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Genetic diversity among populations of Antarctic springtails (Collembola) within the Mackay Glacier ecotone

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Abstract

Past climate changes are likely to have had major influences on the distribution and abundance of Antarctic terrestrial biota. To assess arthropod distribution and diversity within the Ross Sea region, we examined mitochondrial DNA (COI) sequences for three currently recognised species of springtail (Collembola) collected from sites in the vicinity, and to the north of, the Mackay Glacier (77^{o}S). This area is also a transition between two biogeographic regions (northern and southern Victoria Land). We found populations of highly divergent individuals (5-11.3% intraspecific sequence divergence) for each of the three putative springtail species, suggesting the possibility of cryptic diversity. Based on molecular clock estimates, these divergent lineages are likely to have been isolated for 3-5 million years. It was during this time that the Western Antarctic Ice Sheet (WAIS) was likely to have completely collapsed, potentially facilitating springtail dispersal via rafting on running waters and open seaways. The reformation of the WAIS would have isolated newly established populations, with subsequent dispersal restricted by glaciers and ice-covered areas. Given the currently limited distributions for these genetically divergent populations, any future changes in species' distributions can be easily tracked through the DNA barcoding of springtails from within the Mackay Glacier ecotone.

Keywords: *Antarcticinella monoculata*, biomonitoring, climate change, *Cryptopygus nivicolus*, *Gomphiocephalus hodgsoni*

Introduction

Antarctica's terrestrial biota is exposed to cold temperatures, low moisture levels, and steep chemical gradients (Convey et al. 2014; Hogg et al. 2014; Velasco-Castrillon et al. 2014). These conditions, coupled with repeated glacial cycles over the past 80 million years, have shaped a terrestrial landscape characterised by low biodiversity (Adams et al. 2006; Convey 2011). Furthermore, this limited diversity is spread across the highly fragmented 0.32% of Antarctica that is currently considered ice-free (Pugh and Convey 2008). Accelerating rates of climate change, anthropogenic activity, and a heightened risk of invasive species all have the potential to severely disrupt present-day Antarctic biodiversity and thereby ecosystem functioning (Wall 2005; Hogg & Wall 2011; Chown et al. 2012). To understand and monitor changing biodiversity patterns, Terrestrial Observation Networks have been proposed to track environmental changes and consequences for the biota (e.g. Levy et al. 2013). Such networks will require a range of suitable locations to adequately characterise any biotic changes.

Ecotones, transitions from one biological community to another, may be ideal sites to study shifts in species distributions as a consequence of global climate changes (Gosz 1993; Risser 1993). The Mackay Glacier in southern Victoria Land is situated immediately to the north of the McMurdo Dry Valleys. Here, the landscape changes from one dominated by the expansive Dry Valleys to one of small rocky outcrops isolated by glaciers. These changes are also reflected in the communities of invertebrates, particularly springtails and mites. For example, only one species of springtail (*Gomphiocephalus hodgsoni*) is known from the McMurdo Dry Valleys, whereas additional two species (*Antarcticinella monoculata* and *Cryptopygus nivicolis*) are found north of the Dry Valleys (Wise 1967; Hogg et al. 2014). Accordingly, the Mackay Glacier vicinity can be considered an ecotone or transitional zone (*sensu* Risser 1993).

Within the Ross Sea region, three biogeographic zones have been designated (northern Victoria Land, southern Victoria Land, and the Queen Maud Mountains), each with three, and in one case four, reported species of springtail for a total of 10 species within the region (Salmon 1965; Wise 1967; Adams et al. 2006; Sinclair and Stevens 2006). However, the basis of this zonation was derived from early work utilizing classical morphological taxonomy and sampling which had been undertaken in proximity to historical bases or camps. Vast areas were still largely unexplored (e.g. see Peat et al. 2007; McGaughran et al. 2011). Springtails are prominent features of Antarctic terrestrial ecosystems as they are the largest terrestrial invertebrates and are also highly sensitive to environmental disturbances, making them ideal biological indicators of climate change (Hopkin 1997; Hogg et al. 2014; Collins & Hogg 2015). Of the three currently recognised species of springtail within the wider Mackay Glacier region (*Gomphiocephalus hodgsoni* (Carpenter 1921), *Antarcticinella monoculata* (Salmon 1965) and *Cryptopygus nivicolus* (Salmon 1965)), *G. hodgsoni* is relatively widespread and well-studied, while *C. nivicolus* and *A. monoculata* have received comparatively less attention (Adams et al. 2006; Stevens et al. 2006; Hogg et al. 2014). For example, *A. monoculata* was previously known only from two extremely restricted locations (Salmon 1965; Bennett 2013;).

The aim of this study was to assess springtail distribution and genetic (mitochondrial cytochrome *C* oxidase subunit I, COI) diversity within the vicinity of Mackay Glacier. These data will establish the current distributional ranges of species and baseline levels of genetic diversity, against which any future changes can be determined. Such knowledge can then be used to inform, and contribute to, developing Terrestrial Observation Networks (Levy et al. 2013).

Materials and methods

Individuals of three species of springtail were collected in January 2015 from 12 sites in the vicinity of the Mackay Glacier covering a north-south range of approximately 120 km[1] (Fig. 1). Animals were collected directly from rocks using modified aspirators (Stevens and Hogg 2002) or from soil samples. At each of the 12 sites, 3-7 soil samples were collected within a 1 km^2 area (via random trowelling within a metre squared area at each sub-sampling location) . Soil samples weighed between 300-500g and were returned to the laboratory at McMurdo Station with individuals removed via floatation of samples in a sucrose solution (Jenkins 1964). All individuals were preserved in 95% ethanol for subsequent genetic analyses.

Whole individuals were placed into 95-well plates and sent to the Canadian Centre for DNA barcoding (CCDB), University of Guelph for sequencing of the barcode region of the COI gene. Genomic DNA was extracted via the AcroPrep™ PALL Glass Fibre plate method (Ivanova et al. 2006). A 658bp fragment of the mitochondrial COI gene was then amplified in accordance with standard CCDB protocols (see Ivanova et al. 2006; Ivanova and Grainger 2007a, 2007b) using the primer cocktails C_LepFolF (5'ATTCAACCAATCATAAAGATATTGG-3') and C_LepFolR (5'-TAAACTTCTGGATGTCCAAAAAATCA-3') (Folmer et al. 1994; Hebert et al. 2004; Ivanova et al. 2006). Successfully amplified products were then cleaned using Sephadex® before being sequenced in both directions on an ABI 3730xl DNA analyser. Of the 139 springtails extracted, 95 produced complete 658bp sequences. All photographs, collection information, and sequence data have been added to Barcode of Life Data Ssystems (www. boldsystems.org; Ratnasingham and Hebert 2007) and are available from the dataset DS-ANTSP (dx.doi.org/10.5883/DS-ANTSP) and cross referenced to GenBank.

