1   Original Article.

2   **High levels of intra-specific genetic divergences revealed for Antarctic**

3   **springtails: evidence for small-scale isolation during Pleistocene glaciation**

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10   Running Head: Genetic divergences among Antarctic Springtails

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19   **Abstract**

20   **Aim** To examine the levels of genetic variability within and among populations of three

21   Antarctic springtail species (Arthropoda: Collembola) and test the hypothesis that genetic

22   divergences occur among glacially-isolated habitats.

23   **Location** Southern Victoria Land, Ross Dependency, Antarctica.

24   **Methods** Samples were collected from locations in the vicinity of the Mackay Glacier. We

25   analysed mitochondrial DNA (COI) sequence variability for 97 individuals representing three

26   species (*Gomphiocephalus hodgsoni* n=67; *Cryptopygus nivicolus*, n=20; *Antarcticinella*

27   *monoculata*, n=8). Haplotype diversity and genetic divergences were calculated and used to

28   indicate population variability as well as infer divergence times of isolated populations using

29   molecular clock estimates.

30   **Results** Two of the three species showed high levels of genetic divergence.

31   *Gomphiocephalus hodgsoni*, a widespread and common species showed 7.6% sequence

32   divergence on opposite sides of the Mackay Glacier. The more range restricted *Cryptopygus*

33   *nivicolus* species showed 4.0% divergence among populations. The third species,

34   *Antarcticinella monoculata*, was found in only one location. Molecular clock estimates based

35   on sequence divergences suggest that populations separated within the last 4 Mya.

36   **Main Conclusions** Habitat fragmentation resulting from Pliocene (5 Mya) and Pleistocene (2

37   Mya - 10 Kya) glaciations has promoted and maintained high levels of diversity among

38   isolated springtail populations on relatively small spatial scales. The region surrounding the

39   Mackay Glacier has provided refugia for springtail populations during glacial maxima and

40   remains an area of high genetic and species diversity for Collembola within the Ross Sea

41   region.

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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 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*., 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 allow survival in sub-zero temperatures (McGaughran *et al.*, 2011a).

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 & Stevens, 2002; Adams *et al*., 2006). These taxa, which lack survival and dispersal strategies possessed by other invertebrate groups such as nematodes (Adhikari *et* al.,2010; Nkem *et al*., 2006), have been restricted to these fragmented, ice-free zones since the Middle Miocene (14-11 Mya; Stevens & Hogg, 2003; Stevens *et al.*, 2006; McGaughran *et al.*, 2010). At this time, 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). 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

84 glacial history has resulted in extremely low species richness for the Antarctic fauna, with

85 many habitats containing at most one or two arthropod taxa (Janetschek, 1967a). Species are

86 also rarely shared between regions (Gressitt, 1967; Wise, 1971; Sinclair & Stevens, 2006),

87 suggesting limited inter-habitat dispersal. Consequently, the current arthropod taxa are likely

88 to be long-term inhabitants and remnants of, once more widespread species (Convey *et al*.,

89 2009). Even within regions, most species show high levels of genetic divergence across their

90 distributional ranges suggesting the effects of long-term isolation and/or survival in glacial

91 refugia (Frati *et al*., 2001; Stevens & Hogg, 2003; McGaughran *et al*., 2008; Hawes *et al*.,

92 2010; Stevens & D'Haese, 2014). Here, our aim was to extend these studies by focussing on

93 small-scale differences that might occur within faunally-diverse, yet heavily fragmented,

94 landscapes.

95 Ten species of springtail are currently known from the Ross Dependency, four in northern

96 Victoria Land, three in southern Victoria Land and three in the southern Transantarctic

97 Mountains. All species are range-restricted. Species from southern Victoria Land, the focus

98 of our study, consist of three species covering a 3° latitudinal range. Within this region

99 *Gomphiocephalus hodgsoni* is the only relatively widespread species and is common

100 throughout southern Victoria Land (McGaughran *et al*., 2011b). Two additional species,

101 *Cryptopygus nivicolus* (recently redescribed from *Neocryptopygus nivicous* by Greenslade,

102 (2015)) and *Antarcticinella monoculata* are extremely range-restricted and known only from

103 one or two locations near the Mackay Glacier to the north of the Dry Valleys (Wise, 1971)

104 (Fig. 1), suggesting the possibility of a glacial refugium. Recent studies of lichens and mosses

105 also near the Mackay Glacier (Green *et al*., 2011), as well as haplotype diversity for springtail

106 (*G. hodgsoni*) and mite (*Stereotydeus. mollis*) taxa have further suggested this area as a likely

107 refugial zone (Stevens & Hogg, 2003, 2006; McGaughran *et al*., 2008; Demetras *et al*.,

108 2010).

109   In order to determine the geographic scales on which genetic diversity may have been

110   promoted and/or maintained, we focused on small-scale genetic variability in a region of

111   comparatively high species diversity (Mackay Glacier, southern Victoria Land). This glacier

112   is one of only a few outlet glaciers that connect the EAIS with the Ross Ice Shelf in southern

113   Victoria Land (Clapperton & Sugden, 1990). Accordingly, we tested the hypothesis that this

114   region would support genetically divergent springtail populations among isolated habitats.

115   We predicted that high levels of both genetic variability and genetic divergence would exist

116   among these habitats, potentially indicating refugial zones from the Pleistocene glaciations.

117   **Methods**

118   *Study sites and sample collection:*

119   Samples were collected from St John's Ranges near Victoria Valley and on the northern and

120   southern sides of the Mackay Glacier in the northern Dry Valleys region of the Ross

121   Dependency (Fig. 1). Specimens were collected from the undersides of rocks using modified

122   aspirators (Stevens & Hogg, 2002). Soil samples were also taken from each site and returned

123   to the lab where they were suspended a 10% sucrose solution. Invertebrates were then

124   removed from the solution surface under a dissecting microscope (10X magnification) using

125   a fine wire loop. All specimens were stored in 95% ethanol and returned to the University of

126   Waikato for further processing. All specimens were morphologically identified to species

127   level using Gressitt *et al*., (1963) and Salmon, (1965). Specimens not used for DNA analyses

128   were archived at the School of Science, University of Waikato, under the care of IDH.

