High levels of intraspecific genetic divergences revealed for Antarctic springtails: evidence for small-scale isolation during Pleistocene glaciation

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We examined levels of genetic variability within and among populations of three Antarctic springtail species (Arthropoda: Collembola) and tested the hypothesis that genetic divergences occur among glacially-isolated habitats. The study was conducted in southern Victoria Land, Ross Dependency, Antarctica, and samples were collected from locations in the vicinity of the Mackay Glacier. We analyzed mtDNA (cytochrome c oxidase subunit I; COI) sequence variability for 97 individuals representing three species (Gomphiocephalus hodgsoni, N = 67; Cryptopygus nivicolus, N = 20; and Antarcticinella monoculata, N = 8). Haplotype diversity and genetic divergences were calculated and used to indicate population variability and also to infer divergence times of isolated populations using molecular clock estimates. Two of the three species showed high levels of genetic divergence. Gomphiocephalus hodgsoni, a widespread and common species, showed 7.6% sequence divergence on opposite sides of the Mackay Glacier. The more range restricted Cryptopygus nivicolus showed 4.0% divergence among populations. The third species, Antarcticinella monoculata, was found in only one location. Molecular clock estimates based on sequence divergences suggest that populations separated within the last 4 Mya. We conclude that habitat fragmentation resulting from Pliocene (5 Mya) and Pleistocene (2 Mya to 10 Kya) glaciations has promoted and maintained high levels of diversity among isolated springtail populations on relatively small spatial scales. The region surrounding the Mackay Glacier is likely to have provided refugia for springtail populations during glacial maxima and remains an area of high genetic and species diversity for Collembola within the Ross Sea region.


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 & Wall, 2012). The majority of these ice-free areas lie within the Dry Valleys and Transantarctic Mountains of the Ross Dependency (Janetschek, 1967a; Levy, 2012). Even here, exposed ground is often highly fragmented and comprises 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., 2006). This latter requirement is relevant for the soil arthropod fauna, particularly the Antarctic springtails, which lack a desiccation-resistant life stage and, instead, use avoidance and super-cooling methods to enable survival in sub-zero temperatures (McGaughran, Hogg & Convey, 2011a).

The terrestrial arthropods are represented primarily by springtails (Collembola) and mites (Acarina) and are the largest year-round taxa on the continent.
These taxa, which lack the survival and dispersal strategies possessed by other invertebrate groups such as nematodes (Nkem et al., 2006; Adhikari, Wall & Adams, 2010), have been restricted to these fragmented, ice-free zones ever since the Middle Miocene (11–14 Mya) (Stevens & Hogg, 2003; Stevens et al., 2006; McGaughran et al., 2010). At this time, the glaciation of the whole continent reached its fullest extent and the polar ice cap overflowed the Transantarctic Mountains (Lewis et al., 2007). 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 & Sugden, 1990). Subsequently, 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 one or two arthropod taxa at most (Janetschek, 1967a). Species are also rarely shared between regions (Gressitt, 1967; Wise, 1971; Sinclair & Stevens, 2006), suggesting limited inter-habitat dispersal. Consequently, the current arthropod taxa are likely to be long-term inhabitants and remnants of a previously 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, Spinsanti & Dallai, 2001; Stevens & Hogg, 2003; McGaughran, Hogg & Stevens, 2008; Hawes, Torricelli & Stevens, 2010; Stevens & D’Haese, 2014). The present study aimed to extend these studies by focusing on the 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 the present study, consist of three species covering a 3° latitudinal range. Within this region, Gomphiocephalus hodgsoni is the only relatively widespread species and is common throughout southern Victoria Land (McGaughran et al., 2011b). Two additional species, Cryptopygus nivicolaus [recently redescribed from Neocryptopygus nivicous by Greenslade (2015)] and Antarctulinella monoculata, are extremely range-restricted and known only from one or two locations near the Mackay Glacier to the north of the Dry Valleys (Wise, 1971) (Fig. 1), suggesting the possibility of a glacial refugium. Recent studies of lichens and mosses also near the Mackay Glacier (Green et al., 2011), as well as haplotype diversity for springtail (G. hodgsoni) and mite (Stereotydeus mollis) taxa, have further suggested this area as a likely refugial zone (Stevens & Hogg, 2003, 2006; McGaughran et al., 2008; Demetras et al., 2010).