[1] Supplementary collection information is available in Table S1.

Sequences were confirmed as the target species via comparison with BOLD sequences and translated to amino acids to check for the presence of stop codons. Sequences were aligned using MUSCLE in Geneious 7.1.9 and all trimmed to 527 base pairs (bp) to incorporate 69 sequences from a previous study by Bennett (2013) and three Symphypleona outgroups (*Sminthurides aquaticus* IHCO046-03, *S. malmgreni* IHCO047-03, and *Sminthurinus elegans* COONT067-08). This alignment was then used in all subsequent analyses except for the haplotype network construction, which did not include the outgroup taxa. Chi square (χ^2) tests conducted in PAUP* 4.0 (Swofford, 2002) were used to determine whether base frequencies were equal among all sites, identify parsimony-informative sites, and designate first, second, or third codon positions. The most appropriate model of evolution was determined using jModelTest 2.1.1 under the _lowest AIC_ criterion (Posada 2008). Bayesian trees were generated using BEAST software v2 (Bouckaert et al. 2014). A strict clock model and speciation yule process as the tree prior were employed in BEAUTI v2, with the Markov chain Monte Carlo (MCMC) set at 50,000,000 generations, sampling trees every 5,000 generations. The Bayesian analysis was run in BEAST, with the quality of the results evaluated in TRACER v1.5 (Drummond et al. 2010). The burn-in inputted into Tree Annotator v2 was calculated by plotting log-likelihood values against the generation time in TRACER (Rambaut & Drummond, 2007) and determining the point at which normalization occurred. The final tree was visualised in FigTree v1.4.0. Neighbour Joining (NJ) and Maximum Likelihood (ML) analyses were conducted in MEGA v5.05 (Tamura et al. 2011). ML and NJ settings both included 1000 bootstrap replicates, with GTR+I+G used as the model of evolution for ML and Tamura-Nei in NJ. Tamura-Nei was used as it allows unequal base frequencies and multiple substitution types (Simon et al. 2006). All other settings were set to the default options in MEGA. MEGA was also

utilized to create a pairwise distance matrix to calculate intraspecific divergences (uncorrected pair-wise distances), while Barcode Index Numbers (BINs) were assigned by BOLD (Ratnasingham and Hebert 2013) and used as a measure of Molecular Operational Taxonomic Units (MOTUs). An analysis of molecular variance (AMOVA) was performed using Arlequin v3.5 to further evaluate genetic variation. Uncorrected pairwise distances were calculated for haplotypes and analysed with 1600 permutations. Genetic structure was enforced with haplotypes grouped hierarchically into reported species and then BINs. Haplotype networks were constructed in TCS v.1.21 using all 164 sequences. PAUP* was used to determine whether the data were evolving in a clocklike manner. A likelihood ratio test (-2logL = 2(logL0 - logL1), n–2 d.f. where n is the number of terminal taxa) was conducted using the likelihood scores of trees with and without a molecular clock enforced (L0, L1) . Subsequently, a molecular clock was calibrated by dividing uncorrected p-distances by mutation rates of 1.5 to 2.3% sequence divergence per million years (*sensu* Knowlton et al. 1993; Brower et al. 1994; Stevens et al. 2006) and then used to estimate the timing of divergence events. A Bayesian tree with a strict clock and rate set as 0.0115 to simulate 2.3% sequence divergence per MY, with all other settings the same as in the previous Bayesian analysis, is provided[2]. We caution that if dispersal in springtails is male-biased, the maternally inherited COI marker would underestimate gene flow between populations. However, previous analyses of Antarctic springtails (e.g. Stevens & Hogg 2003) have suggested that COI data are congruent with allozyme analyses and that COI is a reliable indicator of genetic differences within and among populations.

[2] Bayesian Inference tree showing estimated divergence time is provided in Supplementary Material 2

Results

Of the 527 nucleotide positions used, 347 positions were constant, 14 were variable but uninformative, and 166 were parsimony informative. No insertions, deletions, or stop codons were detected. There was an overall A-T bias of 62.2% (A = 25.6%, C = 20.9%, G = 16.9%, T = 36.6%). Base frequencies were homogeneous across sequences for all sites considered together (χ^2 = 42.4, df =120, p = 1.0). However, this homogeneity was rejected at variable sites (χ^2 = 173.6, df =120, p = <0.001), informative sites (χ^2 = 183.4, df =120, p = <0.001), and the third codon sites (χ^2 = 148.3, df =120, p = 0.04), which all indicated the presence of an A-T bias. The most appropriate model of evolution was determined as GTR+I+G (-lnL 2,889.306), with a burn in of 20% for the Bayesian Inference analysis inputted into Tree Annotator.

Phylogenetic analyses revealed three previously unreported genetically distinct MOTUs (>2% sequence divergence), one for each of the three recognised species within the region. NJ, ML, and Bayesian analyses all produced concordant results, with all nodes at and above the BIN level having high support (Fig. 2). Maximum intraspecific (within morphologically recognised species) divergences ranged from 5-11.2%, resulting in a total of seven Barcode Index Numbers (BINs) for the wider Mackay Glacier region. Based on the AMOVA, 64.96% of the genetic variation was found among the three recognised species, 32.81% was found among the distinct intraspecific populations (BINs), and the remaining 2.24% was within the divergent populations (BINs). Two of the BINs, one each for *G. hodgsoni* and *C. nivicolus*, were found only at Towle Glacier, indicating a potentially important site for arthropod diversity within the region. *Antarcticinella monoculata* was found at two new locations (Benson Glacier, Pegtop Mountain) and *C. nivicolus* at an additional two locations (Tiger Island, Towle Glacier). The widespread *G.*

hodgsoni, previously reported as far north as Mt Murray (Wise 1967), was not detected or collected at this location.