129   *Genetic analyses:*

130   Genetic analyses were jointly carried out at the University of Waikato and at the Canadian

131   Centre for DNA Barcoding (CCDB) at the University of Guelph. At the University of

132 Waikato total genomic DNA was extracted from the tissue of entire specimens using a

133 Glassfiber Plate DNA Extraction (AcroPrep) method (Ivanova *et al*., 2006) at CCDB, and

134 Red Extract n Amp (Sigma-Aldrich) using 10 µl extraction solution and 2.5 µl tissue prep,

135 following manufacture's protocol. Polymerase Chain Reactions (PCRs) were comprised of a

136 15 µl reaction containing 5.7 µl MQH_2O, 7.5 µl PCR Master Mix Solution (i-Taq, Intron

137 Biotechnology), 0.4 µl of each primer and 1 µl of template DNA. A 658 bp fragment of the

138 mitochondrial COI gene was amplified using the primers HCO2198 (sequence 5'-

139 TAAACTTCAGGGTGACCAAAAAATCA-3') and an altered LCO1490 (sequence: 5'-

140 AGTTCTAATCATTAARGATATYGG-3') (Folmer *et al.*, 1994) for the *G. hodgsoni*

141 specimens. HCO and LepF1 (sequence: 5'-ATTCAACCAATCATAAAGATATTGG-3')

142 (Hajibabaei *et al*., 2006) were used to amplify the *C. nivicolus* and *A. monoculata* specimens.

143 The standard LCO1490 (sequence: 5'-GGTCAACAAATCATAAAGATATTGG-3') was

144 used for both species (in place of the altered LCO1490 and LepF1) at CCDB. Primers were

145 used at 1.0 mM concentration. PCR conditions at CCDB were: initial denaturing at 94ºC for

146 1 min; 5 cycles of 94ºC for 1 min, 45ºC for 1.5 min and 72ºC for 1.5 min; 35 cycles of 94ºC

147 for 1 min, 50ºC for 1.5 min and 72ºC for 1 min followed by a final 72ºC for 5 min. PCR

148 conditions were: initial denaturing at 94ºC for 5 minutes; 36 cycles of 94ºC for 1 min, 52ºC

149 for 1.5 min and 72ºC for 1 min, followed by a final 72ºC for 5 min.

150 PCR products were cleaned using Sephadex (CCDB) or 0.2 µl ExonucleaseI (EXO) and 0.1

151 µl Shrimp Alkaline Phosphate (SAP) with 2.7 µl MQH_2O following manufactures protocol

152 (Global Science & Tech Ltd.) at Waikato. DNA was sequenced in both directions on an

153 ABI3130 sequencer at the University of Waikato DNA sequencing facility using the same

154 primers used for amplification, or on an ABI3730x1 at CCDB. Sequences from the

155 University of Waikato were aligned using Geneious, ver 5.4.2, and confirmed as the target

156 species using the Barcode of Life DataSystems (BOLD; www.boldsystems.org) ver 3 COI

157   animal identification searches. Previous analyses of Antarctic springtails (e.g. Stevens &

158   Hogg 2003), have shown that allozyme analyses were congruent with COI data and that the

159   latter can be used as a reliable indicator of genomic differences occurring among populations.

160   Primer sequences were trimmed from sequence fragments for further analyses. All sequences

161   were uploaded to the BOLD project Antarctic Terrestrial Arthropods (ANTSP) and cross-

162   referenced to GenBank.

163   *Phylogenetic Analysis*

164   COI sequence fragments of 658 bp (219 codons) were obtained for 67 *G. hodgsoni* specimens

165   and 20 *C. nivicolus* specimens. Approximately 560 bp were obtained from single direction

166   reads (using primer LepF1) for eight *A. monoculata* specimens. No stop codons were

167   detected. Sequences of *G. hodgsoni* were unambiguous at 658 bp (no insertions or deletions).

168   However, sequences of *C. nivicolus* and *A. monoculata* contained ambiguous base pair

169   assignments which could not be easily resolved, so sequences were further trimmed at both

170   ends, resulting in sequence fragments of 547 bp (181 codons) for *C. nivicolus* and 527 bp

171   (175 codons) for *A. monoculata*. Two additional *C. nivicolus* sequences were also obtained

172   from GenBank (Accession numbers DQ285403 and DQ285404).

173   Sequences for all species were initially examined in the context of generating a single

174   neighbour-joining tree using a Kimura 2-parameter distance model (Kimura, 1980). All

175   duplicate sequences were identified and removed to include only unique haplotypes in

176   subsequent analyses. Due to the lack of publically available sequence data for taxa that share

177   a recent common ancestor with our ingroup taxa (and that did not approach saturation),

178   analyses were run unrooted among the ingroup taxa. No significant changes were noted in

179   topography between these analyses and ones run previously using *Podura aquatic* as a test.

180   Chi-square tests (X^2) as implemented in PAUP* 4.0 (Swofford, 2002) were used to determine

181   whether the assumption of equal base frequencies among sites was violated on all sites and

182   on third codon positions only. JModel test 2.1.2 (Posada, 2008) was used to determine the

183   most appropriate substitution model for Maximum Likelihood (ML) analysis. Settings were

184   as follows: 11 substitution schemes (88 models), base frequencies +F, rate variation +I, +Γ,

185   set to BioNJ. The model selected for the data set was GTR + I +Γ (-lnL = 1,590.9). Maximum

186   Likelihood heuristic searches were conducted using this model in MEGA 5.10 (Tamura *et al*.,

187   2011) using 1000 bootstrap replicates. Maximum Parsimony (MP) analyses were performed

188   in PAUP* using 1000 full-heuristic search bootstrap replicates.

189   MrBayes 3.2.6 (Huelsenbeck & Ronquist, 2001) was used to conduct a Bayesian Inference

190   analysis. A general time reversal model (GTR +I +Γ) was used, with a log normal relaxed

191   clock model and speciation Yule process as the tree prior. The Markov chain Monte Carlo

192   (MCMC) was set to 1,100,000 generations, sampling trees every 200. A burn in of 100,000

193   trees was determined by plotting log-likelihood values against generation time in TRACER

194   (Rambaut & Drummond, 2007) and checking for the point at which normalization occurred.