To determine the geographical 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 & Sugden, 1990). Accordingly, we tested the hypothesis that this region would support genetically divergent springtail populations among 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 Pleistocene glaciations.

**MATERIAL AND 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 (Stevens & Hogg, 2002). Soil samples were also taken from each site and returned to the laboratory where they were suspended a 10% sucrose solution. Invertebrates were then removed from the solution surface under a dissecting microscope (× 10 magnification) using a fine wire loop. 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, Leech & Wise (1963) and Salmon (1965). Specimens not used for DNA analyses were archived at the School of Science, University of Waikato, under the care of IDH.

**GENETIC ANALYSIS**

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. At the University of Waikato, total genomic DNA was extracted from the tissue of entire specimens using a Glassfiber Plate DNA Extraction (Acro-Prep) method (Ivanova, deWaard & Hebert, 2006) at CCDB, and Red Extract n Amp (Sigma-Aldrich) Prep) method (Ivanova, deWaard & Hebert, 2006) at the University of Waikato. DNA was extracted from the tissue of entire specimens using 10 μL of MQH2o, and Red Extract n Amp (Sigma-Aldrich) using 10 μL of extraction solution and 2.5 μL of tissue preparation, in accordance with the manufacturer’s instructions. Polymerase chain reactions (PCRs) comprised a 15-turer’s instructions. Polymerase chain reactions in accordance with the manufacturer’s instructions (Global Science & Tech Ltd) at Waikato. DNA was sequenced using 10 μL of MQH2o in accordance with the manufacturer’s instructions (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 ABI3730 × 1 at CCDB. Sequences from the University of Waikato were aligned using GENEIOUS, version 5.4.2, and confirmed as the target species using the Barcode of Life Data Systems (BOLD; www.boldsystems.org), version 3, COI animal identification searches. Previous analyses of Antarctic springtails (Stevens & Hogg, 2003) have shown that alzyme analyses are congruent with COI data and that the latter can be used as a reliable indicator of genomic differences occurring among populations. Primer sequences were trimmed from sequence fragments for further analyses. All sequences were uploaded to the BOLD dataset DS-SPMACK (Springtails of Mackay Glacier Region; dx.doi.org/10.5883/DS-SPMACK) and cross-referenced to GenBank (accession numbers KU876787 - KU876880).

**PHYLOGENETIC ANALYSIS**

COI sequence fragments of 658 bp (219 codons) were obtained for 67 G. hodgsoni specimens and 20 C. 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 C. nivicolus and A. monoculata contained ambiguous base-pair assignments that could not be easily resolved, and so the sequences were further trimmed at both ends, resulting in sequence fragments of 547 bp (181 codons) for C. nivicolus and 527 bp (175 codons) for A. monoculata. Two additional C. nivicolus sequences were also obtained from GenBank (Accession numbers DQ285403 and DQ285404).

Sequences for all species were initially examined in the context of generating a single Neighbour-joining tree using a Kimura two-parameter distance
All duplicate sequences were identified and removed to include only unique haplotypes in subsequent analyses. As a result of the lack of publically available sequence data for taxa that share a recent common ancestor with our ingroup taxa (and that did not approach saturation), analyses were run unrooted among the ingroup taxa. No significant changes were noted in topography between these analyses and those run previously using Podura aquatica as a test. Chi-squared tests 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. JMODELTEST, version 2.1.2 (Posada, 2008) was used to determine the most appropriate substitution model for maximum likelihood (ML) analysis. The settings were: 11 substitution schemes (88 models), base frequencies +F, rate variation +L, +Γ, set to BioNJ. The model selected for the dataset was GTR+I+Γ (-lnL = 1590.9). Maximum likelihood heuristic searches were conducted using this model in MEGA, version 5.10 (Tamura et al., 2011) using 1000 bootstrap replicates. Maximum parsimony (MP) analyses were performed in PAUP* using 1000 full-heuristic search bootstrap replicates.