We examined haplotype networks in order to visualise the fine-scale geographic relationships between these 'new' populations and other Mackay Glacier locations. From the 121 *G. hodgsoni* sequences, we found 27 unique haplotypes. Overall, these haplotypes exhibited high levels of genetic connectivity among populations throughout the study area, albeit with some subtle geographic structuring (Fig. 3). Four of the 27 haplotypes occurred in more than one location, with the most common haplotype (Gh1) found at four sites up to an estimated 55 km apart. However, two populations of *G. hodgsoni* were found to be highly divergent with the newly found population at Towle Glacier (Gh23, 24), 28 mutational steps (7.3% sequence divergence) from Mackay Glacier populations (>45 km). In contrast, the other divergent population (Gh25), previously identified at Mount Gran (within the Mackay Glacier vicinity) by Bennett (2013), was 32 mutational steps and 7.3% divergent from other nearby populations.

Cryptopygus nivicolus haplotypes exhibited broadly similar patterns to that of *G. hodgsoni*, although on a smaller scale owing to their more restricted distribution (Fig. 3). A total of seven haplotypes and three BINs were identified from the 21 *C. nivicolus* sequences obtained. The main haplotype (Cn1) was found in three locations separated by distances of up to 30 km, with the distinct Towle Glacier population (Cn5) being 24 mutational steps (5% sequence divergence) away from Mackay haplotypes.

Antarcticinella monoculata were found in very low numbers at all sites where they were found, with the exceptions of Mt Murray and Cliff Nunatak (Fig. 3). Of the 23 individuals we sequenced from these two nearby locations (approximately 6 km apart), all had the identical haplotype (Am1). This haplotype was also 50 mutational steps away, and had sequence

divergences of 11.2%, from the *A. monoculata* collected from the more southern Mackay Glacier locations. Both *G. hodgsoni* and *C. nivicolus* had high levels of genetic variability within the immediate Mackay Glacier area (77°S), including highly divergent haplotypes for each species (Mt Gran haplotype Gh25 at 37 steps, Springtail Point haplotypes Cn6 and Cn7 at 17 steps). In contrast, *A. monoculata* populations found throughout the Mackay Glacier area (excluding northern haplotype) were all similar, separated by a maximum of six mutational steps.

The application of standard molecular clock calibrations of 1.5 to 2.3% sequence divergence per million years (*sensu* Knowlton et al. 1993; Brower et al. 1994; Stevens et al. 2006) suggested that individuals from three of the populations from Towle Glacier and Mount Murray (Gh23 & 24, Cn5, Am1) diverged within the last 5 my. The *C. nivicolus* haplotypes diverged most recently (2.2 - 3.3 mya), followed by *G. hodgsoni* at 3.2 – 4.9 mya, with the most divergent group of *A. monoculata* estimated to have been isolated between 4.9 and 7.5 mya.

Discussion

The mtDNA COI sequences revealed a considerably greater diversity of collembolans than previously known in the Ross Sea region of Antarctica. In particular, we found several highly divergent populations of springtails including three previously unrecognised BINs for the three putative species, for a total of seven BINs within the wider Mackay Glacier area. The high levels of divergence suggest the presence of potentially cryptic species and that diversity in Antarctica, while comparatively depauperate in the global sense, is much more diverse than once thought (Griffiths et al. 2010; Chown et al. 2015). The use of molecular techniques such as DNA barcoding to study diversity has been particularly helpful in detecting genetically divergent populations (e.g. Stevens and Hogg 2003, 2006; Hawes et al. 2010; Stevens & D'Haese 2014). A study by McGaughran et al. (2010) found distinct MOTUs within the springtail *Cryptopygus antarcticus*, while Mortimer et al. (2011) identified two divergent groups within Western Antarctic Peninsula populations of the mite *Alaskozetes antarcticus.* Similarly, Torricelli et al. (2010a, 2010b) found high levels of COI divergence (14.4-17.2%) between Victoria Land and Antarctic Peninsula populations of the 'pan-Antarctic' springtail *Frisea grisea*. Such high levels of variability among springtails and other Antarctic biota could be the result of a number of factors including genetic drift, environmental heterogeneity, and potential diversifying selection in these extreme and variable environmental conditions (Fanciulli et al. 2001).

Habitat fragmentation restricts gene flow, leading to allopatric speciation, and is a likely explanation for the different BINs found only at the Towle Glacier site. The genetic uniformity of all of the northern *A. monoculata* individuals could indicate that the population has also been derived allopatrically, with genetic drift leading to a reduction in diversity and fixation of the single haplotype (e.g. Costa et al. 2013). Alternatively, the population could be comparatively

new, resulting from a founder effect from a more genetically diverse population nearby. Although there was a general trend of increasing divergence with distance, similar to observations of Fanciulli et al. (2001), there were a number of *G. hodgsoni* haplotypes that were shared among locations up to 55 km apart, demonstrating that populations currently exist in both sympatry and allopatry.

The genetically distinct populations (BINs) were all estimated to have diverged within the last 5 my (range: 2.2-7.5 my). While there is controversy surrounding rates of molecular evolution, a number of studies have similarly reported clades of Antarctic invertebrates diverging within the Pliocene (Hawes et al. 2010; McGaughran et al. 2010; Bennett 2013). It was during this time that the Western Antarctic Ice Sheet (WAIS) was thought to have completely collapsed (Pollard and Deconto 2009). This would have resulted in sea levels rising and increased dispersal opportunities for Collembola via rafting in meltwater streams and open seaways (*sensu* Hawes 2011), before the WAIS eventually reformed and glaciers isolated newly established populations (e.g. McGaughran et al. 2010). This could explain why some *G. hodgsoni* haplotypes are shared at sites up to 55 km apart despite being currently isolated by glaciers. Although springtails have been collected in air traps over small-scale distances (< 8 km see Hawes et al. 2007; McGaughran et al. 2011), long-distance wind dispersal is considered unlikely. Further, springtails are prone to desiccation due to their permeable cuticle and have no anhydrobiotic capacity such as that of nematodes, tardigrades, and rotifers which would facilitate Aeolian transport (Freckman and Virginia 1998; Hogg et al. 2014; Velasco-Castrillon et al. 2014a).