195   The majority rule tree was acquired from the 11,004 trees sampled after the burn in period.

196   The tree was then visualized in Tree Annotator (Drummond *et al.*, 2012).

197   Sequences for *G. hodgsoni* and *C. nivicolus* were split into separate data sets for analysis in

198   the program TCS 1.21 (Clement *et al*., 2000) and to construct networks of sequence

199   haploytpes. Single representatives of each haplotype were used in the final analysis to

200   simplify files, and sequences of *C. nivicolus* were trimmed at 547 bp to avoid anomalies, as

201   described above. The *A. monoculata* sequences were not included in these analyses as they

202   were only collected from a single site and consisted of only two similar haplotypes (<0.2%

203   divergence).

204 Uncorrected pair-wise genetic distances between COI sequences for populations at different

205 locations were also calculated for the *G. hodgsoni* and *C. nivicolus* data sets in MEGA 5.10.

206 The likelihood ratio test did not detect evidence of significant rate heterogeneity for *G.*

207 *hodgsoni* (X^2=113.06; *p*<0.001; d.f=14) or *C. nivicolus* (X^2=141.15; *p*<0.001; d.f=10).

208 Approximate geological timing of isolation for the populations was estimated through

209 molecular clock analyses in BEAST 1.8.2 (Drummond *et al.*, 2012). Files generated in

210 BEAUti used a General Time Reversal model (GTR + I + Γ) with speciation Yule Processes

211 as the tree prior and the same MCMC set up as used for the BI tree analysis. A strict clock

212 model with a fixed rate of 0.0115 was used to simulate 2.3% sequence divergence per million

213 years, as determined using insect mitochondrial data (Brower 1994; Juan *et al.*, 1996; Quek *et*

214 *al.*, 2004; McGaughran *et al.*, 2010). Despite being calibrated for insects, the 2.3% sequence

215 divergence per million years was considered a suitable estimate for Collembola as both taxa

216 have similar life cycles (McGaughran *et al.*, 2010).

217 **Results**

218 Of the 658 bp analysed for *G. hodgsoni,* 515 characters were constant, 22 were parsimony

219 informative and the remaining 121 were parsimony uninformative. The nucleotide

220 composition averaged across all sequences showed an A-T bias of 64.0% (A = 27.7%, T =

221 36.7%, C = 19.3%, G = 16.7%). Nucleotide frequencies were not significantly different

222 among sequences for all codon positions (X^2 = 2.19, *p* = 1.0, d.f = 48) or for third codon

223 positions only (X^2 = 7.18, *p* = 1.0, d.f = 48). Of the 549 bp analysed for *C. nivicolus*, 433

224 characters were constant, 22 were parsimony informative and the remaining 94 were

225 parsimony uninformative. The nucleotide composition averaged across all sequences showed

226 an A-T bias of 61.4% (A = 25.8%, T = 35.6%, C = 20.4%, G = 18.2%). Base pair frequencies

227 for *C. nivicolus* were not significantly different among sequences for all codon positions (X^2

228    $= 1.41$, $p = 1.0$, d.f $= 36$) or for third codon positions only ($X^2 = 5.77$, $p = 1.0$, d.f $= 36$). Of the

229   527 bp (175 codons) analysed for *A. monoculata*, 408 characters were constant, 1 was

230   parsimony informative and the remaining 118 were parsimony uninformative. The nucleotide

231   composition averaged across all sequences showed an A-T bias of 59.0% (A = 23.9%, T =

232   35.1%, C = 22.3%, G = 18.7%). Base pairs were not significantly different among sequences

233   for all codon positions ($X^2 = 3.39$, $p = 1.0$, d.f $= 21$) or for third codon positions only ($X^2 =$

234   11.55, $p = 0.95$, d.f $= 21$).

235   *Phylogenetic Analysis*

236   A Maximum Likelihood (ML) tree is shown in Fig. 2. Tree constructions for Maximum

237   Parsimony (Fig.3) and Neighbour Joining (data not shown) showed similar topology and

238   node support. Linking nodes between the haplotype G16 and the rest of the *G. hodgsoni*

239   haplotypes had 100% bootstrap support in the ML and MP trees. The linking node between

240   the *C. nivicolus* haplotypes at Springtail Point and at Mt Gran also received 100% bootstrap

241   support in the ML and MP trees. Bootstrap values for the Mt England *C. nivicolus* haplotypes

242   indicated high support from both the ML and MP trees. The topology for the *G. hodgsoni*

243   haplotypes differed in the ML from both the MP and BI trees. Two clusters were apparent,

244   with 0.99 bootstrap support for the node. Collection locations of haplotypes were mixed

245   between both clusters. The topology of the BI tree was also similar to all other trees for *G.*

246   *hodgsoni*, *C. nivicolus* and *A. monoculata.* Posterior probability values between *C. nivicolus*

247   haplotypes at Springtail Point and at Mt Gran was 1.00, and also 1.00 between the Mt

248   England and Mt Gran group (Fig. 4). The topology and node support of these trees supports

249   the presence of high genetic structuring across the Mackay Glacier.

250   *Haplotype networks*

251   The geographic distribution of sequence haplotypes for *G. hodgsoni* and *C. nivicolus* was

252   investigated using haplotype joining networks. Subsequent haplotype assignments and their

253   collection locations are shown in Table 1. Sixteen haplotypes were found from 67 *G.*

254   *hodgsoni* sequences. Maximum connection steps were fixed at 40 in order to connect

255   haplotype G16 to the rest of the haplotypes (Fig. 5). This network revealed 10 1-step

256   haplotypes, three 2-step haplotypes, two 3-step haplotypes and one 35-step haplotype. The

257   most divergent haplotype shown by this analysis was G16, representing three individuals

258   from Mt Gran. This difference was also supported by divergence values and phylogenetic

259   trees (Figs 2, 3, 4, 7). The remainder of the network which included haplotypes from the St

260   John's Range and Mt Seuss did not show high geographic structure, similar to that observed

261   in the tree-based approaches.