MrBayes, version 3.2.6 (Huelserenbeck & Ronquist, 2001) was used to conduct a Bayesian inference analysis. A general time reversal model (GTR+I+Γ) was used, with a log normal relaxed clock model and speciation Yule process as the tree prior. The Markov chain Monte Carlo (MCMC) procedure was set to 1 100 000 generations, sampling trees every 200. A burn-in of 100 000 trees was determined by plotting log-likelihood values against generation time in TRACER (Rambaut & Drummond, 2007) and checking for the point at which normalization occurred. The majority rule tree was acquired from the 11 004 trees sampled after the burn-in period. The tree was then visualized in TREENANNOTATOR (Drummond et al., 2012).

Sequences for G. hodgsoni and C. niviculus were split into separate datasets for analysis in TCS, version 1.21 (Clement, Posada & Crandall, 2000) and to construct networks of sequence haplotypes. Single representatives of each haplotype were used in the final analysis to simplify files, and sequences of C. niviculus were trimmed at 547 bp to avoid anomalies, as described above. The A. monoculata sequences were not included in these analyses because they were only collected from a single site and consisted of only two similar haplotypes (< 0.2% divergence).

Uncorrected pairwise genetic distances between COI sequences for populations at different locations were also calculated for the G. hodgsoni and C. niviculus datasets in MEGA, version 5.10. The likelihood ratio test did not detect evidence of significant rate heterogeneity for G. hodgsoni ($\chi^2 = 113.06$, d.f. = 14, $P < 0.001$) or C. niviculus ($\chi^2 = 141.15$, d.f. = 10, $P < 0.001$). Approximate geological timing of isolation for the populations was estimated via molecular clock analyses in BEAST 1.8.2 (Drummond et al., 2012). Files generated in BEAUti used a general time reversal model (GTR+I+Γ) with speciation Yule Processes as the tree prior and the same MCMC setup as that used for the Bayesian inference tree analysis. A strict clock model with a fixed rate of 0.0115 was used to simulate 2.3% sequence divergence per Myr, as determined using insect mitochondrial data (Brower, 1994; Juan, Oromi & Hewitt, 1996; Quek et al., 2004; McGaughran et al., 2010). Despite being calibrated for insects, the 2.3% sequence divergence per Myr was considered a suitable estimate for Collembola because both taxa have similar life cycles (McGaughran et al., 2010).

**RESULTS**

Of the 658 bp analyzed 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% ($A = 27.7\%$, $T = 36.7\%$, $C = 19.3\%$, $G = 16.7\%$). Nucleotide frequencies were not significantly different among sequences for all codon positions ($\chi^2 = 2.19$, d.f. = 48, $P = 1.0$) or for third codon positions only ($\chi^2 = 7.18$, d.f. = 48, $P = 1.0$). Of the 549 bp analyzed for C. niviculus, 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 for C. niviculus were not significantly different among sequences for all codon positions ($\chi^2 = 1.41$, d.f. = 36, $P = 1.0$) or for third codon positions only ($\chi^2 = 5.77$, d.f. = 36, $P = 1.0$). Of the 527 bp (175 codons) analyzed for A. monoculata, 408 characters were constant, one 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 not significantly different among sequences for all codon positions ($\chi^2 = 3.39$, d.f. = 21, $P = 1.0$) or for third codon positions only ($\chi^2 = 11.55$, d.f. = 21, $P = 0.95$).

**PHYLOGENETIC ANALYSIS**

The ML tree is shown in Fig. 2. Tree constructions for MP (Fig. 3) and Neighbour-joining (data not
shown) showed similar topology and node support. Linking nodes between the haplotype G16 and the rest of the *G. hodgsoni* haplotypes had 100% bootstrap support in the ML and MP trees. The linking node between the *C. nivicolus* haplotypes at Springtail Point and at Mt Gran also received 100% bootstrap support in the ML and MP trees. Bootstrap values for the Mt England *C. nivicolus* haplotypes indicated high support from both the ML and MP trees. The topology of the *G. hodgsoni* haplotypes showed greater structure in the ML and BI trees. Two clusters were apparent in the ML tree, with node support of 99%. One cluster with the same haplotypes was apparent in the BI tree, with 0.55 posterior probability. Posterior probability values between *C. nivicolus* haplotypes at Springtail Point and at Mt Gran was 1.00, and also 1.00 between the Mt England and Mt Gran group (Fig. 4). The topology and node support of these trees supports the presence of high genetic structuring across the Mackay Glacier.