The region immediately to the north of the Mackay Glacier (<77^{o}S) has received limited sampling attention, with this study providing the first records of species for many of the sites visited. The sampling of *A. monoculata* at Mount Murray and Cliff Nunatak represented the first

time they have been collected there since the original collections in 1958 (Salmon 1965; Wise 1967). Interestingly, *G. hodgsoni* was not collected from the previously recorded northern location (Mt Murray). This absence could suggest that species may have altered their distributions within the past 50 years (e.g. Stevens and Hogg 2002). It is possible that some springtails have been essentially living at their environmental limit and that recent climatic changes may have led to detectable range shifts. However, their absence from Mount Murray could also be attributed to stochastic events at local sites (e.g. natural disturbance). The distribution of springtails is largely governed by the paucity of ice-free habitat, with abiotic factors such as salinity and the availability of liquid water further limiting available habitat (Hogg et al. 2006; Sinclair & Stevens 2006; Velasco-Castrillon et al. 2014b). Historical glacial cycles appear to have influenced the current distribution of springtail populations, with habitat fragmentation and population dynamics refining these distributions. Improving our understanding of genetic variability is fundamental to preserving maximal springtail diversity and thereby the evolutionary potential of natural systems and populations to respond to changing environmental conditions (Adams et al. 2006; Hogg et al. 2006, 2014). Given the presence of several geographically restricted BINs within the Mackay Glacier region, any future changes in species' distributions can be easily tracked through monitoring of DNA barcodes within a site. This finer level of resolution will thereby enhance our capacity to detect potentially subtle biological responses resulting from gradual climate changes.

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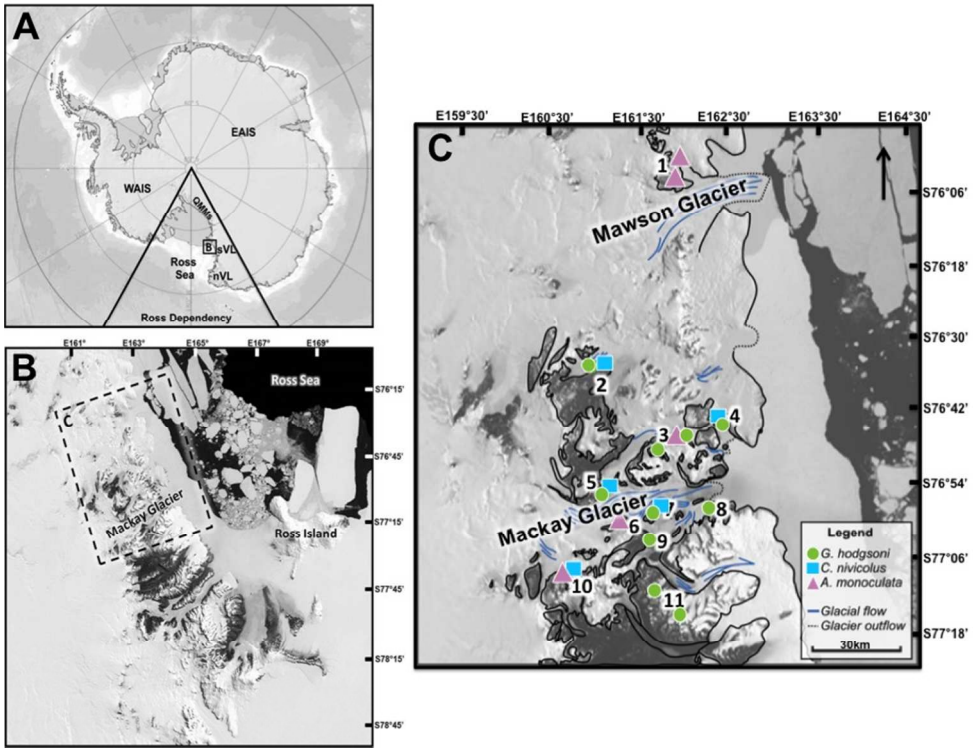
Figure 1A = general map of Antarctica showing the study area (black box) within the Ross Sea region of the Ross Dependency. Also pictured are the Western and Eastern Antarctic Ice Sheets (WAIS, EAIS) in addition to northern and southern Victoria Land (nVL, sVL) and the Queen Maud Mountains. Map adapted from the Antarctic Digital Database v6.0, British Antarctic Survey (http://www.add.scar.org/home/add6). 1B = Map showing the location of the study area around the Mackay Glacier in relation to Ross Island; Map data: Google, DigitalGlobe. 1C = Map of exact collection sites and distribution of the three springtail species. 1= Mount Murray/ Cliff Nunatak, 2= Towle Glacier, 3=Benson Glacier, 4= Tiger Island, 5= Mount Gran, 6= Pegtop Mountain, 7= Mount Seuss, 8= The Flatiron, 9= Sperm Bluff, 10= Springtail Pt, 11= St Johns Range; Map data: Google, DigitalGlobe.

Figure 2: Collated phylogenetic tree of sequences representing 40 unique haplotypes for three springtail species including three Symphypleona outgroups (*Sminthurides aquaticus* IHCO046-03, *S. malmgreni* IHCO047-03, and *Sminthurinus elegans* COONT067-08). Maximum likelihood base tree with support values over 90/ 0.90 displayed in order of ML bootstrap values/ Bayesian posterior probabilities/ NJ bootstrap values. * indicates that the support values were either unavailable or under 90/0.9. Tree is coloured according to the seven BINs present, with bars indicating the location where specific haplotypes were collected. Numbers in brackets refer to specific locations pictured in Fig. 1C. For full Bayesian and NJ trees, see Supplementary Materials 3.

Figure 3: Haplotype network of 27 *Gomphiocephalus hodgsoni* haplotypes, seven *Cryptopygus nivicolus*, and six *Antarcticinella monoculata* haplotypes, all coloured according to collection locations. Numbers beside location names refer to those pictured on Figure 1C. Numbers within boxes indicate the number of mutational steps separating haplotypes, while black dots indicate a single mutational step. The bolded mutational steps highlight how many steps separate the new haplotypes. The *G. hodgsoni* haplotype within the square (Gh1) is the most derived haplotype.