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

263   were fixed at 30 in order to connect the Mt Gran and Mt England haplotypes to the Springtail

264   point haplotypes (Fig. 6). This network revealed nine 1-step haplotypes, two 3-step

265   haplotypes and one 16-step haplotype. This network analysis showed two groups of

266   haplotypes that were connected by 16 missing mutational steps. These two groups

267   corresponded to populations at Springtail Point on the south edge of Mackay Glacier, and Mt

268   Gran and Mt Seuss to the north and in the centre of the glacier respectively. This difference

269   was supported by divergence values and phylogenetic trees. The 2-step link to haplotypes at

270   Mt England was also supported by divergence values and phylogenetic trees.

271   *COI sequence divergence and molecular clock estimates*

272   Genetic distances ranged from 0.0-8% for *G. hodgsoni* and 0.00-4.2% for *C. nivicolus*

273   (Fig.7). Greatest differences were found between haplotype G16 at Mt Gran and the

274   remainder of the *G. hodgsoni* haplotypes, and the genetic distance between *C. nivicolus*

275   haplotypes at Mt Gran and Mt England, and those at Springtail Point. The St John's Range

276   and Mt Seuss *G. hodgsoni* haplotypes showed an average divergence of 0.6% within the

277   group (Fig.7). The single haplotype, G16, at Mt Gran showed an average of 7.6% sequence

278   divergence from the other haplotypes.

279   The average sequence divergences among *C. nivicolus* haplotypes within each location were

280   0.1% at Mt Gran, 0.2% at Springtail Point and 0.2% at Mt England. Sequence divergences

281   between locations showed the haplotypes at Mt Gran to be an average of 4.0% divergent from

282   haplotypes at Mt England. Similarly, Springtail Point haplotypes were an average of 3.8%

283   divergent from those found at Mt Gran. The Mt Gran and Mt England haplotypes were the

284   most similar, with 0.8% sequence divergence between them.

285   Based on a strict molecular clock rate of 2.3% sequence divergence per million years, these

286   populations are all likely to have diverged within the last 4 My (Figs 7, 8). The oldest

287   estimated divergence dated the genetic separation of *G. hodgsoni* haplotypes at Mt Gran

288   (G16) and those in the St John's Range and at Mt Seuss at 3.8 Mya. Divergence dates

289   between the three *C. nivicolus* populations suggested that the Springtail Point haplotypes

290   diverged from the Mt Gran - Mt Seuss population 1.44 Mya. The difference between

291   haplotypes from Mt Gran and Mt Seuss relative to those at Mt England is much more recent

292   by comparison, estimated at 0.38 Mya.

293   **Discussion**

294   Our mitochondrial DNA (COI) analysis of 97 Antarctic springtails from three taxonomic

295   species revealed highly divergent populations across 65 km within the Mackay Glacier.

296   Populations of *Gomphiocephalus hodgsoni* and *Cryptopygus nivicolus* on the lower slopes of

297   Mt Gran were shown to be an average of 7.6% and 3.8% divergent from their nearest

298   neighbours. For *G. hodgsoni*, this represents a considerably greater genetic divergence among

299   populations than the 2.4% divergence previously found for this species throughout the

300   McMurdo Dry Valleys (Stevens & Hogg, 2003; Nolan *et al*., 2006; McGaughran *et al*.,

301   2008). High genetic structure, within both putative species, suggests that populations may

302   have survived *in situ* since the Antarctic continent became fully glaciated. Given the

303   elevations of surrounding mountains it is possible that several locations such as Mt Gran

304   (2235 m) and Mt Seuss (1190 m) protruded above the advancing Mackay Glacier, and

305   remained so since the early Pliocene (Janetschek, 1967a; Clapperton & Sugden, 1990). In

306   particular, this area is known to contain the highest species diversity of springtails in southern

307   Victoria Land, with *G. hodgsoni, C. nivicolus* and *A. monoculata* all known from this area

308   (Gressitt *et al*., 1963). The species diversity of mites, lichens and mosses have also been

309   shown to be high in the Mackay Glacier region relative to other nearby areas such as the Dry

310   Valleys (Demetras *et al*., 2010; Green *et al*., 2011). This suggests that this area has served as

311   a glacial refuge for multiple taxa during the last 5 Mya.

312   We now also highlight the potential for species-level genetic divergences within two

313   springtail taxa for populations on opposite sides of the Mackay Glacier, which may indicate

314   early stages of speciation. Our data suggest that the population of *G. hodgsoni* present on the

315   lower slopes of Mt Gran has been isolated from other known *G. hodgsoni* populations since

316   the Mid-Pliocene (4 Mya). Similarly, the population of *C. nivicolus* from the same location

317   has been isolated from a neighbouring population at Springtail Point by as much as 1.4 Mya.

318   The occurrence of *A. monoculata* at Springtail Point, coupled with the highly divergent

319   populations at Mt Gran supports the notion of high arthropod diversity for this area.

320   The differences in divergence estimates for *G. hodgsoni* (3.8 Mya) and *C. nivicolus* (1.4

321   Mya) may be the result of different evolutionary histories (e.g. later isolation) or possibly

322   differences in mutation rates. For example, Stevens & Hogg (2006) suggested that differing

323   mutation rates may exist between *G. hodgsoni* and the mite *Stereotydeus mollis*. However,

324   little is known about the life history of *C. nivicolus*. The lack of ecological knowledge for *C.*

325   *nivicolus* also makes it difficult to predict its dispersal abilities. Dispersal events in Antarctica

326   are likely to be rare, and often accidental, making it difficult to attribute the presence of a

327   species to ecological gradients (Janetschek, 1967b; Magalhães *et al*., 2012). *G. hodgsoni* is

328   known to survive floating on both sea and fresh water, and dispersal events through wind or

329   accidental carriage by birds is also possible (Stevens & Hogg, 2002; Hawes, 2011;

330   McGaughran *et al*., 2011a, 2011b).

331   As Mackay Glacier is an outlet glacier for the EAIS, it is unlikely to have undergone

332   significant retreat during the interglacial periods of the Pleistocene as many of the alpine

333   glaciers did (Clapperton & Sugden, 1990; Sugden *et al*., 1999). This appears to have isolated

334   the Mt Gran population of *G. hodgsoni* from the populations on Mt Seuss in the centre of the

335   glacier, and those in the St John's Range bordering Victoria Valley. It is possible that the

336   presence of haplotypes from the St John's range in the Mt Seuss population relate to recent

337   dispersal since the last glacial maximum. The sharing of *C. nivicolus* haplotypes between Mt

338   Gran and Mt Seuss also indicates potentially recent dispersal from Mt Gran across the

339   glacier. Hawes, (2011) suggested that potential dispersal mechanisms may work in concert,

340   whereby individuals could be wind-blown onto glaciers and then moved by glacial surface

341   streams. The stochastic nature of dispersal events in Antarctica may explain why *G.*

342   *hodgsoni* has yet to disperse from the Mt Gran population.

