikato	-37.79000092	175.0610046

NZCAD491	Olinga jeanae	North Island	Waikato	-37.79000092	175.0610046
NZCAD492	Olinga jeanae	North Island	Waikato	-37.79000092	175.0610046
NZCAD493	Olinga jeanae	North Island	Waikato	-37.79000092	175.0610046
NZCAD494	Olinga jeanae	North Island	Waikato	-37.79000092	175.0610046
NZCAD497	Pycnocentria sylvestris	South Island	North West Nelson	-41.18899918	172.7440033
NZCAD498	Pycnocentria sylvestris	South Island	North West Nelson	-41.18899918	172.7440033
NZCAD501	Paroxyethira hendersoni	North Island	Hawke`s Bay	-39.21699905	175.8959961
NZCAD503	Paroxyethira eatoni	North Island	Hawke`s Bay	-39.21699905	175.8959961
NZCAD505	Paroxyethira eatoni	North Island	Hawke`s Bay	-39.21699905	175.8959961
NZCAD511	Neurochorema sp. A	South Island	West Coast	-41.65399933	171.9089966
NZCAD512	Neurochorema sp. A	South Island	West Coast	-41.65399933	171.9089966
NZCAD513	Neurochorema sp. A	South Island	West Coast	-41.65399933	171.9089966
NZCAD514	Neurochorema sp. A	South Island	West Coast	-41.65399933	171.9089966
NZCAD515	Neurochorema sp. A	South Island	West Coast	-41.65399933	171.9089966
NZCAD516	Cryptobiosella hastata	North Island	Waikato	-38.5340004	174.9929962
NZCAD517	Cryptobiosella hastata	North Island	Waikato	-38.5340004	174.9929962
NZCAD518	Cryptobiosella hastata	North Island	Waikato	-38.5340004	174.9929962
NZCAD519	Cryptobiosella hastata	North Island	Waikato	-38.5340004	174.9929962
NZCAD520	Cryptobiosella hastata	North Island	Waikato	-38.5340004	174.9929962
NZCAD523	Tiphobiosis indet	South Island	West Coast	-42.2840004	171.4700012
NZCAD524	Tiphobiosis childi	South Island	Lake Brunner	-42.56499863	171.4290009
NZCAD525	Tiphobiosis childi	South Island	Mid Cantebury	-42.93899918	171.473999
NZCAD526	Pycnocentria nr. hawdonia	South Island	North West Nelson	-40.84500122	172.772995
NZCAD527	Pycnocentria nr. hawdonia	South Island	North West Nelson	-40.84500122	172.772995
NZCAD529	Edpercivalia banksiensis	South Island	Canterbury	-43.79499817	172.8399963
NZCAD530	Edpercivalia spaini	South Island	Upper Buller Gorge	-41.76900101	172.1529999
NZCAD531	Edpercivalia fusca	South Island	Eastcoast	-43.4620018	170.0269928
NZCAD532	Hydrochorema tenuicaudatum	South Island	Mid-Canterbury	-42.74000168	171.5110016
NZCAD533	Hydrochorema sp. C	South Island	Marlborough Sounds	-41.30799866	174.003006
NZCAD534	Psilochorema mimicum	North Island	Waikato	-37.42499924	175.0709991
NZCAD535	Pseudoeconesus bistirpis	North Island	Waikato	-37.42499924	175.0709991
NZCAD537	Oeconesus maori	North Island	Waikato	-37.42499924	175.0709991
NZCAD540	Polyplectropus altera	North Island	Waikato	-37.42499924	175.0709991
NZCAD541	Oeconesus maori	North Island	Waikato	-37.42499924	175.0709991
NZCAD542	Psilochorema mimicum	North Island	Waikato	-37.42499924	175.0709991
NZCAD543	Psilochorema mimicum	North Island	Waikato	-37.42499924	175.0709991
NZCAD544	Psilochorema mimicum	North Island	Waikato	-37.42499924	175.0709991
NZCAD547	Psilochorema mimicum	North Island	Waikato	-37.42499924	175.0709991
NZCAD548	Psilochorema mimicum	North Island	Waikato	-37.42499924	175.0709991
NZCAD550	Polyplectropus aurifuscus	North Island	Waikato	-37.42499924	175.0709991