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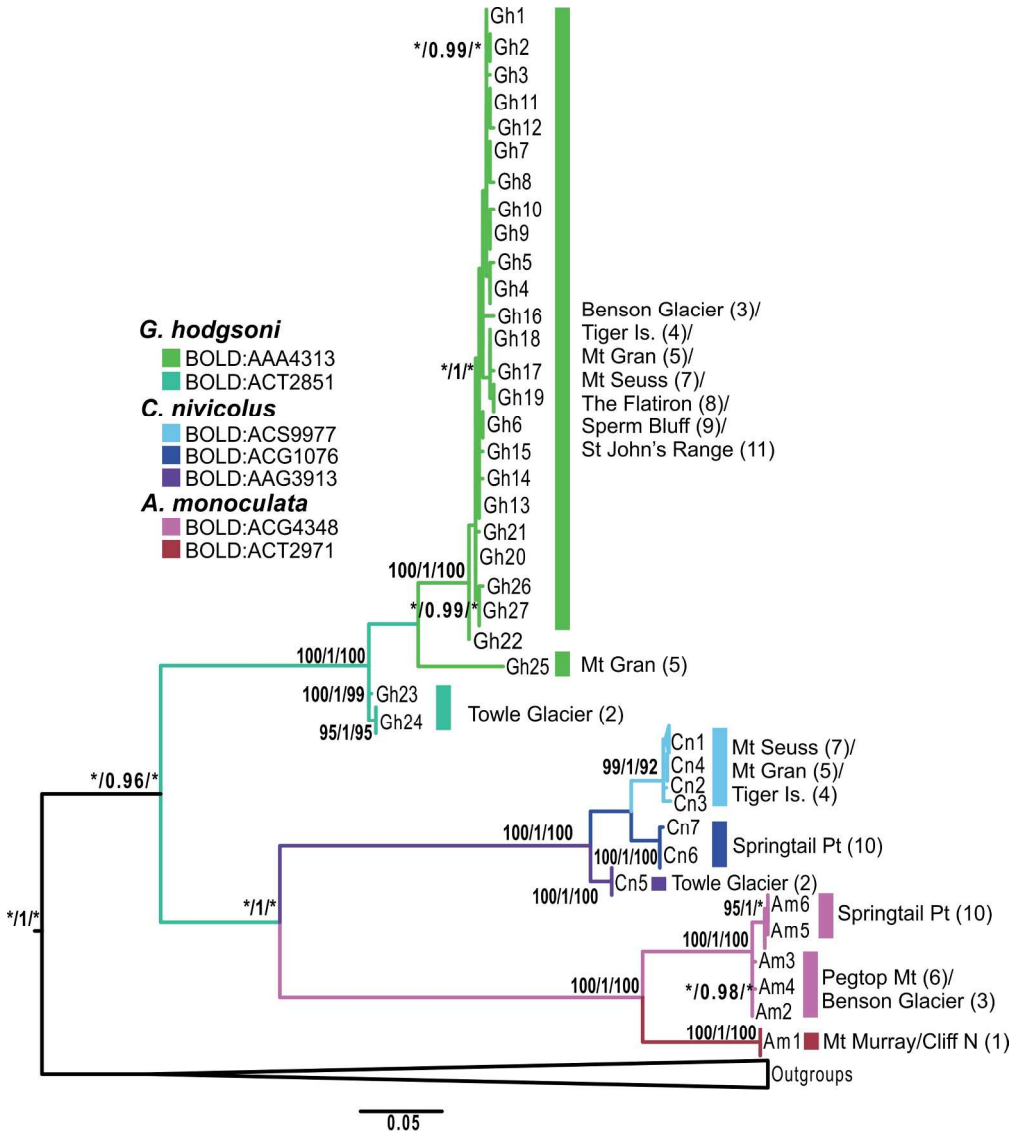

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204x229mm (300 x 300 DPI)

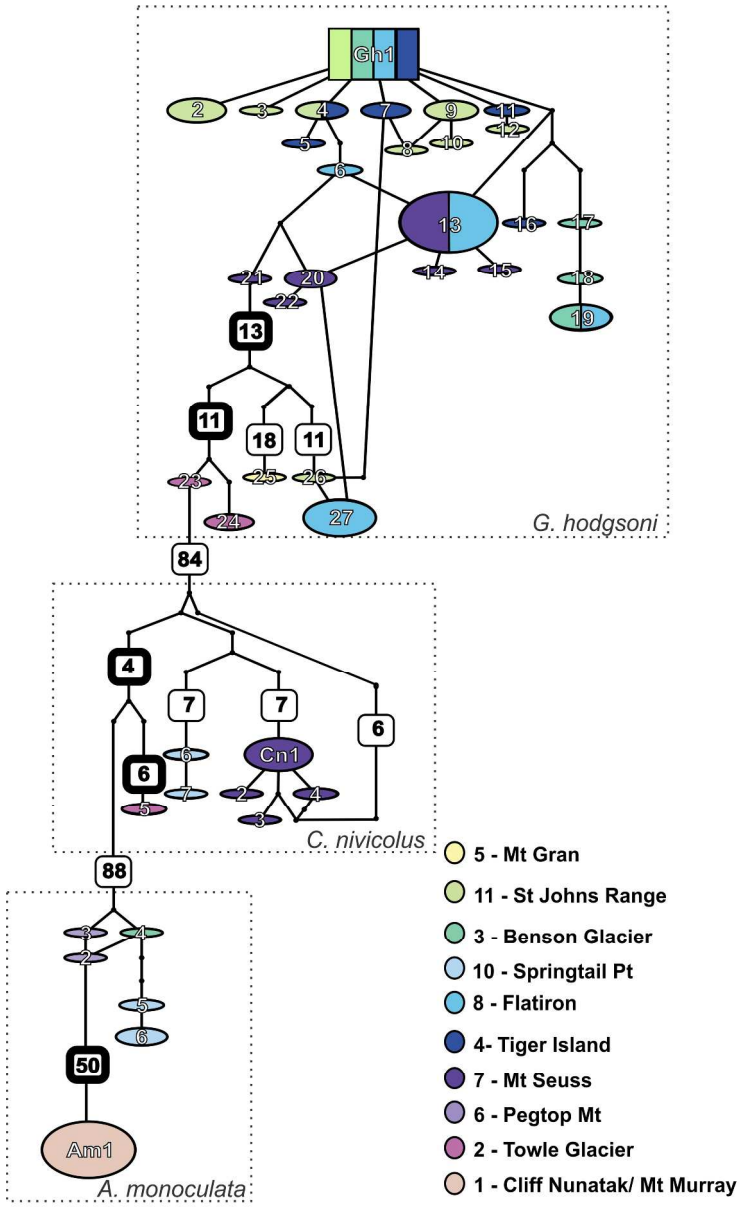


Figure 3: Haplotype network of 27 Gomphiocephalus hodgsoni haplotypes, seven Cryptopygus nivicolus and six Antarcticinella monoculata haplotypes all coloured according to collection locations. Numbers beside location names refer to those pictured on Figure 1C. Numbers within boxes indicate the number of mutational steps separating haplotypes while black dots indicate a single mutational step. The bolded mutational steps highlight how many steps separate the new haplotypes. The G. hodgsoni haplotype within the square (Gh1) is the most derived haplotype.
180x291mm (300 x 300 DPI)