343   One species, *A. monoculata*, was found at only one location in our study area, although

344   another isolated population is known from Mt Murray 150 km to the north (Gressitt *et al*.,

345   1963). Similarly, haplotypes of *C. nivicolus* present at this site were not found elsewhere in

346   our study area. Springtail Point is in an 'up-glacier' position, making dispersal through

347   temporary melt water to more sea-ward locations possible. However, there was no evidence

348   of water courses being formed by temporary streams in this area, and visual assessment of

349   snow banks that surround the site indicate they have changed little since a previous visit

350   (Gressitt *et al.*, 1963). Even with surface water, the dispersal mechanisms used by other

351   springtail species such as wind and stream flow may be limited for *A. monoculata*. The loss

352   of pigmentation, limited tolerance of UV light and presence deeper in the soil profile

353   (Janetschek, 1967a) make it less likely that *A. monoculata* would experience accidental

354   dispersal by water or wind movement.

355   We conclude that the Mackay Glacier has provided a sufficient dispersal barrier to promote

356   and maintain high levels of genetic divergence in two Antarctic springtail species endemic to

357   southern Victoria Land. This isolation likely occurred around the early Pliocene (4 Mya), and

358   has been maintained by on-going glaciations during the Pleistocene. The high genetic

359   diversity, both at the population and species level, suggests that high altitude sites in this

360   region have served as glacial refugia over the past 4 Mya. The isolation of these sites

361   highlights the potential for high genetic diversity to be maintained on a small scale among the

362   fragmented ice-free areas of Antarctica. Accordingly, we suggest that conservation efforts be

363   directed toward maintaining and protecting the integrity of highly fragmented landscapes

364   within the Transantarctic Mountains of the Ross Dependency.

365

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522

523   **Biosketch**

524   KRB is an MSc graduate from the University of Waikato with interests in animal

525   conservation and connectivity among natural populations. Her primary target taxa are

526   terrestrial Collembola and freshwater macroinvertebrates. She is also interested in the

527   evolution of the New Zealand and Antarctic landscapes.

528   Author contributions:

529   IDH, KRB, BJA and PDNH conceived of the research and obtained funding. KRB and IDH

530   conducted the field work and KRB conducted the primary analyses and was lead author of

531   the manuscript in conjunction with IDH BJA and PDNH. All authors reviewed and

532   contributed revisions to the final version of the manuscript.

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

Haplotype #	Location	Co-ordinates (south – east)	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

C. 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				ANTSP156 ANTSP124
	Mt Seuss	-77.034	161.731	
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

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536   List of Figures

537   Figure 1: Sampling sites and Collembola species' locations in the Mackay Glacier vicinity. Two *C.*

538   *nivicolus* specimens were taken from GenBank and were collected from Mt England in 2005. Map

539   adapted from the SCAR Antarctic Digital Database and the Landsat Image Mosaic of Antarctica

540   (LIMA) project.

541   Figure 2: Maximum Likelihood phylogram constructed in MEGA 5.10, based on the GTR+I+Γ model

542   derived from jModelTest, using 97 individual COI sequences reduced to unique haplotypes.Bootstrap

543   values greater than 50 are shown. Tree is drawn to scale and branch lengths are the number of

544   substitutions per site. Collection locations are indicated for genetically distinct groups.

545   Figure 3: Maximum Parsimony Phylogram constructed in PAUP*, using 97 individual COI sequences

546   reduced to unique haplotypes.. Bootstrap values greater than 50 are shown. Tree is drawn to scale and

547   branch lengths are the number of changes over the whole sequence. Collection locations are indicated

548   for genetically distinct groups.

549   Figure 4: Bayesian Inference Phylogram constructed in MrBayes 3.2.6 based on the GTR+I+Γ model

550   derived from jModelTest, using 97 individual COI sequences reduced to unique haplotypes. Posterior

551   probabilities for haplotype group nodes are presented above 0.5. Tree is drawn to scale and branch

552   lengths are measured in the number of changes per site. Collection locations are indicated for

553   genetically distinct groups.

554   Figure 5: Haplotype network analysis for 16 haplotypes from 67 individuals of *G. hodgsoni.*

555   Haplotypes are indicated by their codes as referred to in Table 1. Missing haplotypes or mutational

556   steps are indicated by black dots, or are collapsed into a count of missing steps as in the single white

557   square.

558   Figure 6: Haplotype network analysis for 12 haplotypes from 22 individuals of *C. nivicolus.*

559   Haplotypes are indicated by their codes as referred to in Table 1. Missing haplotypes or mutational

560   steps are indicated by black dots, or are collapsed into a count of missing steps as in the white square.

561   Figure 7: Genetic distances based on mitochondrial COI sequences of 97 springtails covering 30

562   unique haplotypes. Haplotype codes refer to those in Table 1. Collection locations for each haplotype

563   are indicated in the bar at the top and side of the table.

564   Figure 8: Estimated divergence times for populations of *G. hodgsoni* (circle) and *C. nivicolus*

565   (squares). The timeline on the left is in millions of years. Overarching geologic events are presented

566   in the appropriate time zones. Each bar indicates the divergence range between populations as

567   indicated by the associated number pair. Each number refers to haplotypes from geographic locations

568   as follows: 1 = *G. hodgsoni* haplotypes from the St John's range and Mt Seuss; 2 = the *G. hodgsoni*

569   haplotype at Mt Gran; 3 = *C. nivicolus* haplotypes from Springtail Point; 4 = *C. nivicolus* haplotypes

570   from Mt Gran and Mt Seuss; 5 = *C. nivicolus* haplotypes from Mt England.