**HAPLOTYPE NETWORKS**

The geographical distribution of sequence haplotypes for *G. hodgsoni* and *C. nivicolus* was investigated using haplotype-joining networks. Subsequent haplotype assignments and their collection locations are shown in Table 1. Sixteen haplotypes were found from 67 *G. hodgsoni* sequences. Maximum connection steps were fixed at 40 to connect haplotype G16 to the rest of the haplotypes (Fig. 5). This network revealed 10 one-step haplotypes, three two-step haplotypes, two three-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 (Figs 2, 3, 4). The remainder of the network, which included haplotypes from the St John’s Range and Mt Seuss, did not show high geographical structure, similar to that observed in the tree-based approaches.

Twelve haplotypes were found from 22 *C. nivicolus* sequences. Maximum connection steps were fixed at

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**Figure 2.** Maximum Likelihood phylogram constructed in MEGA, version 5.10, based on the GTR+I+Γ model derived from JMODELTEST, using 97 individual cytochrome c oxidase subunit I (COI) sequences reduced to unique haplotypes. Haplotype codes correspond to Table 1 and bootstrap values > 50 are shown. Tree is drawn to scale and branch lengths are the number of substitutions per site. Vertical bars indicate collection locations.
30 to connect the Mt Gran and Mt England haplotypes to the Springtail point haplotypes (Fig. 6). This network revealed nine one-step haplotypes, two three-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 two-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% to 8% for *G. hodgsoni* and from 0.00% to 4.2% for *C. nivicolus* (Fig. 7). The greatest differences were found between haplotype G16 at Mt Gran and the remainder of the *G. hodgsoni* haplotypes, and the genetic distance between *C. nivicolus* haplotypes at Mt Gran and Mt England, as well as those at Springtail Point. The St John’s Range and Mt Seuss *G. hodgsoni* haplotypes showed a mean divergence of 0.6% within the group (Fig. 7). The single haplotype, G16, at Mt Gran showed a mean of 7.6% sequence divergence from the other haplotypes. The mean sequence divergences among *C. 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 a mean of 4.0% divergent from haplotypes at Mt England. Similarly, Springtail Point haplotypes were a mean 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 strict molecular clock rate of 2.3% sequence divergence per Myr, these populations are all likely to have diverged within the last 4 Myr (Figs 7, 8). 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 at 3.8 Mya. Divergence dates between the three *C. nivicolus* populations suggested that the Springtail Point haplotypes diverged from the Mt Gran – Mt Seuss population 1.44 Mya. The difference between haplotypes from Mt Gran and Mt Seuss relative to those at Mt England is much more recent by comparison, estimated at 0.38 Mya.

**DISCUSSION**

Our mtDNA (COI) analysis of 97 Antarctic springtails from three taxonomic species revealed highly divergent populations across 65 km within the Mackay Glacier. Populations of *G. hodgsoni* and *C. nivicolus* on the lower slopes of Mt Gran were shown to be a mean of 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 throughout the McMurdo Dry Valleys (Stevens & Hogg, 2003; Nolan *et al.*, 2006; McGaughran *et al.*, 2008). High genetic structure, within both putative species, suggests that populations may have survived *in situ* ever since the Antarctic continent became fully glaciated. Given the elevations of surrounding mountains, it is possible that several locations, such as Mt Gran (2235 m) and Mt Seuss (1190 m), protruded above the advancing Mackay Glacier, and remained so since the early Pliocene (Janetschek, 1967a; Clapperton & Sugden, 1990). In particular, this area is known to contain the highest species diversity of springtails in southern Victoria Land, with *G. hodgsoni*, *C. nivicolus*, and *A. monoculata* all known from this area (Gressitt *et al.*, 1963). The species diversity of mites, lichens, and mosses has 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 multiple 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, which may indicate early stages of speciation. 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 Mid-Pliocene (4 Mya). Similarly, the population of *C. nivicolus* from the same location has been isolated from a neighbouring population at Springtail Point by as much as 1.4 Mya. The occurrence of *A. monoculata* at Springtail Point, coupled