SUPPLEMENTARY MATERIALS

SM 1 Table of springtail collection information showing the location, coordinates and BOLD sample IDs of sequences for each unique haplotype

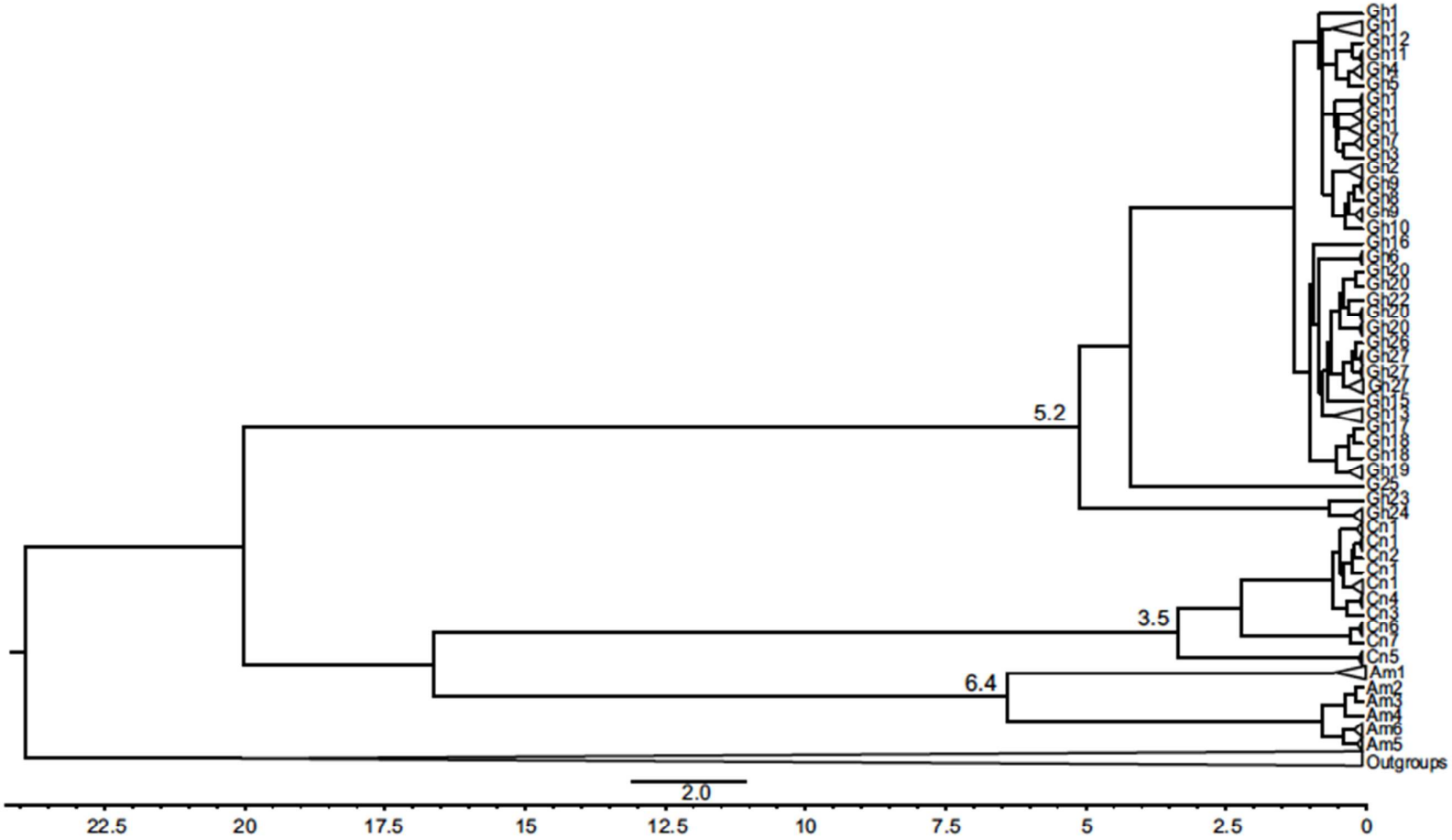
Haplotype #	Location	Coordinates	BOLD Sample IDs
G. hodgsoni			
Gh1	Tiger Island	-76.784 162.452	ANTSP607
	Flatiron	-77.005 162.408	ANTSP585, ANTSP587, ANTSP589, ANTSP605, ANTSP606, ANTSP607, ANTSP628, ANTSP629
	Benson Glacier	-76.822 162.107	ANTSP596, ANTSP597
	St Johns Range	-77.2801 161.731	ANTSP129, ANTSP131, ANTSP134, ANTSP136, ANTSP137, ANTSP138, ANTSP140, ANTSP141, ANTSP143, ANTSP151, ANTSP216
Gh2	St Johns Range	-77.2801 161.731	ANTSP128, ANTSP144, ANTSP145, ANTSP147, ANTSP148, ANTSP149, ANTSP214, ANTSP218
Gh3	St Johns Range	-77.208 161.7	ANTSP210
Gh4	Flatiron	-77.005 162.408	ANTSP584, ANTSP586, ANTSP604
	St Johns Range	-77.208 161.7	ANTSP213, ANTSP215
Gh5	Flatiron	-77.005 162.408	ANTSP631
Gh6	Benson Glacier	-76.87 161.754	ANTSP564, ANTSP565
Gh7	Mount Seuss	-77.015 161.75	ANTSP153, ANTSP166, ANTSP167, ANTSP534
Gh8	St Johns Range	-77.208 161.7	ANTSP209