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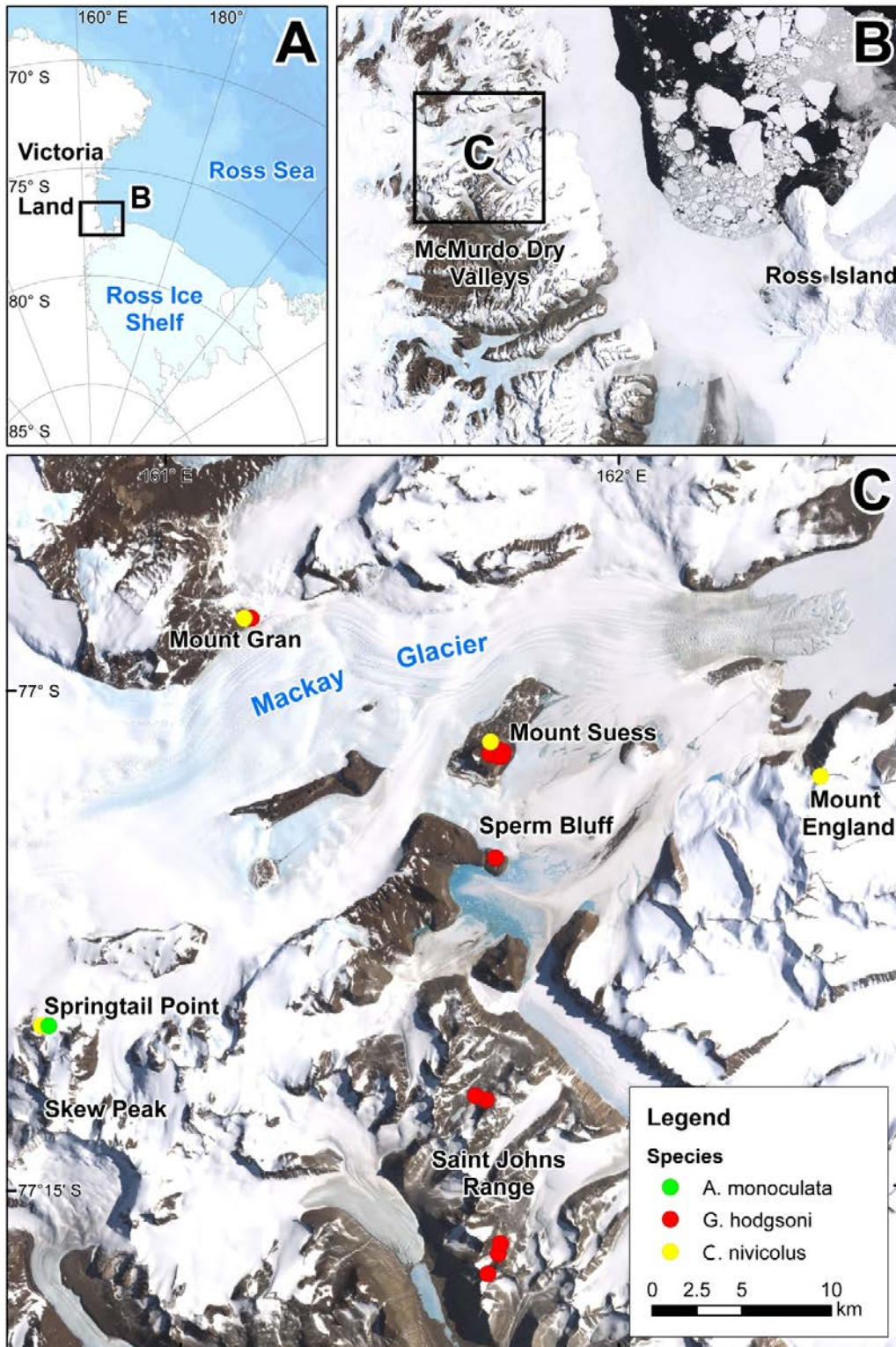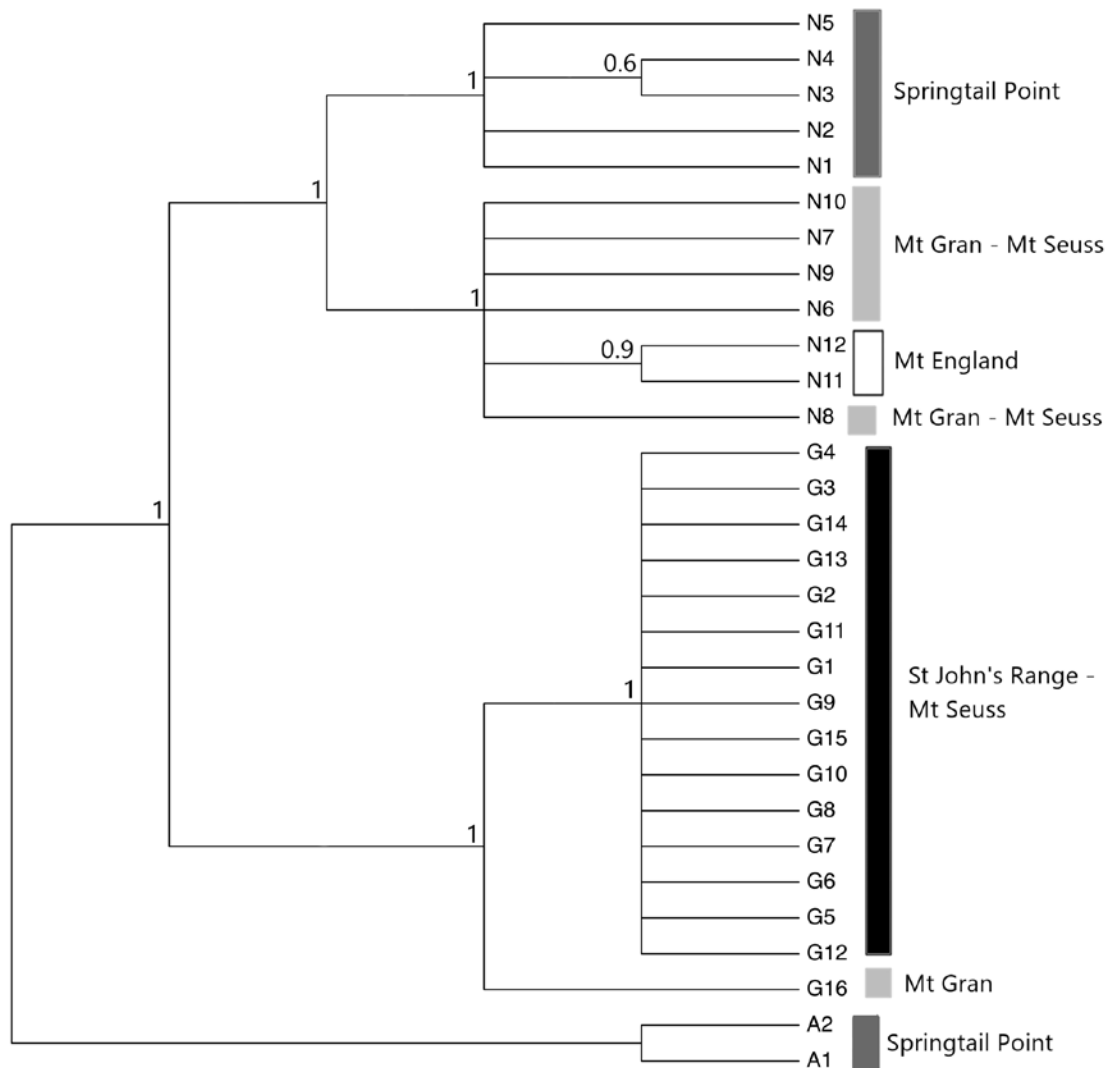