NZCAD552	Hydropsyche catherinae	North Island	Waikato	-37.42499924	175.0709991
NZCAD574	Costachorema psaroptera	South Island	Westland	-41.20600128	172.2879944
NZCAD602	Pycnocentria gunni	North Island	Bay of Plenty	-38.30699921	176.7189941
NZCAD606	Hudsonema alienum	North Island	Waikato	-38.39400101	175.0189972
NZCAD607	Hudsonema alienum	South Island	Fiordland National park	-45.98899841	167.3820038
NZCAD610	Hydrochorema tenuicaudatum	South Island	Fiordland	-45.70500183	167.8840027
NZCAD611	Hydrochorema tenuicaudatum	South Island	Fiordland	-45.70500183	167.8840027
NZCAD613	Hydrochorema crassicaudatum	South Island	Fiordland	-45.70500183	167.8840027
NZCAD618	Confluens olingoides	South Island	Fiordland	-44.71699905	168.2160034
NZCAD619	Hydrobiosis johnsi	South Island	Fiordland	-44.71699905	168.2160034
NZCAD620	Costachorema hebdomon	South Island	Fiordland	-44.50299835	168.6329956
NZCAD621	Costachorema hebdomon	South Island	Fiordland	-44.50299835	168.6329956
NZCAD622	Hydrobiosis torrentis	South Island	Fiordland	-44.48300171	168.6269989
NZCAD623	Hydrobiosis torrentis	South Island	Fiordland	-44.48300171	168.6269989

NZCAD624	Hydrobiosis torrentis	South Island	Fiordland	-44.48300171	168.6269989
NZCAD627	Hydrobiosis charadraea	South Island	Fiordland	-44.52000046	168.5420074
NZCAD628	Costachorema psaropterum	South Island	Fiordland	-44.52000046	168.5420074
NZCAD633	Hydrobiosis silvicola	South Island	Fiordland	-44.52000046	168.5420074
NZCAD636	Costachorema psaropterum	South Island	Fiordland	-45.70500183	168.8800049
NZCAD649	Hudsonema bifid	North Island	Waikato	-38.67100143	174.878006
NZCAD650	Hudsonema alienum	North Island	Waikato	-37.77999878	175.0700073
NZCAD651	Hudsonema cf. alienum	North Island	Waikato	-37.79000092	175.0610046
NZCAD652	Hudsonema alienum				
NZCAD653	Hudsonema alienum				
NZCAD654	Hudsonema alienum				
NZCAD659	Hudsonema amabile	North Island	Waikato	-38.5340004	174.9929962
NZCAD660	Hudsonema amabile	North Island	Waikato	-37.77999878	175.0700073
NZCAD661	Hudsonema amabile	North Island	Waikato	-37.77999878	175.0700073
NZCAD662	Hudsonema amabile				
NZCAD663		North Island	Waikato		
NZCAD664		North Island	Waikato		
NZCAD665		North Island	Waikato		
NZCAD666		North Island	Waikato		
NZCAD667		North Island	Waikato		
NZCAD668		North Island	Waikato		
NZCAD669		North Island	Waikato		
NZCAD670		North Island	Waikato		
NZCAD671		North Island	Waikato		
NZCAD672		North Island	Waikato		
NZCAD673		North Island	Waikato		
NZCAD674		North Island	Waikato		
NZCAD675		North Island	Waikato		
NZCAD676	Costachorema xanthopterum	Waikato			
NZCAD677	Costachorema xanthopterum	Waikato			
NZCAD678	Hydropsyche colonica	Waikato			
NZCAD679	Hydropsyche colonica	Waikato			
NZCAD680	Hydrobiosis soror	Waikato			
NZCAD681	Hydrobiosis soror	Waikato			
NZCAD682	Hydrobiosis soror	Waikato			
NZCAD683	Hydrobiosis copis	Waikato			
NZCAD684	Psilochorema mimicum	Waikato			
NZCAD685	Hydrobiosis parumbripennis	Waikato			
NZCAD686	Hydrobiosis parumbripennis	Waikato			
NZCAD687	Hydrobiosis soror	Waikato			
NZCAD688	Hydrobiosis parumbripennis	Waikato			
NZCAD689	Hydrobiosis gollanis	Waikato			
NZCAD690	Oecetis unicolor	Waikato			