Gh9	St Johns Range	-77.2801 161.731	ANTSP132, ANTSP133, ANTSP135, ANTSP139, ANTSP211, ANTSP212
Gh10	St Johns Range	-77.285 161.726	ANTSP217
Gh11	Flatiron	-77.005 162.408	ANTSP634, ANTSP635
Gh12	St Johns Range	-77.2849 161.726	ANTSP150
Gh13	Mount Seuss	-77.023 161.738	ANTSP152, ANTSP154, ANTSP157, ANTSP158, ANTSP159, ANTSP160, ANTSP161, ANTSP163, ANTSP164, ANTSP165, ANTSP168, ANTSP169, ANTSP172, ANTSP174, ANTSP175, ANTSP225, ANTSP518, ANTSP520, ANTSP521, ANTSP522, ANTSP524, ANTSP532, ANTSP533, ANTSP540, ANTSP541
	Benson Glacier	-76.87 161.754	ANTSP563
Gh14	Mount Seuss	-77.021 161.737	ANTSP521
Gh15	Mount Seuss	-77.015 161.75	ANTSP535
Gh16	Flatiron	-77.005 162.408	ANTSP627
Gh17	Tiger Island	-76.783 162.452	ANTSP639
Gh18	Tiger Island	-76.783 162.452	ANTSP590, ANTSP612
Gh19	Tiger Island	-76.783 162.452	ANTSP592, ANTSP637, ANTSP638, ANTSP640, ANTSP642, ANTSP643, ANTSP644, ANTSP646
	Benson Glacier	-76.822 162.107	ANTSP623
Gh20	Mount Seuss	-77.011 161.768	ANTSP142, ANTSP173, ANTSP519, ANTSP525, ANTSP531

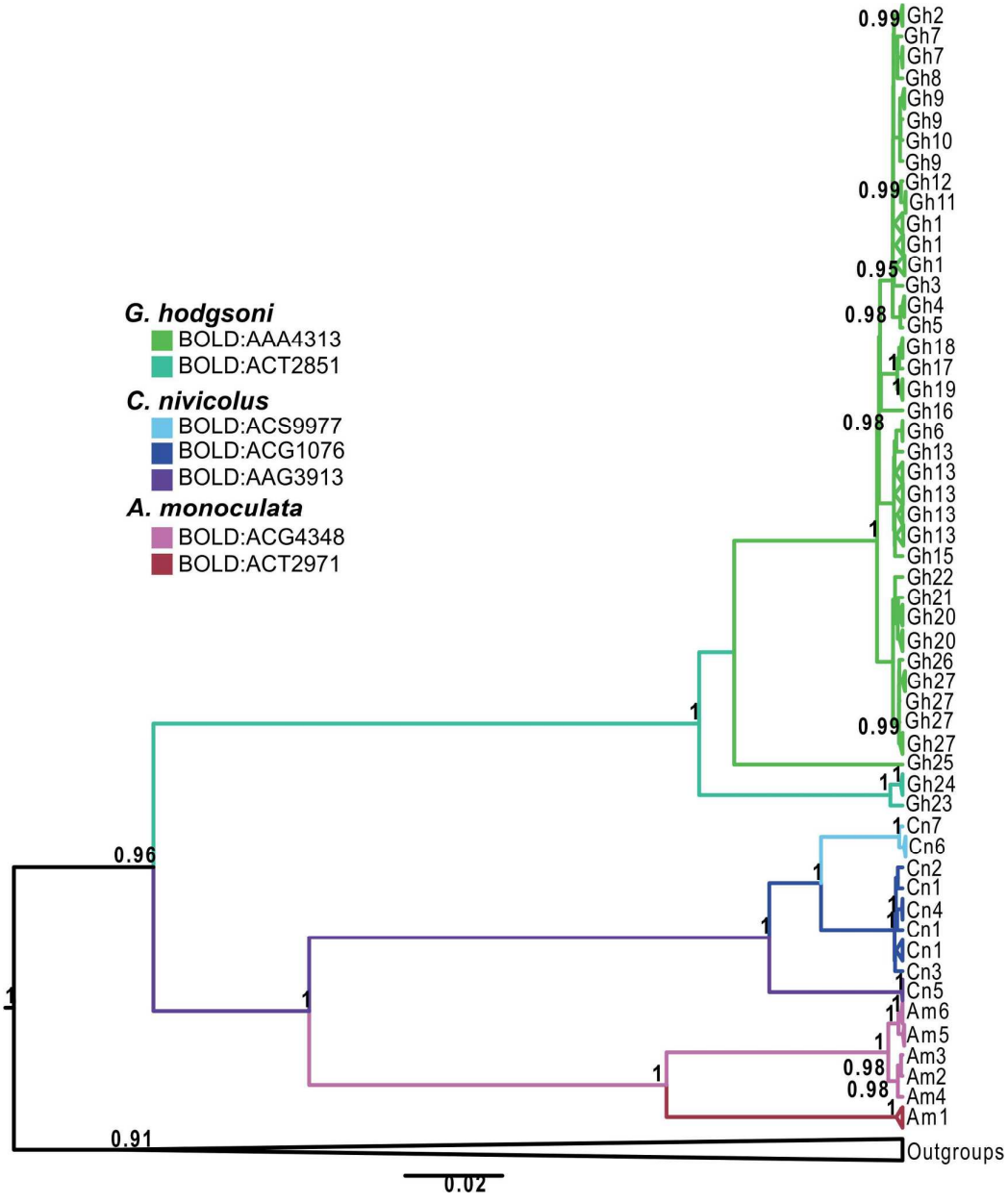
Gh21	Mount Seuss	-77.023 161.738	ANTSP539
Gh22	Mount Seuss	-77.2849 161.726	ANTSP142
Gh23	Towle Glacier	-76.655 161.093	ANTSP561
Gh24	Towle Glacier	-76.729 161.011	ANTSP556, ANTSP557, ANTSP558, ANTSP559
Gh25	Mount Gran	-76.982 161.16	ANTSP202
Gh26	St Johns Range	-77.2849 161.726	ANTSP146
Gh27	Benson Glacier	-76.822 162.107	ANTSP594, ANTSP595, ANTSP598, ANTSP599, ANTSP600, ANTSP601, ANTSP624, ANTSP649, ANTSP651, ANTSP652, ANTSP653, ANTSP654, ANTSP655, ANTSP656
A. monoculata			
Am1	Cliff Nunatak	-76.11 162.015	ANTSP552, ANTSP553, ANTSP554, ANTSP555, ANTSP566, ANTSP567, ANTSP568, ANTSP569, ANTSP579, ANTSP580, ANTSP581, ANTSP582
	Mount Murray	-76.11 162.014	ANTSP570, ANTSP571,ANTSP572, ANTSP573, ANTSP574, ANTSP575, ANTSP576, ANTSP577, ANTSP578, ANTSP613, ANTSP615
Am2	Pegtop Mountain	-77.046 161.362	ANTSP618
Am3	Pegtop Mountain	-77.046 161.362	ANTSP616
Am4	Benson Glacier	-76.822 162.107	ANTSP622
Am5	Springtail Point	-77.168 160.71	ANTSP196, ANTSP235