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583   Fig. 1

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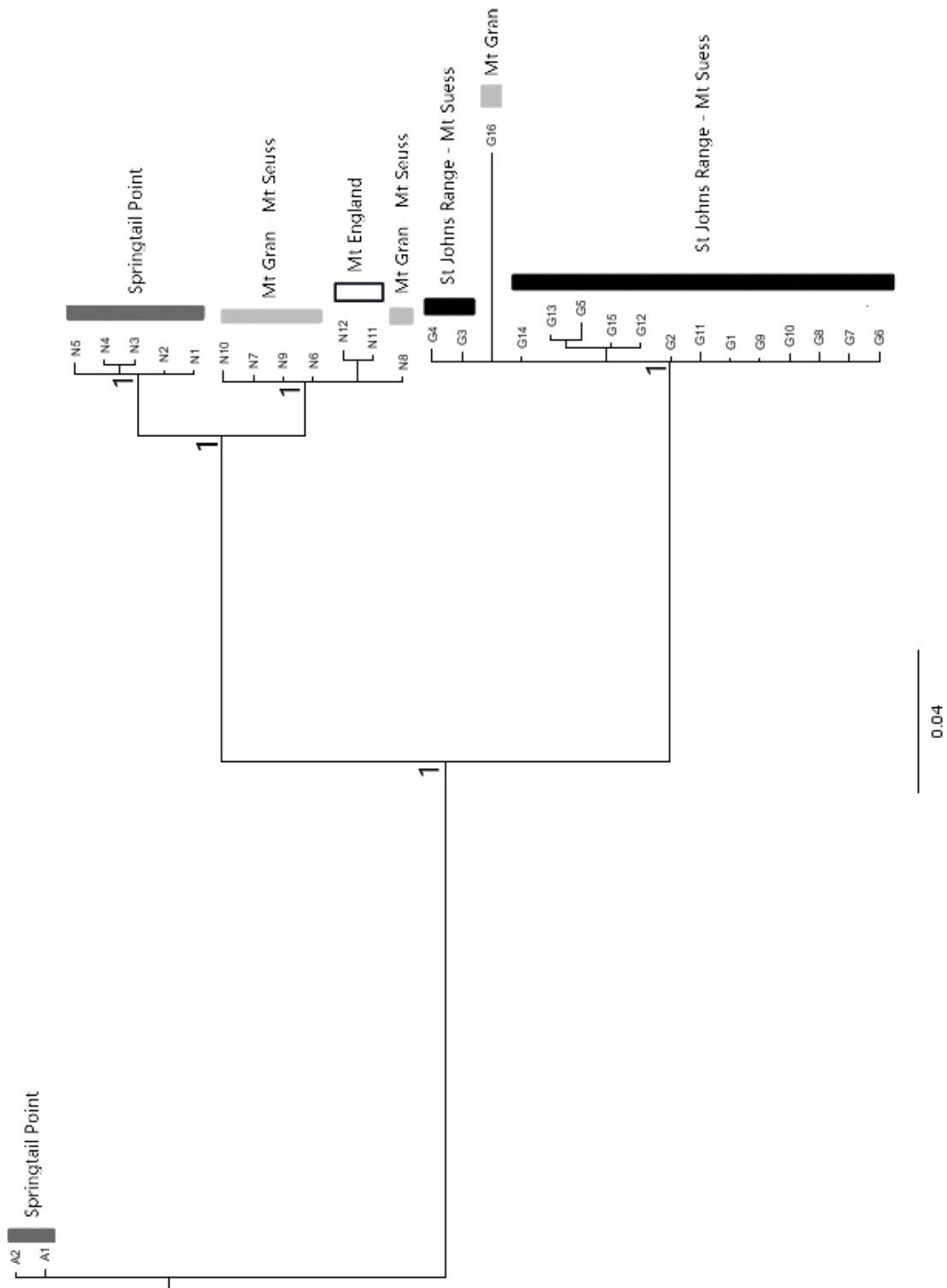611

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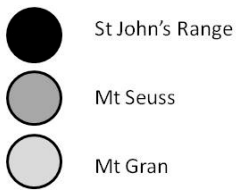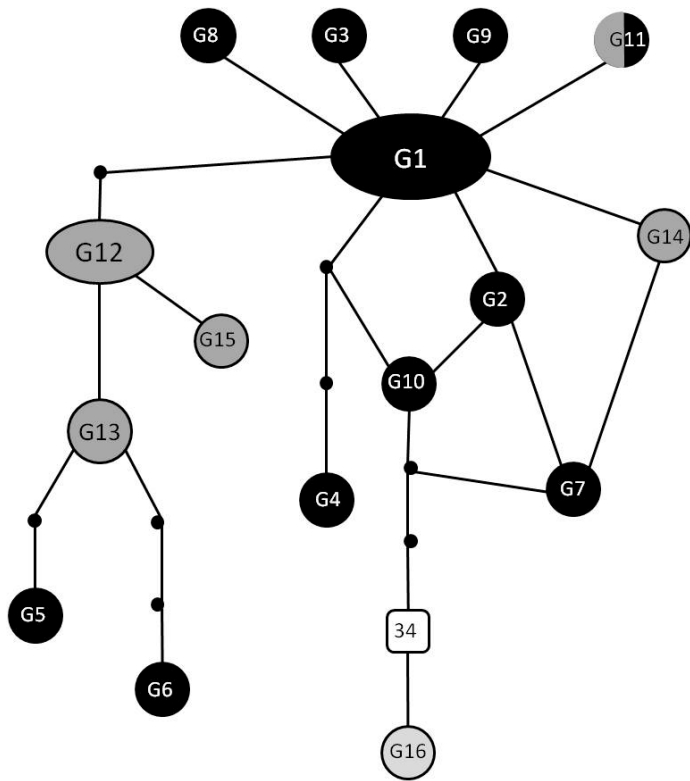613