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**Table 1.** Haplotypes, collection locations, coordinates, and sequences (BOLD Sample Id) associated with each haplotype for three species of Antarctic springtail

<table>
<thead>
<tr>
<th>Haplotype number</th>
<th>Location</th>
<th>Co-ordinates (south – east)</th>
<th>Sample Id's</th>
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<tr>
<td><strong>Gomphiocephalus hodgsoni</strong></td>
<td></td>
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<tr>
<td>G1</td>
<td>St John’s Range</td>
<td>–77.280 161.731</td>
<td>ANTSP129 ANTSP131 ANTSP134 ANTSP136 ANTSP137 ANTSP138 ANTSP140 ANTSP141</td>
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<td></td>
<td></td>
<td></td>
<td>ANTSP143 ANTSP151 ANTSP193 ANTSP211 ANTSP212</td>
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<tr>
<td>G2</td>
<td></td>
<td>–77.208 161.700</td>
<td>ANTSP213 ANTSP215</td>
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<td></td>
<td>–77.285 161.726</td>
<td>ANTSP150</td>
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<td></td>
<td>–77.208 161.700</td>
<td>ANTSP210</td>
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<td>N1</td>
<td>Springtail Point</td>
<td>–77.167 160.710</td>
<td>N2</td>
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<td>N3</td>
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Two Mt England *C. nivicolus* sequences (N11, N12) were retrieved from GenBank.

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* (3.8 Mya) and *C. nivicolus* (1.4 Mya) may be the result of different evolutionary histories (e.g. later isolation) or possibly differences in mutation rates. For example, Stevens & 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 *C. nivicolus*. The lack of ecological knowledge for *C. 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). *Gomphiocephalus hodgsoni* is known to survive floating on both sea and fresh water, and dispersal events through wind or accidental carriage by birds are also possible (Stevens & Hogg, 2002; Hawes, 2011; McGaughran et al., 2011a,b).

Many of the alpine glaciers underwent significant retreat during the interglacial periods of the Pleistocene (Clapperton & Sugden, 1990; Sugden et al., 1999), although Mackay Glacier is unlikely to have done so because it is an outlet glacier for the EAIS. This appears to have isolated the Mt Gran population of *G. hodgsoni* from the populations on Mt Seuss in the centre of the glacier, as well as 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 *C. nivicolus* haplotypes between Mt Gran and Mt Seuss also indicates the potentially recent...
dispersal from Mt Gran across the glacier. Hawes (2011) suggested that potential dispersal mechanisms may work in concert, where individuals could be wind-blown onto glaciers and then moved by glacial surface streams. The stochastic 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

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**Figure 7.** Genetic distances based on mitochondrial cytochrome c oxidase subunit I (COI) sequences of 97 springtails covering 30 unique haplotypes. 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.

**Figure 8.** Estimated divergence times for populations of *Gomphiocephalus hodgsoni* and *Cryptopygus nivicolus*. The timeline on the x-axis is in millions of years. Values at nodes are estimated divergence times in millions of years. Haplotype codes correspond to Table 1 and vertical bars indicate locations of haplotypes.
population is known from Mt Murray 150 km to the north (Gressitt et al., 1963). Similarly, haplotypes of C. 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 seaward 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 indicates they have changed little subsequent to 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 ultraviolet light, and a 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 provided 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 early Pliocene (4 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 4 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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AUTHOR CONTRIBUTIONS

IDH, KRB, BJA and PDNH conceived the research and obtained funding. KRB and IDH conducted the field work and KRB conducted the primary analyses and was lead author of the manuscript in conjunction with IDH BJA and PDNH. All authors reviewed and contributed revisions to the final version of the manuscript.

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