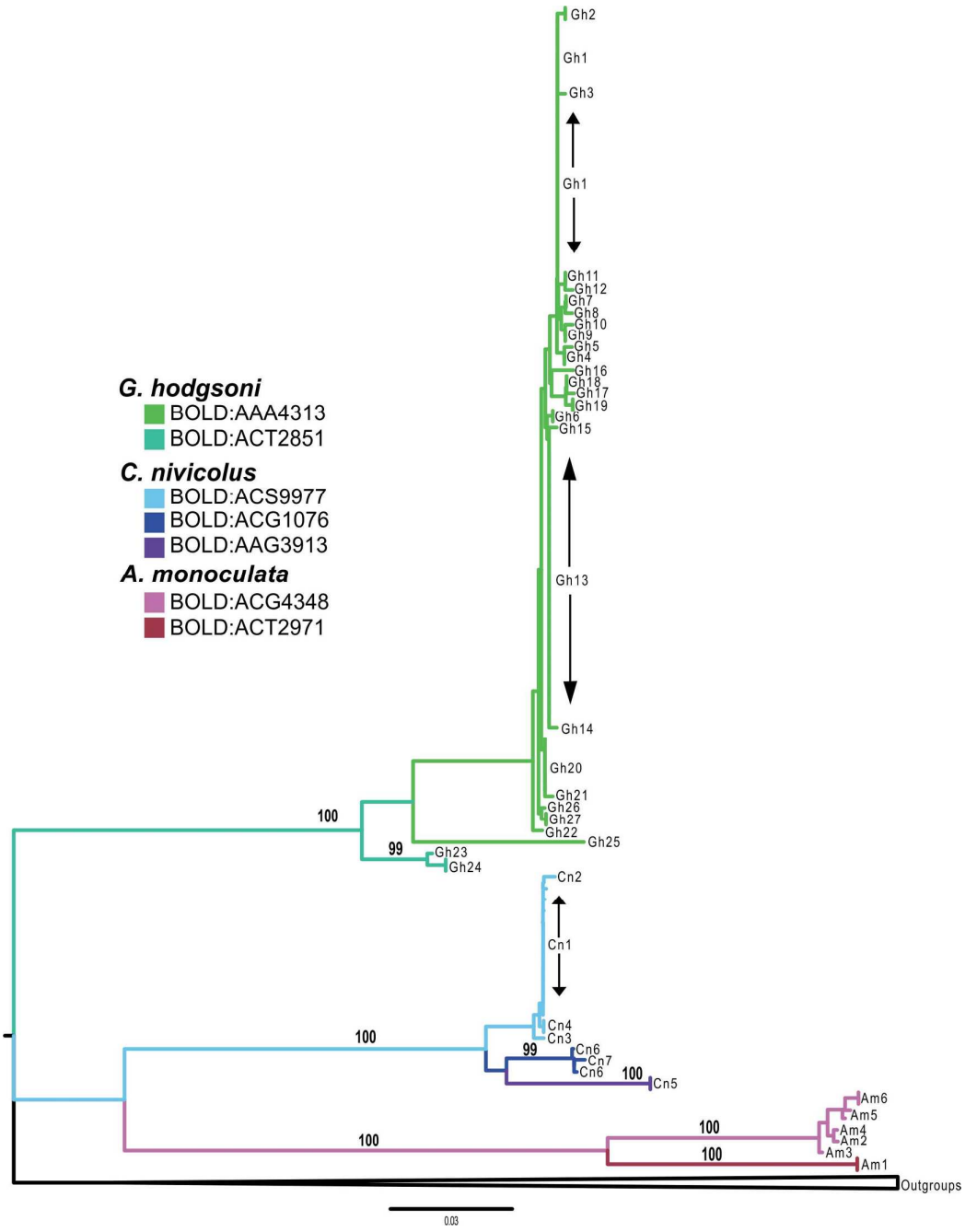
Am6	Springtail Point	-77.168 160.71	ANTSP194, ANTSP195, ANTSP203, ANTSP204, ANTSP205
C. nivicolus			
Cn1	Tiger Island	-76.784 162.452	ANTSP609
	Mount Seuss	-77.023 161.738	ANTSP124, ANTSP155, ANTSP156, ANTSP536, ANTSP543, ANTSP544, ANTSP548, ANTSP611
	Mount Gran	-76.972 161.148	ANTSP619, ANTSP620, ANTSP621
Cn2	Mount Seuss		ANTSP170
Cn3	Mount Seuss	-77.023 161.738	ANTSP547
Cn4	Mount Seuss	-77.023 161.746	ANTSP537, ANTSP550
Cn5	Towle Glacier	-76.655 161.093	ANTSP602, ANTSP603
Cn6	Springtail Point	-77.1676 160.71	ANTSP119, ANTSP121
Cn7	Springtail Point	-77.1676 160.71	ANTSP118

SM2 Bayesian Inference tree showing estimated divergence times for *Gomphiocephalus hodgsoni* , *Cryptopygus nivicolus* and *Antarcticinella monoculata*. Timescale along the bottom is in My. Numbers on nodes refer to estimated divergence times for those populations.



SM3 Supplementary phylogenetic trees (Bayesian followed by Neighbour Joining) of sequences representing 40 unique haplotypes for three springtail species including three Symphypleona outgroups (*Sminthurides aquaticus* IHCO046-03, *S. malmgreni* IHCO047-03 and *Sminthurinus elegans* COONT067-08). Support values over 0.95/95 are displayed with trees coloured according to the seven BINs present.




G. hodgsoni
BOLD:AAA4313
BOLD:ACT2851
C. nivicolus
BOLD:ACS9977
BOLD:ACG1076
BOLD:AAG3913
A. monoculata
BOLD:ACG4348
BOLD:ACT2971
Gh2
Gh1
Gh3
Gh1
Gh11
Gh12
Gh7
Gh8
Gh10
Gh9
Gh5
Gh4
Gh16
Gh18
Gh17
Gh19
Gh6
Gh15
Gh13
Gh14
Gh20
Gh21
Gh26
Gh27
Gh22
Gh25
Gh23
Gh24
Cn2
Cn1
Cn4
Cn3
Cn6
Cn7
Cn6
Cn5
Am6
Am5
Am4
Am2
Am3
Am1
Outgroups
100
99
100
99
100
100
100
100
0.03