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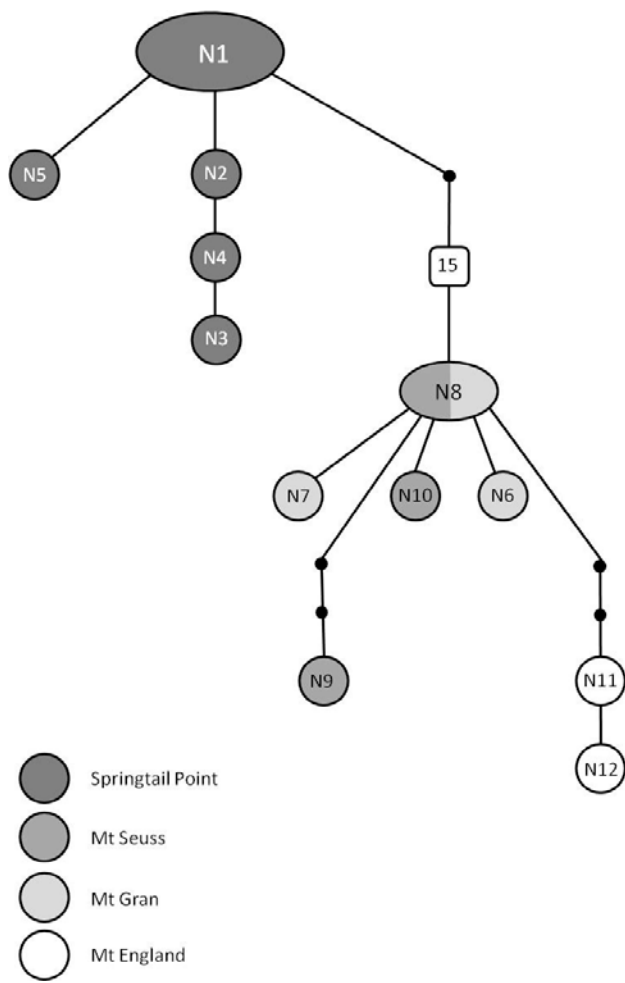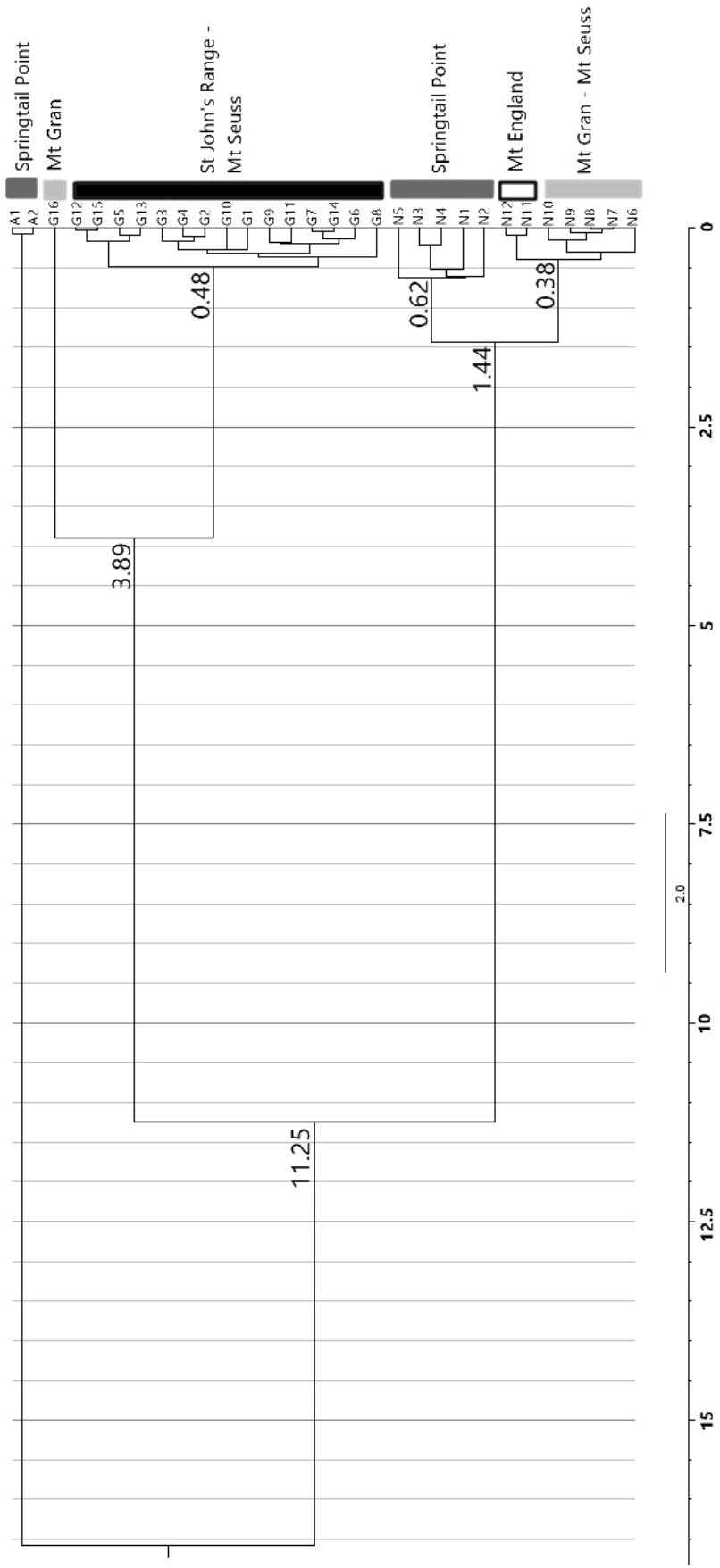
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